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A study of efficacy of disinfectants and bacterial contamination in Al-Hilla Teaching

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Submitted to the Council of the College of Medicine - University of Babylon In Partial Fulfillment of the Requirements For the Degree of Master of Science In Medical Microbiology

By

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١٤٢٧ Tho Alhija

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بسم الله الرحمن الرحيم

﴿ وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي
وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلاَّ قَلِيلاً ﴾

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List of Abbreviations

AIDS	Acquired immunodeficiency syndrome
DNA	Deoxyribonucleic acid
CSOM	Chronic Suppurative Otitis Media
E.coli	***Escherichia coli***
EMB	Eosin Methylene Blue
ENT	Ear-Nose and Throat
IU	International unite
LPS	Lipopolysaccharides
mecA	Methicillin Resistant gene
µg	microgram
µl	micro-liter
ml	milliliter
mm	millimeter
MR	Methyl Red Reagent
MRSA	Methicillin resistant ***Staphylococcus aureus***
MSSA	Methicillin Sensitive ***Staphylococcus aureus***
NCCLs	National Committee for Clinical Laboratories Standards
NIs	Nosocomial Infections
P.aeruginosa	***Pseudomonas aeruginosa***
PBP	Pencillin binding proteins
QAC	Quaternary ammonium compounds
S.*aureus*	***Staphylococcus aureus***
Spp.	Species

TMP-SMX	Trimethoprim-Sulfamethoxazole
KIA	Kligler's Iron agar
UTI	Urinary Tract Infection
VP	Voges-Proskauer Reagent

Abstract

In this study a total of ٢٨٤ samples were investigated for bacterial contamination. The samples included ١١٥ samples being collected from different clinical cases (burns, wounds, chronic suppurative otitis media-CSOM and urinary tract infections-UTI and ١٦٩ samples being collected from Al-Hilla Teaching Hospital environment during a period of eleven months from November ٢٠٠٤ to September ٢٠٠٥.

The study focused on certain bacterial isolates represented by ***Pseudomonas aeruginosa, Staphylococcus aureus*** (including **methicillin resistant *S.aureus* (MRSA))**, ***Escherichia coli*** and ***Bacillus subtilis*** as these organisms are the common causes of nosocomial infections and dominantly distribute in hospital environment.

The results indicated that ***P.aeruginosa*** was detected in a higher frequency in which it accounted for (٣٢٪), (٢١.٤٪) and (٢٠%) in burns, wounds and CSOM infections, respectively, followed by ***S.aureus*** in which it constituted (٢٠%),(٢١.٤٪) and (١٣.٣٪) of these infections, respectively. The sensitivity of ***S.aureus*** to methicillin was detected and it was found that (٤٠٪) and (٣٣.٣%) of ***S.aureus*** causing burns and wounds infections were resistant to methicillin .

E.coli was also found in a remarkable frequency. It constituted (١٦%),(١٧.٨) and (٦.٦٪) in burns, wounds and CSOM infections. ***E.coli*** was found to be the most common agent causing UTI since it accounted for (٣٥.٢٪) of these infections .

The results show that the hospital environment was highly contaminated, that the contamination rate of ENT out patients clinic was (٨٢.٣٪). A

remarkable high contamination rate was observed in emergency ward (٧٥%), burn ward. (٧٢٪), surgical ward (٧٠.٤٪) and operating room (٥٧.٥٪). The contamination rate detected in kitchen and laboratory hospital at the percentage of (٦٠٪) and (٤٠٪) respectively, while for in-use disinfectants antiseptics was (٦٦.٦٪).

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 (S_1)type (while chloroxylenol(S_2)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 $P < ٠.٠٥$.

The effect of the antibiotics on bacterial isolates identified in this study showed that all isolates were resistant to amoxicillin and ampicillin, (٨٢٪)of these isolates were resistant to cephalexine, (٨٠٪) to trimethoprime–

sulfamethaxazole, (٧٤٪) to gentamicin, (٧١٪) to cefotaxime, (٣٥٪) to ciprofloxacine ,(١٦٪) to amikacin.

The study indicate that there are some relation between resistance of ***P.aeruginosa*** to disinfectants and antibiotics.

الخلاصة

تضمنت هذة الدراسة ٢٨٤ عينة تم جمعها و ذلك لغرض التحري عن التلوث البكتيري و قد شملت (١١٥) عينة تم جمعها من إصابات الحروق, الجروح, التهاب ألأذن الوسطى القيحي المزمن و التهاب المجارى البولية بالإضافة إلى (١٦٩) عينة تم جمعها من بيئة المستشفى خلال الفترة من تشرين الثاني ٢٠٠٤ إلى أيلول ٢٠٠٥ من مستشفى الحلة التعليمي .

في هذة الدراسة تم التركيز على عزل أنواع بكتيرية معينة تمثلت ببكتيربا الزائفة الزنجارية ***P.aeruginosa***,العنقوديات الذهبية ***S.aureus*** المقاومة للمثيسلين **(MRSA)**, الاشريكية القولونية ***E.coil*** والعصويات ***B.subtilis*** على اعتبار أن هذة الأنواع من الأنواع الشائعة المسببة لعدوى المستشفيات و تنتشر بصورة واسعة في بيئة المستشفى.

اظهرت النتائج أن بكتريا الزائفة الزنجارية ***P.aeruginosa*** تتواجد بتردد عالي نسبيا آذ شكلت (٣٢٪), (٢١.٤٪) و (٢٠٪) من البكتريا المعزولة من إصابات الحروق, الجروح و ألنهاب ألأذن الوسطى ثم جاءت بعدها بكتريا المكورات العنقودية الذهبية ***S.aureus*** آذ شكلت (٢٠٪) ,(٢١.٤٪)و(١٣.٣٪) من البكتريا المسببة لتلك الإصابات .تم اختبار حساسية بكتريا العنقوديات الذهبية ***S.aureus*** تجاه مضاد المثيسلين و أتضح أن(٤٠٪) و(٣٣.٣٪) من بكتريا العنقوديات الذهبية ***S.aureus*** هي المعزولة من إصابات الحروق ,و الجروح مقاومة للمثيسلين.

وجدت بكتريا الاشريكية القولونية ***E.coli*** بتردد ملحوظ نسبيا حيث بلغت نسبتها (١٦٪)و(١٧.٨٪) و(٦.٦٪) من البكتريا المعزولة من إصابات الحروق, الجروح و التهاب ألأذن الوسطى كما أنها تعتبر من ألأنواع البكتيرية الشائعة المسببة لالتهاب المجارى البولية آذ أنها تشكل (٣٥.٢٪) من البكتريا المسببة لالتهاب المجارى البولية.

لقد أظهرت الدراسة تلوث بيئة المستشفى بصورة كبيرة حيث كان معدل التلوث في استشارية الأنف و الأذن و الحنجرة (٨٢.٣٪) وتم ملاحظة نسبة عالية من التلوث البكتيري في ردهة الطوارىء حيث بلغت (٧٥٪), و في ردهة الحروق(٧٢٪), ردهة الجراحية(٧٠.٤٪) و صالات العمليات الجراحية (٥٧.٥ ٪) في حين بلغت نسبة التلوث البكتيري التي تم ملاحظتها في مطبخ ومختبرات المستشفى (٦٠٪) و(٤٠٪) كما بلغت نسبة التلوث البكتيري للمعقمات والمطهرات قيد الاستعمال في المستشفى (٦٦.٦٪)و في هذة الدراسة تم اختبار كفاءة المعقمات و المطهرات المستعملة في المستشفيات تجاه العزلات البكتيرية بثلاث طرق هي طريقة التخافيف Broth dilution method (و ذلك لتحديد التركيز المثبط ألأدنى) , طريقة ألانتشار بالأقراص Disc Diffusion method و طريقة الانتشار بالحفر Well Diffusion method.

أظهرت نتائج تحديد التركيز المثبط الأدنى أن الهبتين أكثر المعقمات تأثيرا على البكتيريا المفحوصة ثم جاء بعدة من حيث قوة التأثير كل من الديتول النوع (S_1)(بينما اظهر الديتول نوعي(S_1)و(Sp)فعالية اقل),هايبوكلورات الصوديوم (القاصر),الفورمالين ,sodium dichloroisocynurate و الأيودين في حين لم يظهر معقم السافلون أي تأثير على البكتريا المفحوصة.

أبدت بكتريا العنقوديات الذهبية ***S.aureus*** حساسية تجاه معظم المعقمات و المطهرات بينما كانت بكتريا 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 <٠.٠٥.

أما بالنسبة لتأثير بعض المضادات الحيوية على العزلات البكتيرية التي تم التعرف عليها فى هذه الدراسة فقد أظهرت النتائج أعلى نسبة مقاومة للاموكسلين و الأمبيسلين حيث بلغت

(١٠٠٪),(٨٢٪) من العزلات كانت مقاومة للسيفولاكسين,(٨٪) للترامثبريم-سالفاميثوكساسولول,(٧٤٪) للجنتمايسين(٧١٪),للسيفوتاكسيم , (٣٥٪)للسيبروفلوكساسين و(١٦٪) اللأميكاسين.

في هذة الدراسة تم الإشارة إلى أنه توجد علاقة ما بين مقاومة بكتيريا الزائفة الزنجارية ***P.aeruginosa*** للمعقمات و المضادات الحيوية.

١-١:Introduction

Microorganisms occur every where on the surface of the earth and they are able to grow and survive under wide range of environmental conditions. Bacteria are the most successful living organisms and their ubiquity ensure that human are obligated to live in constant and intimate contact with a wide variety of species. Although the number of species capable of causing disease is relatively few but others have the ability to causing diseases if giving right conditions(Atroshi *et.al.*,٢٠٠٤).

During the last ١٠-١٥ years, The use of antibiotic for treatment and prophylaxis has increased (Percival *et.al.*,١٩٩٨). Overuse of antibiotics in human has led to rapid evolution of bacterial resistances to multiple drugs (Fuchs *et.al.*,١٩٩٦),therefore man has been searching for toxic substances which kill or inhibit the growth of microorganisms mainly to prevent their infective or destructive action with lowest possible effect on human, these chemical substances are referred to as Germicides and it's including disinfectants and antiseptics(Andreas *et.al.*,٢٠٠٣).

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(Mckenry and Salerno,٢٠٠١).

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 (MacDonnell and Russell,١٩٩٩).

In the two past decades, evidences have accumulated indicating that the hospital environment can represent an important source of nosocomial pathogens for high risk patient(Muder,١٩٩٥) Nosocomial infections are the - major public health problem through out the world because of their frequency, severity and costs.

An ideal disinfectant to overcome the antimicrobial resistant pathogens should have broad spectrum of antimicrobial activity (Mandell *et.al*.,١٩٩٥) and the efficacy of these agents may be affected by PH, detergent base, temperature, organic matter, ionic and type of the surfactants (Schorer and Eisele,١٩٩٧).

The wide spread use of these agents has promoted some speculation on the development of microbial resistance(Denyer *et.al*.,١٩٨٥) 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. Germicides have multiple target site for their cidal effects on microorganisms while antibiotic have single target site (Russell *et.al*.,١٩٩٧). It will be continued requirement for new and potent antimicrobial agents together with techniques suitable for control and destruction of microbial pathogens(Drexler,١٩٩٤). This study is ***P. aeruginosa*** an attempt to:

١-Detection the common contaminant bacteria among the hospital environment ٢-Determination the type of bacterium being highly resistant to the action of disinfectants and antiseptics.

٣-Determination the level of resistance to the disinfectants and antiseptics

٤-Determination the proper effective concentration of the disinfectants and antiseptics towards tested bacterial isolates .

٥-Determine the most effective antimicrobials agents against pathogenic bacteria isolated from burn patients

١-٢:Literatures review

١-٢-١:Nosocomial infection (NIs) Occurrence and Epidemiology

More people die every day from hospital infection than from automobile crashes, drowing, falls, burns and poisoning combined (Garcia-Martin *et.al.*, ٢٠٠١). Nosocomial infections may be one of five leading causes of death in the United State and must make their prevention a public health priority (MacDonald and Jarvis,١٩٩٨). Nosocomial infections causes or contributes to thousand of death annually(Brownlie *et al.*,١٩٩٠). In USA it has been estimated that more than ٢ million patients are infected in hospital each year(Steed,١٩٩٩). At least ٥٪ to١٠٪ of patients acquire infections during hospitalization (Struelens,١٩٩٩) and more than ٨٠,٠٠٠ deaths each year have been directly linked to the development of NIs(Floom,١٩٩٨).The majority of NIs are not associated with outbreak, they are endemic rather than epidemic infections(Hacek *et.al.*,١٩٩٩).

١-٢-٢:Identification of nosocomial infection (Definition and Origin)

Nosocomial infections occur encompass almost clinically evident infection that don't originate from patient original but it occurs at the time of hospital admission(Jarvis,٢٠٠١). NIs originate from either endogenous or exogenous source. Endogenous infections are caused by microorganisms already part of host flora

While exogenous infections are these caused by microorganisms obtained from animates(from another patients or hospital health workers) or inanimate source (medical device, surgical operation or nursing procedure) of hospital after admission to hospital**(** Garner,١٩٩٦**).**

١-٢-٣:Determination of nosocomial infections(NIs):

There are three principles determinants factors that agiven patient will acquire NIs according to(Baron *et.al.*,١٩٩٤) and these are:

A- The inoculum's and virulence of infecting organisms. B- The susceptibility of the patient for infections. Hospitalized person have increased susceptibility for infection and the risk factors for these as described by (Tasota *et.al.*,١٩٩٨) are:

١-Many patient are immunocompromised

٢-Severity of illness ٣-

Physiological stressor and Psychological stressors ٤-Age

٥- Prophylaxis for stress ulcer ٦-

Sleep disturbance and malnutrition

C-The nature of patients exposure to the infecting organisms .

The primary source of pathogenic organisms in NIs is by direct contact with infected patients, although a symptomatic carriers may transmit such infections in

which health care workers may become persistently colonized with pathogenic flora like ***S.aureus***, Gram negative bacilli such as ***P.aeruginosa*** and the nasal contamination by health care workers represents important hospital reservoir of ***S.aureus***, while the second most common way of spread NIs is by contaminated devices such as needles, catheters and endoscopes (Wenzal,١٩٩٧).

١-٢-٤:Types of nosocomial infections

Platt *et.al*., (١٩٩٨)described four types of NIs they are as follows:

١-Urinary tract infection (UTI) which accounted for ٣٩٪ of NIs .

٢-Surgical wound infection (post operative wound) which accounted for ١٧٪ of NIs.

٣-Respiratory tract infection (Primarily Pneumonia) which accounted for ١٨٪ of NIs.

٤- Blood stream infections (Bacteremia) which accounted for ٧٪ of NIs.

Stephen, (١٩٩٨) has pointed out that the other important types of NIs are :

٥- Skin damage(especially burns)

٧- Gastrointestinal tract infection .

Central nervous system infection

Hospitalized patients commonly acquire nosocomial pathogens such as ***S.aureus, Pseudomonas*** spp. and other Gram- negative and Gram-positive bacteria and most of them were resistant to antimicrobial agents

Nosocomial bacteremia is most often related to the use of intravenous devices such as catheters or cannula in which it may be contaminated by direct contact and spread via health care personal or from the patient own skin flora. In addition, contaminated vechicles such as intravenous fluids or arterial pressure transducer have been important source of nosocomial bacteremia.(Valenti, ١٩٨٠).

Surgical wound infections may acquire at the time of surgery and the reservoirs of bacteria causing these infections must be located in the operating theater and an exception to this are the wounds being not closed primarily but left open(secondary closure) rendering them susceptible to post operative infection(Grinbaum *et.al.*,١٩٩٥). Generally, it is accepted that patient endogenous flora is the most common source of surgical wound infections (Corbach,١٩٩١). It has been reported that the risk of these infections increase when open drain in put incision site. The drain it self act as portal of entry for pathogenic organisms (Ganguly *et.al.*,٢٠٠٣). ***S.aureus*** has been reported to be the dominant species associated with these infections followed by Enterobacteriacea (Speller and Humphreys, ١٩٩٨). In addition, within recent years ,there has been a growing prevalence of Gram–negative bacteria as a causes of serious wounds infections in many hospitals and ***P.aeruginosa*** has been of particular interest (Anderssen *et.al.*,٢٠٠٢).

١-٢-٥:Burns infections

Skin is the normal defense barrier against any microorganisms especially pathogenic organisms and burn injury provide suitable site for bacterial multiplication because of large open wound area containing necrotic tissue (Deich *et.al.*,١٩٨٧**)**. In addition, to general state of immunosuppression caused by

impaired functiong of immune system and in these conditiong microorganisms can easily multiply and colonize wound to high densities(Rastegar Lari *et.al.*, ١٩٩٨).

The most important reservoirs of microorganisms for burns patients is the patients population. Exogenous organisms from the hospital environment are generally more resistant to antimicrobial agents than endogenous organisms. The typical burn wounds are initially colonized predominantly with Gram–positive bacteria which fairly replaced by antibiotic susceptible Gram–negative bacteria usually within weak of burn injury. If wound is delayed and the patient become infected, then the case may require treatment with broad spectrum antibiotics. These organisms may be replaced by antibiotics-resistant bacteria (Pruitt and MacManus,١٩٩٢). The most common bacteria causes NIs is the multi-drugs resistant ***P.aeruginosa*** (Becker*et.al.*,١٩٩١) and ***S.aureus*** especially the ***Methicillin Resistant Staphyllococcus aureus***(**MRSA**) has been reported to be the most frequently pathogens causes NIs and has become an endemic organism in many burn unite.

١-٢-٦:Predominant nosocomial pathogens

Historically, ***Staphylococcus*** spp. **,** ***Pseudomonas*** spp. and ***E.coli*** have been the nosocomial infection troika. During ١٩٥٠ and ١٩٦٠ ***S.aureus*** were the hospital pathogens of major concern and caused major nosocomial problem, while in the ١٩٧٠ Gram–negative bacilli particularly ***P.aerugiosa*** and Enterobacteriaceae became synonymous with NIs and by the late of ١٩٨٠ and early ١٩٩٠, several different classes of antimicrobial drugs that effective against Gram–negative bacilli provided a brief respite in which during this time, **Methicillin Resistant *Staphylococcus*** and **Vancomycin Resistant *Enterococcus*** emerged. Later the three

most common Gram–positive bacteria caused NIs were ***S.aureus,*** **coagulase negative** ***Staphylococci*** and ***Enterococci*** and the Gram–negative bacteria represented by ***E.coli, P.aeruginosa***, ***Enterobacter*** spp. and ***Klebsiella Pnemoniae*** (Weinstein,١٩٩٨). The common Gram–positive and Gram-negative bacteria causing NIs are briefly presented now:

١-٢-٦-١:Gram – positive bacteria

The primary important pathogens belong to Gram- positive bacteria causing NIs are **Mehticillin Resistant *S.aureus* (MRSA**), **Vancomycin–Resistant *Enterococci***, **coagulase negative *Staphylococci*** and multidrug resistant ***Streptococci*** (Ronald and Jones,٢٠٠١).

١-٢-٦-١-١:Methicillin Resistant *Staphylococcus aureus* (MRSA)

S.aureus is always referred to as the common causes of NI especially systemic infection such as bacteremia,endocarditic and wound infections (Katz *et.al*.,١٩٩٨). The resistance among these bacteria is a major concern in which both methicillin and vancomycin resistance occures in staphylococci (Archibald *et.al*.,١٩٩٧)and the most common type of resistance among ***S.aureus*** is the methicillin resistance(Jones and Ptaller,١٩٩٨). **MRSA** become important nosocomial pathogens in the late ١٩٧٠ in the United State and in the two past decades **MRSA** has been spread–world wide and becoming endemic inhabitant of many hospital(Wagenvoort,٢٠٠٢). In USA, **MRSA** was isolated from hospitalized patient including Pneumonia,wounds infections,Bacteremia and urinary tract infections(Jones and Doern,١٩٩٨).

Most infection by **MRSA** is likely to be more sever and require longer hospitalization and treatment than **Methicillin Sensitive *S.aureus*** (**MSSA*). MRSA*** neither more infectious nor more virulent than **MSSA** but it is just more difficult to treat because it can resist to all β-lactam antibiotics and may be resistant to aminoglycosides and quinolnes groups (Boyce *et.al.*,١٩٩٤). In addition, the importance of **MRSA** is not lies in their resistance to all β-lactama but also on it's own resistant to various other important antimicrobial agents including disinfectant and antiseptic(Garner,١٩٩٦). Resistant ***S.aureus*** isolates carrying antiseptic resistant gene has been isolated from clinical cases in which three determinants, the qacA qacB, and qac C gene have been identified that confer resistance to organic cations by means of multidrug efflux and the higher providence of these gene might be found because of interest of selective pressure imposed by the disinfectants agents used in the hospitals (Mayer *et.al.*,٢٠٠١)**. MRSA** has been reported to possessed the qac A /B genes on the wide spreed multiresistance plasmid like epsk I(qacA) and these plasmids frequently carry a number of determinates that responsible for the resistance of wide a rang of antimicrobial agents (Paulsen *et.al.*,١٩٩٧).

١-٢-٦-٢:Gram- negative bacteria

Gram–negative bacteria seem to be frequently associated with NIs, causing clinical antimicrobial problem particularly those of multidrug resistant, represented by ***P.aeruginosa, E.coli, Klebsiella Pnemoniae*** and ***Enterobacter*** ssp. which have been reported to be highly resistant towords pencillins, cephalosporins,aminoglycosides and fluroquinolones (Ronald and Jones,٢٠٠١).

١-٢-٦-٢-١: *P. aeruginosa*

Almost ٥٠ years ago, ***P.aeruginosa*** was rarely considered as real pathogen and in ١٩٧٠ it was recognized as the microorganisms associated with bacterimia in the neutropenic host, while now a day it is a among the most common opportunistic pathogens involved in NI (Pollack,١٩٩٥). In Belgan this organism was the first Gram –negative pathogens causes NIs(Glupczynski *et.al*.,١٩٩٩) while in Turkey it's the third most common nosocomial pathogens (Gencer *et.al*., ٢٠٠٢).

P.aeruginosa has been reported to be associated with nosocomial Pneumonia(Wiblin,١٩٩٧), UTI, surgical wound infection(Kluytmans *et.al*.,١٩٩٧) and bacteremia (Gordon *et.al*.,١٩٩٨) and the outbreak with this bacteria in burn units are still associated with high death rate (٦٠٪). It is also responsible for Pneumonia and septicemia with attributable deaths rates reaching to (٣٠٪)(Van Delden and IgIewski,١٩٩٨).

P.aeruginosa causes sever invasive diseases in critically ill and immunocompromised patients. It is often isolated from patients hospitalized longer than ١week(Ehrlich, ٢٠٠٣), therefore in an expanding AIDS population ***P.aeruginosa*** bacteremia has been associated with (٥٠٪) of deaths(Mendelson *et.al*.,١٩٩٤). Infections with ***P.aeruginosa*** seem to be endemic or sporadic (Davies and Bullock,١٩٨١). ***P.aeruginosa*** has the ability to grow in moist environments with simple nutrients requirement and resistant to antibiotic, disinfectants and antiseptics in which it is known to be colonize the hospital environment (Cortes *et al*.,٢٠٠١) particularly sink, tap water, antiseptic solution, respiratory equipment and bronchoscope(Trautmann *et.al*.,٢٠٠١) In addition, the lower respiratory resistance of mechanically ventilated patients, gastrointestinal tract of patient have anticancer chemotherapy as well as mucosa and skin hospitalized patients

treated with broad spectrum antibiotic can be colonized with this bacteria at rate exceeding ٥٠٪(Giamarellou,٢٠٠٢).

١-٢-٦-٢-١-١:Clinical manifestation of *P.aeruginosa*

P.aeruginosa causes various diseases. localized ***P.aeruginosa*** infection following surgery or burns commonly results in generalized and frequently fatal bacteremia and it also causes corneal infections following eye surgery or injury, It is found in pure cultures, especially in children with middle ear infections, such infections(e.g. ear and eye infections) remain localized while wounds and burns infections and infections in leukemia and lymphoma patients results in sepsis. The difference is most probably due to altered host defense. UTI following introduction of ***P.aeruginosa*** on catheter or in irrigating solutions are not uncommon(Coburn *et.al.*,١٩٨٩).

Most cystic fibrosis patients are chronically colonized with ***P.aeruginosa*** and causes chronic lung diseases, ***P.aeruginosa*** Pneumonia may occur in other patients following the use of contaminated respirators. ***P.aeruginosa*** occasionally causes meningitis following lumber puncture and endocardities following cardiac surgery, Moreover it has been associated with some diarrheal diseases episodes since the first reported case of ***P.aeruginosa*** infections in ١٨٩٠. These organisms has been increasly associated with bacteremia and currently accounts for ١٥٪ causes of Gram–negative and the overall mortality associated with ***P.aeruginosa*** bacteremia a bout ٥٠٪ (Dunn and Wunderink ,١٩٩٥).

١-٢-٦-٢-١-٢:Pathogensis of *P.aeruginosa*

For ***P.aeruginosa*** to initiate infection it usually requires a substantial break in first line defense such a break can result from breach or by pass of normal cutaneous or mucosal barrier (e.g. trauma, surgery, serious burns or indwelling devices), disruption of the protective balance of normal mucosal flora by broad spectrum antibiotics or alteration of the immunologic defense mechanisms (e.g. in chemotherapy induced neutropenia, mucosal clearance from cystic fibrosis, AIDS and diabetes mellitus.

The first step in infection is colonization of altered epithelium and up to ٥٠٪ of hospitalized patient are might risk for ***P.aeruginosa*** colonization(Pollack, ١٩٩٥) in which adherence of ***p.aeruginosa*** to epithelium is probably mediated by ٤pili and flagella which primarily responsible for motility, may also act as adhesive factor to epithelial cells (Gordon *et.al*.,١٩٩٨). Nicas and Iglewski, (١٩٨٥) pointed out that ***P.aeruginosa*** produces several extra cellular products that cause extensive tissue damage, blood stream invasion and dissemination. These virulence factors are summarized as follows:

١-Exotoxin A it is produced by most strains of ***P.aeruginosa*** that causes clinical infection like diphtheria toxin. It is responsible for local tissue damage and bacterial invasion and possibly immunosuppresions (Wick *et.al*., ١٩٩٠).

٢-Exotoxin S it is responsible for direct tissue destruction in lung infections and may be important for bacterial dissemination

٣-Two hemolysines, phospholipase and rhamolipid produced by ***P.aeruginosa*** and this may act synergistically to break down lipid and lecithin and may contribute to tissue invasion by their cytotoxic effects (Van Delden and Iglewski, ١٩٩٨)

٤- Proteases which are assumed to play a major role during the cute pseudomonal infection. Several types of proteases are produced by ***P.aeruginosa*** including las B elastase, las A elastase and alkaline protease.

The role of alkaline protease in tissue invasion and systemic infections is unclear yet (Kernacki *et.al.*,١٩٩٥). Elastolytic activity that destroy protein elastin is major virulent determinate during acute infection and is believed to destroy elastin in human lung tissue resulting in pulmonary hemorrhages. In addition, mucoid strains elaborate mucoid exopolysacchaide (algenate layer) surrounding the cell and offer protection from host immune factors(Turner,٢٠٠٠) .

١-٢-٦-٢-١-٣:Resistance of *P.areuginosa* to antibacterial agents

The increasing emergence of ***P.aeruginosa*** resistance to antibacterial agents is a major challenge in medicine***. P.aeruginosa*** has high intrinsic resistance to many antimicrobial mainly β–lactam(Livermore,٢٠٠٢), Moreover, it may acquire additional resistance mechanisms including decreased outer membrane permeability, penicillin binding protein modification, acquisition of plasmid encoding for production of extended spectrum β–lactamases, acqusion of metallo β–lactmase or others enzymes, increased expression of efflux pumps system and decreased porins expression. These mechanisms of resistance may develop during the course of antimicrobial therapy (Deplano *et.al.*, ٢٠٠٥).

١-٢-٦-٢-٢: *E.coli*

E.coli is the common and famous member of the family Enterobacteriaceae in which it inhibits the intestinal tract of human and animals as a normal flora

(Karmali *et.al*.,١٩٨٣).Consequently, the presence of ***E.coli*** everywhere is an indicators of faecal products (Collee *et.al*.,١٩٩٦). ***E.coli*** causes extra intestinal and intestinal infections in immunocomprimised as well as in healthy individual and these infections included UTI, intra-abdominal infections, diarrhea, meningitis, bacteremia and wound infections(Bettelheim,١٩٩٢). ***E.coli*** was considered one of the most important antimicrobial resistant Gram–negative bacteria that impact on NIs due to it extended spectrum β-lactamases enzyme producsed by these bacteria (Ronald and Jones,٢٠٠١**).**

١-٢-٧:Resistance to antimicrobial agents and biocides

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*** (Braoudaki and Hitton,٢٠٠٤).

١-٢-٧-١:Resistance to antimicrobial agents

Over the past several decades, the frequency of resistance and it association with infectious diseases have increased at alarming rate (Ronald and Jones, ٢٠٠١),therefore bacterial resistance to multiple antibiotics characteristic the present decade (Levy,١٩٩٨). In USA, ٥٠٪ -٦٠٪ patients have acquired NIs which caused by antibiotics resistant pathogens (Weinstein,١٩٩٨).

The first step that contribute to increasing NI caused by resistant pathogens are the selection of resistant mutant strains from the patient own flora during antibiotic treatment or the transfer between bacteria of mobile genetics

determinates of resistance (plasmid and transposons)and then subsequently resistant strains spread among patients in hospitals. In addition, microbial pathogens have the capability to alter their genetic structure possibly allowing them to resist all forms of antimicrobial medications in which mechanisms of antibiotic resistance vary between pathogens.

Bacteria can resist antibiotic by alternating the binding site for antibiotics, by forming lower binding site affinity, by changing the degradative systems and by clearing of the antibiotics from the cell. However, bacteria are highly adaptable and can transfer the genes encode for resistance to other bacteria in population and confer resistance to other antibiotics(Glick,٢٠٠٥).

١-٢-٧-٢:Resistance to disinfectants and antiseptics

The microorganisms have some survival strategies against biocides are either in intrinsic in nature or are acquired(Russell and Chopra,١٩٩٦). These are summarized below:

١-٢-٧-٢-١:A-Intrinsic resistance

Intrinsic resistant is natural, chromosomally controlled property of bacterial cell and it including: impermeability, efflux pump, physiological adaptation (biofilm) and enzymatic inactivation.

١-Impermeability of cell membrane

Among bacteria, biocide sensitivity is based on the permeability of the biocide through the cell wall(Lambert,٢٠٠٢) and impermeability is influenced by the composition of cell wall and physiologic adaptation of the microorganisms to it's environment (Semmelweis, ١٩٩٥).

Gram–negative bacteria are generally less susceptible to biocides because of their complex cell wall(Sheldon,٢٠٠٥) 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. The outer membrane of ***P.aeruginosa*** is responsible of the highly resistance to many antimicrobial agents in comparison with other organisms in which there are some difference in Lipopolysaccharides (LPS) composition and in the cation content of the outer membrane(MacDonnell and Russell,١٩٩٩). The high $Mg^{٢+}$content aid in producing strong Lps–Lps links, furthermore the small size of porins may not permit general diffusion through them in to these bacteria(Pallent *et.al*.,١٩٨٣) .

٢- Physiologic adaptation

As in intrinsic mechanisms, the association of microorganisms with solid surface lead to generate of biofilm which is defined as a consortium of organisms organized with extensive exopolysacchariedexopolymer(Donlan, ٢٠٠١) and indifferent part of biofilm experience different nutrient environment, oxygen tensions and growth rate are likely to be reduced within depth of the biofilm as of nutrient limitation (Poxton,١٩٩٣),therefore bacteria within biofilm may be much less sensitive to antibiotics and biocides(Brown and Gilbert,١٩٩٣).

Biofilms are foci for contamination and colonization occur on implanted biomaterial and medical device, resulting in increased infection rate and possible occurrence of infection.Gram–negative bacteria can grow as biofilm in the catheterized bladder and are able to survive in concentration of chlorohexidine that are effective against organisms in non catheterized individuals (Stickler *et.al*.,١٩٩١). Ntsama-Essomba *et.al*.,(١٩٩٧) have been demonstrated that in the presence of biofilm the resistance of ***E.coli*** increased ٢٥ fold against a pre acetic acid and H_2O_2 mixture, ٥-fold against sodium hypochlorite and more than ٤٠٠ fold against benalkonium chloride In addition, Several years ago reports emerged on the contamination of iodophore antiseptic solution by ***P.cepacia*** and ***P.aeruginosa*** which caused peritoneal infections in infants and Pseudobacteremia in patients in intensive care unite. These organisms that were protected by biofilm matrix showed long–term (٦٨weeks) survival within the antiseptic solutions and had apparent level of resistance that would be expected of bacterial spore and the original source of the contamination appeared to be the water pipes system that colonized within biofilm –containing *pseudomonas* (Anderson and Vess,١٩٩١).

٣- Degradation of biocides

Some microorganisms are able to degrade and inactivate the biocides via enzymatic mechanisms and it has been reported for chlorohexidine,quaternary ammonium compounds (QAC) and phenylethanol (Gilbert and McBain,٢٠٠٣), Moreover, the enzymatic degradation of formaldehyde occurs by species such as ***P.putida*** (Chazal,١٩٩٥).

٤-Pump efflux system

Efflux pump is the major mechanism enable the microorganisms to resist antibiotic and biocides (Thanassi *et.al*.,١٩٩٧). It is intrinsic resistance of microorganisms(Nikaido,١٩٩٨) and efflux pump mediated by plasmid also occur. Efflux pump is encoded in gram–negative and gram-positive bacteria. The expression is induced through sub lethal exposure of agents (Levy, ٢٠٠٢) and expression of efflux pump is enhanced under condition of general stress. There are three separate multi drugs resistance determinants designated as qacA,qacB and qacC have been described in ***S.aureus***. The qacA gene confer the broadest resistance phenotype in which it located predominantly on disinfectants resistance plasmids. The qacA gene has been detected in strains with hospital outbreaks of ***S.aureus*** infection in Australia and the United kingdom, while the qacC gene has been found in ***S.aureus*** strains from clinical staphylococcal isolates being resistant to at least one disinfectant and of additional concern is the fact that many of these strains are **MRSA**(Leelapora *et.al*.,١٩٩٤). In ***P.aeruginosa***, the Mex .AB, Mex .CD and Mex .EF multi drugs efflux system act as transporter for whole rang of biocides and antibiotics and it coupled with the narrow prion channels in the outer membrane of this organisms and restrict diffusion of many antibacterial agent into cell, therefore, this probably responsible for the high intrinsic resistance of this species to antibacterial agents compared with other gram-negative bacteria (Schweizer,١٩٩٨) .

١-٢-٧-٢-٢:B –Acquired resistance to biocides

Such resistance occur by acqusion of plasmids or transposons or by mutation of target site. In gram-negative bacteria, studies described that changes in

permeability by mutation may lead to acquire biocide non susceptibility (Poole, ٢٠٠٢). Plasmid–encoding resistance to biocide had been most extensively investigated with mercurials and these resistance is plasmid borns, inducible and is a common proper of clinical isolates of ***S.aureus*** containing pencillinase plasmids(Kroll and Anagnostopoulos,١٩٨١). In addition, Plasmid-associated non susceptibility to low level biocide has been demonstrated in ***S.aureus*** and ***S.epidermidis***(Behr *et.al*., ١٩٩٤). Some **MRSA** containing plasmid encoding gentamicin resistance (**MGRSA**) also have increased MIC value toward biocides such as QAC, chlorohexidine, ethidium promide and isothionate and gentamicin nucleic acid–binding (GNAB) plasmid conferring chlorohexidine resistant (Philips *et.al*.,١٩٩٠). Kaulfers and Laufs (١٩٨٥) stated that plasmid mediated resistant to formaldehyde has been demonstrated in ***Serria marcessens***. Although plasmid mediated resistance to biocides has been found in Gram–bacteria but intrinsic resistance is of greater significantly (Russell *et.al*.,١٩٩٧). Sutton and Jacoby observed that plasmid dRP, didn't significantly alter the resistance of ***P.aeruginosa*** to QAC, chlorohexidine and chlorinated phenols although increased resistance to hexachlorophene was observed (Sutton and Jacoby,١٩٧٨).

١-٢-٧-٣:Cross resistance between antimicrobial agents and biocides

At the present there are comparatively few reports pointed out that there are cross resistance between clinically used of antibiotic and biocides (Braoudaki and Hitton, ٢٠٠٤). Cross resistance may occur because of the following :

١-Common target side between biocide and antibiotic might lead to selection of mutants altered in such targets site by either agent and the emergence of cross resistance.

٢-Suitable difference in the biocide and antibiotic susceptibility of antibiotic resistant strains might facilate their selection and maintenance in the environment by low sub effective concentrations of biocides and antiseptic as well as the primary antibiotic.

٣- The indiscriminate biocide application might cause the evolution and selection of multi drugs resistant strains through mechanisms such as efflux pumps(Gilbert and McBain ,٢٠٠٣). Cross resistance considered to be intrinsic (Russell *et.al*.,١٩٩٧) and this linkage might be due to a nonspecific reduction in cell permeability, which doesn't allow chemically unrelated molecules into resistant cell (Tattawasart *et.al*.,١٩٩٩). Cross resistance between antibiotics and biocides and between different biocides has been reported for ***P.aeruginosa*** by Lambert and colleagues(٢٠٠١). In addition, isolates of ***Proteus mirabilis*** responsible for hospital outbreak was found to be resistant to CHX and antibiotics. Russell and Chopra,(١٩٩٦) mentioned that extensive use of biocides could lead to the selection of staphylococcal strains showing resistance to both antibiotics and biocides while Russell *et.al*.,(١٩٩٨) has been observed a cross resistance to chlorohexidine, QAC and at least five antibiotics for gram–negative organisms isolated from UTI and proposed that the wide spread use of CHX was the reason for selecting antibiotics resistant strains but Wenzal,(١٩٩٧) stated that the common NIs pathogens representd by ***P.aeruginosa, Klebsiella Pneumonia, E.coli, Saureus S.epidermidis*** and ***Enterococcus*** spp. can be equally susceptible to disinfectants as antibiotic susceptible strains.

١-٢-٨: Disinfectants and Antiseptics

Disinfectants and antiseptics are chemical compounds used in clinical medicine as intervention strategies that prevent the dissemination of nosocomial pathogens and are also used for personal hygiene and to prevent cross–contamination of food–born pathogens in home, restaurants, daycare centers and nursing home (Sheldon,٢٠٠٥). The skin disinfectant for example are used: To protect open wounds and other vulnerable sites from microorganisms transferred on the hand of staff, to protect patients tissue against his or her endogenous flora during surgery or an invasive technique and occasionally to treat carrier and dispersers of multi resistant strains e.g. **MRSA** (Ayliffe *et.al.*,٢٠٠١). Antiseptic and disinfectant are of various types and the choice of the most appropriate antimicrobial compounds for a particular purpose depends upon:

١- **Properties of the chemical agents** .The process of killing or inhibiting growth of microorganisms was a chemical reaction and the rate and the extent of this reaction will be influenced by concentration of agent, temperature, PH and time.

٢-**Microbiological challenge.** since the type of microorganisms present and the level of microbial contamination both have significant effects on the outcome of chemical treatment. Microorganisms them self differ in their susceptibility to biocide in which growing as vegetative cell which are more susceptible whereas a spores are extremely resistant as will as the physiological state and age of culture influence the susceptibility in which young cells are more easily destroyed than old non dividing cells because in old

cells the metabolic activity is slowed down and thickening of on capsulated of cell may increase the resistance of cells (Hugo and Russell, ١٩٩٨).

٣-Enviromental factors

The interaction between the organism and the agents may prevent or enhance by the environment. The presence of organic matters such as blood, animal tissue, feces and organic deposits may reduce the efficacy of biocidal agent because of :

A-The agent may combine with organic mater to form a product which is not microbiocidal i.e. the agent is inactivated **B-**The agent may combine to form a precipitate and then the interaction between the agent and the organisms is prevented **D-** Accumulation of organic matter may provide coating which may prevent interaction, therefore cleaning of medical device and the skin is important before application to disinfectant and antiseptic (Block,١٩٩١).

١-٢-٨-١:Mechanisms of action of disinfectants and antiseptics

Mechanisms of action of biocides on what ever 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 (Brown and Gilbert,١٩٩٣). In addition, the interaction at the cell surface can produce a significant effect on viability(Power,١٩٩٥) but most antimicrobial agents appear to be active intracellularly(Russell and Russell ,١٩٩٥).

١-٢-٨-٢:Spectrum activity of disinfectants and antiseptics

Disinfectant and antiseptic like antibiotic in it's action on microorganisms. Accordingly to Atroshi *et.al.*,(٢٠٠٤) these agents are classified into ٣ categories on it's effect on microorganisms**:**

١- High level biocide that kill 's all microbial pathogens including endospore if exposed for long enough time.

٢- Intermediate level biocide which kill 's all microbial pathogens including mycobacterium except endospore. ٣-

Low level biocide a germicide that kill 's most vegetative bacteria except bacterial endospore and mycobacterium.

١-٢-٨-٣:Overview of commonly used disinfectants and antiseptics

There are various biocides are tradionally used and each one has own unique characteristics which differentiate it from other. It is important to understand these characteristics in order to effectively allocate the most appropriate agent to the task at hand (Block ,٢٠٠١). Some tradional compounds are discussed below :

A-Hydrogen Peroxide (H_2O_2)

It is widely used as biocide for disinfection and antisepsis. It is clear, colorless and commercially available in variety of concentrations vary from ٦٪ to ٢٥٪ and ٦٪ concentration was significantly more used. H_2O_2 was high level disinfectant and it is considered as chemical sterilant (Vesley *et.al.*,١٩٩٢). It is non carcinogenic or mutagenic (Rutala,١٩٩٦),therefore, it considered environmentally friendly because

it is rapidly degraded into innocuous product of water and oxygene. In general,H_2O_2 was bactericidal and greater activity of H_2O_2 seen against Gram-positive than Gram-negative bacteria, virucidal, fungicidal and sporocidal activity achieved at high concentrations with long exposure time.

Hydrogen peroxide strong oxidant act by production of destructive hydroxyl free radicals which can attack membrane lipid, protein, DNA and other essential cell components in which it particularly exposed to sulfhydryl groups and double bound (Lever and Sutton,١٩٩٦). Catalase or other peroxidases produced by some aerobes and facultative anaerobes that possess cytochrome system may protect cell from a metabolical products of H_2O_2 and degrading H_2O_2 into water and O^2 but this can overwhetned by increased concentration used for disinfectant. (Turner,١٩٨٣). H_2O_2 is easily destroyed by heat or enzymes (catalase and peroxides). Concentrated solution of H_2O_2 may irritant the eye, skin and mucous membrane (Andreas *et.al*., ٢٠٠٣).

B-Povidone-Iodine(PVP-I)

Although aqueous or alcoholic (tincture) solution of iodine have been used for ٥٠ years ago(Favero and Bond,١٩٩١) but it is largely replaced by the iodophore because the side effects result from of iodophore solution such as staining, irritation of tissue, resorption and unstability is lower than from the use of aqueous solutions (Gottardi,٢٠٠١). Iodophore are chemical complexes with iodine bound to carrier such as polyvinyl pyrovidin and these complexes gradually release small of free microbiocidal iodine (Gruendemann and Mangum,٢٠٠١) in which iodopher preparation generally contain ٠.١٪ to١٪ iodine. Iodophores solution is intermediate level disinfectant and has broad

antimicrobial spectrum in which it is rapidly bactericidal, virucidal and less active against spore (Davies *et.al*.,١٩٩٣; Larson,١٩٩٥). Iodine is able to penetrate the cell wall of microorganisms quickly and attack key group of protein in particular the amino acid and fatty acid resulting in destruction of cell structure and enzymes (Boyce and Pittet, ٢٠٠٢) and it is seen that free iodine contributes to the bactericidal activity of iodophore(Berkelman *et.al*.,١٩٨١) Iodopher must be diluted according to the manufacturers directions to achieve best antimicrobial activity(Terleckyi and Alxler,١٩٨٧). Iodophores are widely used for antisepsis of skin, mucous membrane and wound. Body surface treated with iodine solution may absorb free iodine and diffuse into deep regions of body, therefore pregnant women and newborn should not be exposed to these agents(Bryant and Zimnerman ,١٩٩٥). The activity of iodine is reduced by organic material (Dxchodala,١٩٩١). Rutala,(١٩٩٦**)** stated that iodophore containing higher concentration of free iodine may be used for disinfection of medical equipments.

C-Chlorine –Releasing Compounds

In addition, to chlorine itself, there are three types of chlorine compounds– the hypochlorite, inorganic and organic chlorine (Joklik *et.al.,*١٩٩٤).The most important and widely used types are hypochlorite and organic N-chloro compounds such as sodium dichloroisocyanurate (NaDCC) (Springthorpe and Sattar,١٩٩٠). Hypochlorite is the oldest and widely used disinfectant(Favero and Bond,١٩٩١). It has been used for more than one century and still being regarded as important disinfectant commonly used in the hospitals. Sodium hypochlorites are usually called household bleach and commonly found in

concentrations of ٥.٢٥٪ -٦٪ (Rutala and Weber,١٩٩٥).

Sodium dichloroisocyanuric, containing the N=Cl group, possesses microbicidal activity and its action is slower than that of the hypochlorites, but can be increased under acidic conditions(Bloomfield *et.al*.,١٩٩٠). All chlorine compounds are powerful oxidizing agents that oxidize thiol group and halogenate amino groups in proteins. In aqueous solution, all chlorine compounds release hypochlorous acid, the most biocidal compound (Rutala , ١٩٩٠) in which inhibition of some key enzymatic bio-reactions in cell and denaturation of protein may play the major role in killing of microorganisms. This process is pH dependent and hypochlorite being more active in acid than alkaline condition, in low PH existence of OHCL the active factor in killing is favored over (hypochlorite ion OCL-) (Dxchodala,١٩٩١).

Chlorine compounds exhibited rapid killing against a wide spectrum of microorganisms including bacteria, virus and fungi and high level of available chlorine will enable it to eradicate acid fast bacilli and bacterial spore (Mckenry and Salerno,٢٠٠١).

Sodium hypochlorite is not staining but it is eye and skin irritant and it is inactivated by organic material and hard water (Andreas *et.al*.,٢٠٠٣). In addition, hypochlorite solution gradually lose strength on storage, therefore fresh solution must prepared before use, while sodium dichloroisocyanurate has a more stable and prolonged effect (Wenzal and Zaidi, ٢٠٠٠). Sodium dichloroisocyanurate has been reported to be less susceptible to inactivation by organic matter (Bloomfield,١٩٩٦).

D-Chloroxylenol

It is halogenated derivative of oxylenol develop in late١٩٢٠ and it is commonly used as antibacterial agent such as Dettol (Larson and Talbot,١٩٨٦). It is used as antiseptic and disinfectant and it is widely available in concentrations of ٠.٥٪ -٤٪ . It has broad spectrum activity that kill fungi, Gram–positive bacteria and it less active against mycobacterium and certain virus (Boyce and Pittet,٢٠٠٢). It's activity mainly duo to disruption of cell membrane and blocking production of adenosine triphosphate(Rotter,١٩٩٩). Chloroxylenol is minimally effected by organic materials, not significantly toxic, mild irritant and may trigger allergic reaction in some individuals (Archibald *et.al.*,١٩٩٧).

E-Formaldehyde

Formaldehyde (in aqueous solution or gas) has been used as disinfectant and sterilant for many decades. It is sold and used principally as a water–based solution called formalin(Akbar *et.al.*,١٩٩٤). It is high to intermediate level disinfectant and wide rang of microorganisms are destroyed by varying concentrations (Sharma,١٩٩٨). It has long been considered to be sporocidal by virtue of its ability to penetrate into the anterior of bacterial spore. It is inactivates microorganisms by alkylation's of amino and sulfhydryl groups of protein and ring nitrogen atoms of purine base (Rutala, ١٩٩٠).

The irritating vapors and pungent odor are produced by formaldehyde even at very low levels and inhalation of formaldehyde vapor may pose a carcinogenic risk, therefore, formaldehyde should in the workplace as a potential carcinogen and mutagenic agent(Wenzal, ١٩٩٧). For all these reasons

the use of this compound in health care institution is very limited despite it's broad spectrum biocidal activity (Wenzal and Zaidi,٢٠٠٠).

F-Chlorohexidine Gluconate (CHX)

It is cationic bisbiguanide, has been widely recognized as an effective and safe antiseptic for more than ٣٠ years ago (Ranganathan,١٩٩٦). It is widely used as antiseptic in surgical washing, oral treatment, wound antisepsis and burn management duo to broad spectrum efficacy and limited irritation. The most common concentration available and used is ٤٪. It exhibits intermediate rapidity of bactericidal action and effective against Gram-positive and Gram-negative bacteria, virucidal and fungicidal but it is not sporocidal (Denton,٢٠٠١). The proper concentration of CHX causes destruction of bacterial cell membrane, leading to leakage of cellular constituents, while high concentration causes coagulation of intracellular constituents.The activity of CHX depends pH and being the greatest antibacterial activity at PH ٧-٨(Russell *et.al.*,١٩٩٨). The activity of CHX is minimally reduced in presence of detergents, blood and other organic materials but it provides persistent antimicrobial action that prevent regrowth of microorganisms for as long as ٦ hours. It is nontoxic if it absorbed through the skin and don't have effect on wound healing but irritating(Gruendemann and Mangum,٢٠٠١).

G-Chlorohexidine Cetramide (Savlon)

It is effective agent against broad spectrum of microorganisms but has minimal effect on mycobacterium and fungi(Larson,١٩٩٥). It is a mixture of

chlorohexidine and cetramid. The ٤٪ of concentration of savlon is proper effective for use as antiseptic(Fakhriddeen,٢٠٠١). The activity of this agents is reduced by blood and organic material and have been and it has been reported to causes irritation (Walsh *et.al*.,١٩٨٧).

١-٢-٨-٤:Contamination of disinfectants and antiseptics

Like other substances, disinfectants and antiseptics are also exposed to microbial contaminations which result in most complication and may deaths Contamination of disinfectants and antiseptics before use can result in clinical infections and outbreaks. Contamination of biocides was usually due to inadequate concentration or inadequate care of containers.Contamination and growth of gram–negative bacteria in disinfectants solution has been reported in Rajavithi General hospital and found that chlorohexidine cetramide was contaminated with ***P.maltophilia*** and the hypochlorite solution was contaminated with ***E.clocacae*** (Kajanahareutai *et.al*.,١٩٩٥). Consequently, one cause of NIs, which has been documented is the use of contaminated disinfectants in which seven cases of UTI in children occurred because of contaminated CHX solution used for disinfecting the bladder–irrigation reservoir. Various bacterial species have been isolated from different containers containing ٠.١% CHX, contaminated stock disinfectants, prolonged usage of disinfectants, and from no washing disinfectants jar before refilling were all reported to be contaminated by bacteria (Keah *et.al*.,١٩٩٥). This is resulted because inappropriate technique of preparation for use in hospital, or is contamination of the products themselves (Oie and Kamiya,١٩٩٦; Kajanahareutai *et.al*.,١٩٩٥).

٢-١:Instruments and Materials

٢-١-١:Laboratory instruments

The Laboratory instruments used in this study are shown in Table ٢.١.

Table (٢.١):- Laboratory instruments used in this study

No.	Instruments	Company
١	Sensitive electronic balance	A &D, Japan.
٢	Autoclave	Stermite, Japan.
٣	Incubator	Memmert,Germany
٤	Distillator	GFL-Germany
٥	Centrifuge	Hermle, Japan
٦	Oven	Memmert,Germany
٧	Refrigerator	Concord, Italy
٨	Light microscope	Olympus, Japan.
٩	Micropipette	Oxford, USA.
١٠	pH meter	Hoeleze & Cheluis, KG, Germany.
١١	Inoculating loop	Japan
١٢	Inoculating needle	Japan
١٣	Benson burner	Germany

٢-١-٢: Materials

A-Culture Media

The following culture media were used according to the requirements: ١-**Neutrient agar media (Oxiod)** It was used for isolation and cultivation of bacteria from their source and to study of their morphological characteristics (Baron *et.al.*, ١٩٩٤).

٢- MacConkey' s agar media (Mast)

It was used to isolate Gram–negative bacilli and to differentiate lactose fermenter from nonfermenter bacteria (Baron *et.al.,* ١٩٩٤).

٣- Blood agar (Mast)

It was used for cultivation of bacterial strains and determining of hemolytic reactions.

The medium was prepared by adding ٥% of human blood to autoclaved and cooled to ٥٠C˚ blood agar base (Collee *et.al*., ١٩٩٦).

٤- Mannitol Salt agar (Oxiod) It was used as selective media for ***S. aureus*** (Vall *et.al.,* ١٩٩٩).

٥-Eosin Methylene Blue (EMB) agar (Oxiod)

The medium was used to cultivate ***E.coli***, which produced special color''Green metallic shine''for these bacteria (Collee *et.al*., ١٩٩٦).

٦- Methyl red –Vogas proskauer medium (Oxiod) The medium was used to detect the ability for bacteria to complete or partial fermentation of glucose(MacFaddin ,٢٠٠٠).

٧- Kligler's Iron agar (KIA) (Biolife)

The medium was used to detect the ability of microorganisms to produce (H_2S)and fermented sugars with producing acid and gas(Vall *et.al.,* ١٩٩٩).

٨-Simmon Citrate agar (Oxiod)

It was used as differential medium to detect the ability of bacteria to use citrate as a sole source of carbon (MacFaddin ,٢٠٠٠).

٩- Brain –Heart Infusion Broth (B.H.I.B.)(Mast)

It is enrichment medium used for cultivation of bacteria from their source(Collee *et.al*., ١٩٩٦).

١٠-Neutrient broth (Oxiod)

It is general medium and it's used in general experiments .The medium was also used to maintain the bacterial isolates(Collee *et.al*., ١٩٩٦).

١١- Pepton Water (Mast)

The medium was used to detect the ability of bacteria to produce indol from tryptophane(MacFaddin ,٢٠٠٠).

١٢-Urea agar medium (Biolife)

The base medium was prepared according to manufacturing company, autoclaved and cooled to ٥٠C° then added ٥ml of ٢٠٪ filtration sterilized

urea solution to ٩٥ml of media in which this media insured that their pH was (٦.٨-٦.٩) and tubed the medium as deep slopes. This medium was used for detecting bacterial ability to produce urease enzyme (MacFaddin, ,٢٠٠٠).

١٣-Sugars Fermentation medium It was used to determine the ability of organism to ferment sugars with or without gas production. It was prepared according to MacFaddin(٢٠٠٠) and it consist of :

A-Base medium

It was prepared by dissolving ١٠gm of peptone, ١gm of beef extract, ٥gm of NaCl and ٠.٠١٨ of phenol red indicator in ١٠٠٠ml distilled water ,then neutralized pH to ٧.٤ and distributed in test tube of ٥ml in each test tube and then Durham's tube was inserted in each tube to detect gas production, then the tubes were sterilized by autoclaving.

B-Sugar solution

The sugars used in this test were glucose,sucrose, lactose, mannitol and arabinose at concentration (١٪) for each one then added ٠.١ml of each sugar sterilized by chloroform to each tube containing the base medium.

١٤-Motility test medium (Semisolid media)

It was used to detect the motility of microorganisms .It was prepared according to the method suggested by MacFaddin(٢٠٠٠) by added ٤gm of agar-agar to the ١٠٠ml of neutrient broth, sterilized by autoclaving and dispensed in tubes of ٥ml in each.

١٥-Gelatin agar medium (Oxiod)

It was used to detect bacterial ability to hydrolysis gelatin by producing gelatinases enzymes (Vall *et.al.,* ١٩٩٩).

١٦- Muller –Hinton agar (Mast)

It was used for detection the antimicrobial susceptibility test as recommended by Vall *et.al.,*(١٩٩٩).

B- Reagents and Chemical solutions

١- Catalase test reagent This reagent was prepared in ٣٪ concentration of H_2O_2 and it was used to identify bacterial ability to produce catalase enzyme (MacFaadin,٢٠٠٠).

٢- Oxidaese test It consists of ١٪ tetra-methyl p.phenylene diamine dihydrochloride (Baron *et.al.,*١٩٩٤).

٣-Methyl red reagents

It was prepared according to collee *et.al*.,(١٩٩٦). It was used for detection of complete glucose hydrolysis.

٤-Vogas –Proskauer reagent

It was prepared according to collee *et.al.,* (١٩٩٦) and it consist of two solution:

Solution A: α-naphthol indicator,which was prepared by dissolving ٥gm of α-naphthol in ١٠٠ml of absolute ethylic alcohol.

Solution B: KOH,it was prepared by dissolving ٤٠gm of KOH in ١٠٠ml of distilled water. It was used to detect of partial hydrolysis of glucose.

٥-Kovac's reagent The reagent was prepared by dissolving ٥gm from (dimethylamino benzaldehyde) in ٧٥ml ethyl alcohol and ٢٥ml of concentrated HCL. This reagent was used for the detection of indole (MacFaddin,٢٠٠٠).

٦-Normal saline solution

It was used for for suspending bacterial cells and general experiments and it was prepared by dissolving ٩٩gm of NaCl in ١٠٠٠ml of distilled water and autoclaved at (١٢١ for ١٥ minutes)(Al-Muhanah,١٩٩٤).

٧-**Frazeir' s reagent**

The reagent was prepared by dissolving ١٥gm $HgCL_٢$ in ٢٠ml of concentrated HCL, then ١٠٠ml of distilled water were added. It was used to detect the bacteria ability to hydrolysis gelatin (Collee *et.al*.,١٩٩٦). **٨-MacFarland standard solution**

The solution of tube No.٠.٥ was prepared according to Baron *et.al*.(١٩٩٤) by mixing ٠.٠٥ml of barium chloride with ٩.٩٥ml of concentrated sulfunic acid in which result in turbidity approximately equal to bacterial cells density of $١.٥x١٠^{٨}$ cell/ml.

٩- Gram Stain solutions The solutions were prepared according to the Baron *et.al.*,(١٩٩٤).

C-Disinfectants and antiseptics

Different types of disinfectants and antiseptics as shown in table (٢-٢) are used to test susceptibility of bacteria

Table (٢-٢)disinfectants and antiseptics used in this study .

Source	**Name**
١- Hydrogen peroxide ($H_٢O_٢$) ٦٪	Al-Teeba Company_ Baghdad, Iraq.
٢- formaldehyde (formalin)١٠٪	United Kingdom
٣-Sodium hypochlorite (bleach chlorox)٦٪	product of Al- Baher factor of detergent .Baghdad,Iraq.
٤-Chlorohexidine gluconate (Hibitene)	Al-Rahma pharmaceutical co. Aman –Jordan
٥-Chlorohexidine cetramide (Savlon)	Ministry of health –Iraq
٦-Povidone –iodin (Betadine) ٤٪ ١٠٪,١٨٪	Mourad Est(Syria) Syria
٧-Choroxylenol (Dettol) ٥٪(٣ type) ١,٢ type($S_١$,$S_٢$) ٣ type(Sp)	The Iraqi company for drugs industries and medical requirements-Samara– Iraq Spartin company
٨-Chlorine compound (Large disc)٣٪	Syria

D-Antibiotics

The antibiotics discs used to detected susceptibility of bacteria shown in table(٢-٣)

Table (٢-٣):- Antibiotics

No.	Antibiotic	Symbol	Potency	Manufacture	Zone diameter nearest whole mm		
					Sensitive	Medium	Resistant
١	Pencillin G	P	١٠ IU	Bioanalysis	>٢٩	□	<٢٨
٢	Ampicillin	Amp	١٠ µg/ml	Bioanalysis	>٢٩	□	<٢٨
٣	Amoxicillin	Amx	٢٥ µg/ml	Bioanalysis	>٢٠	□	<١٩
٤	Co-trimoxazole	SXT	٥ µg/ml	Bioanalysis	>١٦	١١_١٥	<١٠
٥	Cefotaxime	CTX	٣٠ µg/ml	Bioanalysis	>٢٣	١٥_٢٢	<١٤
٦	Cephalexin	CL	٣٠ µg/ml	Bioanalysis	>١٨	١٥_١٧	<١٤
٧	Amikacin	AK	٣٠ µg/ml	Bioanalysis	>١٧	١٥_١٦	<١٤
٨	Gentamicin	CN	٣٠ µg/ml	Oxiod	>١٥	١٣_١٤	<١٢
٩	Ciprofloxacine	C	٣٠ µg/ml	Oxiod	>٢١	١٦_٢٠	<١٥

١٠	**Oxacillin**	**OX**	**١ µg/ml**	**Al-Razii**	>١٣	١١-١٢	<١٠

٢-٢:Methods ٢-٢-

١:Samples collection

The samples were collected from different departments of Hilla Teaching hospital from hospital environment and from patients:

١-Patients

The samples being collected from this group included: wound infections (surgical and burns wound),chronic suppurative otitis media and urinary tract infections).

٢-Hospital environment

The samples being collected from hospital environment included the following sites :

A-Operating room(Surgical operative beds, surgical instruments, tables, sink,wall and floor).

B-Burn ward (patients beds, floor, wall and bath room)

C-Surgical ward (floor, patients beds, washbasine, tables and wall). **D-Emergency Ward**(beds, floor, wall and sucker).

E- Ear-nose-throat out patients clinic(testing instruments,auroscope, tables and wall).

F-Labrotary (bench , and wall) **G-Kitchen**

(sink, food preparing tables and floor)

Other sources of samples including disinfectant and antiseptics

٢-٢-٢: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.

The bacterial isolates were isolated and identified according to their diagnostic characteristics and then compared with their being reported in referential references Baron *et.al*., (١٩٩٤), Collee *et.al*., (١٩٩٦) and MacFaddin,(٢٠٠٠). The characteristics being investigated are:

١-Cultural characteristics (colony property, hemolysis, pigment and other characteristics).

٢-Cellular characteristics (Microscopic examination)

Smear of bacterial isolates were prepared on clean slide and stain with gram stain in order for identification of staining type ,shape ,arrangement and presence of spore.

٣-Biochemical test

These tests included :

١- Catalase test

Transfer a colony of the microorganism to clean slide and adding drop of ٣٪ H_2O_2, the formation of gas bubbles indicates for positive results (Collee *et.al.*,١٩٩٦).

٢-Oxidase test

This test was done by transfer pure colony of under test bacteria (١٨-٢٤h) to filter paper which moisten with several drops of tetra-methyl p.phenylene diamine dihydrochloride, when the colonies were colored with deep violet color the result was regarded as positive result (Baron *et.al.*,١٩٩٤). **٣-**

Methyl red test

MR-VP broth tubes inoculated with tested bacteria and incubated at ٣٧ C^{o} for ٤٨hr. About ٥ drops of methyl red reagent were added. The change of color to red indicates for positive result, while the yellow color indicates for negative result(Collee *et.al.*, ١٩٩٦).

٤-Vogas –Proskauer test

MR-VP medium was inoculated with tested bacteria and incubated at (٣٧C) for (٤٨h), then result was read by adding (٠.٦ml)of α-napthol and (٠.٢ml)of ٤٠٪KOH with shaking,then allow the mixture to for up to ٣٠ min. The positive reaction indicates red color due to partial hydrolysis of glucose (Collee *et.al.*, ١٩٩٦).

٥- Indol test

The peptone water media was inoculated with the tested bacteria and incubated at ٣٧C° for ٤٨hr,then few drops of kovac's reagent were added. Appearance of red ring color indicates positive result (MacFaddin,٢٠٠٠). **٦-**

Citrate utilizating test

The tube contained slant of simmon citrate agar were inoculated with tested bacteria by stabbing in the bottom and streaking on the slant, then incubate it at (٣٧C°)for ٩٦hr.Change of the medium from green to blue indicate for positive result (Collee *et.al.*, ١٩٩٦).

٧- Coagulase test This enzyme has been tested by two methods

A-Slide test for bound coagulase (clumping factor)

A drop of human plasma was placed on a clean, dry glass slide. A drop of distilled water was placed next to the drop of plasma as a control. By a sterile loop, an a mount of the isolated colony was emulsified with each drop, when clumping was observed in the coagulase plasma drop indicate the organism agglutinates(Baron *et.al.*,١٩٩٤).

B-Tube test for free coagulase

Several colonies of bacteria were transferred with the loop to tube containing ٠.٥ml of plasma. The tube incubated at ٣٧C for overnight. The test was read by tilting the tube and observing for clot formation in the plasma and test considerd negative when clot not observed (Baron *et.al.*,١٩٩٤).

٨-Urease test

The tube that contained a slant of (urea agar) was seeded with tested bacteria and incubate at ٣٧C° for (٢٤hr). Change the color of medium to deep pink indicate for positive result (Collee *et.al.*,١٩٩٦).

٩-Motility test

Inoculated the tube that contained semisolid media with tested bacteria by stabbing method and incubate at ٣٧C° for ٢٤-٤٨hr. The disseminating of growth outer the the stabbing line indication for positive result (MacFaddin, ٢٠٠٠).

١٠- Fermentation test

Inoculation the tube contained the required sugar with tested bacteria and incubation at ٣٧C° for (٢-٥) days. Change the color of indicator to yellow indicates for positive result (MacFaddin ,٢٠٠٠).

١١- Gelatin test

The gelatin media plates were seeded by tested organism and incubated under appropriate conditions for (٢-٣)days and then treated with Frazier's reagent. The positive test resulted in pale zones a round the gelatin liquefying colonies (MacFaddin,٢٠٠٠).

١٢-Blood hemolysis test

Blood agar medium was streaked with a pure colony of bacteria and incubated at ٣٧°c for ٢٤–٤٨ hours. The appearance of a clear zone around the colony indicates for ß–hemolysis while the presence of green–color indicates for α–hemolysis (Baron, *et al.,*١٩٩٤).

١٣-Mannitol Salt agar (selective test for *S. aureus)*

The media was inoculated with tested bacteria and incubated for (٢٤hr) at ٣٧C°.Change the color from pink to yellow indicates for positive result (MacFaddin,٢٠٠٠).

١٤- Kligler' s Iron agar (KIA)

The media was inoculated with tested bacteria by stabbing the butt of the tube and streaking the surface of slant and incubated for (٤٨hr). Changing the color of the media to yellow indicates for positive result due to sugar fermentation with or without gas formation at butt of slant (MacFaddin, ٢٠٠٠).

Growth of *P.aeruginosa* at ٤٢C°

Neutrient broth tube inoculated with ***P.aeruginosa*** and incubated at ٤٢ C°. The growth was observed after ٣days.The growth at this degree of temperature is characteristic for ***P.areuginosa*** (Ayoub,١٩٨١).

٢-٢-٣:Antibiotics sensitivity test

This test was performed on Muller –Hinton agar with the antibiotics discs in table(٢-٢) using the Bauer *et.al.,* (١٩٦٦) method. It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from ٥ isolated colonies grown on a blood agar plate to ٥ ml of broth. This culture was then incubated for ٢ hours to produce a bacterial suspension of moderate turbidity. A sterile swab was used to obtain an inoculum from the standardized culture. This

inoculum was the streaked on a Muller-Hinton plate. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps or a disc applicator. Incubation was usually overnight with optimal time of ١٤ hours at ٣٧ **C°**. Antibiotics inhibition zones were measured using a caliber. Zone size was compared to standard zones to determine the susceptibility or resistance of organism to each antibiotic according to (NCCLS,٢٠٠٠) criteria.

٢-٢-٤:Stock culture The identified isolates were kept as stock culture in neutrient broth tube supplemented with ٥٪ glycerol at (-٢٠)C° for ٦months(Al-Muhanah,١٩٩٤).

٢-٢-٥: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 ٣٧C° for ٧ days(Dawaf,١٩٩٣).

٢-٢-٦:Disinfectants and antiseptics susceptibility test

The antimicrobial activity of disinfectant and antiseptic was tested against ٤ 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 :

١-Minimum Inhibitory Concentration (MIC)

٢-Disc Diffusion method

٣- Well Diffusion method

١-Minimum Inhibitory Concentration (MIC) method

The MIC test was determinated according to the method suggested by Baron *et.al.*(١٩٩٤). depending on the turbidity of the bacterial growth. The MIC was recorded as the lowest concentration prevents the bacteria under test to grow.

١. Preparation of the Culture Media

The brain–heart infusion broth was used mentioned in the paragraph number (٢–١–٢).

٢. Preparation of the bacterial Suspension

The bacterial suspension which specific for MIC tests were prepared by transport a pure bacterial growth from overnight growth on blood agar plate directly suspended in screw tube contain ٥ ml of normal saline. The broth was incubated at ٣٧°c for ٤–٦ hours until it's turbidity compared to that of the recommended turbidity standard

٣. Preparation Stock Solution

The initial dilutions of the disinfectants and antiseptics used in this study were prepared from the original stock solutions prepared from the commercial concentrations mentioned in table(٢-٢). Serial dilutions from the initial concentration were prepared by the two fold dilution method in the liquid culture media (BHI) for seven disinfectants and antiseptics.

٤. Procedure

In this study the macro-dilution method was used as follows :

- Nine sterile test tubes (for each disinfectants) were numbered from ١ to ٩.

- ١ ml of BHI broth was dispensed in each tube.١ml of the disinfectants solution was added to tube No.١ using a sterile pipette and mixed well. ١ ml of the mixture was transferred to tube No.٢ . The contents of tube ٢ were then mixed thoroughly and ١ ml was transferred to tube ٣ . This process was continued up to tube No.٩ from which ١ ml was discarded, consequently, the final volume in each tube was ١ ml. The ١ ml of the bacterial suspension previously prepared was added for each tubes. In this experiment, two tubes were used as control. The first one contained ١ ml of BHI broth plus ١ ml of bacterial suspension, while the second control called negative growth control which contained only ٢ ml of BHI broth.

٢-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, (١٩٨٥) in which discs of ٦mm diameter were prepared from filter paper (whatman No.٣)by using paper borer. These discs (every ١٠٠ discs) were placed in a clean test tube for each prepared concentration of tested disinfectants and antiseptics and were closed with cotton plaques and sterilized by autoclave. After this ١ml of each concentration of these chemicals prepared from original stock was added to one test tube containing sterilized discs and this done for every concentration of used disinfectants and antiseptics and these concentrations were ٥٠,١٠٠, ٢٠٠, ٤٠٠,٥٠٠,٨٠٠,١٠٠٠, ٣٠٠٠,٥٠٠٠ and ٨٠٠٠µ/disc for each hydrogen peroxide, sodium hypochlorite, formaldehyde, chloroxylenol(S١) and chlorohexidine cetramid. The

susceptibility test of these disinfectants and antiseptics discs was determinated according to Bauer *et.al*.,(١٩٦٦) method mentioned previously.

٣- Well Diffusion method

In this method neutrients agar plate was prepared by cut equally spaced well (٦mm),then the plates inoculated with cotton swab dipping into screw tube bacterial suspension with turbidity equal to the turbidity of o.٥ MacFarland stander tube for each tested bacterial isolate by streaking over the surface of plates. After this neutrient agar wells were filled with ١٠٠µl (٠.١ml) of prepared concentrations for each disinfectants and antiseptics and incubate the plates at ٣٧C^{o} for ٢٤hr. The susceptibility to these chemicals biocide was determinated by measuring the zone inhibition a round the wells for each concentration (Norrel and Messely, ١٩٩٧)(Hangnga *et.al*.,٢٠٠٢).

Statistical analysis

Differences between the percentage of bacteria isolated from UTI and bacteria isolated from others infections (burn, wound and chronic supperative otitis media) were analysed using Z distribution analysis

Differences between contamination rates of different sites of hospital environment were analysed using chi squared analysis

٣.١ Diagnostic Features of Bacterial Isolates

٣.١.١.Gram-Positive Bacteria

Table(٣-١and ٣-٢) show the results of biochemical tests being used for identification and isolation of gram positive and gram negative bacteria .The result were compared with referential reported by Baron *et.al.*,١٩٩٤)

Table (٣-١):-Biochemical tests for Identification G+ve Bacteria

Test	***S.aureus***	***B.subtilis***
Gram stain	G+ve cocci (clusters)	*G+ve Bacilli(Chain)*
Haemolysis	+	*V*+
Presences of spore	_	+
Oxidase	—	N
Catalase	+	V+
Coagulase	+	N
Sugar fermentation		
Glucose	+	+
Lactose	+	N
Arabinose	-	+
Sucrose	+	N
Mannitol fermentation	+	+
VP	N	+
Indol	N	_
Simon Citrate	N	+

+ = **positive result** , □ = **Negative result**

٣.١.٢ Gram-Negative Bacteria

Table (٣-٢):-Biochemical tests for Identification G-ve Bacteria

Test	***E.coli***	***P.aeruginosa***
Gram stain	G¯ve, short rods	G¯ve rods
Hemolysis	—	+/-
EMB	Metalic	pale
oxidase	-	+
Catalase	+	+
MR	+	-
VP	-	-
Indole	+	-
TSI	A/A/G	ALK/no change
Motility	+	+
Simon Citrate	-	+
Urease	-	-
Growth at ٤٢ C	-	+
Gelatin liqufication	+	+

٣-٢:Isolation and identification of bacteria

In this study a total of ٢٨٤ samples were collected. These samples included ١١٥ samples of different clinical cases (burns, wounds, chronic otitis media and urinary tract infections). In addition, ١٦٩ samples were collected from hospital environments(operating room, burns ward,surgical ward, emergency ward, ENT out patients clinic, laboratory and kitchen) through period of ١١ months (from November ٢٠٠٥ to September ٢٠٠٦).

١- Samples collection from patients

The study focused on certain bacterial types represented by ***P.aeruginosa, S.aureus*** (including **Methicillin Resistant *S.aureus* (MRSA))**, ***E.coli*** and ***B.subtilis*** as these organisms are the common causes of nosocomial infections and dominantly distribute in hospital environment.

High infection rate (٨٣.٣٪) was observed in burns of hospitalized patients (table٣-١).This result was expected since patients are immunosupp- ressed and with long stay in hospital, during which they may be submitted to

endotracheal intubation and /or catheterization of blood vessels and bladder in burns ward,therefore, these patients were more susceptible for infections (Oncul *et.al.*,٢٠٠٢ ; Xue *et.al.*,١٩٩٩).

The results showed that the infection rate in burn patients with ***P.aeruginosa*** accounted for (٣٢٪). It was high compared with the infection rate of ***S.aureus*** which accounted for (٢٠٪) and ***E.coli*** which accounted for (١٦٪) as shown in table (٣-٣) .

Table (٣-٣) Frequency of bacterial types isolated from different cases

Source of isolation(No. of samples**)**	**No. of Positive samples(%)**	***P.aeruginosa***	***S.aureus***	***E.coli***	others bacteria
Burn infections(٣٠)	٢٥(٨٣.٣٪)	٨(٣٢%)	٥(٢٠%)	٤(١٦%)	٨(٣٢٪)
Wound infections (٣٥)	٢٨(٨٠٪)	٦(٢١.٤٪)	٦(٢١.٤٪)	٥(١٧.٨٪)	١١(٣٩٪)
chronic suppurative Otitis media(٣٠)	٣٠(١٠٠٪)	٦(٢٠٪)	٤(١٣.٣٪)	٢(٦.٦٪)	١٨(٦٠٪)
Urinary tract infections(٢٠)	١٧(٨٥٪)	٠	٠	٦(٣٥.٢٪)	١١(٦٤.٧٪)
Total	١٠٠	٢٠	١٥	١٧	٤٨

It has been reported in local studies that the most dominant organism in burn infections was ***P.aeruginosa*** followed by ***S.aureus*** (Fakhriddeen, ٢٠٠١;Ayoub, ١٩٨١). Other results being reported by other studies seemed to be in agreement with the results of this study that ***Paeruginosa*** was most common pathogen in burn infections. (Donati *et.al*.,١٩٩٣;Pandit *et.al*.,١٩٩٣; Martin,١٩٩٣;Tredget *et.al*.,١٩٩٢). In Iran, Rastegar-Lari *et.al*.,(١٩٩٨) pointed out that ***P.aeruginosa*** accounted for (٧٣.١٪) of burns infections followed by ***S.aureus*** (١٠.٣٪). Recently, Savas and colleagues (٢٠٠٥) stated that ***P.aeruginosa*** was the dominant bacterial types being isolated from burns which constitute (٢٦.٩٪) of these infections.

On other hand,Wenzal,(١٩٩٧) stated that ***S.aureus*** accounted for (٢٦٪), while ***P.aeruginosa*** and ***E.coli*** accounted for (٢١٪) and (٨٪) of burns infections respectively. The variable results regarding infections rate and the density of bacterial isolates from burns infections can be attributed to the hospital policy in management of such cases. Moreover, geographic climatic, and hygienic factors may also be correlated with the relative variability of results among different area (Memmel *et.al*., ٢٠٠٤).

P.aeruginosa was reported as the ''classic pathogen '' in burns infections in many countries such as Jordan(Al-Akayleh,١٩٩٩), Libya (Husain *et.a.*,١٩٨٩) and Turkey (Oncul *et.al*.,٢٠٠٢), while other studies pointed out that ***S.aureus*** being the most predominat microorganism in burns infections(Becker *et.al*.,١٩٩١). However, it can be concluded that ***P.aeruginosa*** is the most common pathogen causing burns infection and complications since it is inherently resistant to many antibacterial agents and able to acquire resistance for all antibacterial agents hence it is referred to as multidrug resistant (Pirnray

et.al.,٢٠٠٣),This character enable this organism to distribute every where in the environment particularly hospital environment.

The **MRSA** was recently emerged worldwide as the major nosocomial pathogen and most important types of ***S.aureus*** causing sever and relatively most seriously infections particularly in hospitalized patients. ***S.aureus*** was isolated in a percentage of (٢٠٪). Among this, **MRSA** constituted (٤٠٪) as shown in table (٣-٤). Al-Hadithi and Yousif,(٢٠٠٣) have reported that **MRSA** accounted for (٣٣.٣٪) of all ***S.aureus*** isolates recovered from burns, while Danchivijitr *et.al*.,(١٩٩٥) found in their study that the **MRSA** frequency was (٤٨.٣٪) of burns isolates.

The major reservoirs of **MRSA** are infected patients. The transient flora on hands of health care workers was important mode for transmission morganisms to the patients. In addition, colonization on environmental surfaces also serves as reservoir for **MRSA**. Airborne infections was also possible (Sachdev *et.al*.,٢٠٠٣). Furthermore, bacteria usually gain access to burn patients through cross contamination of burn wound (Gang *et.al*.,١٩٩٩).

Table (٣-٤) Distribution of **MSSA** and **MRSA** according to the site of infections

Types of infections	**MSSA**	**MRSA**	Total	Total positive sample
Burn infections	٣(٦٠%)	٢(٤٠%)	٥	٢٥
Wound infections	٤ (٦٦.٦٪)	٢(٣٣.٣٪)	٦	٢٨
Ear infections	٤(١٠٠٪)	٠	٤	٣٠
Urinary tract infections	٠	٠	٠	١٧
Total	١١	٤	١٥	١٠٠

The observations indicate that burns infections are common and an increasing problem in Hilla Teaching Hspital. The presence of high number of burn patients in limited burn care facilities and unavailabilities of single isolation room definitely increase the risk of infections in those patients

The results showed that infection rate with wound infections was (٨٠٪) with a remarkable dominance of ***P.aeruginosa***(٢١.٤٪), ***S.aureus*** (٢١.٤٪) and ***E.coli*** (١٧.٨٪) as shown in table(٣-٣). The results being reported by Jibran, (١٩٨٦) revealed that ***P.aeruginosa*** was the major cause of wound infections in which it accounted for (٤٥.٦٪) while ***S.aureus*** and ***E.coli*** constituted (١٠.٤٪) and (٨.٢٪) respectively. Oguntibeju and Rau, (٢٠٠٤) have also reported that the infection rate of wounds by ***P.aeruginosa*** was (٣٣.٣٪), ***S.aureus*** (٢١.٧٪) while by ***E.coli*** it was (١١.٧٪). Maki, (١٩٨٢) found that ***E.coli*** was the most

common organism in wound infections. Consequently, the results varied from area to area Bennett and Brachman,(١٩٩٢) found that ***S.aureus*** accounted for (١٥-٣٣٪) of wound infections whereas ***E.coli*** constituted (١٢-٩٪). Mahmood,(٢٠٠٠) found that ***S.aureus, P.aeruginosa*** and ***E.coli*** constituted (٥٠.٣٪), (١٦.٣٪)and (١٤.٣٧٪) of wound infections respectively. Kampf and Kramer,(٢٠٠٤); Wenzal,(١٩٩٧) stated that ***S.aureus*** was the most common bacteria in surgical wound infections. Maki,(١٩٨٢) has been concluded that among the enteric bacilli ***E.coli*** was the most common cause of wound infections which indicated for contamination with faecal matter***. E.coli*** was considered as the major cause of surgical wound infections in which it constituted (٢١.٨٪) in England (Vilar–Comple *et.al*.,٢٠٠٠) .

In the present study **MRSA** accounted for (٣٣.٣٪) of all ***S.aureus*** isolates being detected in wounds as shown in table (٣-٤). Al-Sahlawi, (٢٠٠٢) reported that there was significant increase in **MRSA** infections in wounds patients.**MRSA** was found to cause (٢١٪) of surgical wound infections in Canada (Simor *et.al*.,٢٠٠٥). Al Hadithi and Yousif,(٢٠٠٣) reported that **MRSA** constituted (١١.١٪) of ***S.aureus*** isolates being recovered from wound infections. In spite of **MRSA** strains are opportunistic pathogen but can cause sever wound infections particularly surgical wounds infections caused by endogenous strains of ***S.aureus***(Kluytmans *et.al*., ١٩٩٧). These types of ***S.aureus*** are difficult to treat and difficult to eradicate from the infected sites because they are highly resistant to various antibiotics.

The variation in proportion of wound infections and variation in distribution of bacterial isolates in wound infections between studies may be related to the site and type of operation, period of operation, period of stay in hospital, bacterial types present on the skin and clothing at the site of wound,

presence of invasive medical devices and the types of antibiotics used for treatment (Oguntibeju and Rau,٢٠٠٤) .

The results obtained from this study showed that ***P.aruginosa, S.aureus*** and ***E.coli*** were isolated from chronic suppurative otitis media in a percentage of (٢٠٪),(١٣.٣٪) and (٦.٦٪) respectively (table٣-٣). The results agreed with recent results by Al-kabi,(٢٠٠٦) since he found that ***P.aeruginosa*** accounted for (٢١.٦٪), ***S.aureus*** (١٨٪) and ***E.coli*** (٨.٤٪) of bacteria that isolated from otitis media. These results came to confirm the results of other studies,since Khalil,(١٩٨٠) found that ***P.aeruginosa*** accounting for (٢٣.٩٪), ***S.aureus*** (١٣.٥٪) and ***E.coli*** (٦.٢٪)of otitis media infections. Al-khafaji (١٩٩٣) pointed out that ***P.aeruginosa, S.aureus*** and ***E.coli*** were found to constitute (٤٦.٥٪),(٢٠.٨٪) and (٢.٢٨٪) of otitis media respectively. Similar results have been reported by Ul-Haq *et.al.*,(٢٠٠٢) who reported that ***P.aeruginosa*** accounted for (٣٧.١٪) and ***S.aureus*** (٣١.%) of these infections. The domination of ***P.aeruginosa*** as infectious pathogen can be ascribed to it's ability to resist wide range of antibiotics and disinfectants and it's ability to produce various types of extracellular products such as toxin, alkaline protease and phospholipase (Stenfors and Raisanen,١٩٩١).

E.coli was isolated from UTI in a frequency of (٣٥.٢٪) as shown in table (٣-٣). Many studies have been reported that ***E.coli*** was the most common infecting pathogen in UTI. ***E.coli*** was isolated in a percentage of (٢٤٪) by Subhi,(٢٠٠٣) and (٢٦٪) by Weinstein *et.al.*,(٢٠٠٥).

No significant difference found between bacteria isolated from burn,wound and CSOM but there significant difference between bacteria isolated from UTI and others infections at level of significant (P<٠.٠٥)

٢-Samples collection from hospital environment

In the last two decades, evidences have accumulated which indicating that the hospital environment can represent an important source of nosocomial infections, that the hospital environment including floor, walls, beds, tables, bath and air can be the main sources of the nosocomial pathogens. Consequently, some samples were selected from different locations of the hospital environment. The results shown in table (٣-٥) indicated that ENT patient clinic was the most site being exposed for contamination, since the contamination rate was relatively high (٨٢.٣٪), while contamination rate in laboratory was the lowest, since it accounted for (٤٠٪). The contamination rate detected in Kitchen and Disinfectants (In-use) were (٦٠٪) and (٦٦.٦٪) respectively, whereas burns ward, surgical ward and emergency ward revealed close contamination rate, that ranged from(٧٠.٤%) to (٧٥٪). The contamination rate of different sites of hospital eenvironment differed significantly at the level of significant (P <٠.٠١)

It was found that the contamination rate of operating room was (٥٧.٥٪) as shown in table(٣-٥) and the frequencies of contamination of beds, floor and tables were (٧٧.٧٪), (٧٥٪) and (٥٠٪) respectively as shown in table(٣-٦). Mashouf and Vala,(٢٠٠٦) found that contamination rate of operating room was (٦٦.٩٪) while Al-Waznee *et.al*., (٢٠٠٣) found this rate to be (٢٠٪). The density of contaminant microorganisms vary largely depending on the number of people present in the operating room as well as the staff and sub staff of

activity and the type of disinfectants and antiseptics being used for disinfection.

Dawaf,(١٩٩٣) in her study in Baghdad found that contamination rate in beds and floors of operating room were (٦٩.٥٪) and (٨٦.٩٪) respectively. Wenzal,(١٩٩٧) stated that floors were the most heavily contaminated sites in the operating room in which sedimentation of airborne bacteria or contact with shoes, trolleys wheels and other solid objects contaminate these floors.

Table (٣-٥) Percentage of contamination in hospital environment and frequency of bacterial types isolated from hospital environment in hospital environment

Location	**Total sample**	**No. of Positive result**	**(%) of total contamination**	***P.aeruginosa***	***S.aureus***	***E.coli***	***B.subtilis***
ENT patient clinic	١٧	١٤	٨٢.٣٪	٠	٣(٢١.٤٪)	١(٧.١٪)	٠
Emergency ward	٢٨	٢١	٧٥٪	٢(٩.٢٪)	٢(٩.٢٪)	٢(٩.٢٪)	٠
Burn ward	٢٥	١٨	٧٢٪	٤(٢٢.٢٪)	٠	١(٥.٥٪)	٠
Surgical ward	٢٧	١٩	٧٠.٤٪	٢(١٠.٥٪)	٢(١٠.٥٪)	١(٥.٢٪)	١(٥.٢٪)
Disinfectants(in- use)	٩	٦	٦٦.٦٪	١(١٦.٦٪)	٠	٠	٢(٣٣.٣٪)
Kitchen	١٥	٩	٦٠٪	٠	٠	٢(٢٢٪)	٠
Operating room	٣٣	١٩	٥٧.٥٪	٢(١٠.٥٪)	٠	١(٥.٢٪)	١(٥.٢٪)

Laboratory	١٥	٦	٤٠٪	٠	٠	٠	١(١٦.٦٪)
Total	١٦٩	١١٢	٦٥.٥٪	١١	٧	٨	٥

Table (٣-٦) The frequency of bacterial types detected in operating room:

Site of isolates (**No. of samples**)	No .and % of contamination	No. of isolates	Type of isolates	others
Beds(٩)	٧ (٧٧.٧%)	١	***P.aeruginosa***	٦
Floor(٤)	٣ (٧٥٪)	١	***E.coli***	٢
Tables(٦)	٣ (٥٠٪)	١ ١	***P.aeruginosa*** ***B.subtilis***	١

In addition, many equipments and instruments used in the operating room has been linked to surgical wound infections. Surgical instruments are considered sterile when first received in the operating room, but can become secondary sources of infection when the air or punctured gloves contaminate them(Blakemore *et.al.*,١٩٧٩). Al-Gersha,(١٩٨٨) showed that strict aseptic technique was essential in the operating room to maintain sterility and most (٩٨٪) of technicians were considered as causing break in a septic technique.

P.aeruginosa was detected in a percentage of (١٠.٥٪) of bacteria contaminated operating room, while both ***E.coli*** and ***B.subtilis*** accounted for (٥.٢٪) as shown in table(٣-٥). Mashouf and Vala,(٢٠٠٦) also isolated ***P.aeruginosa, E.coli*** and ***B.subtilis*** from beds, wall, floor and tables in operating room. These microorganisms are usually brought into operating room by the wheel of trolleys coming from wards (Wenzal,١٩٩٧).

Samples taken from burns ward indicated for high contamination rate reached up to (٧٢٪), while the contamination rates of beds, floor and bathroom were (٥٠٪)(٥٠٪) and (١٠٠٪) respectively table(٣-٦). Similar results have been reported by Fakhriddeen,(٢٠٠١) who found that contamination rate in burns ward was (٧٦.٧٪). Table (٣-٥) showed that ***P.aeruginosa*** was detected

in a remarkable frequency(٢٢.٢٪) in which it isolated from beds and bathroom. The results were in agreement with that results obtained by Kolmos *et.al*.,(١٩٩٣) who stated that bath equipments were the main sources of nosocomial infection resulted by ***P.aeruginosa***.

Table (٣-٧)The bacterial types and percentage of contamination detected in burns ward

Site of isolates**(No. of samples)**	No. and % of contamination	No. of isolates	Type of isolates	others
Beds(٨)	٤ (٥٠٪)	١	***P.aeruginosa***	٣
Floor(٦)	٣ (٥٠٪)	١	***E.coli***	٢
Bathroom (beds and instruments)(٥)	٥ (١٠٠٪)	٣	***E.coli***	٢

Generally, ***P.aeruginosa*** seem to be the common organism causing hospital infection. This organism is always described as a highly adaptable organism for environmental conditions and has minimal nutritional requirements in which it is known to colonize hospital environment, particularly moist sites such as sink, soap, antiseptic solution, respiration equipment and hydrotherapy and it can be found in tap water and even in distilled water(Ehrlich,٢٠٠٣). In addition, ***P.aeruginosa*** colonized food especially vegetables (regarding hospital kitchen) and represent a source of endemic infection with ***P.aeruginosa*** for hospital acquired infections(Correa

et.al.,١٩٩١). ***E.coli*** accounted for (٥.٥٪) of bacteria contaminated burns ward table(٣-٥).

The mode of transmission of organism to the burn patients directly and/or indirect via either hands of health workers or by contact with inappropriately decontaminated equipments. In addition, patient own flora may spread through water during washing or other activity to colonize other sites in the surrounding environment that greatly increased the risk of infections (Goldman and Pier,١٩٩٣).

High frequency of contamination was observed in surgical and emergency ward and it accounted for (٧٠.٤٪) and (٧٥٪) respectively as show in table(٣-٥).

The contamination rate being found in this study regarding beds, floor and washbasine of surgical ward were (٥٥.٥٪), (١٠٠٪) and (١٠٠٪) respectively (table٣-٨). Al-Waznee *et.al*.,(٢٠٠٣) reported that contamination rate of hospital beds was (٥٠٪). The increased contamination rates of such sites can be attributed to the overcrowding in these ward resulting in increase spread of organisms from bed to bed and some part of mattresses may contain various types of bacteria and moving these mattresses will spread the organisms all around the ward. Ayliffe,(١٩٩٨) mentioned that mattresses were the most contaminated area in the hospital ward. In addition, Al–waznee *et.al*.,(٢٠٠٣) found that contamination rate of hospital ward floor was (١٠٪) while Al-Janabi,(٢٠٠٠) stated that contamination rate of ward floor was (٦٢.٢٪) .

Table (٣-٨) The bacterial types and percentage of contamination detected in surgical ward

Site of isolates(**No. of samples)**	No. and % of contamination	No. of isolates	Type of isolates	others
Beds(٩)	٥ (٥٥.٥%)	١	***E.coli***	٤
Floor(٦)	٦ (١٠٠٪)	١	***E.coli***	٥
Wash basine(٥)	٥ (١٠٠٪)	٢ ١	***P.aeruginosa*** ***S.aureus***	٢

The contamination frequencies of beds, tables and sucker in emergency ward were (٨١٪),(٦٦٪) and (٥٠٪) respectively(table٣-٩). Over-worked staff, more transfer of patients between wards, increased use of invasive devices and poor hygienic services all these factors were found to be highly increase the rate of contamination in hospital ward (Ayliffe,١٩٩٨).

Table (٣-٩) The bacterial types and percentage of contamination detected in emergency ward

Site of isolates **(No.of samples)**	No. and % of contamination	No. of isolates	Type of isolates	others
Beds(١١)	٩ (٨١٪)	٢ ١ ١	***P.aeruginosa*** ***S.aureus*** ***E.coli***	٥
Tables(٣)	١ (٦٦.٦٪)	١	***E.coli***	٠
Sucker(٦)	٣ (٥٠٪)	١	***S.aureus***	٢

The data shown in table(٣-٥) indicate that the frequencies of ***P.aeruginosa*** in surgical ward and emergency ward were (١٠.٥٪) and (٩.٢), ***S.aureus*** (١٠.٥٪) and (٩.٢٪)***, E.coli*** (٥.٢٪) and (٩.٢٪) respectively, while ***B.subtilis*** was detected in surgical ward only in a percentage of(٥.٢٪).

In attempt to detect the bacterial contamination in Ear-Nose-Throat (ENT) patient clinic several samples were taken from this clinic. The results indicated for high contamination rate (٨٢.٣٪) as shown in table (٣-٥). The high contamination rate in ENT clinic may due to the over crowding in this clinic and to low level of aeration in which it permits for high density of microorganisms in the space of the clinic.In addition, non sterilization,non use of effective

antiseptics and decrease number of instruments lead to the high level of contamination .

The contamination rate of auroscope, testing instrument and tables in ENT clinic were (١٠٠٪),(١٠٠٪) and (٦٠٪) respectively(table٣-١٠) in which the frequency of ***S.aureus*** was (٢١.٤٪) and ***E.coli*** (٧.١٪) as shown in (table ٣-٥). The high contamination rate of auroscope and other instruments may related to the repeated use of these objects and the use of inactive disinfectant.

Table (٣-١٠)The bacterial types and percentage of contamination detected in ENT patients clinic

Site of isolates **(No. of samples)**	No. and % of contamination	No. of isolates	Type of isolates	others
Auroscope(٤)	٤ (١٠٠٪)	١ ١	***S.aureus*** ***E.coli***	٢
Testing instrument(٥)	٥ (١٠٠٪)	١	***S.aureus***	٤
Tables(٥)	٣ (٦٥%)	١	***S.aureus***	٢

From kitchen environment several bacterial isolates were obtained. ***E.coli*** was detected in remarkable frequency (٢٢.٢%) as shown in table (٣-٥) in which it was isolated from sink of the kitchen. ***E.coli*** is the usual habitant as normal flora of human and animal intestinal tract (Karmali *et.al.*,١٩٨٣). Therefore, the presences of this organism strongly indicates for faecal contamination of

environment and water supplies(Collee *et.al*.,١٩٩٦) .***E.coli*** causes various gastrointestinal diseases, UTI and wound infections (Baron *et.al*.,١٩٩٤). The ability of Gram–negative bacilli to survive in wet environment for long periods of time (**>**٢٥٠ days) and quickly proliferate (generation time for ***E.coli*** is ٣٠ min), may explain their common occurrence in the environment (Perryman and Flournory,١٩٨٠). The contamination rate in sink of the kitchen was (١٠٠٪). The overall contamination rate of Kitchen environment was (٦٠٪). This may due to the aviability of organic materials in the kitchen, the moist atmosphere and the optimum temperature which lead to such relatively high contamination.

Table (٣-١١) The bacterial types and percentage of contamination detected in laboratory, kitchen and disinfectants and antiseptics

Site of isolates (**No. of samples)**	No.and % of contamination	No. of isolates	Type of isolates	others
Laboratory bench(٧)	٣ (٤٢.٨%)	١	***B.subtilis***	٢
Kitchen sink(٥)	٥ (١٠٠٪)	٢	***E.coli***	٣
Disinfectants and antiseptics antiseptics**(chloroxylenol and chlorohexidine cetramide**) **(**٩)	٦ (٦٦.٦٪)	١ ٢	***E.coli*** ***B.subtilis***	٣

The contamination rate of hospital laboratory was (٤٠٪) as mentioned previously in table (٣-٥) and the contamination rate of laboratory bench was

(٤٢.٨٪).The results agreed with that results observed by Ogunsola *et.al*., (٢٠٠٠) who indicated for higher contamination rate (٤٨٪) of laboratory. It is not surprising that an increased contamination rate of medical laboratory, because working with microorganisms in the laboratory may release microorganisms to the environment (Harrington and Shannon,١٩٧٧).

Disinfectants are usually used in medicine to eradicate or to minimize the harmful microorganisms, but the medical problem becomes big when these disinfectants become contaminated. This was observed in this study since relatively high contamination rate (٦٦.٦٪) of disinfectants (in-use) was recovered as shown in table(٣-٥). ***P.aeruginosa*** and ***B.subtilis*** were the common bacterial types can tolerate and resist these disinfectants(table٣-١٠). Contamination of disinfectants and antiseptics seemed to be common. The contamination rate for these agents was (٣٤.٤٪) in Nigeria hospitals, ٤٣٪ in Japan hospitals and ٧.٩٪ in Malaysian hospital, Resistance of microorganisms toward disinfectants and antisepticss varies widely according to some factors including type of microorganism, type and concentration of disinfectants(Gajadhar *et.al*.,٢٠٠٣)

Failure of disinfectants to kill bacteria may reflect the poor quality of disinfectants or improper preparation and inadequate care of containers (Kajanahareutai *et.al*.,١٩٩٥) in which refilling and repeated use of same containers without cleaning and drying for long period of time strongly contribute to this contamination(Keah *et.al*.,١٩٩٥).

Fakhriddeen,(٢٠٠١) referred that tap water used to dilute these chemical may contain different types of bacteria included ***Acintobacter, pseudomonas, Micrococcus***, ***E.coli*** and ***Bacillus***. Some of these bacteria particular ***P.aeruginosa*** and ***E.coli*** found to be strongly tolerate the chlore in chlorinated

water(Al-Azzawy,١٩٩٧). Disinfectants should be prepared according to manufacture directions to achieve potent antimicrobial activity therefore, fresh solution must be prepared and used within the validity period (Oie and Kamiya,١٩٩٦).

P.aeruginosa spp. have been reported to be the predominant bacterial types recovered from contaminated disinfectants and antiseptics since it was found to be able to resist chlorohexidine gluconate ,chlorohexidine cetramide, chloroxylenol and PVP-I (MacDonnell and Russell,١٩٩٩). In addition, spores of Bacillus spp. were also detected in disinfectants and antiseptics because of their ability to resist such chemical agents (Russell,١٩٩٠).

P.aeruginosa was detected in a remarkable frequencies in clinical cases and hospital environment. This can be attributed to it's high adaptability to extreme environmental conditions and hence tolerates and survives in such conditions**(**Pirnray *et.al.*,٢٠٠٣). Furthermore, this orgaism was described as highly resistant to high concentrations of salt ,dyes and many antimicrobial agents (Ehrlich,٢٠٠٣). Hamza,(٢٠٠٦) described ***P.aeruginosa*** as the drugs resistant organism which can resist antibacterial agents by various mechanisms. The contamination rate observed in hospital environment through this study may reflect the relaxation in general hygienic measures.

٣-٣:Disinfectants and Antiseptics

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 :

١- **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 (Nicoletti *et.al*., ١٩٩٣). The MIC values of Chlorohexidine gluconate (CHX) toward these bacteria were ١٢٨,١٢٨ ٣٢and ٣٢µg/ml respectively as show in figure(٣-١) while the MIC values of $H_٢O_٢$ for ***P.aeruginosa***, ***E.coli***, **MRSA** and ***B.subtilis*** were ١٠٢٤, ٥١٢, ٥١٢ and ١٢٨µg/ml respectively figure(٣-٢) whereas the MIC values of sodium hypochlorite for these bacteria were ٢٠٤٨,٢٠٤٨,٥١٢ and ١٢٨ µg/ml respectively Figure(٣-٣). Sodium dichloroisocyanurate revealed the following MIC values ٣٢٧٦٨,١٦٣٨٤, ١٢٨ and ١٢٨µg/ml for ***P.aeruginosa***, ***E.coli,*** **MRSA** and ***B.subtilis*** respectively as shown in figure (٣-٤).

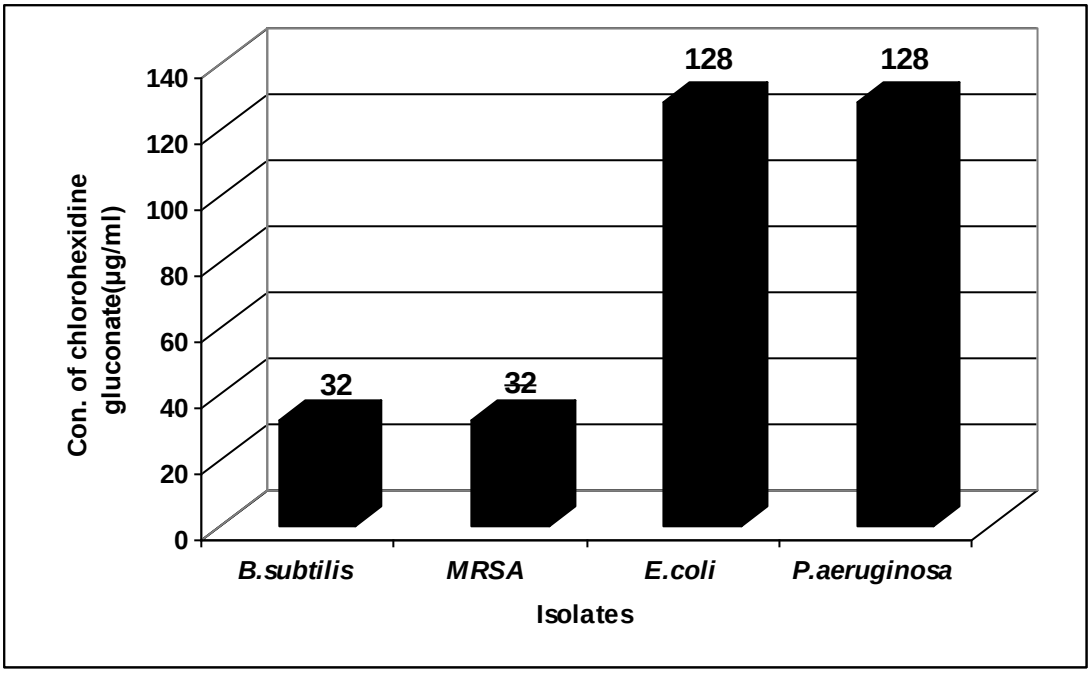


Figure (٣-١) The MIC values of chlorohexidine gluconate on different bacterial isolates

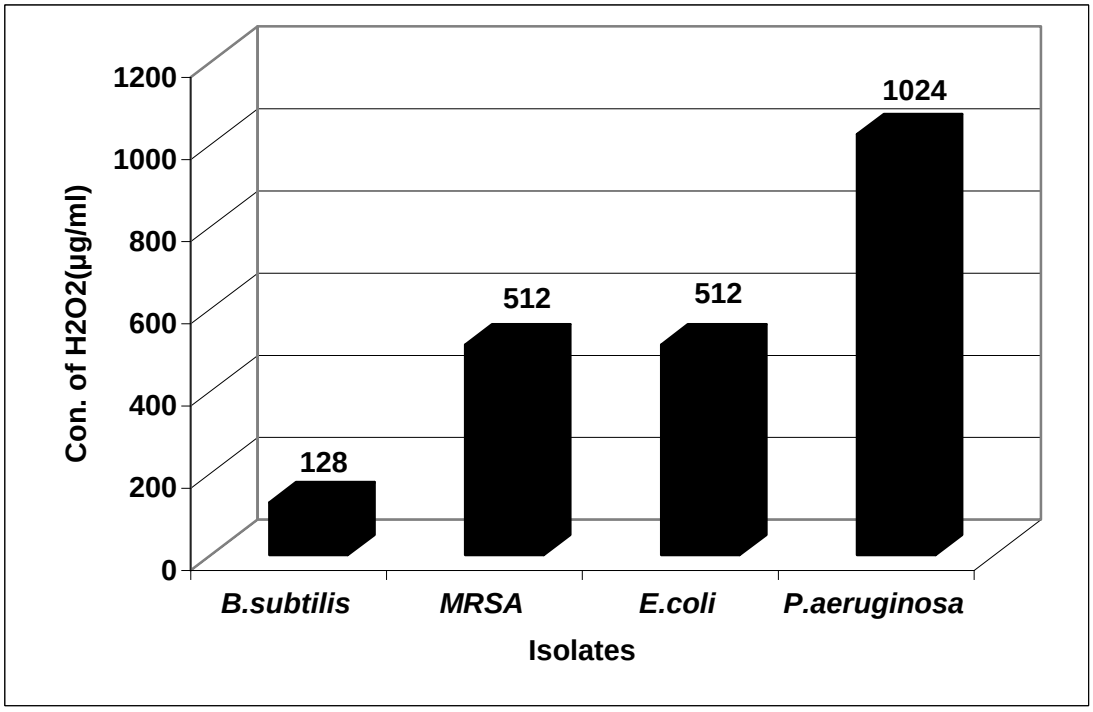
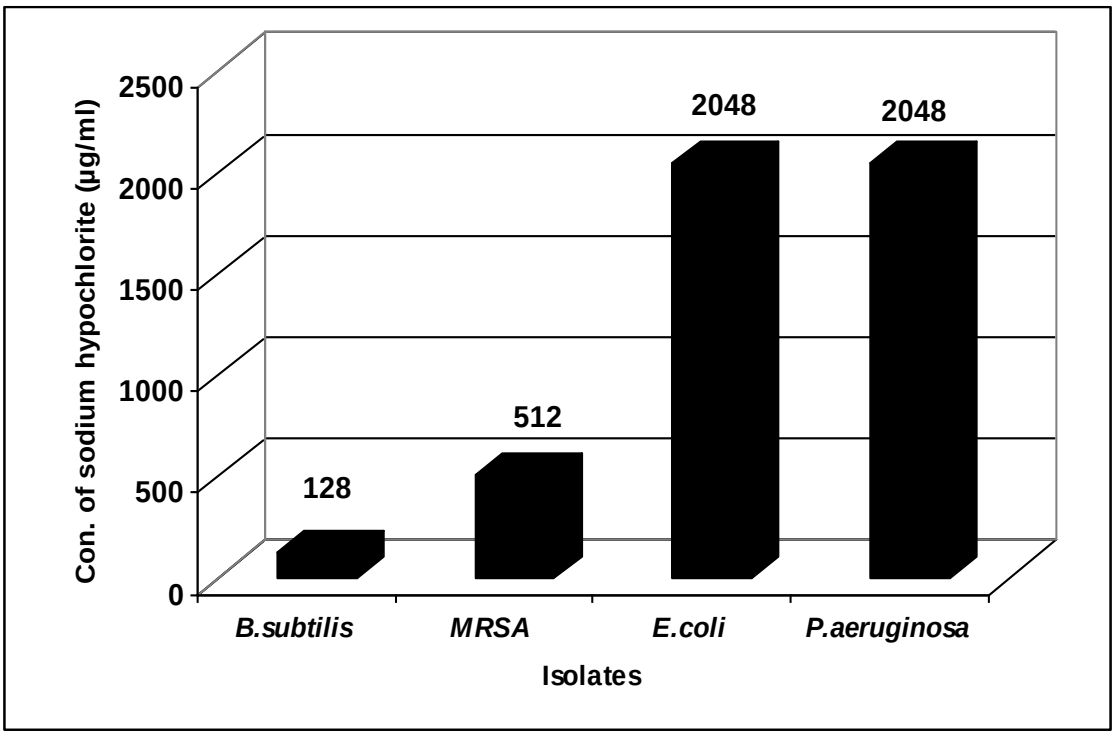
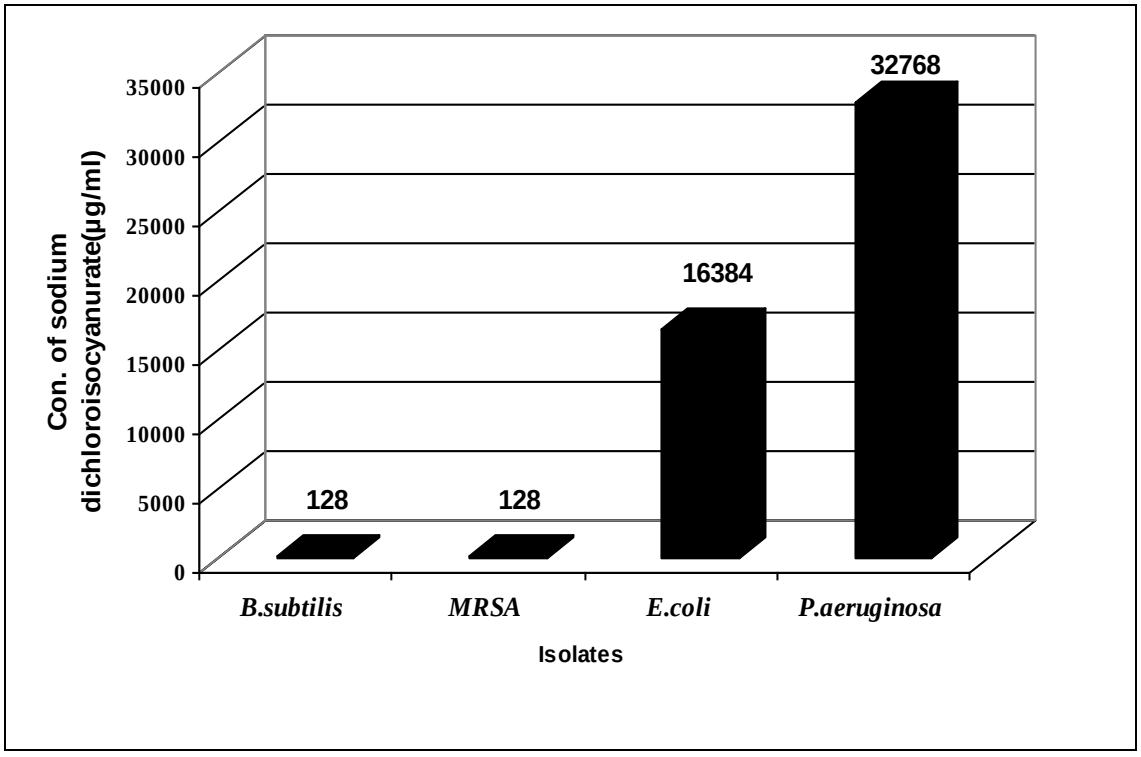


Figure (٣-٢) The MIC values of H_2O_2 on different bacterial isolates



Figure(٣-٣)The MIC values of sodium hypochlorite on different bacterial isolates



Figure(٣-٤)The MIC value

s of sodium dichloroisocynurate on different bacterial isolates

The MIC values for Formaldehyde were ٤٠٩٦,٢٠٤٨,١٠٢٤ and ٢٥٦µg/ml for ***P.aeruginosa***, ***E.coli,*** **MRSA** and ***B.subtilis*** respectively figure(٣-٥).

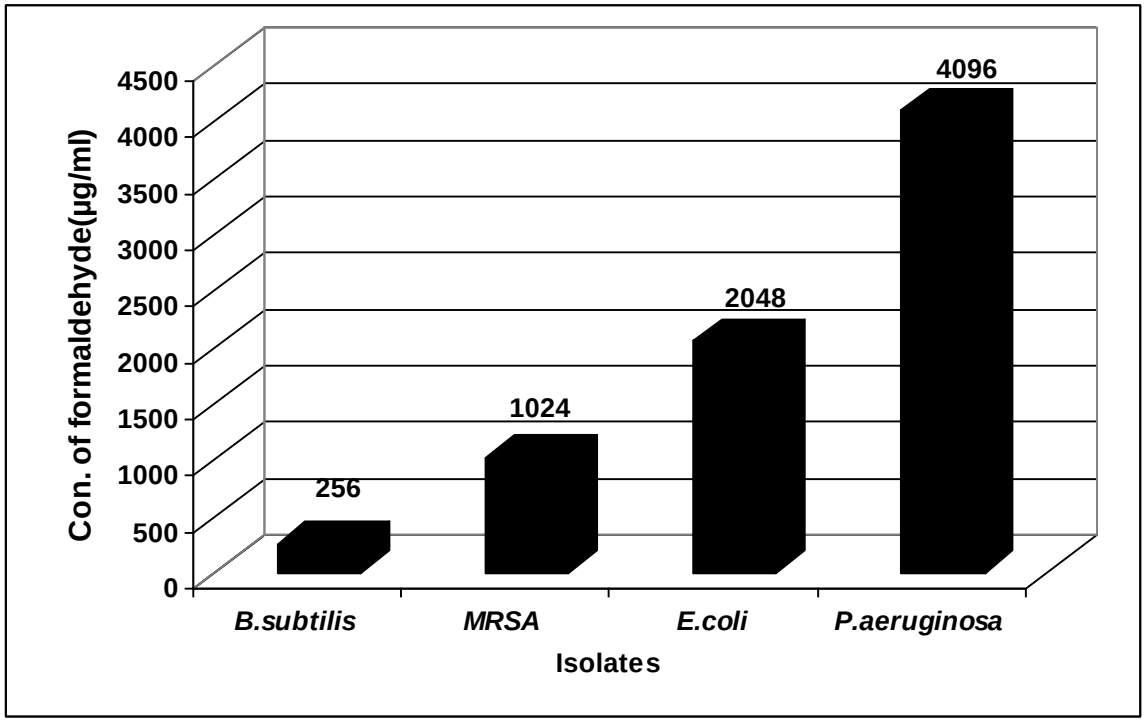


Figure (٣-٥) The MIC values of formaldehyde on different bacterial isolates

In the this study ٣ types of chloroxylenol from different origins were tested. The first two types of chloroxylenol termed ($S_1$and S_2) from the Iraqi company for drugs industries and medical requirements(Samara) have MIC values towards ٤ types of bacteria as shown in figure (٣-٦). 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 ٢٠٤٨ and ١٠٢٤µg/ml respectively. Chlorohexidine cetramid

tested in this study exhibited no any efficiency against all bacterial tested even in high concentration reach to ١gm/ml.

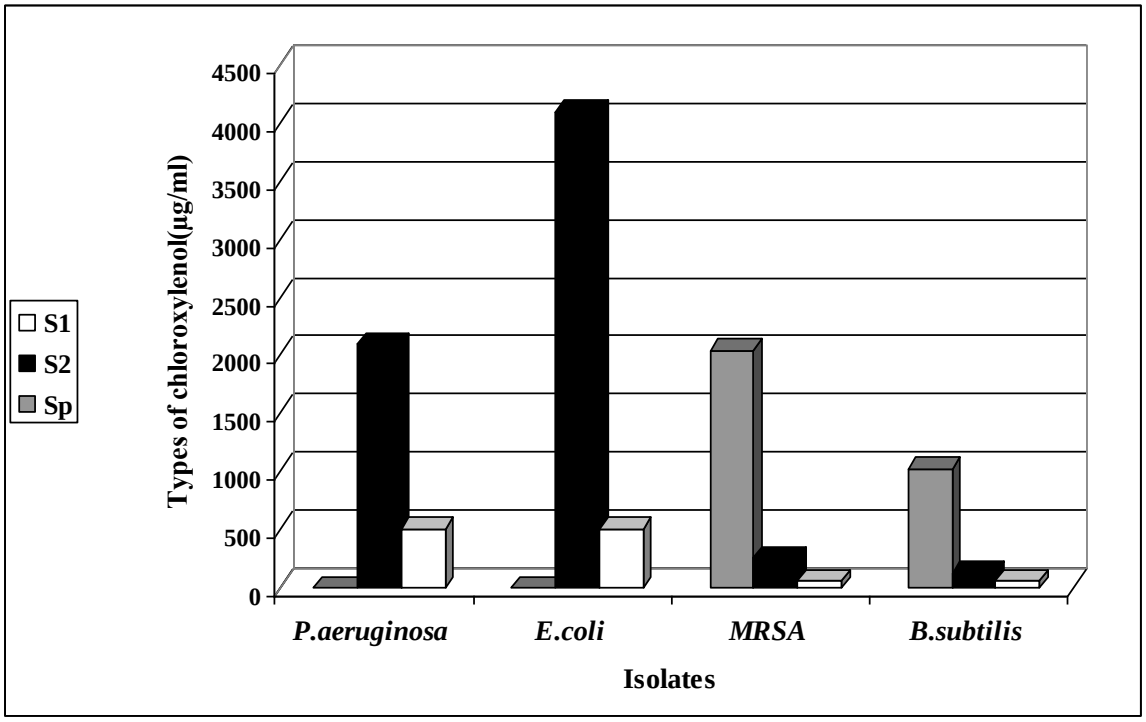
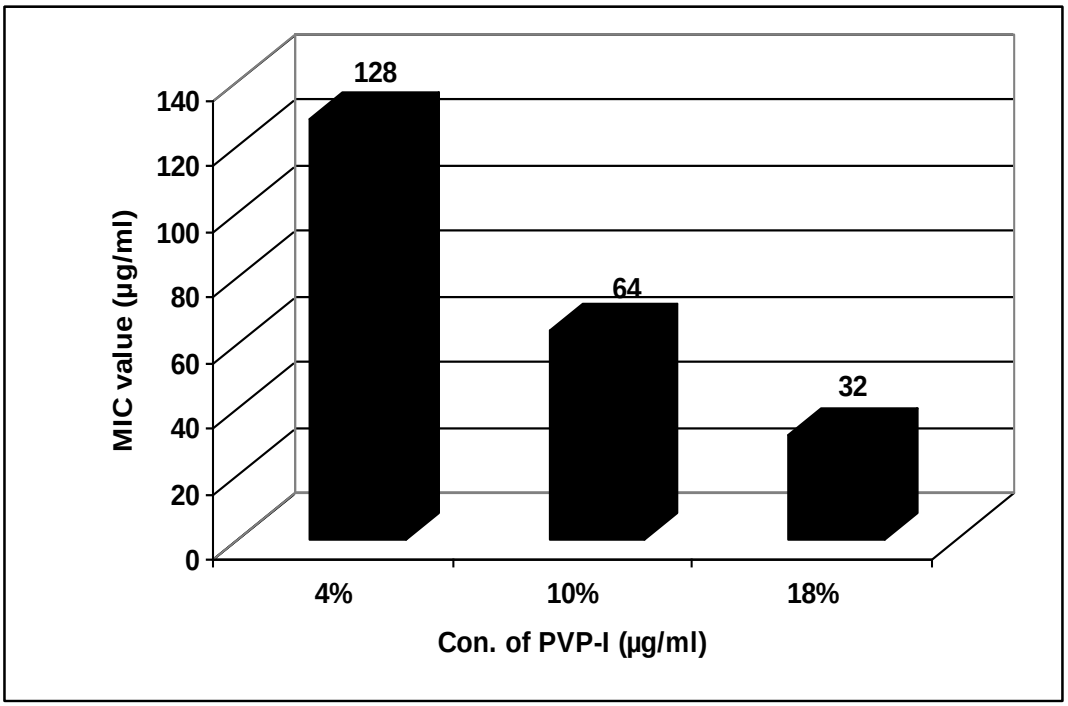


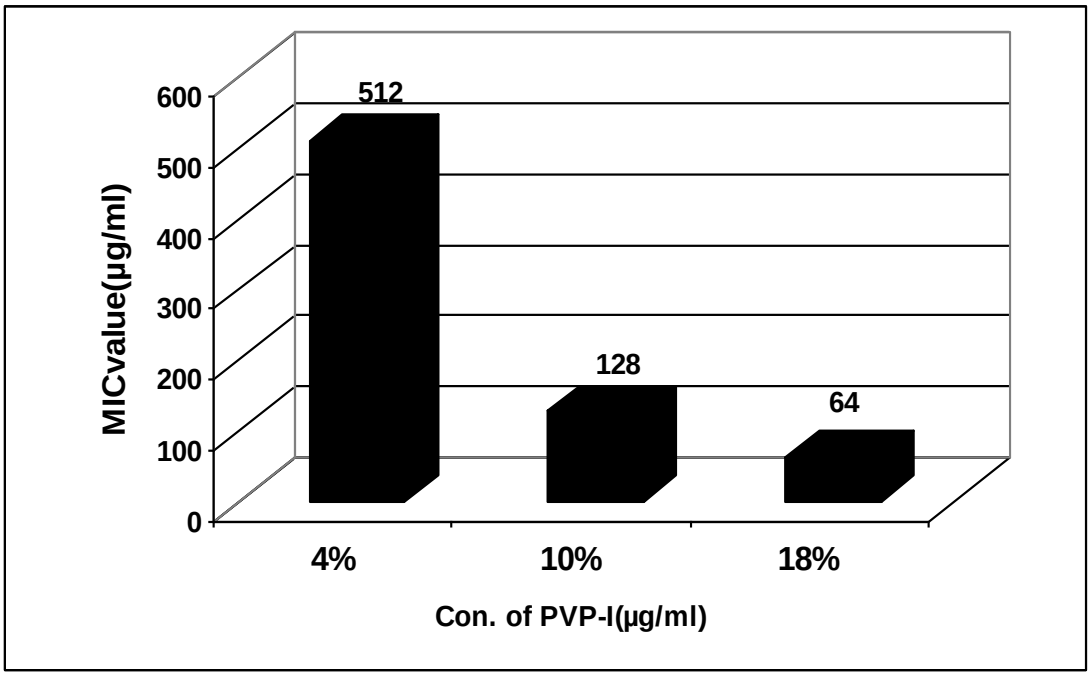
Figure (٣-٦) The MIC values of three types of chloroxylenol ($S_١$,$S_٢$,Sp) on different bacterial isolates .

The susceptibility test organism towards PVP-I was widely variable, for this, test organism were investigated separately towards the appropriate MIC at (٤٪, ١٠٪ and ١٨٪) of PVP-I, hence the MIC for test bacteria are shown in (figure ٣-٧,٣-٨,٣-٩ and ٣-١٠).

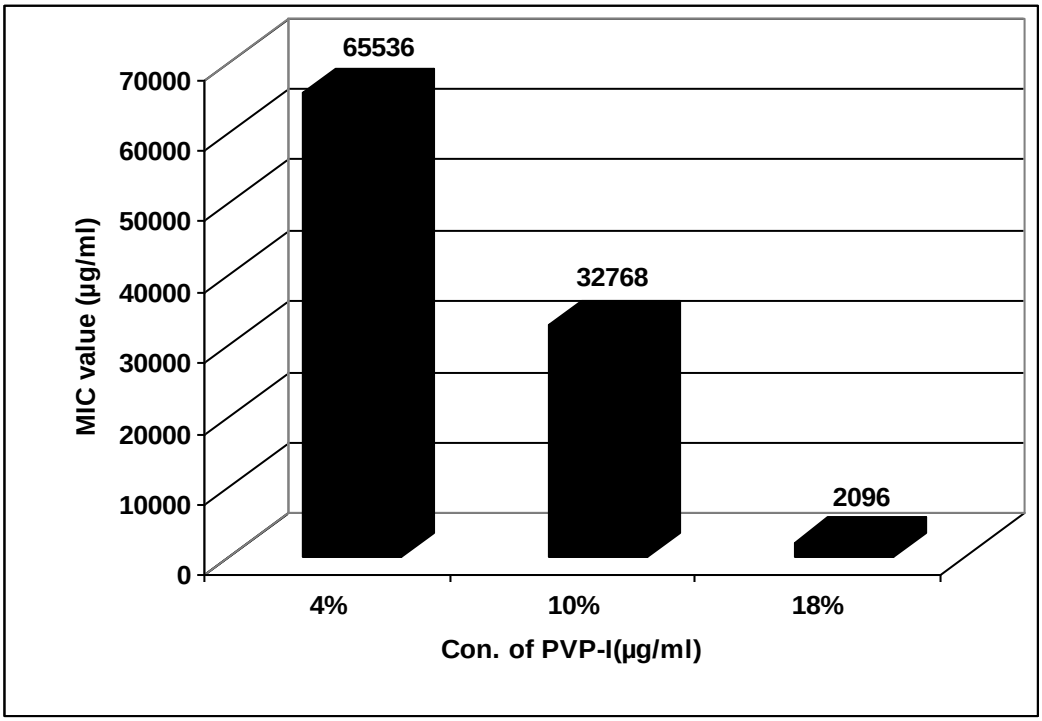
B.subtilis exhibited a remarkable sensitivity while ***P.aeruginosa*** was strongly resistant against PVP-I since the MIC regarding ***B.subtilis*** ranged from ٣٢-١٢٨ µg/ml versus ٣٢٧٦٨ to ١٣١٠٧٢ µg/ml for ***P.aeruginosa***, while for MRSA ranged between ٦٤-٥١٢ µg/ml, while for E.coli ranged between ٢٠٩٦-٦٥٥٣٦ µg/ml.



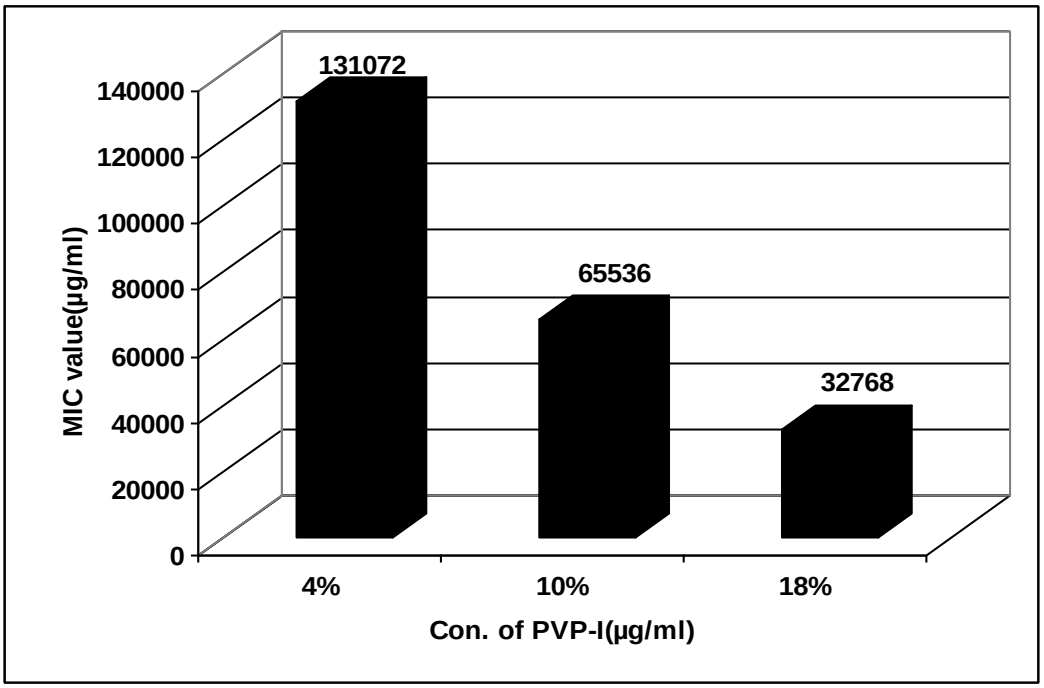
Figure(٣-٧)The MIC values of different concentrations of PVP-I on ***B.subtilis***



Figure(٣-٨) The MIC values of different concentrations of PVP-I on **MRSA**



Figure(٣-٩) The values of MIC of different concentrations of PVP-I on ***E.coli***



Figure(٣-١٠)The MIC values of different concentrations of PVP-I on ***P.aeruginosa***

Chlorohexidine gluconate (CHX) was the most potent and effective disinfectant according to this study followed by chloroxylenol(S_1)type while chloroxylenol(S_2) and (Sp) types exhibithed lower activity H_2O_2, sodium hypochlorite, formaldehyde, sodium dichloroisocyanurate and PVP-I. According to the results obtained by Fakhriddeen,(٢٠٠١). Chlorohexidine cetramid was the potent disinfectants against bacteria. He arranged the disinfectants according to their potency as chlorohexidine cetramid, CHX, PVP-I, chloroxylenol, formaldehyde and H_2O_2. 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,(١٩٦٦) reported that many disinfectants used in the hospital lost it's activity while it's still in primary jar in which all the factor mentioned above affected the efficiency of those agents. Nicoletti *et.al.*,(١٩٩٣) pointed out that MIC values of CHX for ***P.aeruginosa***, ***E.coli*** and ***S.aureus*** were ٦٤-١٢٨,٤-٨ and ٤-٨µg/ml respectively. Mengistu *et.al.*,(١٩٩٩) found that all **(**١٠٠%**)**of ***E.coli*** isolates and (١٩.٢%) of ***P.aeruginosa*** isolates were inhibited only by high concentration of CHX in which the MIC ≥١٠٠٠µg/ml. ***S.aureus*** has been reported to be susceptible ≤١٠٠ µg/ml for CHX, while Brumfit *et.al.*,(١٩٨٥) found that **MRSA** isolates were more resistant than **MSSA** isolates to CHX, cetramid and quaternary ammonium compound, while Wenzal,(١٩٩٧) reported that **MRSA** and **MSSA** strain were equally susceptible to CHX.

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*** (Kampf and Kramer,٢٠٠٤) 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 ,RP١) 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 (Gilbert and McBain,٢٠٠٣)**.** ***P.aeruginosa*** has been derscribed to be highly resistant to CHX.

Recent studies suggested that mutation or over expression of CHX target sites resulted in non susceptible microorganisms (Sheldon,٢٠٠٥). ***S.aureus*** was

reported to harbor antiseptic resistance genes. Such isolates have been isolated from clinical samples and three determinants qacA,qacB and qacC have been identified which confer resistance to biocide agents (Mayer *et.al*., ٢٠٠١).

Fakhriddeen,(٢٠٠١) pointed out that MIC values of H_2O_2 for ***P.aeruginosa***, ***E.coli***, ***S.aureus*** were ١٠٢٤ ,٥١٢ and ٥١٢µg/ml respectively while Penna *et.al*., (٢٠٠١) mentioned that MIC values of (H_2O_2) for ***E.coli*** ranged between ١٢٥٠-٣٧٠٠µg/ml and for ***S.aureus*** it ranged ٦٢٥-٩٣٨µg/ml. Many aerobic microorganisms have developed intrinsic defense system that confer tolerance to peroxide stress particularly H_2O_2 and this includes the production of neutralizing enzymes including peroxidase, catalase and glutathione reductase to prevent cellular damage (Demple and Harrison, ١٩٩٤; Storz and Altuvia,١٩٩٤). Moreover, ***Bacillus*** spp. tolerance to H_2O_2 has been described to vary during the growth phase and in mutant strains (MacDonnell and Russell,١٩٩٩).

Penna *et.al*., (٢٠٠١) 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 (١١٠٩-١٤٩٧)µg/ml, while the Penna *et.al*.,(٢٠٠٢) found that MIC values of sodium hypochlorite for ***P.aeruginosa*** was ٢٥٠٠µg/ml.

Dawaf,(١٩٩٣) demonstrated that MIC values of chloroxylenol for ***P.aeruginosa***, ***E.coli and S.aureus*** were ١٠٢٤ ,١٠٢٤ and ١٢٨µg/ml respectively. Davies *et.al*.,(١٩٨٠) referred that chloroxylenol has good activity against Gram–positive than Gram–negative bacteria and was less active against ***P.aeruginosa***.

The MIC values of formaldehyde for ***P.aeruginosa***, ***E.coli*** and ***S.aureus*** were ١٠٢٤,٥١٢ and ٥١٢µg/ml respectively as it has been reported by Fakhriddeen in (٢٠٠١) while lower relatively MIC for formaldehyde(١٥٦ µg/ ml) against both ***E.coli*** and ***S.aureus*** have been found by(Penna and coworkers in (٢٠٠١). ***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 (Wenzal and Zaidi,٢٠٠٠). In addition, alterations in the cell surface (outer membrane proteins) and presence of at least one additional high molecular mass protein in the formaldehyde tolerant strains lead to these resistance (Zachi *et.al*.,١٩٩٦) .

Fakhriddeen,(٢٠٠١) stated that MIC values of pvp-I (١٠٪) for ***P.aeruginosa***, ***E.coli*** and ***S.aureus*** were ٥١٢,١٢٨ and ٣٢µg/ml respectively while Penna *et.al*., (٢٠٠١) have reported that MIC values of pvp-I (١٠٪) for ***E.coli*** was ١٥٦٠µg/ml. The result of study done by Yz *et.al*.,(٢٠٠٤) showed that **MRSA** isolates were more resistant to iodophore than **MSSA** and there was obvious difference in MIC values between **MRSA** and **MSSA** in which there was a remarkable increase (٥,٠٠٠ fold) in **MRSA** tolerance to PVP-I compared with **MSSA**(Al-Masaudi *et.al*.,١٩٩١) .

This study observed that ***P.aeruginosa*** and ***E.coli*** exhibited high MIC values against pvp-I. These bacteria were isolated from burns and wounds inspite of disinfecting those patients by PVP-I. This may indicate that PVP-I was inactive perhaps because of an improper preparation. The problem of inactive disinfectant or improper (sublethal) concentration of disinfectants lead to the emergence of mutants which develop resistance against such disinfectants.

Moreover, the increasing and mis use of disinfectants strongly contribute for emergence and /or selection of resistant population of pathogenic microorganism in the hospital environment (Guimaraes *et.al*.,٢٠٠٠) .

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(Russell *et.al*.,١٩٩٨). The changes in permeability system may lead to acquire resistance to biocidal compounds(Sheldon ,٢٠٠٥).

Luzzaro *et.al*.,(٢٠٠١) in his study found that ***P.aeruginosa*** have high MIC values against pvp-I reached upto ١٩٠٠٠ µg/ml. 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 (Pallent *et.al*.,١٩٨٣). Furthermore, ***P.aeruginosa*** possesses active efflux pump system acts as wide transporters for a whole rang 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 (Schweizer,١٩٩٨)..

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.(Russell and Chopra,١٩٩٦).

٢-Disc Diffusion method

To perform this experiment, discs for chloroxylenol(S١),hydrogen peroxide($H_٢O_٢$), sodium hypochlorite, sodium dichloroisocyanurate and formaldehyde of different concentrations were prepared according to method recommended by Wage and Hedin, (١٩٨٥) and tested towards ***P.aeruginosa***, ***E.coli*** and **MRSA**.

MRSA isolates exhibited considerable resistance to $H_٢O_٢$ at concentration below ١٠٠٠ µg/disc but it was sensitive at higher concentrations as shown in figure (٣-١١). ***E.coli*** was resistant to the concentration of $H_٢O_٢$١٠٠٠(µg/disc) but it was sensitive at concentrations ٣٠٠٠, ٥٠٠٠ and ٨٠٠٠ µg/disc with inhibition zone ٦, ٧ and ١١ mm, while ***P.aeruginosa*** exhibited resistance to most concentrations but it was sensitive to $H_٢O_٢$ only at concentrations ٣٠٠٠,٥٠٠٠ and ٨٠٠٠ µg/disc with inhibition zone ٧,١١ and ١٥ mm(figure٣-١١). Fakhriddeen, (٢٠٠١) found in his study that ***S.aureus***, ***E.coli*** and ***P.aeruginosa*** were resistant to lower concentrations ٦٠٠ µg/disc of $H_٢O_٢$ than concentration being used in this study.

Figure(٣-١٢) shows that **MRSA** exhibited relative sensitivity to chloroxylenol (S_1) at concentrations of ١٠٠,٢٠٠,٣٠٠,٤٠٠ and ٥٠٠ µg/ml with inhibition zone of ٨,١٤,١٨,٢١ and ٢٥ mm but it was not affected by concentrations below ١٠٠ µg/disc while ***E.coli*** and ***P.aeruginosa*** revealed full resistance to all concentration of chloroxylenol (٥٠-٥٠٠ µg/disc). Fakhriddeen,(٢٠٠١) showed that ***S.aureus*** and ***E.coli*** were resistant to chloroxylenol in ١٠-١٠٠µg/disc ,but they were sensitive to this disinfectants at increased concentrations whereas ***P.aeruginosa*** was full resistant to all concentrations of chloroxylenol up to ٥٠٠µg/disk .

The results of this study showed that **MRSA** was resistant to all concentration of sodium hypochlorite below ٣٠٠٠µg/disc while it was sensitive to the concentrations of ٣٠٠٠,٥٠٠٠ and ٨٠٠٠ µg/disc with inhibition zone ٤,٧ and ١١ mm respectively as shown in figure(٣-١٣), while ***E.coli*** and ***P.aeruginosa*** were resistant to all sodium hypochlorite concentrations. **MRSA** was inhibited only at concentration ≥١٠٠٠ µg/disc (figure ٣-١٤) of formaldehyde, while ***E.coli*** and ***P.aeruginosa*** were resistant to all concentrations of formaldehyde (٥٠- ٨٠٠٠µg/disc).All bacterial isolates showed full resistance to sodium dichloroisocyanurate at concentrations (٥٠-٨٠٠٠ µg/disc) .

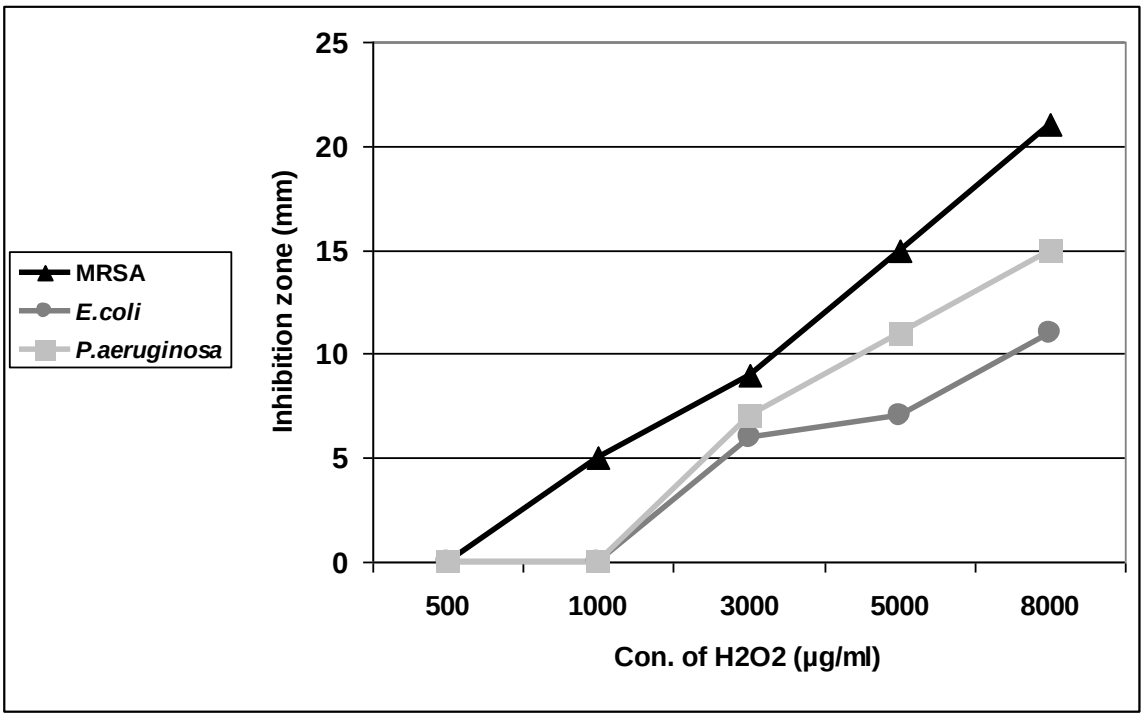


Figure (٣-١١) Inhibition zones of discs containing different concentrations of H_2O_2 on bactertia

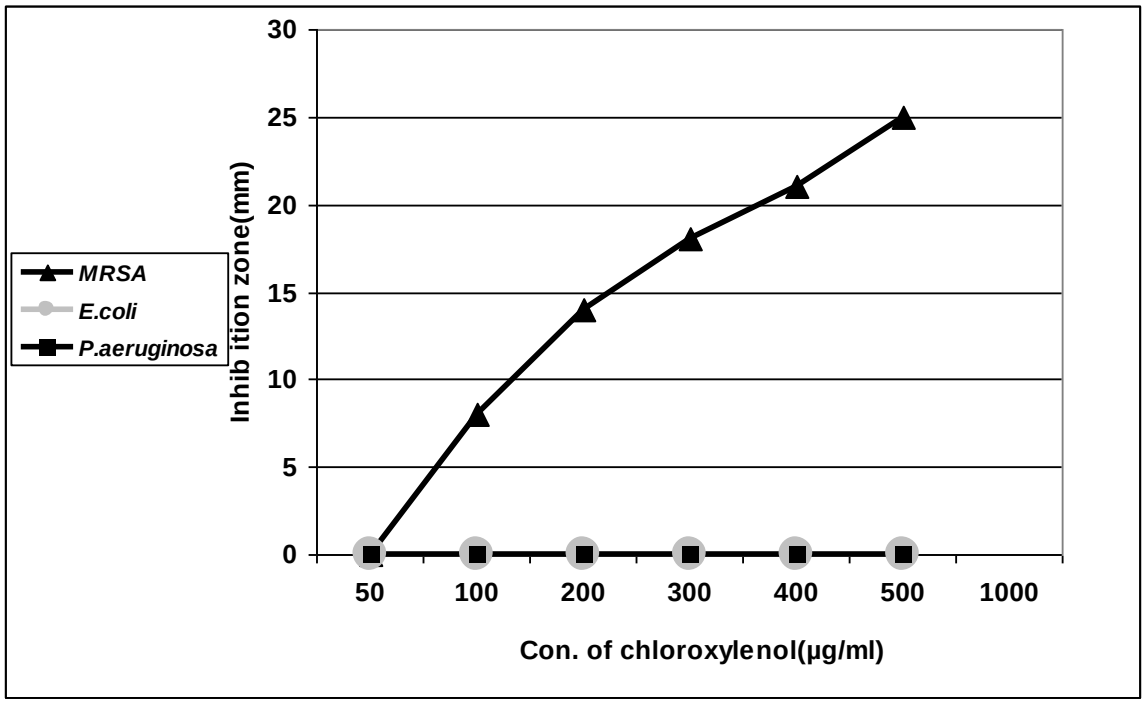


Figure (٣-١٢) Inhibition zones of discs containing different concentrations of Chloroxylenol on bactertia

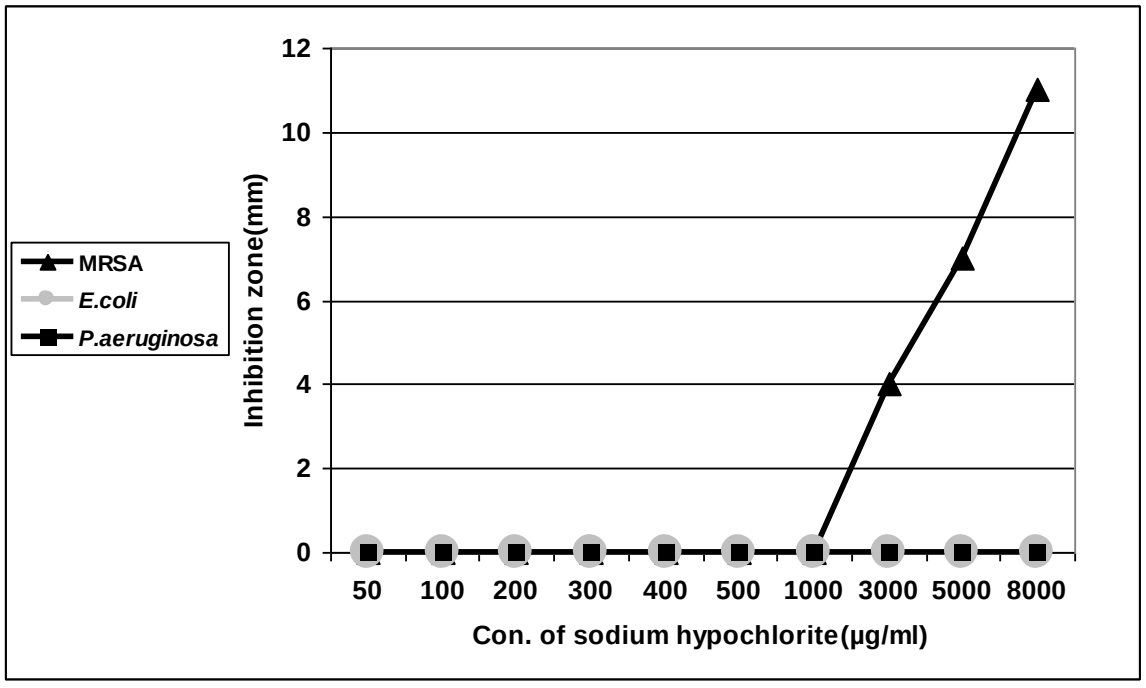


Figure (٣-١٣) Inhibition zones of discs containing different concentrations of sodium hypochlorite on bactertia

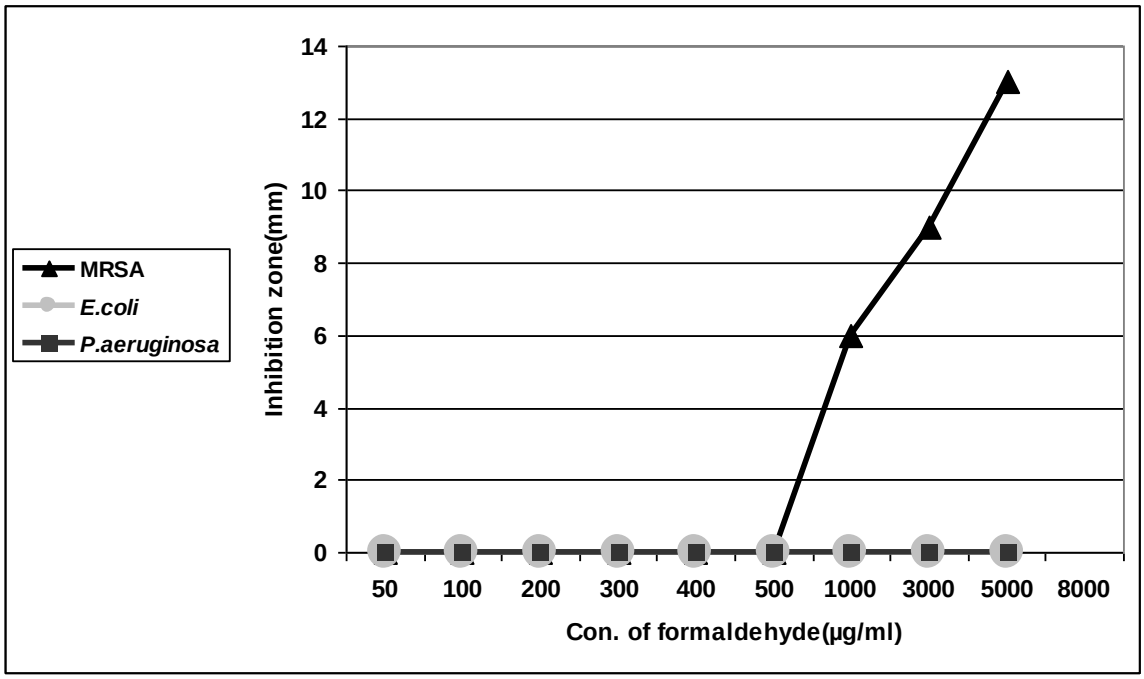


Figure (٣-١٤) Inhibition zones of discs containing different concentrations of Formaldehyde on bactertia

٣- Well Diffusion method

According to the results shown in figure (٣-١٣) **MRSA** isolates were sensitive to H_2O_2 at concentrations from ٧٨ to ٥٠٠٠ µg/ml and ***B.subtilis*** affected at concentration above ٧٨µg/ml while ***E.coli*** show resistance to the concentration below ٢٥٠٠ µg/ml while ***P.aeruginosa*** were affected at concentration up to ٢٥٠٠µg/ml. **MRSA** was sensitive to chloroxylenol (S_2) at concentration ٦٢٤µg/ml with inhibition zone ٦ mm and inhibition zone increased with increased concentrations reaching up to ١٦mm at ٥٠٠٠ µg/ml. ***B.subtilis*** was sensitive to the same disinfectant at concentration ≥ ٦٢٤ µg/ml

whereas ***E.coli*** was sensitive to chloroxylenol only at high concentrations ١٢٥٠,٢٥٠٠ and ٥٠٠٠ µg/ml and ***P.aeruginosa*** effected only at concentrations up to ١٢٥٠ µg/ml (figure٣-١٤).

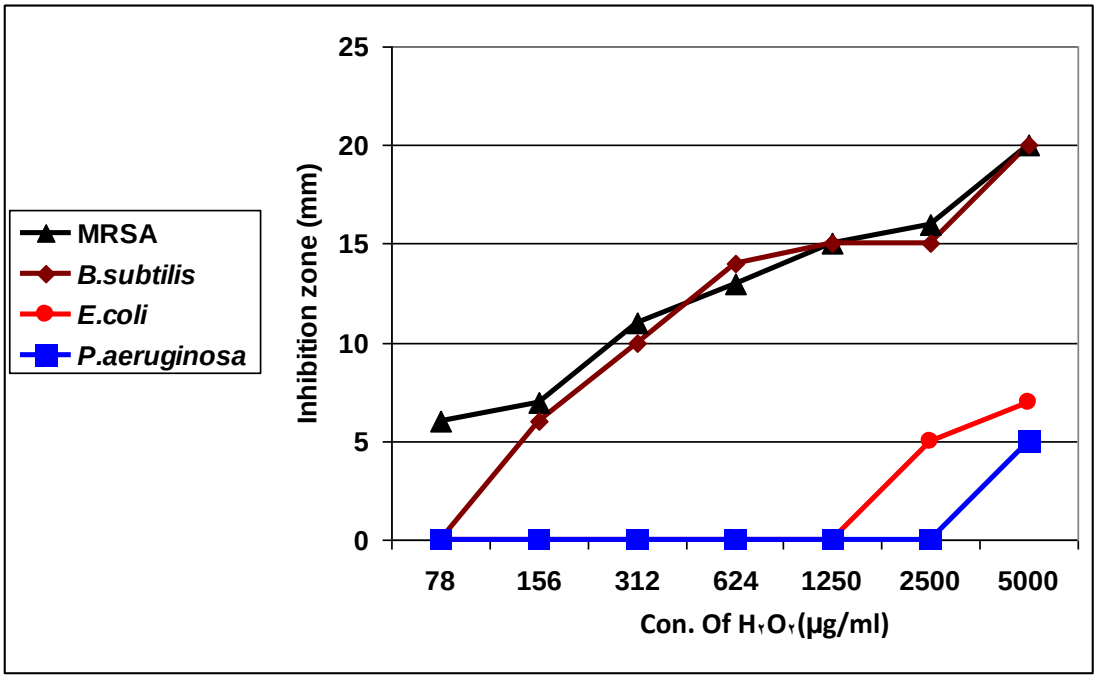
Resistance of ***E.coli*** to sodium hypochlorite was observed at concentration below ٢٥٠٠µg/ml but it was sensitive at higher concentration as shown in figure(٣-١٥). **MRSA** and ***B.subtilis*** revealed relative sensitivity compared with ***E.coli*** while ***P.aeruginosa*** exhibited full resistance to all concentrations of this disinfectant.

The results shown in figure(٣-١٦) indicate show that ***P.aeruginosa*** and ***E.coli*** were inhibited by ١٢٥٠µg/ml concentration of formaldehyde while ***B.subtilis*** and **MRSA** were sensitive to formaldehyde at lower concentrations, since they inhibited at concentration of ٣١٢ and ٦٢٤µg/ml respectively.

The results regarding PVP-I exhibited that **MRSA** was sensitive to pvp-I (٤%) at concentration ١٢٥٠ and ٢٥٠٠µg/ml with inhibition zone ٥ and ٧ mm while ***B.subtilis*** was sensitive to the concentrations ٦٢٤, ١٢٥٠ and ٢٥٠٠µg/ml. Both ***E.coli*** and ***p.aeruginosa*** was exhibited full resistance to the all concentration of pvp-I (٤٪,١٠٪ and١٨٪) figure (٣-١٧) .

In our study we noticed that **MRSA** and ***B.subtilis*** sensitive to pvp-I (١٠٪) at concentrations ٣١٢µg/ml and it was also sensitive to other highest concentrations (figure ٣-١٨)...

Figure(٣-١٩) showed that MRSA was sensitive to pvp-I (١٨%) at concentrations ٣١٢µg/ml with inhibition zone ٥mm and it also sensitive to other concentration while ***B.subtilis*** was effected at concentration below.



Figure(٣-١٥) Inhibition zones of different concentrations of H_2O_2 on test bacterial isolates.

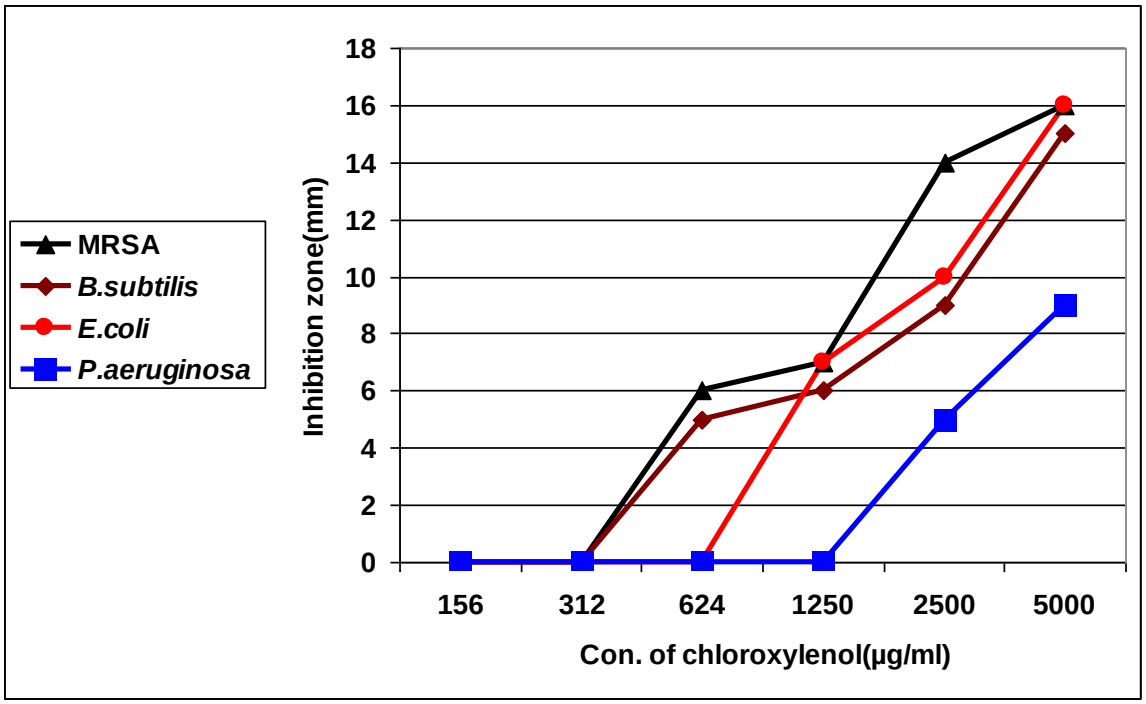


Figure (٣-١٦) Inhibition zones of different concentrations of chloroxylenol ($S_٢$) on bacteria

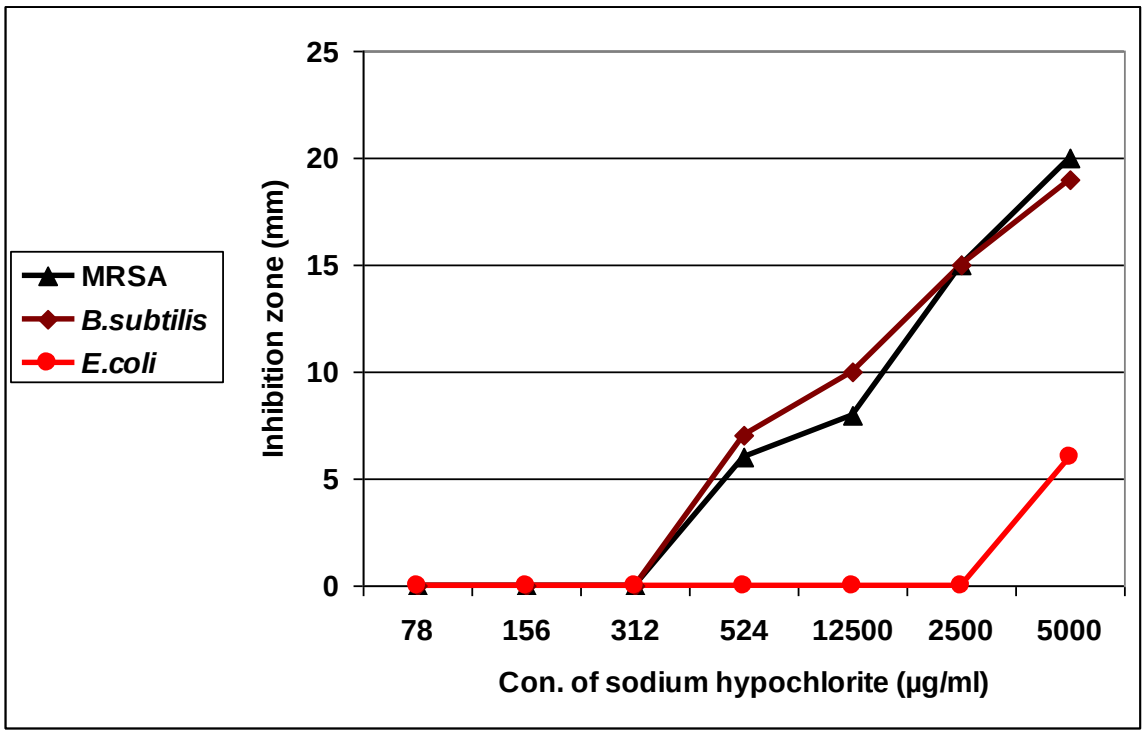


Figure (٣-١٧) Inhbition zone of different concentrations of sodium hypochlorite on bacteria

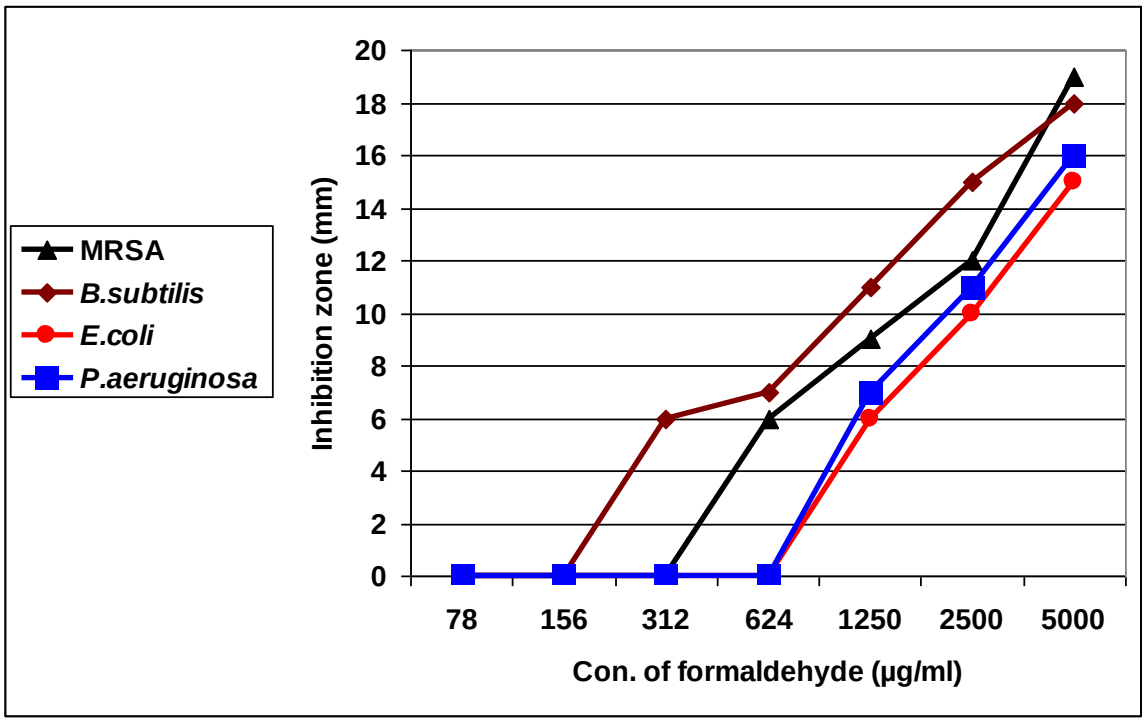


Figure (٣-١٨) Inhibition zones of different concentrations of formaldehyde on bacteria

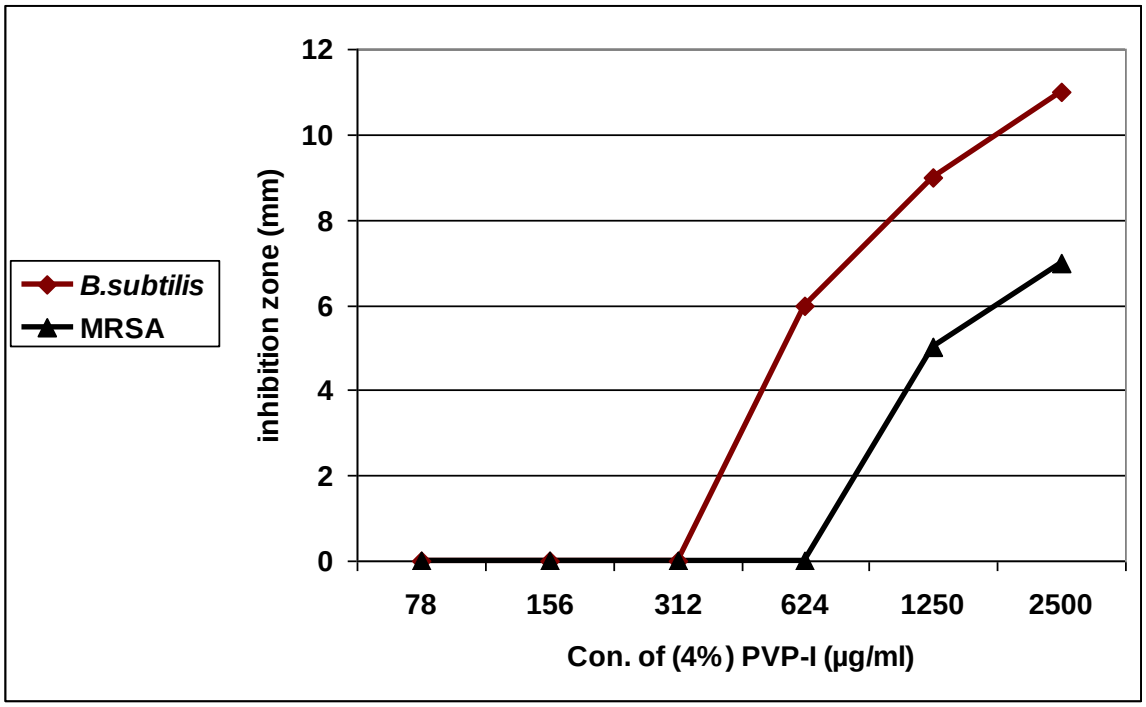


Figure (٣-١٩) Inhibition zone of different concentrations of PVP-I (٤٪) on bacteria

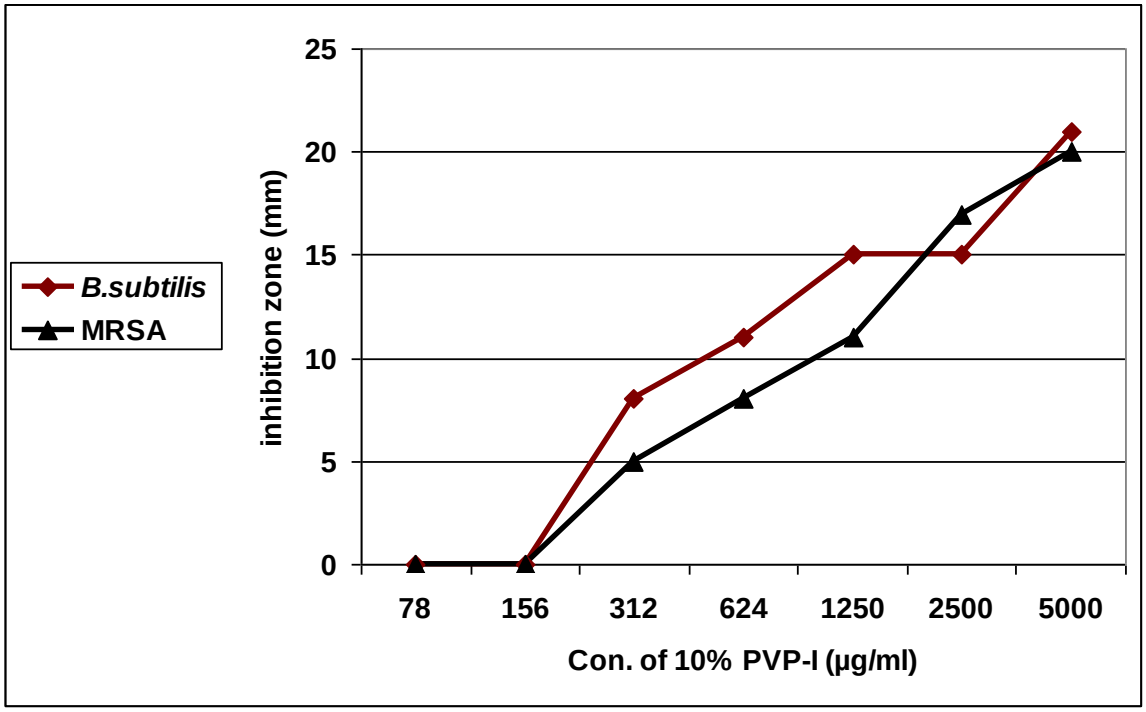


Figure (٣-٢٠) Inhibition zone of different concentrations of PVP-I (١٠٪) on bacteria

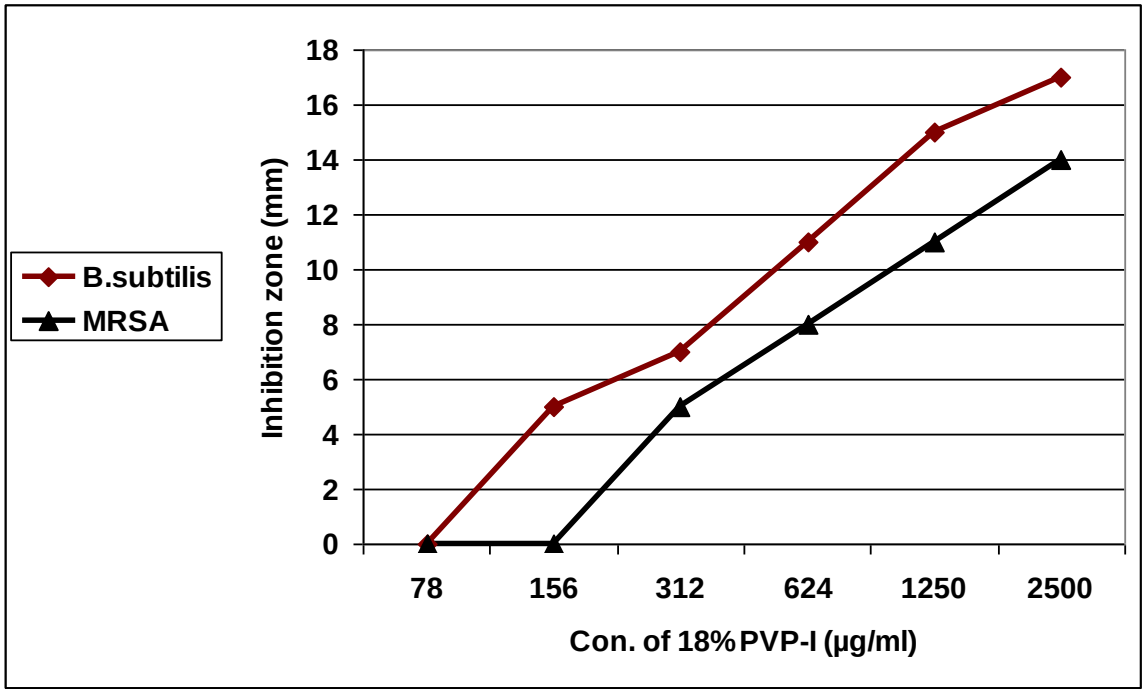


Figure (٣-٢١) Inhibition zones of different concentrations of PVP-I (١٨%) on bacteria

٣-٤:The sensitivity of bacteria to the antibiotics:

The sensitivity of ***S.aureus*** (**MSSA** and **MRSA**),***P.aeruginosa*** and ***E.coli*** isolated from different clinical cases to antibiotics was detected by Bauer-Kirby method. These Bacteria exhibited remarkable resistance to most antimicrobial agents in which they exhibited full resistance to β-lactam compounds and this may be due to the widespread use of these compound lead to emergence of resistance.

In this study ***S.aureus*** revealed resistance against most antibiotics in which both **MSSA** and **MRSA** were fully resistant ١٠٠٪ to penicillin, ampicillin and amoxicillin. This results was in agreement with results being reported by Fang and Hedin,(٢٠٠٣) who stated that ***Staphylococci*** were found to be resistant to methicillin or oxacillin should be generally resistant to all β-lactam antibiotics. The primary mechanism for resistance to β-lactam is the enzymatic hydrolysis of the β-lactam ring by β-lactamases. Failure of antibiotic to penetrate to pencillin binding proteins (PBP) target site and low affinity binding of antibiotic to PBP also confer resistance to these antibiotics(Ang *et.al.*,٢٠٠٤).

Figure (٣-٢٠) shows that **MRSA** was more resistant than **MSSA** to the antibiotic in which the resistance of **MRSA** for gentamicin, cefotaxime and trimethoprime–sulfamethaxazole was ٨٠٪ for each versus with ٦٦.٦٪, ٦٦.٦٪ and ٧٥% of **MSSA** resistance to these antibiotics respectively. **MRSA** showed relatively low resistance to the ciprofloxacin ٤٠٪ and amikacin ٢٠٪ while **MSSA** revealed sensitivity to these antibiotics and only ٢٥٪ and ٨.٣٪ of them were resistant to ciprofloxacin and amikacin .

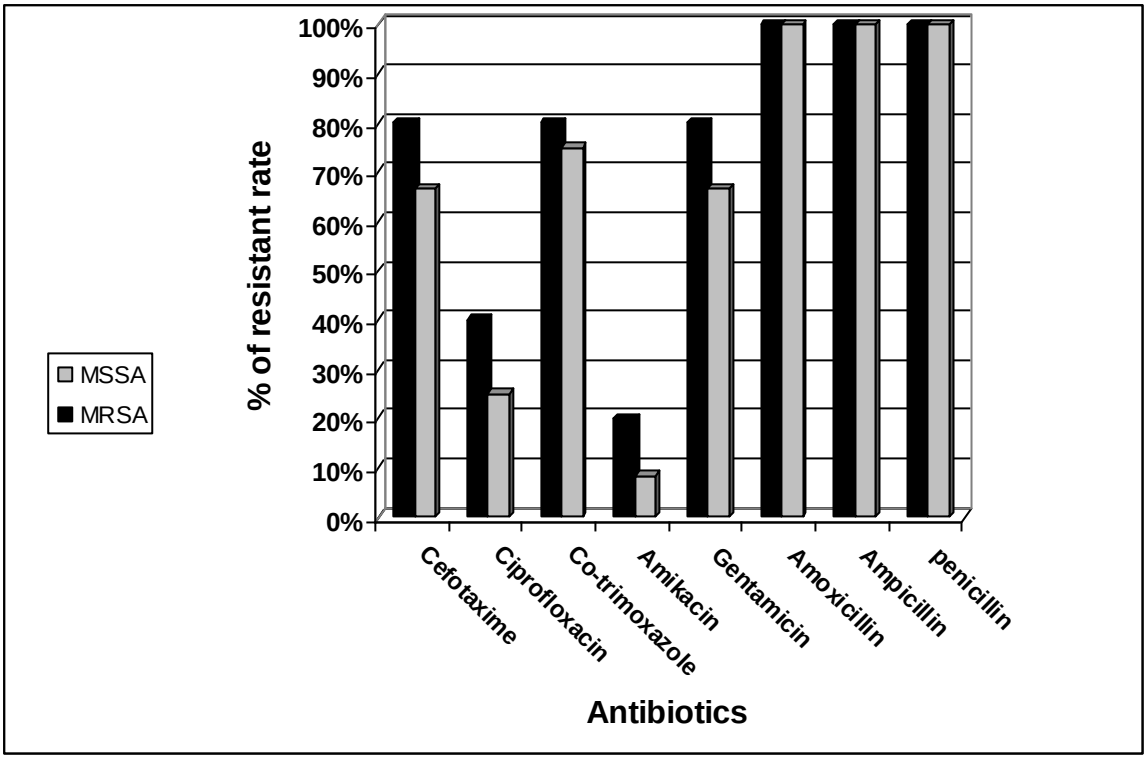


Figure (٣-٢٢) The resistant rate (%)of **MRSA** and **MSSA** isolates to the antibiotics

In ٢٠٠٣,(Hanumanthappa *et.,al*) found that the resistance of **MRSA** and **MSSA** to amoxicillin was ١٠٠٪ and ٦٤ %, gentamicin ٨٢.٤٪ and ٢.٨٪, cefotaxime ٧٧٪ and ٠ and ciprofloxacin ٦٦.١٪ and ٩.١٪ respectively while Shehanel–Din *et.,al*.,(٢٠٠٣) mentioned that the resistance of **MRSA** and **MSSA** to penicillin, gentamicin, trimethprime–sulfamethaxazole and ciprofloxacin was ١٠٠٪ and ٢٥٪, ٥٨٪ and ٥٠٪, ٢٥٪ and ٢٥٪ and ٣٣.٣٪ and ٢٥٪respectively. The results observed in this study were in accordance with that results found by Al-Hadithi and Yousif,(٢٠٠٣) who pointed out that all **MRSA** isolates were resistance to ampicillin and cefotaxime. Melter *et. al*.,(١٩٩٩) stated that all **MRSA** isolated were fully resistant to ampicillin, while ٩٠٪ of them were

resistant to trimethoprime–sulfamethaxazol. In ٢٠٠٠,Alborzi and his colleagues stated that ٩١٪ of **MRSA** were resistant to penicillin while amikacine was found to be effective against **MRSA** as well as he mentioned that ciprofloxacin has antistaphylococcal activity with resistance rate ٤٠٪ to **MRSA** .

Resistance to methicillin in ***S.aureus*** conferred by the possession of a chromosomal copy of modified Penicillin binding proteins (PBP) and this proteins has low affinity for most β-lactam antibiotics and mediated cross-resistance to other antibiotics(Hawkey,٢٠٠١).

P.aeruginosa exhibited high resistance to most antibiotics including ampicillin١٠٠٪, amoxicillin١٠٠٪, gentamicin٨٥.٧٪ and trimethoprime – sulfamethaxazol ٩٠٪,cefotaxime ٩٠% and cephalexine ٨٥٪ whereas has lower resistance toward amikacin ٢٨٪ and ciprofloxacine ٣٨%(Figure ٣-٢١). These results were in accordance with that results being reported by Karlowsky *et.al*.,(٢٠٠٣) who pointed out that most ***P.aeruginosa*** isolates were resistant to ampicillin, amoxicillin and trimethoprime-sulfamethaxazo.

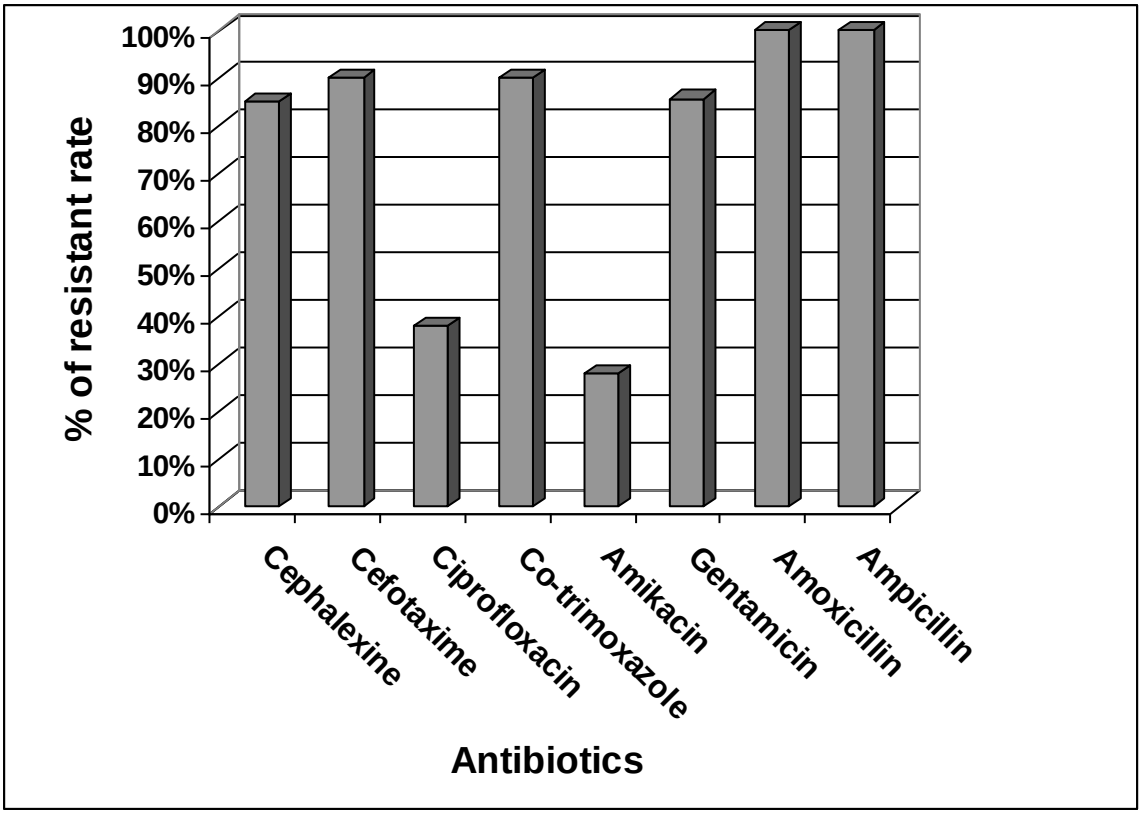


Figure (٣-٢٣) The resistant rate (%)of ***P.aeruginosa*** isolates to the antibiotics

In ٢٠٠٥, (Bisiklis *et.al*) showed that the resistance of ***P.aeruginosa*** to ampicillin was ١٠٠٪, cefotaxime ٩٠٪ and gentamicin ٧٨٪. The resistance of bacteria to β-lactam and cephalosporin mediated mainly by β-lactamases enzymes as well as loss of PBP by mutation confer resistance to these antibiotics(Livermore,٢٠٠٢). Brooks *et.al*.,(٢٠٠١) and Eggman *et.al*.,(١٩٩٧) noted that the strains of *pseudomonas* isolated from hospitals environment had multiple antibiotic exposure and are almost always resistant to oral cephalosporins.

Hsu *et.al*,(٢٠٠٥) referred that resistance of ***P.aeruginosa*** was ٥٠٪ to ciprofloxacin, ٥٨٪ to gentamicin and ٢٣٪ to amikacin. Gencer *et.al*.,(٢٠٠٢) mentioned that ٧٥٪ and ٧٣٪ of ***P.aeruginosa*** isolates were susceptible to ciprofloxacine and amikacin respectively.

Antibiotics resistance phenomenon is widely common among clinical isolates of ***P.aeruginosa*** and it possibly due to the intrinsic or acquired resistance in which these bacteria are highly inherently resistant and this arises from combination of unusually restricted outer membrane permeability and chromosomally encoded β-lactamases (Hankock and Speert**,** ٢٠٠٠)

Regarding ***E.coli***, it exhibited full (١٠٠٪) resistance to both ampicillin and amoxicillin while ٦٣.%of ***E.coli*** were resistant to gentamicin, ٥٢.٦٪ to cefotaxime, ٧٨.٩٪ to trimethoprime–sulfamethaxazol and ٨٩.٤% to cephalexine while it showed lower resistance toward ciprofloxacine ٣٦.٨٪ and amikacin ١٠.٥% (Figure ٣-٢٢). Alora and Manaloto, (١٩٨٣) found that resistance of ***E.coli*** to ampicillin was ٩٨٪, gentamicin ٤١٪ and amikacin ٢٥٪.

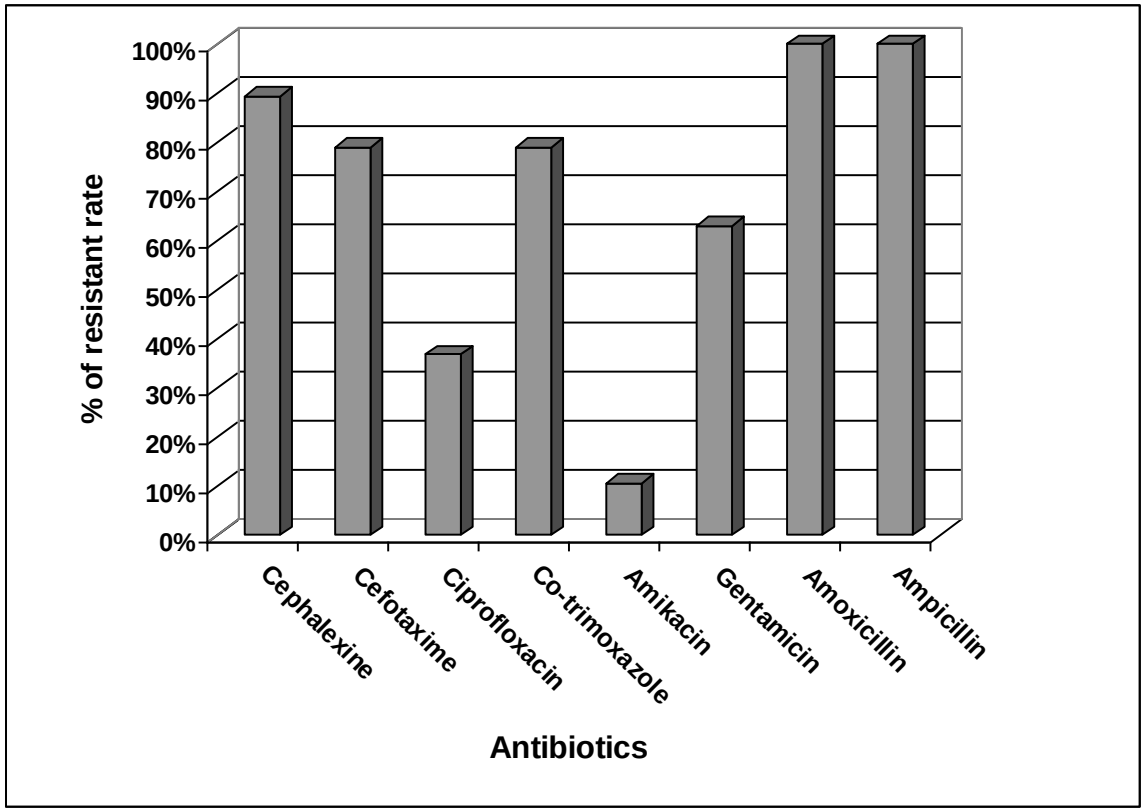


Figure (٣-٢٤) The resistant rate (%)of ***E.coli*** isolates to the antibiotics

In ١٩٨٨, (Marre and schulz) observed that there is upward trend in the resistance of ***E.coli*** to amoxicillin/ampicillin. Nyquist and Reo, (٢٠٠٢) pointed out that ٤٨٪ and ٩٪ of ***E.coli*** strains were resistant to amoxicillin and cephalexin respectively. Jacoby and Medeiros,(١٩٩١) stated that most members of ***Enterobacteriacea***e including ***E.coli*** produce β-lactamases that hydrolysis cefotaxime as well as new cephalosporines compounds. The outer membrane found in ***E.coli*** and other gram–negative bacteria was important barrier of drug penetration and accumulation in these bacteria and responsible of high resistance in these bacteria (Mandell *et.al.*,١٩٩٥).

Oplustil *et.al.*,(٢٠٠١)found that ٤٧٪ and ١.٢٪ of of ***E.coli*** stains were resistant to trimethoprime–sulfamethaxazol and amikacin respectively while

(Karbasizaed *et.al*.,٢٠٠٣) reffered that resistance of ***E.coli*** to trimethoprime – sulfamethaxazol was ٧٣.٣٪.The developing resistance of bacteria to the trimethoprime–sulfamethaxazol caused by changes in cell permeability and overproduction or alteration of dihydrofolate reductase enzyme making such bacteria less affected by this drug (Houvinen,١٩٨٣)and the increase in trimethoprime–sulfamethaxazol resistance, particularly among enteric group, is especially marked in developing countries with up to ٢٠٪ of nosocomial clinical isolates being resistant to trimethoprime–sulfamethaxazol (Reves *et.al*.,١٩٩٠). In, ١٩٩٦ (Shehabi and Baadran) menoited that ***E.coli*** sensitivity to gentamicin and cefotaxime was ٣٣٪ and ٣٣٪ respectively. Sabtcheva *et.al*.,(٢٠٠٣) stated that resistance to aminoglycosides is mainly mediated by enzymatic modification and inactivation of the antibiotic,reduced permeability and accumulation of the drug also responsible for this resistance.

Lesseva and Hadjiisk,(١٩٩٨) found that ٤٤.٤٪ and ١٠٠٪ of ***E.coli*** isolates were sensitive to ciprofloxacine and amikacin respectively. The resistance rate to ciprofloxacine was increased this may be due to abuse of antibiotics leading to transferring the resistance through genetic factors such as plasmids and transposons or due to changing in cell wall permeability (Mims *et.al*.,٢٠٠٤).

The study may conclude that there are some relation between resistance of ***P.aeruginosa*** to disinfectants and antibiotics. This phenomenon was previously reported by Lambert and colleagues(٢٠٠١) who stated that there is relation between antibiotics and biocides resistance. Russell *et.al*.,(١٩٩٨) also observed these relation between chlorohexidine, QAC and at least five antibiotics for gram–negative organisms isolated from UTI and proposed that the wide spread use of CHX was the reason for selecting antibiotics resistant strains. However, other studies stated that this relation is abdout since Wenzal,(١٩٩٧) stated that the

common NIs pathogens represented by ***P.aeruginosa, K.Pneumonia, E.coli, S.aureus S.epidermidis*** and ***Enterococc*** spp. can be equally susceptible to disinfectants as antibiotic susceptible strains.

Conclusions

١-***P.aeruginosa, S.aureus and E.coli*** distribute around the hospital environment causing what is called nosocomial infections.

٢-Among Staphylococci**,** Methicillin Resistant ***S.aureus*** (**MRSA**) was also resident causing infections to the hospitalized patients. Burns and wounds are more susceptible for infection by this organism.

٣-Hospital environment was highly contaminated with different bacterial species, hospital wards and ear-nose-throat (ENT) out patients clinic are more Susceptible for bacterial distribution.

٤-Chlorohexidine gluconate was the most effective biocidal agent against tested bacteria.

٥-Chlorohexidine cetramid seems to act weakly and deficiently on bacteria, since, no effect for this compound or observed was show no efficiency against all bacterial isolates in the present study.

٦-Disinfectants and Antiseptics potency is quitely dependent upon origins(company), storage condition and concentration

٧-Bacterial isolates especially ***P .aeruginosa*** and ***E.coli*** showed high resistanceto disinfectants and antiseptics used in the hospital and this led to wound and hospital contamination.

٨-Amikacin and ciprofloxacine seem to be the most effective antibiotics against ***P.aeruginosa***,***S.aureus***(**MSSA** and **MRSA**) and ***E.coli***

٩- The MIC method seems to be more reliable and more acceptable compared with others method.

Recommendations

١-Application of strict an extreme rules for visiting the patients by their relative.

٢-Limitation the tour of staff,substaff and patients attendants inside hospital.

٣-Separation of the hospital units (kitchen, out patients clinic, sterilization room, washing room ect., away from each other.

٤-Improving hygiene measure including hand washing of medical staff, appropriate sterilization for all equipment and infection control practices. ٥-Foundation a committee in hospital responsible for reporting nosocomial infections,resistant patterns of the hospital flora and continuing education of medical staff.

٦-Application of molecular typing to understanding of the mechanism of aFurther studies should be achieved to detect the source of resistant of antimicrobials in hospital.

٧-Use chlorohexidine gluconate (Hibitine)for routine disinfection and antisepsis in the hospital.

٨-Use amikacine for treatment infections caused by ***P.aeruginosa.S.aureus*** and ***E.coli***.

٩-From the results of this work, it may recommend that further studies be conducted on nosocomial infections taking large samples and study both aerobic and anaerobic bacteria associated with these infections as well as testing the bacterial isolates against disinfectants and antiseptics with others method such as phenol coefficient.

Akbar, F.; Vaguerano, M.U.; Akbarkhan, Z. and Bisesi, N.S.(١٩٩٤). Formaldehyde exposure, acute laboratory. **Am.J.Med.,٢٦٢:٦١-٧٥.**

Al-Akayleh, A.T.(١٩٩٩). Invasive burn wound infection. **Ann.Burns. Fire.Disasters,١٢:٦-٢٠٤.**

Al-Azzawy, A.H.(١٩٩٧). A study of contaminating bacteria of drinking water in Babylon Province. M.SC. Thesis in Microbiology. College of Science, Babylon Unvi.

Alborzi, A.; Pourabbas, B.; Salehi, H. and Panjehshahin, B.O.(٢٠٠٠). Prevalence and pattern of antibiotic sensitivity of methicillin sensitive–resistant ***Staphylococcus aureus*** in Shiraz-Iran. **Irn.J. Med.Sci.,٢٥(١):١-٨.**

Al-Gersha, K.A.M.(١٩٨٨). Evalution of praties and knowleage of scrub nurses concerning some aspeticts of aseptic technique at main teaching hospitals in Baghdad. M.SC.Thesis.College of Nursing, Baghdad Univ.

AL-Hadithi, H.T. and Yousif, Y.Y.(٢٠٠٣). Tolerances of methicillin- resistant ***staphylococcus aureus*** to Dryness,Ultra violet light and resistance to antibiotic. **Med.J.Bas.,٢١(١):٢٢-٤٧.**

Al-Janabi, I.Al.J.(٢٠٠٠). Pathogenic aerobic bacteria causing hospital infection in Sadam General Hospital–Najaf. M.SC. Thesis in Microbiology. The Leader's College of Education for girls,Kufa Univ. (In Arabic).(cited by Fakhriddeen,٢٠٠١)

Al-Kabi, S.J.(٢٠٠٦). Bacteriology of chronic otitis suppurative media. **Kufa.Med.J.,٩(١):٣١٢-٣٢٥.**

Al-Khafaji, F.H. (١٩٩٣). Clinical and micobiology study in chronic

supportive otitis media. M.SC.Thesis, College of Medicine, Baghdad Univ.

Al-Masaudi, S.B.; Day, M.J. and Russell, A.D.(١٩٩١**).** Antimicrobial resistance and gene transfer in ***Staphylococcus aureus***.**J.Appl. Bacteriol.,**٧٠**:**٢٧٩-٢٩٠**.**

Al-Muhanah, A.M.(١٩٩٤**).** Isolation and identification of antibiotic-producing bacteria from different sources. M.Sc.Thesis in Microbiology. College of Science, Babylon Univ.(In Arabic). (cited by Fakhriddeen,٢٠٠١).

Alora, B.D. and Manaloto, D.(١٩٨٣**).** Nosocomial infection: clinical features of two hundred forty three (٢٤٣) cases in Santo Tomas University Hospital. **Phil.J.Microbiol.Infect.Dis.,**١٢**(**١**):**٣١-٣٩**.**

Al-Sahlawi, Z.S.R.(٢٠٠٢**).** Acomparative study on local isolates of methicillin resistant and methicillin sensitive ***Staphylcoccus aureus***. M.Sc.Thesis in Microbiology. College of Science, Kufa Univ.(In Arabic).

Al-Waznee, W.S.; Moslemawi, T.A. and Al-Tarfi, B.A.(٢٠٠٣**).**A study of bacterial contamination sources in delivery rooms and wards of maternity and children hospital in Kerbala province**. J. Kerbala.Unvi.,**١**(**٣**):**١٨٣-١٩٠**.**

Anderson, R.L. and Vess, R.W.(١٩٩١**).** Investigation of intrinsic ***Pseudomonas Cepactia*** contamination in commercially manufactured providine-iodin. **Infect.Cont.Hosp.Epidemiol.,**١٢**: ٢٩٧-٣٠٢.**

Andreas, F.; Widmer and Frei, R.(٢٠٠٣**).**Decontamination,Disinfection and Sterilization. In: Murray, P.R.; Baron, E.J.; Jorgensen, J.H.; Pfaller, M.A. and Yoiken, R.H. **Mannual of Clinical Microbiology**.٨thed., American Society for Microbiology, Washington :٧٧-١٠٨.

Anderssen, J.J.; Korsager, B.; Alstrup, P. and Jepsen, O.B.(٢٠٠٢). Post-operative wound infection: indicator of clinical quality?. **Dan.Med.Bul.,٤٩: ٤-٢٤٢.**

Ang, J.Y.; Ezike, E. and Asmar, B.(٢٠٠٤). Antibacterial resistant**. Indian.J.Pediatrics.,٧١(Issue-٣):٢٢٩-٢٣٩.**

Archibald, L.; Phillips, L. and Monnet, D.(١٩٩٧). Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit**. Clin. Infect.Dis.,٢٤:٢١١-٢١٥.**

Atroshi, F.; Augustin, M. and Ali-Vehmas, V.(٢٠٠٤). **Assement of enzymatic cleaning agents and disinfectants against bacterial biofilm**. J.Pharm.Pharmaceut.Sci.,٧(١):٥٥-٦٤.

Ayliffe,G.(١٩٩٨). **History and future impact of MRSA/antibiotic-resistant species. Revised guidelines for the control of methicillin-resistant** *Staphylococcus aureus* **in hospitals**. J.Hosp.Infect.,٣٩:٩٠-٢٥٣.

Ayliffe, G.A.J.; Babb, J.R. and Taulor, L.J.(٢٠٠١).Hospital-Acquired Infection Priciples and Prevention .٣rded.,Arnold, London.

Ayoub, N.S.(١٩٨١). Epidemiological markers of *Pseudomonas* in general hospitals (in Baghadad). M.SC. Thesis in Microbiology. College of Medicine, Baghdad Univ.(cited by Jibran,١٩٨٦).

Baron, E.J.; Peterson, L.R. and Finegold, S.M.(١٩٩٤). Bailey and Scott'S Diagnostic Microbiology .٩thed.,The C.V. Mosby, Co., USA.

Bauer, A.W.; Kirby, W.M.; Sherris, J.C. and Turk, M. (١٩٦٦).

Antibiotic susceptibility testing by standardized single disk method. **Am.J.Clin.Pathol.,٤٥:٤٩٣-٤٩٦.**

Becker, W.K.; Cioffi, W.G. and McManus, A.T.(١٩٩١). Fungal burn wound infection**. Arch.Surg.,١٢٦:٤-٤٤.**

Behr, H.; Reverdy, M.E.; Mabilat, C.; Freney, J. and Fleurette, J. (١٩٩٤). Relation entrele niveau des concentrations minimales inhibitrices de cinq antiseptiques et la présence du gène *qac*A chez ***Staphylococcus aureus***.**Pathol.Biol.,٤٢:٤٤-٤٣٨**.

Bennett, J.V. and Brachman, P.S. (١٩٩٢). Hospital infections, ٣rd ed.,Little Brown,NewYork.

Berkelman, R.L.; Lewin, S. and Allen, J.R.(١٩٨١). Pseudobacteremia caused by povidin-iodin solution contaminated with ***Pseudomonas cepacia*****. N.Engl.J.Med.,٩٥:٣٢-٣٦.**

Bettelheim, K.A.(١٩٩٢). The genus *Escherichia*. In: Balows, A.; Truper, H.G.; Dworkin, M.; Harder, W. and Sctleifer, K.H.(١٩٩٢). **The prokaryotes**, ٢nd ed., Springer –Verlag KG, Berlin, Germany.

Bisiklis, A.; Tsiakiri, E. and Alexiou-Daniel, S.(٢٠٠٥). Phenotypic detection of ***Pseudomonas aeruginosa*** producing metallo-β-lactamases. European Society of Clinical Microbiology and Infectious Diseases, ١٥th European Congress of Clinical Microbiology and Infectious Diseases,١١٣٤:١-١١.

Blakemore, W.S.; Garrity, G.J. and Thurer, R.J.(١٩٧٩). Infection by air-borne bacteria with cardio- Plmonary by pass. **Surg.,٧٠:٨٣٠-٨٣٨.**

Block,S.S.(١٩٩١). Peroxygen compounds. In: **Disinfection, Sterilization, and Preservation**, ٤thed., Lea and Febiger, Philadelphia.

Block, S.S.(٢٠٠١). Disinfection, Sterilization and Preservation,٥th ed., Lippincott Williams and Wilkins,Philadelphia.

Bloomfield, S.F.; Smith-Burchiell, C.A. and Dalgleish, A.G. (١٩٩٠). Evalution of hypochlorite–releasing agents against the human in immunodeficiency virus(HIV)**. J.Hosp.Infect.,١٥:٨-٢٧٣.**

Bloomfield, S.F.(١٩٩٦). Chlorine and iodine formulations. In: Ascenzi, J.M. **Handbook of Disinfectants and Antiseptics**. Marcel Dekker,Inc.,New York.pp:١٣٣-١٥٨.

Boyce, J.M. and Pittet, D.(٢٠٠٢). Guideline for and Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/ SHEA/ APIC/IDSA Hand Hygiene Task Force.**CDC.,٥١(١٦):١-٤٤.**

Boyce, J.M.; Jackson, M.M. and Pugliese, G.(١٩٩٤). Methicillin-resistant ***Staphylococcus aureus*** (**MRSA**): A briefing for acute care hospitals and nursing facilities. **Infect.Control.Hosp. Epidemiol.,١٥:١٠٥-١١٥.**

Braoudaki, M. and Hitton, A.C. (٢٠٠٤). Adaptive resistance to biocides **in *Salmonella enterica*** and ***Escherichia coli*** O١٥٧ and cross-resistance to antimicrobial**. J.Clin.Microbiol.,٤٢ (١):٧٣-٧٨.**

Brooks, G.F.; Butel, J.S. and Morse, S.A.(٢٠٠١). Jawetz, Melnick and Aldelberg's Medical Microbiology.٢٢nd ed. McGraw-McGraw-Hill.PP:٧٠-١٥٦.

Brown, M. and Gilbert, P. (١٩٩٣). Sensitivity of biofilms to antimicrobial agents. **J.Appl.Bacteriol.Symp.Suppl.,٤:٨٧-٩٧.**

Brownlie, L.; Stephenson, J. and Cole, J.A.(١٩٩٠). Effect of growth rate on plasmid maintenance by ***E.coli*** HB١٠ (PA١٥٣)**. J.Gen. Microbiol.,١٣٦:٢٤٧١-٢٤٨٠.**

Brumfit, W.; Dixson, S. and Hamilton-miller, J.M.(١٩٨٥). Resistance to antiseptics in methicillin and gentamicin resistant ***staphylococcus aureus***.**Lancet, I:١٤٤٢-١٤٤٣.**

Bryant, W.P. and Zimnerman, D.(١٩٩٥). Iodin Induced hyperthyroidismina newbhorn**. Ped.,٩٥:٤٣٤-٤٣٦.**

Chazal, P.M. (١٩٩٥). Pollution of modern metalworking fluid containing biocides by pathogenic reexamination of chemical treatments accuracy. **Eur.J. Epidemiol.,١١:١-٧.**

Coburn, J.; Wyatt, R.T.; Iglewski, B.H. and Gill, D.M.(١٩٨٩). Several GTP-binding proteins, including o٢٤ C-H-ras, are preferred substrates of ***Pseudomonas aeruginosa*** exoenzyme S. **J.Biol. Chem.,٢٦٤:٩٠٤.**

Collee, J.G.; Fraser, A.G.; Marmoin, B.P. and Simmous, A.(١٩٩٦). Mackie and Mccartney Practical Medical Microbiology. ١٤th ed.,Churchill livingstone,USA.

Corbach, S.I.(١٩٩١). Antimicrobial propylaxis for appendectomy and colorectal surgery. **Rev.Infect.Dis.,١٣(١٠):٨١٥-٨٢٠.**

Correa, C.M.; Tibana, A. and GantijoFiloh, P.P.(١٩٩١). Vegetabables as a source of infection with *Pseudomonas* in a university and oncology hospital of Riode Ganeiro**.J.Hosp.Infect.,١٨(٤): ٣٠١ -٣٠٦.**

Cortes, P.; Mariscal, D.; Valles, J.; Rello, J. and Coll, P.(٢٠٠١). Presence of polyclonal ***Pseudomonas aeruginosa*** in an intensive care unit: a ٢٧-month prospective study on molecular epidemiology. **Infect.Control.Hosp.Epidemiol.,٢٢:٧٢٠-٧٢٣.**

Danchivijitr, S.; Chantrasakull, C.; Chokloikaew, S. and Trakool-somboon, S.(١٩٩٥). An outbreak of methicillin-resistant ***Staphylococcus aureus*** **(MRSA**) in a burn unite **.J.Med. Assoc. Thai.,٧٨(suppl-١):٥١١-٥١٤.**

Davies, A.J. and Bullock, D.W.(١٩٨١). ***Pseudomonas aeruginosa*** in

two special care baby unites. Patterns of colonization and infection. **J.Hosp.Infect.,٢:٧-٢٤.**

Davies, J.G.; Babb, J.R.; Bradley, C.R. and Ayliffe, G.A.(١٩٩٣). Preliminary study of test methods to assess the virucidal activity of skin disinfectants using poliovirus and bacteriophages. **J.Hosp.Infect.,٢٥:٣١-١٢٥.**

Davies, J.; Babb, J.; Ayliffe, G. and Wilkins, M.(١٩٨٠). Disinfection of the skin of the abdomen. **Br.J.Surg.,٦٥:٨-٨٥٥.**

Dawaf, H.M.(١٩٩٣). The effect of chemical disinfectants on bacterial contaminations in surgical theaters. M.Sc. Thesis in microbiology. College of Science, Baghdad Uni.(In Arabic). (cited by Fakhridden, ٢٠٠١).

Deich, E.A.; Bridges, R.M. and Dobkle, M.(١٩٨٧). Burn antibacterial host defenses**. Ann.Surg.,٢٠٦:٣٤٠-٣٤٨.**

Demple, B. and Harrison, **L. (١٩٩٤).** Repair of oxidative damage to DNA: enzymology and biology**. Annu.Rev.Biochem.,٦٣:٩١٥-٩٤٨.**

Denton, G.E.(٢٠٠١). **Chlorohexidine. In: Block, S.S.** Disinfection, Sterilization and Preservation**. ٥th ed.,Lippincott Williams and Wilkins, Philadelphia.**

Denyer, S.P.; Hugo, W.B. and Harding, V.D. (١٩٨٥**). Synergy in preservative combinations**. J.Pharm.,٢٥:٢٤٥-٢٥٥.

Deplano, A.; Denis, O.; Poirel, L.; Hocquet, D.; Nonhoff, C.; Byl, P. and Struelens, B.(٢٠٠٥). Molecular characterization of an epidemic clone of panantibiotic-resistant ***Pseudomonas aeruginosa*****. J.Clin.Microbiol.,٤٣(٣):١١٩٨-١٢٠٤.**

Donati, L.J.; Scamazzo, F.; Gervasoni, M.; Magliano, A.; Stankov, B. and Fraschini, F.(١٩٩٣). Infection and antibiotic therapy in ٤٠٠٠ burned patients treated initially between ١٩٧٦ and ١٩٨٨**. Burns,١٩(٤):٨-٣٤٥.**

Donlan, R.M.(٢٠٠١). Biofilms and device-associated infections. **Emerg. Infect.Dis.,٧(٢):٢٧٧-٢٨١.**

Drexler, K.E.(١٩٩٤). Molecular nanomachines: physical principles and implementation strategies.**Ann.Rev.Biophys.Biomol.Structure .,٢٣:٣٧٧-٤٠٥.**

Dunn, M. and Wunderink, R.G.(١٩٩٥). Ventilator-associated pnemonia caused by *Pseudomonas* infection. **Clinics.Chest.Medicine, (١٦):٩٥.**

Dxchodala, G.R.(١٩٩١). Chlorine and chlorine compounds. in: Block, S.S**. Disinfection, Sterilization and Preservation**. ٤th ed.,Lea and Febiger, Philadelphia.

Eggman, S.; Lofler, S. and Burmn, L.(١٩٩٧). An allelic variant of the chromosomal class A-β-lactamas K٢ spesific for ***Klebsiella pneumoniae***. **J.Antimicrob.Agents.Chem.,٤١(١٢):٩-٢٧٠٥.**

Ehrlich, R.L. (٢٠٠٣). *Pseudomonas aeruginosa* Apublication of Marland. **State Public Health Laboratory,٧(١٢):١-١٧.**

Fakhriddeen, A.J.(٢٠٠١). A bacteriological study of the pathogens causing nosocomial infections. M.SC. Thesis in Microbiology. College of Science,Kufa Univ.

Fang, H. and Hedin, C.(٢٠٠٣). Rapid screening and identification of methicillin–resistant ***Staphylococcus aureus*** from clinical samples by selective–broth and real-time PCR Assay**.J.Clin. Microbiol.,٤١(٧):٢٨٩٤-٢٨٩٩.**

Favero, M.S. and Bond, W.W.(١٩٩١). Sterilization, disinfection and antisepsis in the hospital . In: Balows, A.; Hausler, W.J. and Herrmann, K.L. **Manual of Clinical Microbiology**. ٥th ed., American Society for Microbiology, Washington.pp:١٨٣-٢٠٠.

Floom, J.B.R.(١٩٩٨). It's not rocket science but it can save lives. **Science,٢٨٠:١٥٠٧.**

Fuchs, S.; Haritopoulou, T. and Wilhelmi, M. (١٩٩٦). Biofilms in freshwater ecosystems and their use as a pollutant monitor. **Water.Sci.Technol.,٣٤:١٣٧–١٤٠.**

Gajadhar, T.; Lara, A.; Sealy, P. and Adesiyun ,A.A.(٢٠٠٣). Microbial contamination of disinfectants and antiseptics in four major hospitals in Trinidad. **Rev.Panam.Salud.Publica.,١٤(٣):٢١٩-٢٢٢.**

Gang, R.K.; Bang, R.L.; Sanyal, S.C.; Mokaddas, E. and Lari, A.R. (١٩٩٩).*Pseudomonas aeruginosa* septicaemia in burns. **Burns, ٢٥:٦-٦١١.**

Ganguly, P.S.; Khan, Y. and Malik, A.(٢٠٠٣). Nosocomial infections and hospital procedures.**Indian.J.community.Med.,٢٥(١):١٠١-١٠٣.**

Garcia-Martin, M.; Lardelli-claret, P. and Jimenes-Moleon, J.(٢٠٠١). Proportion of hospital deaths potentially attributable to nosocomial infections**. J.Infect.Con.Hosp.Epidemiol.,٢٢:٧٠٨-٧١٤.**

Garner, J.S.(١٩٩٦). Hospital infection control practices advisory committee. Guideline for isolation precautions in hospitals**. Infect.Control.Hosp.Epidemiol.,١٧:٥٣-٨٠.**

Gençer, S.; Ak, Ö.; Benzonana, N.; Batırel, A. and Özer, S.(٢٠٠٢). Susceptibility patterns and cross resistances of antibiotics against ***Pseudomonas aeruginosa*** in a teaching hospital of Turkey. **Annals.Clin.Microbiol.Antimicrob.,١(١):٢.**

Gilbert, P. and McBain, A.J.(٢٠٠٣). Potential impact of increased Use of biocides in consumer products on prevalence of antibiotic resistance**.Clin.Microbiol.Rev.,١٦(٢):١٨٩-٢٠٨.**

Glick, M.(٢٠٠٥). Antibiotics .**Am.Dent.Assoc.,١٣٦(٧):٨٤٤-٨٤٥.**

Glupczynski, Y.; Delmee, M.; Goossens, H. and Struelens, M.J.(١٩٩٩)

.Distribution and prevalence of antimicrobial resistance among gram-negative isolates in intensive care units (ICU) in Belgian hospitals between ١٩٩٦ and ١٩٩٩. **Acta.Clin.Belg.,٥٦: ٢٩٧-٣٠٦.**

Goldman, D.A. and Pier, G.B.(١٩٩٣). Pathogensis of infection related intravascular catheterization**. Clin.Microbiol.Rev.,٦:١٧٦-١٩٢.**

Gordon, S.M.; Serkey, J.M.; Keys, T.F.; Ryan, T.; Fatica, C.A. and Schmitt, S.K.(١٩٩٨). **Secular trends in nosocomial bloodstream infections in a ٥٥-bed cardiothoracic intensive care unit.** J.Ann. Thorac.Surg.,٦٥:٩٥-١٠٠.

Gottardi, W.(٢٠٠١). Iodin and iodine compound. In: Block, S.S., **Disinfection, Sterilization and Preservation**. ٥th ed., Lippincott Williams and Wilkins, Philadlphia.

Grinbaum, R.S.; Mendoica, J.S. and Cardo, D.M.(١٩٩٥). An outbreak of hand-scrubbing related surgical site infections in vascular surgical procedures.**Infect.control.Hosp.Epidemiol.,١٦:١٩٨-٢٠٣.**

Gruendemann, B.J. and Mangum, S.S.(٢٠٠١). **Infection Prevention in Surgical Settings.** Saunders Company, Philadelphia,USA .

Guimaraes, M.A.; Tibana, A.; Nunes, M.P. and Santos, K.R.(٢٠٠٠). Disinfectan and antibiotic activites: A comparactive analysis in Brazilian Hospital bacterial isolates**. J.Microbiol.,٣١:١٩٣-١٩٩.**

Hacek, D.M.; Suriano, T.; Noskin, G.A.; Kruszynky, J.; Reiberg, B. and Peterson, L.R.(١٩٩٩). Medical and economic benefit of a comprehensive infection control clonality**. Am.J.Clin.Pathol., ١١١:٦٤٧-٦٥٤.**

Hamza, L.F.(٢٠٠٦). The resistance of ***Pseudomonas aeruginosa*** isolated from clinical cases for antibiotics. M.Sc.Thesis in Microbiology . College of Medicine, Babylon Unvi.

Hangnga, V.U.; Lana, F.; Washington, J.; Dang, T.; Villarreal, C.; Rosenblatt, J.; Maness, C.; Coodheart, R. and Heggers, J.(٢٠٠٢). **Burn Wound Infection Susceptibilities to Topical Agents: The Nathan's Agar Well Diffusion Technique.**

Hankock, R.E. and Speert, D.P.(٢٠٠٠). **Antibiotic resistance in** *P.aeruginosa***: mechanism and impact on treatment.** Drug Resistance updates, ٣:٢٤٧-٢٥٥.

Hanumanthappa, A.R.; Chandrappa, N.R. and Rajasekharappa, M. G.(٢٠٠٣).**Prevalence of methicillin resistant** *Staphylococcus aureus* **in Karnataka.** Indian.J.Pathol.Microbiol.,٤٦(١):٣٢-١٢٩.

Harrington, J.M. and Shannon, H.S.(١٩٧٧). Survey of safety and health care in British medical laboratories**. Br.Med.J.,١:٦٢-٦٢٨.**

Hawkey, P.(٢٠٠١). The enemy within hospital–acquired, antibiotic resistant bacteria. **Microbiology Today,٢٢:٧-٩.**

Houvinen, P.(١٩٨٣). Trimethoprime resistance. **Antimicrob.Agents. Chemother.,٣١:١٤٥١-١٤٥٦.**

Hsu, D.I.; Okamoto, M.P.; Murthy, R. and Beringer, A.W. (٢٠٠٥).

Fluoroquinolone–resistant pseudomonas aeruginosa: risk factors for acquisition and impact on outcomes. **J. Antimicrob. Chemother.,٥٥(٤):٥٣٥-٥٤١.**

Hsu, D.I.; Okamoto, M.P.; Murthy, R. and Beringer, A.W. (٢٠٠٥).

Fluoroquinolone–resistant ***pseudomonas aeruginosa***: risk factors for acquisition and impact on outcomes**. J. Antimicrob.Chemother.,٥٥(٤):٥٣٥-٥٤١.**

Hugo, A.B. and Russell, W.A. (١٩٩٨). Pharmaceutical Microbiology. ٦th ed.,Black well Ltd,USA.

Husain, M.T.; Karim, Q.N. and Tajuri, S.(١٩٨٩). Analysis of infection in a burn ward**. Burns,١٥:٢٩٩-٣٠٢.**

Jacoby, G.A. and Medeiros, A.A.(١٩٩١). More extended–spectrum β-lactamases. **Antimicrob.Agents.Chemother.,٣٥:١٦٩٧.**

Jarvis, W.R.(٢٠٠١). Infection control and changing health–care delivery systems. **Emerg.Infect.Dis.,٧:١٧٠-١٧٣.**

Jibran, S.(١٩٨٦). Isolation and identification of bacteria from traumatic wound and their sensitivity patterns to antibiotics. M.SC. Thesis in Medical Microbiology. College of Medicine, AL-Mustansiriya Univ.

Joklik, W.K.; Willett, H.P.; Amos, D.B. and Wilfert, C.M.(١٩٩٤). Zinser Microbiolog. ٢٠th ed., Appletone and Lange.pp:١٨٨.

Jones, M.A. and Doern, R.N.(١٩٩٨). Bacterial pathogens isolated from patients with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the sentry antimicrobial surveillance program (United States and Canada, ١٩٩٧). **Antimicrob.Agents.Chemother.,٤٢:١٧٦٢-١٧٧٠.**

Jones, R.N. and Ptaller, M.A.(١٩٩٨) Bacterial resistance: a worldwide problem. **Diagn.Microbiol.Infect.Dis.,٣١:٣٧٩-٣٨٨.**

Kajanahareutai, S.; S irikulsatien, P.; Yospol, P.; Rahule, S. and Sangkasuwan, S.(١٩٩٥). Efficacy and contamination of in-use disinfectants in Rajavithi General Hospital. **J.Med.Assoc. thai.,٨٧(Suppl-١):٣٦-٣٩.**

Kampf, G. and Kramer, A.(٢٠٠٤). Epidemiologic Background of Hand Hygiene and Evaluation of most important Agents for Scrubs and Rubs .**Clin.Microbiol.Rev.,١٧(٤):٨٦٣-٨٩٣.**

Karbasizaed, V.; Badami, N. and Emtiazi, G.(٢٠٠٣). Antimicrobial, heavy metal resistance and plasmid profile of coliforms isolated from nosocomial infections in a hospital in Isfahan, Iran. **Afric.J.Biotech.,٢ (١٠):٣٧٩-٣٨٣.**

Karlowsky, J.A.; Draghi, D.C.; Jones, M.E.; Thornsberry, C.; Friedland, I.R. and Sahm, D.F.(٢٠٠٣). Surveillance for Antimicrobial Susceptibility among Clinical Isolates of ***pseudomonas aeruginosa*** and

Acinetobacter baumannii from Hospitalized Patients in the United States, ١٩٩٨ to ٢٠٠١**. Antimicrob.Agents.Chemother.,٤٧(٥):١٦٨١-١٦٨٨.**

Karmali, M.A.; Steele, B.T.; Petric, M.; Lim, C.(١٩٨٣). Sporadic cases of haemolytic uremic syndrome associated with faecal cytotoxin and cytotoxin-producing ***Escherichia coli*** in stools. **Lancet.١(٨٣٢٥):٦١٩-٦٢٠.**

Katz, S.L.; Gershon, A.A. and Hotez, P.J.(١٩٩٨). Krugman's Infectious Diseases of Children. ١٠th ed.,Mosby Inc,USA.

Kaulfers, P.M. and Laufs, R.(١٩٨٥). Transmissible formaldehyde resistance in ***Serratia marcescens***. **ZbIBakt.Hyg I Abt.Org., ١٨١:٣٠٩-٣١٩.**

Keah, K.C.; Jegathesan, M.; Tan, S.C.; Chan, S.H.; Chee, O.M.; Cheong, Y.M. and Suleiman, A.B.(١٩٩٥). Bacterial contamination of Hospital disinfectants**. Med.J.Malaysia,٥٠ (٤):٢٩١-٢٦٧.**

Kernacki, K.A.; Hobden, J.A.; Hazlett, L.D.; Fridman. R. and Berk, R.S.(١٩٩٥). *In vivo* bacterial protease production during ***Pseudomonas aeruginosa*** corneal infection**. Invest. Ophthalmol.Vis.Sci.,٣٦:٨-١٣٧١.**

Khalil, H.E.(١٩٨٠). The bacteria causing otitis media in different age-groups in Mosoul area. Thesis. College of Medicine, Mosoul Univ.(cited by Al-Khafaji,١٩٩٣).

Kluytmans, J.; Belkum, A. and Verbrugh, H.(١٩٩٧). Nasal carriage of ***Staphylococcus aureus***. **Clin.Microbiol.Rev.,١٠:٥٠٥-٥٢٠.**

Kolmos, H.J.; Thuesen, B.; Nielsen, S.V.; Lohmann, M.; Kristoffersen, K. and Rosdahi, V.T.(١٩٩٣). Outbreak of infection in a burn unite due to ***Pseudomonas aeruginosa*** originating from contaminated tubing used for irrigation of patients**. J.Hosp.Infect.,٢٤:١١-٢١.**

Kroll, R.G. and. Anagnostopoulos, **G.D.(١٩٨١).** Potassium leakage as a lethality index of phenol and the effect of solute and water activity. **J.Appl.Bacteriol.,٥٠:١٣٩-١٤٧.**

Lambert, P.A.(٢٠٠٢). Cellular impermeability and uptake of biocides and antibiotic in gram-positive bacteria and *mycobacteria.* **J.Appl.Microbiol.Symposium.,٩٢:٤٦-٥٤.**

Lambert, R.; Joynson, J. and Forbes, B.(٢٠٠١). The relationships and susceptibilities of some industrial, laboratory and clinical isolates of ***Pseudomonas aeruginosa*** to some antibiotics and biocides. **J.Appl.Microbiol.,٩١:٩٧٢-٩٨٤.**

Larson, E. and Talbot, G.H.(١٩٨٦). An approach for selection of health care personnel hand washing agents. **Infect.Control,٧:٢٤-٤١٩.**

Larson, E.L.(١٩٩٥). APIC Guidelines Committee. APIC guideline for hand washing and hand antisepsis in health care settings. **Am. J.Infect.Control.,٢٣:٦٩-٢٥١.**

Leelapora. A.; Paulsen, H.; Tennent, J.M.(١٩٩٤). Multdrug resistance to antiseptics and disinfectants in Coagulase–negative staphylococci. **J.Med.Microbiazl.,٤٠:٢١٤-٢٢٠.**

Lesseva, M. and Hadjiisk, O.(١٩٩٨). Treatment of serious infections complications in burned patients with emipenem/cilastatin. **Annals.Burns.Fire.Disasters,XI(٢):٣٣-٤٠.**

Lever, A.M. and Sutton, S.V.(١٩٩٦). Antimicrobial effects of hydrogen peroxide as antiseptic and disinfectant. In: Assenzim, J.M**. Handbook of Disinfectants and Antiseptics.** Marcel Dekker, New york.pp:١٥٩-١٧٦.

Levy, S.B.(٢٠٠٢). Active efflux, a common mechanism for biocide and antibiotic resistance. **J.Appl.Microbiol.,٩٢:٦٥-٧١.**

Levy, S.B.(١٩٩٨). Antimicrobial resistance: bacteria on the defense**. BMJ.,٣١٧:٦١٢-٦١٣.**

Linton, K.B. and George, E.(١٩٦٦). Inactivation of chlorohexidine (Hibitane) by bark corks.**Lancet,١:١٣٥٣-١٣٥٥.**

Livermore, D.M. (٢٠٠٢). Multiple Mechanisms of Antimicrobial Resistance in ***Pseudomonas aeruginosa:*** Our Worst Nightmare?. **Clin.Infect.Dis.,٣٤:٦٣٤-٦٤٠.**

Luzzaro, F.; Mantengoli, E.; Perilli, M.; Lombardi, G.; Orlandi, V.; Orsatti, A.; Amicosante, G.; Rossolini, G.; and Toniolo, **A. (٢٠٠١).** Dynamics of a Outbreak of Multidrug-Resistant ***Pseudomonas aeruginosa*** Producing the PER-١ Extended-Spectrum-β-Lactamase. **J.Clin.Microbiol.,٣٩(٥):١٨٦٥-١٨٧٠.**

MacDonnell, G. and Russell, D.(١٩٩٩). Antiseptics and Disinfectants: Activity, Action and Resistance. **Clin.Microbiol.Rev.,١٢(١): ١٤٧-١٧٩.**

MacDonald, L.C. and Jarvis, W.R.(١٩٩٨). Linking Antimicrobial Use to Nosocomial Infections: The role of a Combined Laboratory – Epidemiology Approach. Hospital Infections Program.**CDC., ١٢٩(Issue ٣):٢٤٥-٢٤٧.**

MacFaddin, J.F.(٢٠٠٠).**Biochemical test for Identification of Medical Bacteria**. The Williams and wilkins Co.,USA.

Mahmood, M.(٢٠٠٠). Bacteriology of surgical site infections and antibiotic susceptibility pattern of the isolates at a tertiary care hospital in Karachi**. J.Pak.Med.Assoc.,٥٠(٨):٩-٢٥٦.**

Maki, H.A.K.(١٩٨٢). Isolation and identification of pathogenic bacteria encountered in cases of wound infections with their antibiogram pattern. M.Sc. Thesis in Medical Microbiology. College of Medicin, Univ of Baghdad.

Mandell, Z.; Nikaido, H. and Pool, K.(١٩٩٥).Role of MexA-Mex B-Opr Min antibiotic efflux in ***Pseudomonas aeruginosa.*** **Antimicrob.Agents.Chemother.,٣٩:١٩٨٤-١٩٥٣.**

Marre, Z.A. and Schulz, Y.L. (١٩٨٨). *In vitro* activity of methicillin and amoxicillin-clavulanic acid against strains of ***Escherichia coli*** producing TEM-١, OXA-١ and chromosomal β-lactamases. **J. Microb.,٣٨:٦٥-٣٦٣.**

Martin, S.O.(١٩٩٣). Nosocomial infections in intensive care units: an overview of their epidemiology out come and prevention. **New-Horiz.,١(٢):**٧١-١٦٢.

Mashouf, R.Y. and Vala, S.S.(٢٠٠٦). Study on bacterial contamination of surgical rooms and delivery centers of Humadan hospitals and health care centers. ١٦th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

Mayer, S.; Boos, M.; Beyer, A.; Fluit, A. and Schmitz, F.(٢٠٠١). **Distribution of the antiseptic resistance genes *qacA, qacB* and *qacC* in ٤٩٧ methicillin-resistant and susceptible European isolates of** *Staphylococcus aureus*. J.Antimicrob.Agents., ٤٧:٨٩٦-٨٩٧.

Mckenry, L.M. and Salerno, E.(٢٠٠١). **Mosby's pharmacology in nursing. ٢١st ed.,Mosby,In C.,USA.pp:** ١١٩٨-١٢٠٨.

Melter, O.; Sanches, I.S.; Schyindler, M.; Sousa, M.A.; Mato, R.; Kovarova, V.; Zemlickova, H. and Lencastre, H.(١٩٩٩). Methicillin–resistant ***Staphylococcus aureus*** clonal types in the Czech Republic**.J.Clin.Microbiol.,٣٧(٩):**٢٧٩٨-٢٨٠٣.

Memmel, H.; Kowal-Vern, A. and Latenser, B.(٢٠٠٤**).** Infections in Diabetic Burn Patients**. Diabetes Care, ٢٧:٢٢٩-٢٣٣.**

Mendelson, M.H.; Gurtman, A.; Szabo, S.; Neibart, E.; Meyers, B.R. and Policar, M.(١٩٩٤). *Pseudomonas aeruginosa* bacteremia in patients with AIDS. **Clin.Infec.Dis.,١٨:٩٥-٨٨٦.**

Mengistu, Y.; Erhie, W. and Bellete, B.(١٩٩٩). *In vitro* susceptibility of staphylococci to chlorohexidine and antibiotics. **Ethiop.J. Health.Dev.,١٣:(٣):٢٨-٢٢٣.**

Mims, C.; Docknell, H.M.; Goering, R.V. and Roitt, I.(٢٠٠٤). Medical Microbiology.٣rd ed.,Elsevier Limited.

Muder, R.R.(١٩٩٥). Other *Legionella* species. In: Mandell, G.L.; Bennatt, J.E. and Dolin, R**. Principles and Practice of Infectious disease.** ٤th ed.Newyork:٢٠٩٧-٢١٠٣.

NCCLS.(٢٠٠٠). Performance Standards for Antimicrobial Disk Susceptibility Tests, National Committee for Clinical Laboratory Standards.

Nicas, T.I. and Iglewski, B.H.(١٩٨٥). **The contribution of exoproducts to virulence of** *Pseudomonas aeruginosa*. Can.J.Microbiol., ٣١:٩٢-٣٨٧.

Nicoletti, G.; Boohossion, V.; Gurevitch, F.; Borland, R. and Morgenroth, P.(١٩٩٣). The antimicrobial activity *in vitro* of chlorohexidine, a mixture of isothiazolinones,''Kathan'' (G) and cetyltrimethyl ammonium bromide (CTAB). **J.Hosp. Infect.,٢٣:٨٧-١١١.**

Nikaido, H.(١٩٩٨). Antibiotic resistance caused by gram-negative multidrug efflux pumps. **Clin.Infect.,Dis.,٢٧:٣٢-٤١.**

Norrel, S.A. and Messely, K.A.(١٩٩٧). Microbiology Laboratory Manual Principles and Applications Prentice. Hall Upper saddle River, New Jercey.

Ntsama-Essomba, C.; Boutter, S. and Ramaldes, M.(١٩٩٧). Resistance of ***Escherichia coli*** growing as biofilms to disinfectants**. Vet. Res.,٢٨:٣٥٣-٣٦٣.**

Nyquist, A.M. and Reo, M.(٢٠٠٢). Bugs and Drugs. **Childrens Hospital Association, XVIII(٣):٢٧٦-٢٧٩.**

Ogunsola, T.; Utulu, P.; Mabayoje, O.; Odugbemi, T. and Mabadeje, A.F.(٢٠٠٠). Reservoirs of Nosocomial Pathogens at a University Teaching Hospital in Nigeria. **J.Nig.Infect.Iont. Associ.,٣(٢).**

Oguntibeju, O.O. and Rau, N.(٢٠٠٤). Occurrence of ***Pseudomonas aeruginosa*** post-operative wound infections**. pak.J.Med. Sci.,٢٠(٣):١٨٧-١٩١.**

Oie, S. and Kamiya, A.(١٩٩٦). Microbial contamination of antiseptics and disinfectants. **Am.J.Infect.Control,٢٤:٣٨٩–٣٩٥.**

Oncul, O.; Yksel, F.; Altunay, H.; Açikel, C.; Ielikٍz, B. and Iavuslu, S. (٢٠٠٢). The evaluation of nosocomial infection during ١-year period in the burn unit of a training hospital in Istanbul, Turkey. **Burns,٢٨:٤٤-٧٣٨.**

Oplustil, C.P.; Nunes, R. and Mendes, C.(٢٠٠١). Multicenter Evaluation of Resistance Patterns of ***Klebsiella pneumoniae, Escherichia coli, Salmonella*** spp. and ***Shigella*** spp. isolated from Clinical Specimens in Brazil: Resistant Surveillance Program. **Braz.J. infect.Dis.,٥(١):١٥-٢٤.**

Pallent, L.J.; Hugo, W.B; Grant, D.J. and Davies, A.(١٩٨٣). ***Pseudomonas cepacia*** and infections. **J.Hosp.Infect.,٤:٩-١٣.**

Pandit, D.V.; Gore, M.A.; Saileshwar, N. and Deodhar, L.P. (١٩٩٣).

Laboratory data from the surveillance of a burns ward for the detection of hospital infection. **Burns.١٩(١):٥-٥٢.**

Paulsen, I.T.; Firth, N. and Skurray, R.A. (١٩٩٧). Resistance to antimicrobial agents other than ß-lactams. In: Crossley, K.B. and Archer, G.L. The Staphylococci in Human Disease, Churchill Livingston,New York.

Penna, T.C.V.; Mazzola, P.G. and Martins, A.M.S.(٢٠٠١). The efficacy of chemical agents in cleaning and disinfection programs**. J. BMC.Infect.Dis.,١:١٦-٣١.**

Penna, V.T.C.; Martins, S.A.M. and Mazzola, P.G.(٢٠٠٢). Identification of bacteria in drinking and purified water during the monitoring of a typical water purification system. **BMC Public Health.,٢:١٣-٢٨.**

Percival, S.L.; Knapp, J.S.; Edyvean, R. and Wales, D.S. (١٩٩٨). Biofilm development on stainless in mains water. **Water Res.,٣٢:٢٤٣–٢٥٣.**

Perryman, F.A. and Flournory, D.J.(١٩٨٠). Prevalence of gentamicin and amikacin–resistant bacteria in sink drains. **J.Clin. Microbiol.,١٢:٧٩-٨٣.**

Philips, L.G.; Heggers, J.R.; Robson, M.C.(١٩٩٠). Burn and trauma units as sources of methicillin resistant ***Staphyllococcus aureus*****. J.Burn.Care.Rehabil.,١٣:٧-٢٩٣.**

Pirnray, J.; Vos, D.; Cochez, C.; Bilocq, F.; Pirson, J.; Struelens, M.; Duinslaeger, L.; Cornelis, P.; Zizi, M.; and Vanderkelen, A.(٢٠٠٣). Molecular Epidemiology of ***Pseudomonas aeruginosa*** Colonization in a Burn Unit: Persistence of a Multidrug-Resistant Clone and a Silver Sulfadiazine-Resistant Clone**. J.Clin.Microbiol.,٤١(٣١):١٩٢-١٢٠٢.**

Platt, R.; Goldmann, D.A. and Hopkins, G.C.(١٩٩٨). Epidemiology of nosocomial infections. In: Corbach, L.; Bartlett, G. and Blacklow, R. **Infecions Diseases**,٢nd ed.,W.B.Saunders Co., pp:١٠٨.

Pollack, M. (١٩٩٥). ***Pseudomonas aeruginosa***. In: Mandell, G.L.; Bennett, J.E.; Dolin, R. **Principles and Practice of Infectious Diseases,** ٤th ed, Churchill Livingstone,London,UK.

Poole, K.(٢٠٠٢). Mechanisms of bacterial biocide and antibiotic resistance. **J.Appl.Microbiol.Symp.,:٥٥-٦٤.**

Power, E.G.M.(١٩٩٥). Aldehydes as biocides. **Prog.Med.Chem.,٣٤: ١٤٩-٢٠١.**

Poxton, I.R.(١٩٩٣). Prpkaryote envelope diversity**. JAPPL.Bacteriol. Sym.,(Suppl-٦٧):٨-٩١.**

Pruitt, B.A. and MacManus, A.T.(١٩٩٢). The changing epidemiology of infection in burn patients. **World.J.Surg.,١٦:٥٧-٦٧.**

Ranganathan, N.S.(١٩٩٦).**Disinfection, Sterilization and preservation**. Lippincott William and Wilkins,Philadelphia.

Rastegar Lari, A.R.; Alaghehbandan, R. and Akhlaghi, L.(١٩٩٨). Burn wound infections and antimicrobial resistance in Tehran, Iran: an increasing problem. **Burns,٢٤(٧):٤١-٦٣٧.**

Reves, R.R.; Fong, M. and Pickering, L.K.(١٩٩٠). Risk factors for faecal colonization with trimethoprime-resistant and multi resistant ***Escherichia coli*** among children in day care centers in Houston ,Texas. **Antimicrob.Agents.Chemother.,٣٤:١٤٢٩ -١٤٣٤.**

Ronald, N. and Jones, R.N.(٢٠٠١). Resistance Patterns Among Nosocomial Pathogens Trends Over the Past Few Years. **Chest.,١١٩:٣٩٧-٤٠٤.**

Rotter, M.(١٩٩٩). Hand washing and hand disinfection .In: Mayhall, C.G. **Hospital Epidemiology and Infection Control.** ٢nded., Lippincott Williams and Wilkins,Philadelphia.

Russell, A.D. and Russell, N.J. (١٩٩٥). Biocides: activity, action and resistance. **Symp.Soc.Gen.Microbiol.,٥٣:٣٢٧-٣٦٥.**

Russell, A.D. and Chopra, I.(١٩٩٦). Understanding antibacterial action and resistance, Ellis Horwood, Chichester, United Kingdom.

Russell, A.D.; Furr, J.R. and Maillard, J.Y. (١٩٩٧). Microbial susceptibility and resistance to biocides. **ASM News,٦٣:٤٨١-٤٨٧.**

Russell, A.D.(١٩٩٨). Possible Link between Bacterial Resistance and Use of Antibiotics and Biocides**. Antim.Agents.Chemo.,٤٢(٨): ١٥١-٢١٥.**

Russell, A.D.(١٩٩٠). The bacterial spore and chemical sporicides. **Clin. Microbial.Rev.,٣:٩٩-١١٩.**

Russell, A.D.; Tattawasart, U.; Maillard, J. and Furr, J.R.(١٩٩٨). Possible Link between Bacterial Resistance and Use of Antibiotics and Biocides. **J.Antimicrob.Agents.Chemother., ٤٢(٨):٢١٥١-٢١٥٥.**

Rutala, W.A. and Weber, D.(١٩٩٥). Uses of inorganic hypochlorite (bleach) in health–care facilities. **Clin.Microbiol.Rev.,١٠:٥٩٧ -٦١٠.**

Rutala, W.A.(١٩٩٠). APIC guidelines for selection and use of disinfectants**. Am.J.Control,١٨:٩٩-١١٧.**

Rutala, W.A.(١٩٩٦). APIC guideline for selection and used disinfectants. **Am.J.Infect.Con.,٢٤:٣١٣-٣٤٢.**

Sabtcheva, S.; Galimand, M.; Gerbaud, P.; Courvalin, P. and Lambert, T.(٢٠٠٣). Aminoglycoside resistant gene ant(٤)-llb of ***Pseudomonas aeruginosa*** BM٤٤٩٢, a clinical isolate from Bulgaria. **Antimicrob.Agents.Chemother.,٤٧(٥):١٥٨٤-١٥٨٨.**

Sachdev, D.; Amladi, S.; Natraj, G.; Baveja, S.; Kharkar, V.; Mahajan, S. and Khopkar, U.(٢٠٠٣). An outbreak of methicillin-resistant ***Staphylococcu aureus*** (**MRSA)** infection in dermatology indoor patients. **Indian.J.Derma.Venero. Lepro.,٦٩(Issue-٦):٣٧٧-٣٨٠.**

Savas, L.; Duran, N.; Savas, N.; Nlen, Y. and Ocak, S.(٢٠٠٥). The prevalence and resistant patterns of ***Pseudomonas aeruginosa*** in intensive care units in a University Hospital Turk**y. J.Med. Sci.,٣٥:٣١٧-٣٢٢.**

Schorer, M. and Eisele, M.(١٩٩٧). Accumulation of inorganic and organic pollutants by biofilms in the aquatic environment. **Water Air Soil Pollut.,٩٩:٦٥١–٦٥٩.**

Schweizer, H.P.(١٩٩٨). Intrinsic resistance to inhibitors of fatty acid biosynthesis in ***Pseudomonas aeruginosa*** is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. **Antimicrob.Agents.Chemother.,٤٢:٣٩٤-٣٩٨.**

Semmelweis, I.P.(١٩٩٥). The etiology, concept, prevention of childbed fever. Reprinted Amer**. J.Obstet.Gynecol.,١٧٢ (١):٧-٢٣٦.**

Sharma, S.(١٩٩٨). Efficacy of paraformaldehyde tablets in sterilization of polyvinyl-chloride tubing. **Indian.J.Ophthalmol.,٤٦(Issue -٣):١٧٤-١٧٥.**

Shehabi, A.A. and Baadran, I.(١٩٩٦). Microbial infection and antibiotic resistance patterns among Jordanian intensive care patients. **Eastern Mediterran-Ean Health.J.,٢(Issue-٣):٥١٥-٥٢٠.**

Shehanel-Din, S.A.; El-Shafey, E.; El-Hadidy, M.R.; Bahaa El-Din, A.; El-Hadidy, M. and Zaghloul, H.A.(٢٠٠٣). Methicillin resistant ***Staphylococcus aureus***: A problem in the Burns Unite.**Egypt.J.Plast.Reconstr.Surg.,٢٧(١):١-١٠.**

Sheldon, A.T.(٢٠٠٥). Antiseptic Resistance: What Do We Know and What Does It Mean?.**Clin.Lab.Sci.,١٨(٣):١٨١-١٨٨.**

Simor, A.E.; Agostini, M.; McGeer, A.; Bryce, E.; Loeb, M. and Mulvey, M.(٢٠٠٥). Surveillance for Methicillin-Resistant ***Staphylococcus aureus*** in Canadian Hospitals-A Report Update from the Canadian Nosocomial Infection Surveillance Program. Public Health Agency of Canada.Canada Communicabl Diseases Report.٣١(٣).

Speller, D.C. and Humphreys, H.(١٩٩٨). Hospital–acquired infection. In: Collier, L.; Balows, A. and Sussman, M. Topley and Wilson's Microbiology and Microbial Infections.,Vol.٣. Arnold,London.pp:١٨٧-٢٢٩.

Springthorpe, V.S. and Sattar, S.A.(١٩٩٠). Chemical disinfection of virus. **Clin.Rev.Environ.Control.,٢٠:١٦٩-٢٢٩.**

Steed, C.J.(١٩٩٩). Common infections acquired in the hospital: the nurse's role in prevention. **Nurs.Clin.North.Am.,١(٣٤):٤٤٣.**

Stenfors, L.E. and Raisanen, S.I.(١٩٩١). Immunoglobulins coated bacteria in effusion from secretary and chronic suppurative otitis media. **Am.J.otolarnyoll.,١٢:١٦١-١٦٤.**

Stephen, T.A.(١٩٩٨). Nosocomial infections**. Infect.Con.Hosp. Epidemiol.,٢٣:٣٤-٤٢.**

Stickler, D.J.; Dolman, J.; Rolfe, S. and Chawla, J.(١٩٩١). Activity of antiseptics against urinary tract pathogens growing as biofilms on silicone surfaces. **Eur.J.Clin.Microbiol.Infect.Dis.,١٠: ٤١٠-٤١٥**.

Storz, G. and Altuvia, S. (١٩٩٤). OxyR regulon methods. **Enzymol., ٢٣٤:٢١٧-٢٢٣.**

Struelens, M.J. (١٩٩٩). Hospital Infection Control. In: Armstrong, D. and Cohen, J. **Infectious Diseases**. ٣rd ed., Mosby, Philadelphia.pp:١٠٢.

Subhi, M.D. (٢٠٠٣). Urinary tract infections in children. **J.Basic.Med. Scien.,٣(٢):١١١.**

Sutton, L. and Jacoby, G.A. (١٩٧٨). Plasmid-determined resistance to hexachlorophene in ***Pseudomonas aeruginosa***. **Antimicrob. Agents.Chemother.,١٣:٦٣٤-٦٣٦.**

Tasota, F.J.; Fisher, E.M.; Coulson, C.F. and Hoffman, L.A.(١٩٩٨). Protecting ICU patients from nosocomial infections: practically. **Gritical.care.Nurse.J.,١٨(١):٥٤-٦٧.**

Tattawasart, U.; Maillard, J.Y.; Furr, J.R. and Russell, A.D.(١٩٩٩). Development of resistance to chlorhexidine diacetate and

cetylpyridinium chloride in ***Pseudomonas stutzeri*** and changes in antibiotic susceptibility**. J.Hosp.Infect.,٤٢:٢١٩-٢٢٩.**

Terleckyi, B. and Alxler, D.A.(١٩٨٧). Quantitive neutralization assay of fungicidal activity of disinfectants. **Antimicrob.Agents. Chemother.,٣١:**٧٩٤-٧٩٨.

Thanassi, D.; Cheng, L.W. and Nikaido, H.(١٩٩٧). Active efflux of bile salts by ***Escherichia coli*****. J.Bacteriol.,١٧٩:٢٥١٢-٢٥١٨.**

Trautmann, M.; Michalsky, T.; Wiedeck, H.; Radosavljevic, V. and Ruhnke,V.(٢٠٠١). Tap water colonization with ***Pseudomonas aeruginosa*** in a surgical intensive care unit (ICU) and relation to *Pseudomonas* infections of ICU patients. **Infect.Control. Hosp.Epidemiol.,٢٢:٤٩-٥٢.**

Tredget, E.F.; Shankowsky, H.A.; Joffe, A.M.; Inkson, T.I.; Volpel, K.; Paranchych, W.; Kibsey, C.; Alton, J.D. and Burke, F. (١٩٩٢). Epidemiology of infections with ***pseudomonas aeruginosa*** in burn patients: the role of hydrotherapy. **Clin. Infec.Dis.,١٥(٦):**٩-٤٤١**.**

Turner, F.J.(١٩٨٣). Hydrogen peroxide and other oxidant disinfectants. In: Block, S.S**. Disinfection, Sterilization and Preservation**. ٣rd ed.,Lea and Febiger,Philadelphia.

Turner, P.J.(٢٠٠٠). MYSTIC (Meropenem Yearly Susceptibility Test Information Collection): a global overview. **J.Antimicrob. Chemother.,٤٦(٢):**٩**–**٢٣**.**

Ul-Haq, M.M.; Trariq, T.M. and Ul-Jalil, S.(٢٠٠٢). Chronic suppurative otitis media: A study in children of female hospital workers. **Pak.Ped.Assoc.,٢٦(٤).**

Valenti, W.M.(١٩٨٠). Nosocomial viral infections, Epidemiology and significance.**Infect.control,١:٣٣.**

Vall, J.S.; Nacente, R.B. and Coll, M.S.(١٩٩٩). Hand book of microbiology culture media, ٥th ed.,Scharlau Chemie, S.A., Barcelona,Spain.

Van Delden, C. and Iglewski, B.H.(١٩٩٨). Cell-to-Cell Signaling and ***Pseudomonas aeruginosa*** infection**. J.Emerg.Infect.Dis.,٤(٣) :١٤٢٠.**

Vesley, D.; Norlien, K.G. and Nelson, B.(١٩٩٢). Significant factors in

the disinfection and sterilization of flexible endoscopes. **Am. J.Infect.Con.,٢٠:٢٩١-٣٠٠.**

Vilar-Comple, D.; Mohar, A.; Saudoval. S.; Rosa, M.; Gordillo, P. and Volkow, P.(٢٠٠٠). Surgical site infection at the national cancer institute in Mexico: a case-control study**. Am.J.Infect. Cont.,٢٨(١):١٤-٢٠.**

Wage, S.K. and Hedin, P.A.(١٩٨٥). Quercetin ٣-o-Galactosyl Clucoside a compound from narrow leafleted with antimicrobial activity.**Phytochemistry.,٢٤:٢٤٣-٢٤٥.**

Wagenvoort, J.H.T.(٢٠٠٠). Dutch measures to control **MRSA** and the expanding European Union. **Euro.Surveill.,٥(٣):٢٦-٢٨.**

Walsh, B.; Blakemore, P.H. and Drabu, Y.(١٩٨٧).The effect of hand cream on the antibacterial activity of chlorhexidine gluconate**. J.Hosp.Infect.,٩:٣٠-٣.**

Weinstein, R.A.(١٩٩٨). Nosocomial infection update. **Emerg.Infect. Dis.,٤:٤١٦-٤٢٠.**

Weinstein, R.; Gaynes, R. and Edwards, J.(٢٠٠٥). Overview of nosocomial infections caused by gram–negative bacilli. **Health.Care.Epidemiol.,٤١:٨٤٨-٨٥٥.**

Wenzal, R. and Zaidi, M.(٢٠٠٠). Disinfection, sterilization and control of Hospital waste. In: Mandell, G.L.; Beanetts, J.E. and Dolin, R. **Principles and Practice of Infectious Diseases** ,٥th ed.,Vol.٤.Churchill livingstone, Philadelphia.pp:٢٩٩٥-٣٠٠٥.

Wenzal, R.P.(١٩٩٧). Prevention and control of Nosocomial Infections, ٣rd ed., Wiliams and Wilkins, Baltimore, USA.

Wiblin, R.T.(١٩٩٧). Nosocomial pneumonia. In: Wenzal, R.P. **Prevention and Control Nosocomial Infections**. ٣rd ed.,Williams and Wilkins, Baltimore.

Wick, M.J.; Hamood, A.N. and Iglewski, B.H.(١٩٩٠). Analysis of the structure-function relationship of ***Pseudomonas aeruginosa*** exotoxin. **Mol.Microbiol;٤:٣٥-٥٢٧.**

Xue, B.; Liu, X. and Tang, M.(١٩٩٩).The change in bacterial flora and antibiotic resistance of bacteria of burn patients in our hospital

during ١٩٨٦-١٩٩٦. **Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi,١٥:١٢-٣٠٩.**

Yz, Y.; LL, Z.; Xy, L. and Yh, Z.(٢٠٠٤). Study on the resistance of methicillin-resistant ***staphylococcus aureus*** to iodophore and chlorohexidine.**Zhonghua Liu Xing. Bing Xue Za Zhi,٢٥(٣):٥٠٢٤٨.**

Zachi, M.; Heni, S.; Shapira, R. and Oren, A.(١٩٩٦). The role of the outer membrane in formaldehyde tolerance in ***Escherichia coli*** VU٣٦٩٥ and *Halomonas* spp**. MAC.Microbial.,١٤٢:١٢٤٩-١٢٥٤.**