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Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza - Palestine

الجودة الميكروبية للصابون وفعالية المطهرات المستخدمة في مستشفيات قطاع غزة – فلسطين

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Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza - Palestine

الجودة الميكروبية للصابون وفعالية المطهرات المستخدمة في مستشفيات قطاع غزة – فلسطين

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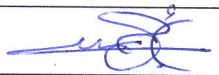
Signature:		التوقيع:
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الجودة الميكروبية للصابون وفعالية المطهرات المستخدمة في مستشفيات قطاع غزة – فلسطين

Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza - Palestine

وبعد المناقشة التي تمت اليوم الأربعاء 13 ربيع الثاني 1438هـ، الموافق 2017/01/11م الساعة العاشرة صباحاً، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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واللجنة إذ تمنحه هذه الدرجة فإنها توصيه بتقوى الله ولزوم طاعته وأن يسخر علمه في خدمة دينه ووطنه.

والله ولي التوفيق،،،

نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

Abstract

"Microbiological Quality of Soaps and Efficacy of Antiseptics and Disinfectants Used in Hospitals in Gaza – Palestine"

The aim of the present study is a determine microbiological (bacteria and fungi) quality of antiseptics and soap samples, identify bacteria that contaminate antiseptics and soaps that used in hospitals in Gaza – Palestine, measure the efficacy of antiseptics on bacteria, and determine the chemical efficacy of antiseptics and disinfectants.

To determine microbiological (bacteria and fungi) quality of antiseptics and soap samples and identify bacteria that contaminate antiseptics and soaps that used in hospitals in Gaza – Palestine, I used plated media method, to measure the efficacy of antiseptics on bacteria, I used stainless steel cylinder method, and to determine the chemical efficacy of antiseptics and disinfectants I used pH measurements and chemical concentration test.

The soap results shown as the percentage of samples that complied with the standards (passed) was 15/15 (100%) in European Gaza Hospital, and the lowest passing result as 1/14 (6.7%) in Kamal Adwan Hospital and the total passing value as 73/105 (69.5%) and the total failing value as 32/105 (30.5%), and The results showed that the percentage of contaminated samples by bacteria and fungi was 18/105 (17.1%) and total percentage value was 32/105 (30.5%), the most common contaminant was coliform 13/105 (13.4%), *Pseudomonas* spp. 12/105 (11.4%) and *Bacillus* spp. 6/105 (5.7%). The contamination with *yeast* 11/105 (10.5%) was more than the mold 7/105 (6.7%), and The pH test results showed that the average of pH of soap samples in hospitals indicate the highest passing value as 18/20 (90%) in Nasser Hospital, the lowest passing value as 0/15 (0%) in European Gaza hospital, and the total passing value as 43/105 (41%), and the total failing value as 62/105 (59%).

The antiseptics/disinfectants results shown as the average of zone of inhibition is 28.2 mm for *E. coli*, 20.8 mm for *P. aeruginosa*, and 20.7mm for *S. aureus*, the total percentage passing samples by concentration is 140/233 (60.09%) and the percentage of failing sample in all hospitals is 93/233 (39.91%), and the highest percentage of passing results is 79.66% for Chlorhexidine, and lowing percentage of passing results is 48.28% for Chlorine, and in pH parameter that total percentage of passing results is 141/144 (97.9%), and that the total percentage of falling results is 3/144 (2.1%).

In conclusions, the results of the present study show that more than half samples collected from biggest central hospital in Gaza stripe failed in tests, and the percentage of fail in Antiseptics/Disinfectants samples were 40.3%, and in soap samples were 74.3%.this results may become a potential human health hazard, the main causes were using locally manufactured soaps and disinfectants and over dilution the antiseptics/disinfectants before using.

Keywords: microbiological quality, effectiveness, antiseptics, disinfectants, soap, hospitals in Gaza, Palestine, *S. aureus, P. aeruginosa, E. coli.*

الملخص

"الجودة الميكروبية للصابون وفعالية المطهرات المستخدمة في مستشفيات قطاع غزة – فلسطين"

تهدف هذه الدراسة لتحديد الجودة الميكروبية (بكتيريا وفطريات) للمطهرات وللصابون، ولتحديد البكتيريا الملوثة لهذه المواد المستخدمة في مستشفيات قطاع غزة – فلسطين، وكذلك لقياس فعالية المطهرات على البكتيريا، وتهدف أيضا لتحديد الفعالية الكيميائية للمطهرات والمعقمات المستخدمة.

لتحديد الجودة الميكروبية (بكتيريا وفطريات) للمطهرات والصابون، وتحديد أنواع البكتيريا الملوثة لهذه المواد المستخدمة في مستشفيات قطاع غزة – فلسطين استخدمت طريقة زراعة الأطباق البكتيرية. ولقياس فعالية المعقمات على البكتيريا استخدمت طريقة اسطوانات الستانلس ستيل، ولتحديد الفعالية الكيميائية للمطهرات والمعقمات استخدمت اختبار قياس درجة الحموضة واختبار التركيز الكيميائي.

أظهرت نتائج الصابون أعلى نسبة نجاح (100%) في مستشفى غزة الأوروبي، وأدنى نسبة نجاح (6.7%) في مستشفى كمال عدوان، وكانت نسبة النجاح الكلي (69.5%) ونسبة الرسوب الكلي (30.5%)، وتظهر النتائج أن نسبة العينات الملوثة بالبكتيريا والفطريات (17.1%) وبنسبة كلية بلغت (30.5%)، وأكثر مسببات التلوث كانت القولونية (13.4%)، الزائفة الزنجارية (11.4%)، والعصية النيابية (5.7%). والتلوث بالخمائر (10.5%) أكثر من التلوث بالأعفان (6.7%)، وفي اختبار درجة الحموضة كانت أعلى نسبة نجاح (90%) في مستشفى ناصر، وأقل نسبة نجاح (0%) في مستشفى غزة الأوروبي، بنسبة نجاح عامة بلغت (41%) وبنسبة رسوب عامة بلغت (59%).

أظهرت نتائج المطهرات والمعقمات أن متوسط منطقة التثبيط بلغت 28.2 ملم للإشريكية القولونية، و 20.8 ملم للزائفة الزنجارية، و 20.7 ملم للمكورة العنقودية الذهبية. وكانت نسبة نجاح العينات في اختبار التركيز الكيميائي (60.1%) وكانت نسبة الفشل في العينات (39.9%)، وكانت أعلى نسبة نجاح (79.7%) للكلورهكسدين، وأقل نسبة نجاح (48.3%) للكلور، وفي اختبار درجة الحموضة كانت نسبة النجاح الكلي (97.9%) ونسبة الرسوب الكلي (2.1%).

في الاستنتاجات فإن نتائج هذه الدراسة تظهر أنه أكثر من نصف العينات المجموعة من أكبر المستشفيات المركزية في قطاع غزة قد رسبت في الاختبارات وأن نسبة الرسوب في عينات المطهرات والمعقمات بلغت (40.3%) وفي عينات الصابون بلغت (74.3%). وهذه النتائج قد تشكل خطرا عل صحة الانسان، الأسباب الرئيسية هي استخدام الصابون المصنع محليا، وتقليل تركيز المطهرات والمعقمات قبل استخدامها.

كلمات مفتاحية: الجودة الميكروبية، الفعالية، المطهرات، المعقمات، الصابون، مستشفيات قطاع غزة، فلسطين، المكورة العنقودية الذهبية، الزائفة الزنجارية، الإشريكية القولونية.

قال الله تعالى:

"قَالُوا سُبحَانَكَ لَا عِلمَ لَنَا إِلا مَا عَلمتَنَا إِنكَ أَنتَ العَلِيمُ الحَكِيم"

(البقرة: 32)

They said: "Glory to Thee, of knowledge We have none, save what Thou Hast taught us: In truth it is Thou Who art perfect in knowledge and wisdom."

(Al-Baqarah: 32)

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List of Abbreviations

BPA	Baird Parker Agar
MAC	MacConkey agar
VRBA	Violet Red Bile Agar
MHA	Mueller Hinton Agar
RBA	Rose Bengal Agar
CA	Cetrimide Agar
MYP	Mannitol Egg Yolk Polymyxin Agar
USA	United States of America
pH	Power of Hydrogen
TPC	Total plate count
QAQ	Quaternary Ammonium Compounds
G+ve	Gram positive
G-ve	Gram negative
Anti/Dis	Antiseptics and Disinfectants
CHX	Chlorhexidine
CFU	cell forming unit
CDC	Center for Disease Control
SDS	Sodium Dodecyl Sulfate
TBC	Total Bacterial Count
NA	Nutrient Agar
PCA	Plate Count Agar
VRBA	Violet Red Bile Agar
API	Analytical Profile Index

Chapter 1
Introduction

Chapter 1

Introduction

1.1 Overview

Microbiological quality is the acceptability of a product lot based on the absence or presence of a number of microorganisms, including parasites and/or a quantity of their toxins/metabolites per unit of mass, volume, area or lot **(Cordier, 2004).**

Soap is a salt of a fatty acid. Soaps are mainly used as surfactants for washing, bathing, and cleaning, yet they are also used in textile spinning, as they are important components of lubricants. Soaps for cleansing are obtained by treating vegetable or animal oils and fats with a strongly alkaline solution **(Cavitch, 1995).**

Soap has a little value as an antiseptic, but it does have an important function in the mechanical removal of microbes through scrubbing. The skin normally contains dead cells, dust, dried sweat, microbes, and oily secretions from oil glands. Soap breaks the oily film into tiny droplets, a process called emulsification, and the water and soap together lift up the emulsified oil and debris and float them away as the lather is washed off. In this sense, soaps are good degerming agents (Degermation refers to the process of mechanically removing microbes from the skin) **(Alberts, Wilson, & Hunt, 2008; Tortora, Funke, & Case, 2013).**

Medical applications quite often require sterility, especially with regard to invasive instruments such as scalpels, clamps, dental hand tools, and the like, this absolute level of microbial control is often unwarranted and perhaps even unwanted. In many cases, it is remarkably important to focus on reducing the size of a microbial population, or its microbial load. Sanitization refers to any cleansing technique that removes debris, microorganisms, and toxins, and in this way reduces the potential for infection and spoilage. Soaps and detergents are the most commonly employed sanitizers. It is important to note that sanitization is often preferable to sterilization. **(Kathleen P. Talaro & Chess, 2012).**

There are many types of microorganisms isolated from soaps in hospitals and public places such as *Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumanii,*

Proteus penneri, Flavimonas oryzihabitans, Enterobacter aerogenes, Enterobacter cloacae, Staphylococcus aureus, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia marcescens, Citrobacter koseri, candida **(J. A. Caetano, Lima, Di Ciero Miranda, Serufo, & Ponte, 2011; M. Chattman, S. L. Gerba, & C. P. Maxwell, 2011; V. Chaturvedi & Kumar, 2011; S. M. H. Zeiny, 2009).**

Efficacy is the ability to produce a desired or intended result **(Stevenson, 2010).** Antibiotics are defined as naturally occurring substances which inhibit or destroy selective bacteria or other microorganisms, generally at low concentrations. Antiseptics are biocides or products that destroy or inhibit the growth of microorganisms in or on living tissue and disinfectants are similar but generally are products or biocides that are used on inanimate objects or surfaces. Sterilization refers to a physical or chemical process that completely destroys or removes all microbial life, including spores (Sanitization reduces microbial numbers on inanimate objects to safe levels by physical or chemical means.). Preservation is the prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods. A number of biocides are also used for cleaning purposes; cleaning in these cases refers to the physical removal of foreign material from a surface **(Seymour Stanton Block, 2001a).**

The Food and Drug Administration in the United States regulates the formulation, manufacture, and use of antiseptics and germicides because these agents involve direct human exposure and contact **(Madigan, Martinko, Bender, Buckley, & Stahl, 2012).**

Antisepsis include preparing the skin before surgical incisions with iodine compounds, swabbing a wound with hydrogen peroxide, and ordinary hand washing with a germicidal soap **(Kathleen Park Talaro & Rhoads, 2012).** Disinfectants can be sporostatic but are not necessarily sporicidal **(Johnston, Lambert, Hanlon, & Denyer, 2002).**

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. They are an essential part of infection control practices and aid in the prevention of nosocomial infections

(Control & Epidemiology, 1996; William A Rutala, 1996). Mounting concerns over the potential for microbial contamination and infection risks in food and general consumer markets have also led to increased use of antiseptics and disinfectants by the public. A wide variety of active chemical agents (or "biocides") are found in these products, many of which have been used for hundreds of years for antisepsis and disinfection **(S.S. Block, 1991).** In general, antiseptics and disinfectants have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, antiseptics and disinfectants may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular cross- resistance to antibiotics **(McDonnell & Russell, 2001).**

There has been a dramatic increase in the usage of chemical biocides (i.e. disinfectants and antiseptics) in the food, water and pharmaceutical industries, and in the healthcare and domiciliary environments. The need to reduce and control nosocomial infection **(Favero, 2002)** and to improve product quality and overall hygiene, for example, in the hospitals **(Solveig Langsrud, Maan Singh Sidhu, Even Heir, & Holck, 2003)** health authorities and the public. Public knowledge in particular and a better commitment to overall hygiene **(Bloomfield, 2002)** have contributed to the increased usage of antiseptics and disinfectants in the home environment **(Levy, 2001).** Protocols for testing the antimicrobial efficacy of disinfectants and antiseptics are essential to provide reliable information on the efficacy of an antimicrobial product and provide assurance for the end users **(J. Holah, 2003).**

It is important to note that many of these antiseptics and disinfectants may be used singly or in combination in a variety of products which vary considerably in activity against microorganisms. Antimicrobial activity can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method **(Fraise, Maillard, & Sattar, 2012).**

The concept of testing or checking disinfectants is very old. Robert Koch described a disinfectant test in the article (Über Desinfektion), in 1881 **(Gerald Reybrouck, 1998).** Testing disinfectant helps to find the cause of the spread of the infection **(Wadhwa et al., 2007).** Disinfectants should remove or inactivate known or possible

pathogens from inanimate objects **(Gerald Reybrouck, 1998)**, hospitals should have their inbuilt test method that can be easily applicable **(Hume et al., 2009) (Akinsanya, 1993).**

An unpublished study in Gaza strip investigated the contamination of liquid soaps in hospitals. It showed alarming results with high percentage of the tested liquid soap samples was found to be contaminated with coliforms (29%), a considerable percentage of samples contained yeast (21%) and molds (5%).

1.2 Objectives

1.2.1 General Objective

To assess the microbiological quality and effectiveness of antiseptics and soap used in hospitals in Gaza – Palestine.

1.2.2 Specific Objectives

1. To determine microbiological (bacteria and fungi) quality of antiseptics, disinfectants and soap samples.
2. To identify bacteria that contaminate antiseptics, disinfectants and soaps that used in hospital in Gaza – Palestine
3. To measure the efficacy of antiseptics and disinfectants on bacteria.
4. To determine the chemical concentration of antiseptics and disinfectants.

1.3 Significance

Hospitals should exert their utmost efforts to prevent contamination to avoid health care associated infection (HAI), which is becoming a major threat because of the high possibility of the spread of multiple drug resistant pathogens in hospitals. Therefore, soaps, detergents, and antiseptics are commonly used in hospital to minimize risks of pathogens transmission. As with any commercial products, these materials may be

contaminated during manufacturing, processing, storage and use, thus, complicating the process and increasing risks of HAI.

Determination of the microbiological quality of antiseptics and soaps in general hospitals in the Gaza strip would generate the first original data and shed light on the general quality. This is of utmost need, because most of these soaps are manufactured locally with no standards or guidelines to ensure their quality. Antiseptics are imported as concentrates and are diluted locally, no data available on the effectiveness of such products. This study will be the first to tackle this issue.

It is expected that the results of this cross sectional study would help the hospital`s infection control committees in reviewing the process of purchasing such products and the policy of testing, use and storage. In general, hospitals would benefit from this study and is expected to contribute to the reduction of HAI. The local industry will also benefit from the findings of this study.

Chapter 2
Literature review

2.1 Soap

A substance used with water for washing and cleaning, made of a compound of natural oils or fats with sodium hydroxide or another strong alkali. Typically having perfume and coloring added (Oxford University Press.). They are defined as salts formed through the reaction of fatty acids obtained from vegetal and animal fats with metals or basic radicals (sodium, potassium, ammonia etc.), and exert detergent action, i.e. they permit the removal of dirt, remains and viable (non-colonizing) microorganisms **(J. A. Caetano et al., 2011).** Chemically, soap is a salt of a fatty acid **(McNaught, Wilkinson, & International Union of Pure and Applied Chemistry., 1997).** Soaps are mainly used as surfactants for washing, bathing, and cleaning, but they are also used in textile spinning and are important components of lubricants. Soaps for cleansing are obtained by treating vegetable or animal oils and fats with a strongly alkaline solution. Fats and oils are composed of triglycerides; three molecules of fatty acids are attached to a single molecule of glycerol. The alkaline solution, which is often called lye (although the term "lye soap" refers almost exclusively to soaps made with sodium hydroxide), brings about a chemical reaction known as saponification. In this reaction, the triglyceride fats are first hydrolyzed into free fatty acids, and then these combine with the alkali to form crude soap, an amalgam of various soap salts, excess fat or alkali, water, and liberated glycerol (glycerin) **(Cavitch, 1995).** Soaps are key components of most lubricating greases, which are usually emulsions of calcium soap or lithium soaps and mineral oil. These calcium- and lithium-based greases are widely used. Much other metallic soap is also useful, including those of aluminum, sodium, and mixtures of them. Such soaps are also used as thickeners to increase the viscosity of oils. In ancient times, lubricating greases were made by the addition of lime to olive oil **(Bohnet, 2003).**

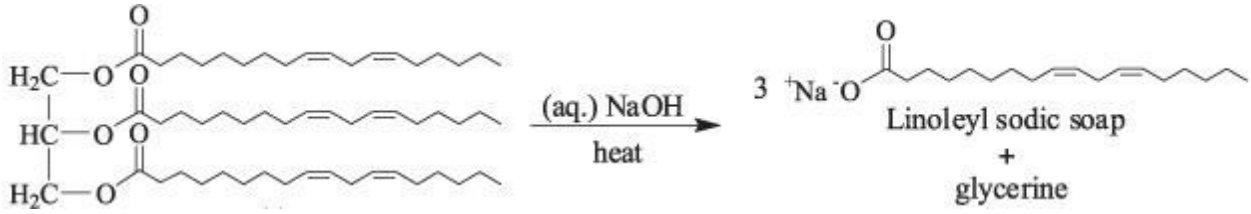


Fig (2.1): Saponification reaction

2.2 Antiseptic and Disinfectant

Microbial control methods involve the use of physical and chemical agents to eliminate or reduce the numbers of microorganisms from a specific environment. Microbial control methods are used to prevent the spread of infectious agents, retard spoilage, and keep commercial products safe. The population of microbes that cause spoilage or infection varies widely in species composition, resistance, and harmfulness. This means that microbial control methods must be adjusted to fit individual situations. The type of microbial control is indicated by the terminology used. Sterilization and -cidal agents destroy all viable organisms, including viruses. Antisepsis, disinfection, sanitization, -static agents reduce the numbers of viable microbes to a specified level **(Kathleen P. Talaro & Chess, 2012).**

An antiseptic agent or products that destroy or inhibit the growth of microorganisms in or on living tissue **(Kathleen P. Talaro & Chess, 2012; Wijesinghe & Weerasinghe, 2012)** (e.g. health care personnel hand washes and surgical scrubs)**(Seymour Stanton Block, 2001a; McDonnell & Russell, 1999).** A disinfectant agent is used on inanimate objects to destroy vegetative pathogens but not bacterial endospores **(Guralnik, 1980; Kathleen P. Talaro & Chess, 2012).** Disinfectants can be sporostatic but are not necessarily sporicidal **(Johnston et al., 2002)**. Effectiveness of Disinfectants depends on four factors **(Bennett, Jarvis, & Brachman, 2007; Seymour Stanton Block, 2001a).**

- Temperature of water: Most effective when the environmental temperature rises. The same can be achieved by mixing it in hot water.
- Strength of solution: The stronger the solution, the effectiveness of a disinfectant increases in a shorter period.

- Duration of exposure: The longer a disinfectant remains on the surface the more effective it will be.
- Cleanliness of surface: The most important thing to remember is that the disinfectant works the best on clean surface.

Sanitization refers to a physical or chemical process that completely destroys or removes all microbial life, including spores and reduces microbial numbers on inanimate objects to safe levels **(Seymour Stanton Block, 2001a; Kathleen P. Talaro & Chess, 2012)**.

Preservation is the prevention of multiplication of microorganisms in formulated products, including pharmaceuticals and foods **(Seymour Stanton Block, 2001a; McDonnell & Russell, 1999)**.

Degermation refers to the process of mechanically removing microbes from the skin **(Kathleen P. Talaro & Chess, 2012)**.

Microbial death is defined as the permanent loss of reproductive capability in microorganisms **(Kathleen P. Talaro & Chess, 2012)**.
Antimicrobials are described according to their ability to destroy or inhibit microbial growth. **Microbicidal agents** cause microbial death. They are described by what they are -cidal for: sporocides, bactericides, fungicides, viricides **(Kathleen P. Talaro & Chess, 2012)**.

2.2.1 Commonly Used Antiseptic and Disinfectant in Gaza

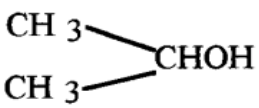
Four major groups of antiseptics and disinfectants

2.2.1.1 Alcohols: are among the most widely used disinfectants and antiseptics **(Seymour Stanton Block, 2001a; Wallen, 2002).** They are colourless hydrocarbons with one or more hydroxyl functional groups. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and

spore germination **(Trujillo & Laible, 1970; Yasuda-Yasaki, Namiki-Kanie, & Hachisuka, 1978)**, but this effect is reversible **(Trujillo & Laible, 1970),** Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Many alcohol products include low levels of other biocides (in particular chlorhexidine), which remain on the skin following evaporation of the alcohol, or excipients (including emollients), which decrease the evapora- tion time of the alcohol and can significantly increase product efficacy **(Bush, Benson, & White, 1986)**. Alcohols are bactericidal and fungicidal but not sporicidal. Some lipid containing viruses are also destroyed by alcohol **(Prescott, Harley, & Klein, 2005).** The two most popular alcohol germicides are ethanol and isopropanol usually used in about 70-80% concentration **(Collins, Allwood, Bloomfield, & Fox, 1981; McDonnell & Russell, 2001)**. In general, isopropyl alcohol is considered slightly more efficacious against bacteria **(Price, 1939)** and ethyl alcohol is more potent against viruses **(Wallen, 2002)**, however, this is dependent on the concentrations of both the active agent and the test microorganism **(Wallen, 2002)**.

Fig (2.2): Ethanol and Isopropanol

Ethanol
(Antisepsis)



Isopropanol
(Disinfection)

2.2.1.2 Halogens (iodine and chlorine): are important antimicrobial agents **(Prescott et al., 2005)**, Most halogens exert their antimicrobial effect primarily in the non-ionic state. They are highly effective components of disinfectants and antiseptics. Halogens are strong oxidizing agents. They are sporicidal with longer exposure. The major forms used in microbial control among chlorine compounds and Iodine compounds **(Kathleen P. Talaro & Chess, 2012)**.

Chlorine compounds: are liquid and gaseous chlorine, hypochlorites (OCl) and chloramines (NH2-Cl). They destroy vegetative bacteria and fungi, but not their spores **(Gerald Reybrouck, 1998)**. The most important types of chlorine compounds are sodium hypochlorite, chlorine dioxide, and the N-chloro compounds such as sodium dichloroisocyanurate (NaDCC), with chloramine-T being used to some extent. Sodium

hypochlorite solutions are widely used for hard-surface disinfection (household bleach) and can be used for disinfecting spillages of blood containing human immunodeficiency virus or HBV. NaDCC can also be used for this purpose and has the advantages of providing a higher concentration of available chlorine and being less susceptible to inactivation by organic matter. In water, sodium hypochlo- rite ionizes to produce Na1 and the hypochlorite ion, OCl2, which establishes an equilibrium with hypochlorous acid, HOCl (Ascenzi, 1995). Between pH4 and 7, chlorine exists predominantly as HClO, the active moiety, whereas above pH9, OCl2 predominates. Although chlorine compounds have been predominantly used as hard-surface disinfectants, novel acidified sodium chlorite (a two-component system of sodium chlorite and mandelic acid) has been described as an effective antiseptic **(McDonnell & Russell, 1999)**.

Fig (2.3): Chlorine compound

$\Rightarrow OCl^-, HOCl, Cl_2$

Chlorine compound
(Disinfection)

Iodine compounds: have the broadest spectrum of all topical anti-infectives with action against bacteria, fungi, viruses, spores, protozoa and yeast. Iodine is used mainly as a skin antiseptic. But less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal, virucidal, and sporicidal **(Seymour Stanton Block, 2001a)**. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I_2) being primarily responsible for antimictrobial efficacy **(Seymour Stanton Block, 2001a)**.

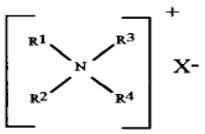
Fig (2.4): Iodine compounds

$\Rightarrow I_2$

Iodine compounds
(Antisepsis, Cleaning)

2.2.1.3 Quaternary Ammonium Compounds (QAC): Surface-active agents (surfactants) have two regions in their molecular structures, one a hydrocarbon, water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surfactants are classified into cationic, anionic, nonionic, and ampholytic (amphoteric) compounds. Of these, the cationic agents, as exemplified by quaternary ammonium compounds (QACs), are the most useful antiseptics and disinfectants **(Hugo, 1971)**. They are sometimes known as cationic detergents. QACs have been used for a variety of clinical purposes (e.g., preoperative disinfection of unbroken skin, application to mucous membranes, and disinfection of noncritical surfaces). In addition to having antimicrobial properties, QACs are also excellent for hard surface cleaning and deodorization. have positively charged quaternary nitrogen and a long chain hydrophobic aliphatic chain **(Prescott et al., 2005)**. They are used as low level disinfectants **(McDonnell & Russell, 2001)**. If used in medium concentrations, they are effective against some Gram-positive bacteria, viruses, fungi and algae. In low concentrations, they have microbistatic effect. QACs are ineffective against the tubercle bacillus, the hepatitis virus, Pseudomonas and spores at any concentration **(Kathleen P. Talaro & Chess, 2012)**.

Fig (2.5): Quaternaty Ammonium Compounds



Quaternary Ammonium Compounds (QACs)
(Antisepsis, cleaning, Disinfection, preservation)

2.2.1.4 Biguanides: three substances under this type are Chlorhexidine, Alexidine and polymeric biguanides

Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific **(Seymour Stanton Block, 2001a)**. Despite the advantages of

chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter **(A. D. Russell & Day, 1993)**.

2.3 Definition of Activity

There is no ideal disinfectant and the best compromise should be chosen according to the situation. A disinfectant solution is considered appropriate when the compromise between the antimicrobial activity and the toxicity of the product is satisfactory for the given application. Another consideration may well be the cost. The more active disinfectants are automatically the more toxic ones; potentially toxic products can be applied to inanimate objects or surfaces, whereas for disinfection of human tissues only the less toxic disinfectants can be considered. For antisepsis, different disinfectants are used for application to the intact skin (e.g. alcoholic solutions) and to mucous membranes or wounds (only aqueous solutions of non-toxic substances). Cost is a less important consideration for an antiseptic than for a disinfectant **(Yves Chartier et al., 2014)**.

The principal requirements for a good antiseptic are absence of toxicity and rapid and adequate activity on both the natural flora and, especially, pathogenic bacteria and other microorganisms after a very short expo- sure time. Essential requirements for a disinfectant are somewhat different: there must be adequate activity against bacteria, fungi, and viruses that may be present in large numbers and protected by dirt or organic matter. In addition, since disinfectants are applied in large quantities, they should be of low ecotoxicity.

In general, use of the chosen disinfectant, at the appropriate concentration and for the appropriate time, should kill pathogenic microorganisms, rendering an object safe for use in a patient, or human tissue free of pathogens to exclude cross-contamination **(Yves Chartier et al., 2014)**.

Table (2.1): Antiseptics and disinfectants and microorganism's activity

	Bactericidia		Mycobactericida	Sporicidia	Fungicidia		Virucidia
	Gram+ bacteria	Gram- bacteria	Mycobacteria	Bacterial spores	Yeast	moulds	Viruses
Alcohols	VA	VA	VA	NA	A	A	A
QACs	VA	A	NA	NA	A	A	A
Guanidines	VA	VA	NA	NA	A	A	A
Sodium hypochlorite and other chlorine compounds	VA	VA	A	A	A	A	A
Iodine	VA	VA		VA	A	A	A
Chlorhexidine	VA	A	NA	NA	LA	LA	LA

VA: Very active; A: Active; LA: Less active; NA: No active; *: Less active against *staphylococci* and *enterococci* **(Masri et al., 2013; Wijesinghe & Weerasinghe, 2012; Yves Chartier et al., 2013)**

2.4 Factors effects on Antiseptics and disinfectants activity

Several factors can affect the antimicrobial efficacy of chemical biocides. These factors have usually been well characterised for many of these compounds **(A. D. Russell, 2004)**. However, their practical significance for the end- product and its usage is rarely discussed. Failure of a disinfection/sanitisation process often reflects the non-respect or lack of understanding of these factors. Hence, it is important to combine the use of a suitable antimicrobial product/ formulation for a specific task with the training of the end user. Since compliance to the manufacturer's instructions is particularly important, the efficacy of an antimicrobial product should be evaluated with standard protocols that investigate a range of conditions. Many antimicrobial tests, notably practical tests, include various parameters in their design, such as concentration, contact time, tempera- ture, soiling, type and number of microorganisms. Generally, these factors can be divided into those inherent to the biocide and those inherent to the microorganisms.

Table (2.2): Factors effects on Antiseptics and disinfectants activity

Factors	Comments
Concentration	The main cause for failure of disinfection. Dilution can inactivate biocides, notably those with a high concentration exponent.
Contact time	Non-respect of, or poor compliance with, contact time can result in the survival of microorganisms. Contact time needs to be adapted to the condition of usage.
pH	Can affect both the microorganisms and the agents, especially if it is an acid or a base.
Organic load	Particularly important in the food industry, and in the clinical context with blood spillage. Can severely reduce the antimicrobial efficacy of biocides.
Factors	Comments
Temperature	Could be an issue when biocides are used in cold conditions; e.g. cold room, chilled food production, or with the efficacy of preservatives (i.e. products kept at a low temperature).
Microorganisms Type	Microorganisms vary in susceptibility to biocides, prions and spores being the most resistant to disinfection.
Number	Heavy microbial contamination is more difficult to disinfect/ sanitise.
Phenotype	Microorganisms grown as biofilms, or with a low metabolism are more resistant to antimicrobials than planktonic grown microorganisms and microorganisms grown on `rich' media.
Neutralisation/ incompatibility	Can inactivate completely or partially the activity of a biocide. Knowledge of the products is important.

2.4.1 Concentration is probably the most important factor to consider when antimicrobial efficacy is concerned **(A. D. Russell & McDonnell, 2000)**. There have been several published reports of microbial contamination following chemical disinfection, or microbial survival within biocidal products/formulations **(Poole, 2002; A. D. Russell, 2002)**. For example, many reports concern the failure of QAC disinfectants, although in many cases inappropriate concentrations were used **(Ehrenkranz, Bolyard, Wiener, & Cleary, 1980)**. Holah and colleagues (2002) pointed out that when the concentration of QACs remains high (i.e. 1000mgL^{-1}), survival of vegetative microorganisms is unlikely. Likewise, failure of high-level disinfectants such as glutaraldehyde to eliminate all microorganisms from endoscope washer disinfectors have been reported **(Griffiths, Babb, Bradley, & Fraise, 1997)**. The effect of changes in concentration on antimicrobial efficacy can be estimated by the concentration exponent (η) and is given by the equation:

$$\eta = \frac{\log t2 - \log t1}{\log C1 - \log C2}$$

Where C_1 and C_2 represent two concentrations and t_1 and t_2 the respective times to reduce the population to the same level. The concentration exponent varies among biocides. It gives an indication of the effect of diluting an in-use concentration; i.e. biocides with high concentration exponent will rapidly lose activity upon dilution, whereas those with a low concentration exponent will retain activity upon dilution. This in effect allows the selection of appropriate concentrations to be evaluated with antimicrobial test protocols.

Table (2.3): Examples of concentration exponent η

Antiseptics and Disinfectants	Exponent
Alcohol	
Benzyl alcohol	2.6 – 4.6
Aliphatic alcohols	6.0 – 12.7
Antiseptics and Disinfectants	Exponent
Cationic Antiseptics and Disinfectants	
Chlorhexidine	2
Polymeric biguanides	1.5 – 1.6
QACs	0.8 – 2.5
Crystal violet	0.9

2.4.2 Contact time is an important factor of all antimicrobial testing protocols and the choice of time of exposure usually reflects conditions in practice. There is no simple relationship between activity and contact time, although longer exposure time is usually associated with better activity and might be essential to eliminate the `resistant' clones of a microbial population. Standard antimicrobial test protocols, for manufacturers' and hygienic guidelines usually specify a set con- tact time or the minimum contact time required. For example, the European Standard for the testing of surface disinfectants (CEN1276, 1997a) stipulates that 5 $\log_{10}$ reduction in bacterial concentration must be attained within 5 minutes of exposure time. Likewise, the hygienic hand-wash procedure (CEN1499, 1997b) recommends a minimum of 1 minute contact time, which reflects acceptable hand-washing time in practice.

2.4.3 The effect of pH on antimicrobial activity is complex and can affect the microorganism as well as the compound **(A. D. Russell, 2004)**. For some biocides, their active state is the non-ionised form (e.g. phenols, acetic acid, benzoic acid) and increase pH decreases their activity. Others (e.g. cationic biocides, glutaraldehyde) show an enhanced activity at an alkaline condition. However, testing for antimicrobial efficacy at different pH is usually not recommended since the pH is usually set for a given antimicrobial formulation and cannot be altered easily without affecting the stability of the formulation.

Table (2.4): pH for Antiseptic and disinfectants

Antiseptic and Disinfectants	pH
Halogens Sodium hypochlorite, chlorine, Iodine	<7
Biguanides Chlorhexidine, Alexidine, PHMB	>7
QACs	>7
Alcohols	7

2.4.4 Organic load or soiling (e.g. serum, blood, pus, earth, food residues, faecal materials) contributes to decreasing biocidal activity by either `mopping up' the active concentration or/and offering some protection to the microorganisms. Indeed the antimicrobial efficacy of some biocides can be deeply affected by soiling. Practical tests now reflect the importance of soiling by stipulating testing under clean and dirty conditions, usually by the addition of serum albumin (e.g. $3gL^{-1}$ for testing under dirty condition) in the reaction vessel (e.g. CEN1276, 1997a). The effect of soiling also emphasises the necessity of cleaning surfaces and equipment before a biocidal product is used, or combining a disinfectant with a detergent. In the food and dairy industry, a reduction in biocidal activity may occur with the presence of organic matter and effective pre-cleaning prior to disinfection is recommended. Some chemical biocides may exert a detergent action, whereas some detergents exhibit some biocidal activity. In this respect the surface to be treated is important to consider (see below) as it affects the efficacy of a biocide or biocide/detergent combination.

Tabel (2.5): Soiling in Antiseptics and disinfectants

Antiseptic and Disinfectants	Soiling
Halogens Sodium hypochlorite, chlorine, Iodine	+
Biguanides Chlorhexidine, Alexidine, PHMB	+
QACs	+
Alcohols	+

2.4.5 Temperature: The activity of biocides usually increases with a rise in **temperature** and this principle is used, when combining biocide and steam sterilisation. Other equipment, such as some automated washer disinfectors, also combine biocides and elevated temperature. On the other hand, low temperature may decrease the antimicrobial efficacy of biocides. Temperature is particularly an issue during storage of a biocidal formulation/product, especially upon preservation, and where chilled food is produced **(Taylor, Rogers, & Holah, 1999)**. The effect of temperature on activity can be calculated with the temperature coefficient (θ) and more conveniently by the Q_{10} value (change in activity following a rise of 10°C). The Q_{10} value is given by the equation:

$$Q_{10} = \frac{Time\ to\ Kill\ at\ T°C}{Time\ to\ Kill\ at\ (T+10)°C}$$

Standard testing protocols recommend testing at a temperature of 20°C ± 1°C (e.g. CEN1276, 1997a) or around ambient temperature (18-25°C) (e.g. CEN13697, 2001). However, this does not reflect product usage at low temperature, although the activity of a compound at additional temperature can be tested.

2.4.6 Neutralizing Agents

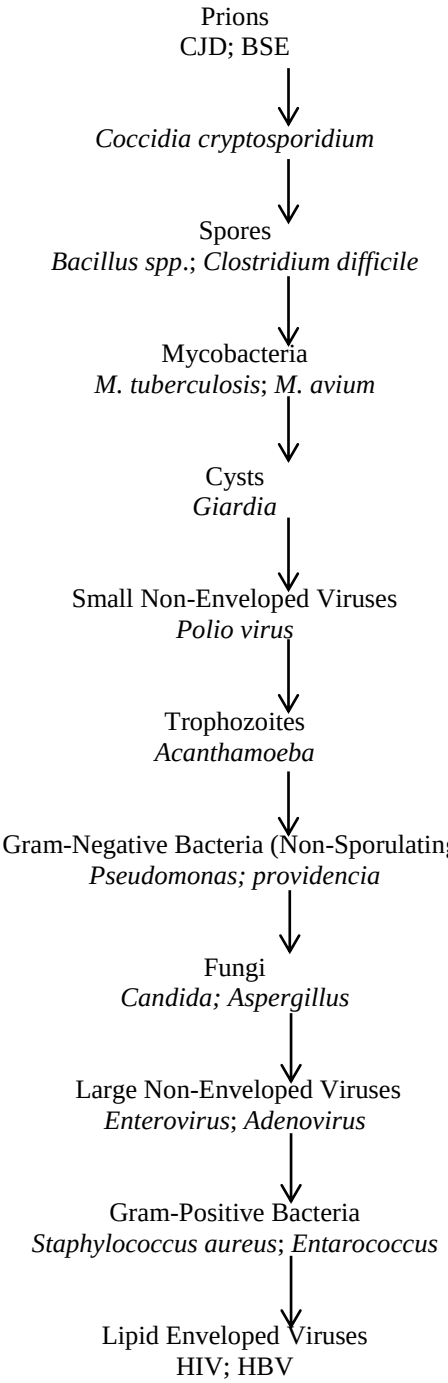
Table (2.6): Examples of neutralizing agents for antiseptics and disinfectants

Antiseptics and Disinfactants	Possible neutraliser	comment
Alcohols	None (dilution)	
Cationic compounds Chlorhexidine QACs	 Lecithin + tween Lethicin + Lubrol	
Halogens Chlorine, Sodium hypochlorite, Iodine	Sodium thiosulphate	Sodium thiosulphate might be toxic to some bacterial species.

2.4.7 The surface to be disinfected is not usually listed as a factor influencing the activity of a biocide as such, but needs to be considered here. The antimicrobial efficacy of disinfectants or sanitisers will depend to some extent on the surface upon which they are used. Surfaces can vary greatly, particularly whether they are porous or non-porous. Porous surfaces will have a tendency to entrap and protect microbial contaminants, whereas non-porous surfaces can reduce bacterial adhesion and facilitate a cleaning or a disinfection process.

2.4.8 Different types of microorganisms present different levels of sensitivity to a given antimicrobial biocide. Attempts have been made to classify microorganisms according to their overall susceptibility to biocides. Usually, such classification relies upon information on the intrinsic property of a microorganism but is not designed to give a definite answer about the susceptibility of a type of microorganism, since variation within species and even strains might occur. Practically, the type of microorganisms expected on a given surface help in the selection of an appropriate disinfectant or sanitiser. Antimicrobial test protocols usually include testing against a range of bacteria and fungi, which are selected depending upon the expected usage of the biocide, i.e. food industry, hospital environment, etc. However, the number of test protocols available to evaluate virucidal and mycobactericidal activity is limited. In addition, there is no standardisation and these protocols tend to vary greatly between countries **(Campos et al., 2012; Fraise et al., 2012; Sauerbrei et al., 2012)** notably with the test organisms. a similar protocol for the healthcare and veterinary environment has not been published yet **(J. Holah, 2003)**. The choice of the viral indicator is particularly contentious **(Jean-Yves Maillard, 2004)**.as seen in figure (2.6)

Fig (2.6): Microorganisms and antiseptics and disinfectants



(Masri et al., 2013; Kathleen P. Talaro & Chess, 2012)

2.4.9 Bacterial phenotype can affect the activity of antimicrobial biocides. Growth conditions including physical (e.g. temperature, gas) and chemical conditions (e.g. pH), nutrient limitation and diet (i.e. excess of lipids), but also whether the cells are grown as a biofilm or in suspension will produce microorganisms with a different phenotype. The metabolic status of the cell is particularly important since bacteria with

a `low metabolism' or quiescent bacteria are particularly resilient to the antimicrobial effects of biocides **(Gilbert, McBain, & Rickard, 2003; J. T. Holah, Taylor, Dawson, & Hall, 2002)**.

2.4.10 The number of microorganisms that should be used in standard tests has long been debated and differs between test protocols. It is generally accepted that the higher the level of microbial contaminant, the more difficult the disinfection. Predicting the level of contamination might be difficult and often the worst case scenario is considered, i.e. a high-inoculum. Most tests work on the basis of reducing the number of microorganisms to an acceptable level (e.g. a 5 $\log_{10}$ reduction on surface), but not to the complete elimination (i.e. sterilisation) of the microorganisms. If this is generally acceptable for most microorganisms, a problem can arise with highly infectious or virulent microorganisms such as the hepatitis B virus, Escherichia coli O157 for which a complete elimination would be recommendable **(Masri et al., 2013; Steinhauer, 2010; Wijesinghe & Weerasinghe, 2012).**

2.5 Limitations in the use of Antiseptics and disinfectants

The limitations in using biocidal products usually refer to their toxicity, to the alteration of the surface/equipment onto which they are used (e.g. corrosiveness, colour formation), to their incompatibility with other components of a formulation, but also to their overall efficacy against a given predicted microorganism. For example, high-level disinfectants are needed for the disinfection of critical surfaces in the hospital environment **(W. A. Rutala & Weber, 1999).** Toxicity is also important to consider not only for the end user (e.g. with antisepsis and preservation) but also for the environment. For example, the use of high concentrations might not be acceptable because of the high toxicity for the environment. Within the food industry, consideration must also be given to the potential for any biocide residues to taint or otherwise change the organoleptic properties of the foodstuffs produced.

Limitations related with antiseptic and disinfectants activity **(Lelieveld, Mostert, & Holah, 2005)**:

- ✓ Broad spectrum activity including activity against bacteria, fungi, viruses
- ✓ Rapid antimicrobial activity

- ✓ Retain stability (product) and antimicrobial efficacy over a wide range of pH
- ✓ Retain stability (product) and antimicrobial efficacy over a wide range of temperature
- ✓ Retain activity in the presence of organic load and hard water
- ✓ Retain activity upon dilution
- ✓ Residual activity: The presence of remaining low concentration (below the minimum inhibitory concentration, MIC) of a biocide on a surface is the subject of much debate with current evidence on emerging microbial resistance to biocides.

Limitations related with safty:

- ✓ No or low toxicity
- ✓ Degradable in the environment

Limitations related with formulation and usage:

- ✓ No or low corrosiveness
- ✓ Non-staining
- ✓ No odour
- ✓ Good wetting and detergency
- ✓ Easily combined with liquid or powder
- ✓ Compatible with other chemicals (e.g. surfactants)
- ✓ Cost-effective

Table (2.7): Advantages and Disadvantages of Antiseptics and disinfectants.

Antiseptics and disinfectants	Advantages	Disadvantages
Alcohols (60–90%) including ethanol or isopropanol	Fast acting No residue No staining Low cost Readily available in all countries	Volatile, flammable, and irritant to mucous membranes Inactivated by organic matter May harden rubber, cause glue to deteriorate, or crack acrylate plastic
Chlorine and chlorine compounds: the most widely used is an aqueous solution of sodium hypochlorite 5.25–6.15% (household bleach) at a concentration of 100–5000 ppm free chlorine	Low cost, fast acting Readily available in most settings Available as liquid, tablets or powders	Corrosive to metals in high concentrations (>500 ppm) Inactivated by organic material Causes discoloration or bleaching of fabrics Releases toxic chlorine gas when mixed with ammonia Irritant to skin and mucous membranes Unstable if left uncovered, exposed to light or diluted; store in an opaque container

2.6 Mechanism of Action of Common Antiseptics and Disinfectants

The mechanisms of antimicrobial action of a range of chemical agents that are used as antiseptics or disinfectants or both are discussed. Different types of microorganisms are considered, and similarities or differences in the nature of the effect are emphasized. The mechanisms of action of antiseptics and disinfectants on microorganisms, especially bacteria **(Denyer & Stewart, 1998)**. These include examination of uptake **(Ioannou, Hanlon, & Denyer, 2007; Yeaman & Yount, 2003)**, lysis and leakage of intracellular constituents **(J-Y Maillard, 2002)**, perturbation of cell homeostasis **(Dodd, Sharman, Bloomfield, Booth, & Stewart, 1997)**, effects on model membranes **(Lambert, 2004)**, inhibition of enzymes, electron transport, and oxidative phosphorylation **(J-Y Maillard, 2002)**, interaction with macromolecules **(Setlow, 2006)**, effects on macromolecular biosynthetic processes **(J-Y Maillard, 2002)**, and microscopic examination of biocide-exposed cells. Additional and useful information can be obtained by calculating concentration exponents (n values **(Denyer & Stewart, 1998)**) and relating these to membrane activity **(Denyer & Stewart, 1998)**. Many of these procedures are valuable for detecting and evaluating antiseptics or disinfectants used in combination **(A. Russell, 2004)**. Similar techniques have been used to study the activity of antiseptics and disinfectants against fungi, in particular yeasts. Additionally, studies on cell wall porosity **(De Nobel, Klis, Priem, Munnik, & Van Den Ende, 1990)** may provide useful information about intracellular entry of disinfectants and antiseptics **(S. Hiom, Furr, Russell, & Hann, 1995)**. Mechanisms of antiprotozoal action have not been widely investigated. One reason for this is the difficulty in culturing some protozoa (e.g., Cryptosporidium) under laboratory conditions. However, the different life stages (trophozoites and cysts) do provide a fascinating example of the problem of how changes in cytology and physiology can modify responses to antiseptics and disinfectants. Khunkitti et al. **(Khunkitti, Avery, Lloyd, Furr, & Russell, 1997; Lloyd et al., 2001)** have explored this aspect by using indices of viability, leakage, uptake, and electron microscopy as experimental tools. Some of these procedures can also be modified for studying effects on viruses and phages (e.g., uptake to whole cells and viral or phage components, effects on nucleic acids and proteins, and electron microscopy) **(Rodgers, Hufton,**

Kurzawska, Molloy, & Morgan, 1985). Viral targets are predominantly the viral envelope (if present), derived from the host cell cytoplasmic or nuclear membrane; the capsid, which is responsible for the shape of virus particles and for the protection of viral nucleic acid; and the viral genome. Release of an intact viral nucleic acid into the environment following capsid destruction is of potential concern since some nucleic acids are infective when liberated from the capsid **(Brul & Coote, 1999; A. Russell, 1991)**, an aspect that must be considered in viral disinfection. Important considerations in viral inactivation are dealt with by Klein and Deforest and Prince et al. **(Prince, Prince, & Prince, 1991)**, while an earlier paper by Grossgebauer is highly recommended **(Grossgebauer, 1970)**.

Table (2.8): Summary of mechanism of action of antiseptics and disinfectants

Target	Antiseptics and Disinfectants	Mechanism of Action
Cell envelope (cell wall, outer membrane)	Glutaraldehyde EDTA, other permeabilizers	Cross-linking of proteins. Gram-negative bacteria: removal of Mg21, release of some LPS.
Cytoplasmic (inner) membrane	QACs Chlorhexidine Diamines PHMB, alexidine Phenols	Generalized membrane damage involving phospholipid bilayers. Low concentrations affect membrane integrity, high concentrations cause congealing of cytoplasm. Induction of leakage of amino acids. Phase separation and domain formation of membrane lipids. Leakage; some cause uncoupling.
Cross-linking of macromolecules	Formaldehyde Glutaraldehyde	Cross-linking of proteins, RNA, and DNA. Cross-linking of proteins in cell envelope and elsewhere in the cell.
DNA intercalation	Acridines	Intercalation of an acridine molecule between two layers of base pairs in DNA.
Interaction with thiol groups	Silver compounds	Membrane-bound enzymes (interaction with thiol groups).
Effects on DNA	Halogens Hydrogen peroxide, silver ions	Inhibition of DNA synthesis. DNA strand breakage.
Oxidizing agents	Halogens Peroxygens	Oxidation of thiol groups to disulfides, sulfoxides, or disulfoxides. Hydrogen peroxide: activity due to from formation of free hydroxy radicals (zOH), which oxidize thiol groups in enzymes and proteins; PAA: disruption of thiol groups in proteins and enzymes.

2.6.1: Mechanism of Action of Alcohols

Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water, it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis **(E. Larson & Morton, 1991)**.

The mode of action of alcohol depends upon its concentration. Alcohol with a concentration of 50% and higher dissolves membrane lipids, disrupts cell surface tension and compromises membrane integrity. An alcohol that has entered the protoplasm denatures protein through coagulation but only in alcohol-water solution of 50-95%. Absolute alcohol (100%) dehydrates cells and inhibits their growth. Some of its effectiveness as surface disinfectants can be attributed to its cleansing or detergent action, which helps in the mechanical removal of microorganisms. Solutions of 70-95% alcohol are used as skin degerming agents **(McDonnell & Russell, 2001)**.

2.6.2 Mechanism of Action of Halogens

Chlorine compounds In solution these compounds combine with water and release hypochlorus acid (HOCl), that oxidises the sulfhydryl (S-H) group on the amino acid cysteine, that interferes with the disulfide (S-S) bridges of numerous enzymes. The resulting denaturation of the enzymes is irreversible and suspends metabolic reactions **(Kathleen P. Talaro & Chess, 2012)**.

CRAs are highly active oxidizing agents and thereby destroy the cellular activity of proteins **(Chlorine, 1995)**; potentiation of oxidation may occur at low pH, where the activity of CRAs is maximal, although increased penetration of outer cell layers may be achieved with CRAs in the unionized state. Hypochlorous acid has long been considered the active moiety responsible for bacterial inactivation by CRAs, the OCl2 ion having a minute effect compared to undissolved HOCl **(Seymour Stanton Block, 2001a)**. This correlates with the observation that CRA activity is greatest when the percentage of undissolved HOCl is highest. This concept applies to hypochlorites, NaDCC, and chloramine-T.

Deleterious effects of CRAs on bacterial DNA that involve the formation of chlorinated derivatives of nucleotide bases **(Dukan & Touati, 1996)**. Hypochlorous acid has also been found to disrupt oxidative phosphorylation **(Barrette Jr, Hannum,**

Wheeler, & Hurst, 1989) and other membrane-associated activity **(Camper & McFETERS, 1979)**. inhibition of bacterial growth by hypochlorous acid. At 50 mM (2.6 ppm), HOCl completely inhibited the growth of E. coli within 5 min, and DNA synthesis was inhibited by 96% but protein synthesis was inhibited by only 10 to 30%. Because concentrations below 5mM (260ppm) did not induce bacterial membrane disruption or extensive protein degradation, it was inferred that DNA synthesis was the sensitive target. In contrast, chlorine dioxide inhibited bacterial protein synthesis **(McDonnell & Russell, 2001)**.

CRAs at higher concentrations are sporicidal **(A. Russell & Day, 1995)**; this depends on the pH and concentration of available chlorine **(Allan Denver Russell, 1982)**. During treatment, the spores lose refractivity, the spore coat separates from the cortex, and lysis occurs **(Kulikovsky, Pankratz, & Sadoff, 1975)**. In addition, a number of studies have concluded that CRA-treated spores exhibit increased permeability of the spore coat **(Allan Denver Russell, 1982)**.

CRAs also possess virucidal activity **(Seymour Stanton Block, 2001b)**. chlorine inactivated naked f2 RNA at the same rate as RNA in intact phage, whereas f2 capsid proteins could still adsorb to the host. the RNA of polio virus type 1 was degraded into fragments by chlorine but that poliovirus inactivation preceded any severe morphological changes. And in other studies found that the capsid of poliovirus type 1 was broken down **(Sharp & Leong, 1980)**.

Iodine compounds Iodine rapidly penetrates the cells of microorganisms where it apparently disturbs a variety of metabolic functions by interfering with disulfide bonds of protein. It also iodinates cell proteins.

the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins (in particular the free- sulfur amino acids cysteine and methionine **(Seymour Stanton Block, 2001a)**, nucleotides, and fatty acids **(Seymour Stanton Block, 2001a)**, which culminates in cell death . Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses **(Prince et al., 1991)**. Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds **(Springthorpe & Sattar, 1990)**.

2.6.3 Mechanism of Action of Quaternary Ammonium Compounds

QACs lower cellular surface tension. This can have several effects but chief among them is the disruption of the cell membrane and the loss of its selective permeability. QACs kill micro-organisms by causing a leakage of microbial protoplasm, precipitating proteins and inhibiting metabolism **(Kathleen P. Talaro & Chess, 2012).**

2.6.4 Mechanism of Action of Biguanides

Chlorhexidine is a bactericidal agent **(Denyer & Stewart, 1998)**. Its interaction and uptake by bacteria, the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using [^{14}C] chlorhexidine gluconate, the uptake by bacteria **(Hageman & Havinga, 2006)** and yeasts **(S. J. Hiom, Furr, Russell, & Dickinson, 1992b)** was shown to be extremely rapid, with a maximum effect occurring within 20 s. Damage to the outer cell layers takes place **(El Moug, Rogers, Furr, El-Falaha, & Russell, 1986)** but is insufficient to induce lysis or cell death. The agent then crosses the cell wall or outer membrane, presumably by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In yeasts, chlorhexidine "partitions" into the cell wall, plasma membrane, and cytoplasm of cells **(S. J. Hiom, Furr, Russell, & Dickinson, 1992a)**. Damage to the delicate semipermeable membrane is followed by leak- age of intracellular constituents, which can be measured by appropriate techniques. Leakage is not per se responsible for cellular inactivation but is a consequence of cell death **(A. Russell & Hugo, 1988)**. High concentrations of chlorhexidine cause coagulation of intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage **(Davies, 1973)**, so that there is a biphasic effect on membrane permeability. An initial high rate of leakage rises as the concentration of chlorhexidine increases, but leakage is reduced at higher biocide concentrations because of the coagulation of the cytosol. an inhibitor of both membrane-bound and soluble ATPase as well as of net K^+ uptake in *Enterococcus faecalis*. However, only high biguanide concentrations inhibit membrane-bound ATPase **(Chopra, Linton, Hugo, & Russell, 1987)**, which suggests

that the enzyme is not a primary target for chlorhexidine action. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects **(McDonnell & Russell, 2001)**.

The effects of chlorhexidine on yeast cells are probably similar to those previously described for bacteria **(S. Hiom, Hann, Furr, & Russell, 1995)**. Chlorhexidine has a biphasic effect on protoplast lysis, with reduced lysis at higher biguanide concentrations. Furthermore, in whole cells, the yeast cell wall may have some effect in limiting the uptake of the biguanide **(S. Hiom, Furr, et al., 1995)**. an effect on the fungal plasma membrane but with significant actions elsewhere in the cell **(Bobichon & Bouchet, 1987)**. Increasing concentrations of chlorhexidine (up to 25 mg/ml) induce progressive lysis of *Saccharomyces cerevisiae* protoplasts, but higher biguanide concentrations result in reduced lysis **(S. J. Hiom et al., 1992a)**.

chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive **(Khunkitti, Lloyd, Furr, & Russell, 1998)**. the effects of chlorhexidine and other biocides on *Acanthameoba* and showed that membrane damage in these protozoa is a significant factor in their inactivation.

Mycobacteria are generally highly resistant to chlorhexidine **(Selvaraju, Khan, & Yadav, 2005)**. Little is known about the uptake of chlorhexidine (and other antiseptics and disinfectants) by mycobacteria and on the biochemical changes that occur in the treated cells. Since the MICs for some mycobacteria are on the order of those for chlorhexidine-sensitive, gram-positivecocci, the inhibitory effects of chlorhexidine on mycobacteria may not be dissimilar to those on susceptible bacteria. *Mycobacterium avium-intracellulare* is considerably more resistant than other mycobacteria **(Bradley & Fraise, 1996)**.

Chlorhexidine is not sporicidal (discussed in "Mechanisms of resistance"). Even high concentrations of the bisbiguanide do not affect the viability of Bacillus spores at ambient temperatures **(Shaker, Dancer, Russell, & Furr, 1988)**, although a marked sporicidal effect is achieved at elevated temperatures **(Shaker, Russell, & Furr, 1986)**. Presumably, sufficient changes occur in the spore structure to permit an increased uptake of the biguanide, although this has yet to be shown experimentally. Little is known about the uptake of chlorhexidine by bacterial spores, although coatless

forms take up more of the compound than do "normal" spores **(Shaker, Furr, & Russell, 1988)**. Chlorhexidine has little effect on the germination of bacterial spores **(Poole, 2002)** but inhibits outgrowth. The reason for its lack of effect on the former process but its significant activity against the latter is unclear. It could, how- ever, be reflected in the relative uptake of chlorhexidine, since germinating cells takeup much less of the bisbiguanide than do outgrowing forms **(A. Russell, Jones, & Milburn, 1985)**. Binding sites could thus be reducedin number or masked in germinating cells.

The antiviral activity of chlorhexidine is variable. Studies with different types of bacteriophages have shown that chlorhexidine has no effect on MS2 or K coliphages **(J.-Y. Maillard, Beggs, Day, Hudson, & Russell, 1994)**. High concentrations also failed to inactivate *Pseudomonas aeruginosa* phage F116 and had no effect on phage DNA within the capsid or on phage proteins **(J-Y Maillard, Beggs, Day, Hudson, & Russell, 1995)**; the transduction process was more sensitive to chlorhexidine and other biocides than was the phage itself. The chlorhexidine bound poorly to F116 particles. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses **(Park & Park, 1989)**. Chlorhexidine does not inactivate nonenveloped viruses such as rotavirus **(Springthorpe, Grenier, Lloyd-Evans, & Sattar, 1986)**, HAV **(Mbithi, Springthorpe, & Sattar, 1990)**, or poliovirus **(McDonnell & Russell, 2001)**. Its activity be restricted to the nucleicacid core or the outer coat, althoughit is likely that the latter would be a more important target site.

Table (2.9): Mechanisms of action of chlorhexidine.

Target microorganism	Chlorhexidine action
Bacteria	Membrane active agent, causing protoplast and spheroplast lysis, high concentrations cause precipitation of proteins and nucleic acids.
Spores	Not sporicidal but prevents development of spores; inhibits spore outgrowth but not germination.
Mycobacteria	Mycobacteristatic (unknown) but not mycobactericidal
Yeasts	Membrane active agent, causing protoplast lysis and intracellular leakage; high concentrations cause intracellular coagulation
Protozoa	against *A. castellanii* demonstrate membrane activity (leakage) toward trophozoites, less toward cysts
Viruses	Low activity against many viruses; lipid enveloped viruses more sensitive than nonenveloped viruses; effect possibly on viral envelope, perhaps the lipid moieties

2.7 Mechanism of Microorganism Resistance

Many studies considerable progress has been made in understanding more fully the responses of different types of bacteria (mycobacteria, nonsporulating bacteria, and bacterial spores) to antibacterial agents. As a result, resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (self-replicating, extrachromosomal DNA) or transposons (chromosomal or plasmid integrating, transmissible DNA cassettes). Intrinsic resistance is demonstrated by gram- negative bacteria, bacterial spores, mycobacteria, and, under certain conditions, *staphylococci* **(Table5)**. Acquired, plasmid-mediated resistance is most widely associated with mercury compounds and other metallic salts. In recent years, acquired resistance to certain other types of biocides has been observed, notably in staphylococci **(Percival, Bowler, & Russell, 2005)**.

Table (2.10): mechanisms of innate bacterial resistance to antiseptics and disinfectants.

Type of resistance	Examples	Mechanism of resistance
Gram positive bacteria	Chlorhexidine	
Gram negative bacteria	QACs, triclosan, diamines.	Barrier presented by outer membrane may prevent uptake of antiseptic or disinfectant; glycocalyx may also be involved.
Spores	Chlorhexidine, QACs, phenolics	
Mycobacteria	Chlorhexidine, QACs. Glutaraldehyde.	Barrier presented by outer membrane may prevent uptake of antiseptic or disinfectant; glycocalyx may also be involved. Reason for high resistance of some strains of *M. chelonae*
Inactivation (chromosomally mediated)	Chlorhexidine.	Breakdown of chlorhexidine molecule may be responsible for resistance

2.8 Nosocomial Infection

Nosocomial infections (also known as hospital-acquired infections, hospital-associated infections and hospital infections) are infections that are not present in the patient at the time of admission to a health-care facility but develop during the course of the patient's stay **(Yves Chartier et al., 2014)**.

Nosocomial infections occur as a result of medical procedures performed on patients that lead to infections from a patient's own (endogenous) flora or as a result of

exposure to items contaminated with infectious agents. Additionally, the risk of acquiring an infection increases for patients with altered or compromised immunity **(Bennett et al., 2007)**.

Human beings are reservoirs of numerous types of microorgansims. Faeces contain approximately 10^{13} bacteria per gram, and the number of microorganisms on skin varies between 10^2 and 10^4 per cm^2. Many species of microorganisms live on mucous membranes and are considered normal flora. When the integrity of these barriers is challenged (e.g. microorganisms penetrate the skin or the mucous membrane), this creates an opportunity for an infection to occur **(Yves Chartier et al., 2014)**.

2.8.1 From Exposure to Infection

Whether an infection will develop after an exposure to microorganisms depends upon the interaction between the microorganisms and the host. Healthy individuals have a normal general resistance to infection. Patients with underlying disease, newborn babies and the elderly have less resistance and are at greater risk to develop an infection after exposure **(Health Canada & Occupational, 2012)**.

Local resistance to infection also plays an important role: the skin and the mucous membranes act as barriers in contact with the environment. Infection may occur when these barriers are breached. Local resistance may also be overcome by the long-term presence of an irritant, such as a cannula or catheter. The likelihood of infection increases daily when a patient has a catheter attached **(Filipe, 2010)**.

The most important determinants of infection are the nature and number of the infectious agents. Microorganisms range from the completely innocuous to the extremely pathogenic; the former will never cause an infection even in immunocompromised individuals, while the latter will cause an infection in virtually every case of exposure **(Andersen, 2010)**.

When only a few organisms are present, an infection will not necessarily develop. However, when a critical number is exceeded, it is very likely that an infection will become established. For every type of microorganism, the *minimal infective dose* can be determined. This is the lowest number of bacteria, viruses or fungi that cause the first clinical signs of infection in a healthy individual. For most causative agents of nosocomial infections, the minimal infective dose is relatively high. For example, for

Klebsiella and *Serratia* spp. and other Enterobacteriaceae, it is more than 10^5 colony-forming units (CFUs)/gram, but for hepatitis B virus it is less than 10 plaque-forming units (PFUs)/ gram **(Yves Chartier et al., 2014)**.

2.8.2 Source of Infection

In a health-care facility, the sources of infectious agents may be the personnel, the patients or the inanimate environment **(Prüss, Giroult, & Rushbrook, 2014)**.

The hospital environment can be contaminated with pathogens. *Salmonella* or *Shigella* spp., *Escherichia coli* O157:H7 or other pathogens may be present in the food and cause an outbreak, just as they can in a community outside the hospital. Waterborne infections may develop if the water-distribution system breaks down. In more sophisticated facilities, the water-cooling system of air-conditioning equipment may become contaminated with *Legionella pneumophilia*, causing Legionnaires' disease in susceptible patients. Pharmaceuticals may become contaminated during production or preparation; an outbreak of infection by *Pseudomonas aeruginosa*, *Burkholderia cepacia* or *Serratia marcescens* may occur as a consequence. In all these examples, it may be possible to isolate the same causative agent in several patients, which would suggest a common source. All possible measures should be taken to prevent the recurrence of such incidents.

The source of a nosocomial infection may also be a health-care worker who is infected or colonized (a carrier) with an infectious agent. The symptoms of infection will make the potential transmission apparent to the health-care worker and/or to managerial staff, and infected personnel are usually taken off patient care duties. Sometimes a carrier may be symptomless (i.e. is colonized by potentially pathogenic organisms but does not develop any infection). A typical example is methicillin-resistant *Staphylococcus aureus*, which may be carried in the nasal passages of 30–60% of health-care personnel. Faecal carriage of enteropathogens such as *Salmonella* spp. also occurs frequently, but the prevalence varies according to the region. Other conventional pathogens that can be found in symptomless carriers include *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Neisseria meningitidis*, hepatitis B virus and cytomegalovirus. Exposure of patients to carriers can give rise to an outbreak of disease. Careful investigation and isolation of the same organisms from

a cluster of patients as well as the carrier should reveal the cause of the outbreak **(Prüss et al., 2014)**.

The source of most hospital epidemics is infected patients; that is, patients infected with pathogenic microorganisms. These microorganisms are often released into the environment in very high numbers, depending on the disease, exceeding the minimal infective dose, and exposing other patients, who subsequently develop hospital-acquired infections. The recent case of severe acute respiratory syndrome and its impact on health-care waste-generation rates **(Chiang, Sung, Chang, & Tsai, 2006)** is a classic example of hospital-based epidemics relating to a respiratory disease.

2.8.3Nosocomial Infection Pathogens

2.8.3.1 Conventional pathogens

Cause disease in healthy individuals in the absence of specific immunity **(Prüss et al., 2014)**.

Examples: Methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes* (beta strep group A), *Salmonella* spp., *Shigella* spp., vancomycin-resistant *Enterococcus*, *Corynebacterium 34iphtheria*, *Mycobacterium tuberculosis*, *Bordetella pertussis*, hepatitis A and B viruses, rubella virus, rotaviruses, human immunodeficiency virus (HIV) **(Prüss et al., 2014)**.

2.8.3.2Conditional pathogens

Cause disease, other than trivial local infections, only in persons with reduced resistance to infection (including newborn infants) or when implanted directly into tissue or a normally sterile body area **(Prüss et al., 2014)**.

Examples: *Streptococcus agalactiae*, *Enterococcus* spp., *Clostridium tetani*, *Escherichia coli*, *Klebsiella* spp., *Serratia marcescens*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Candida* spp **(Prüss et al., 2014)**.

2.8.3.3Opportunistic pathogens

Cause generalised disease, but only in patients with profoundly diminished resistance to infection **(Prüss et al., 2014)**.

Examples: Atypical mycobacteria, *Nocardia asteroides, Pneumocystis carinii* ***(Gerberding, 1998)***.

2.9 Reference Studies

2.9.1 Bacterial contamination in liquid soap for hospital and public use

In exploratory, cross-sectional study was developed at the hospitalization units of a medium-sized hospital in Fortaleza, Ceará, Brazil. Data were collected between May and July 2007. Fifty-nine liquid soap dispensers were analyzed, of which 33 contained the following microorganisms: *Burkholderia cepacia* (14), *Pseudomonas putidas* (9), *Pseudomonas aeruginosa* (3), *Klebsiella pneumoniae* (3), *Enterobacter clocae* (2), and *Pseudomonas luteola* (2). The units with the largest number of contaminated samples were the surgical (n=7) and the dermatological clinics (n=4). Contamination was also found in an original flask of the same lot of liquid soap used to fill up the dispensers. In conclusion, there is a need to regulate and control the quality of these products in the production lines as well as during use in hospital services, mainly because they are used to prevent hospital infection**(J. A. Caetano et al., 2011)**.

Burkholderia species move through one single polar flagellum, or flagellum cluster. The best-known species is Burkholderia cepacia, which is aerobic, gram-negative and rod-shaped, capable of growing even in disinfecting solutions. This species has an extraordinary nutritional spectrum and can degrade more than 100 different organic molecules. This ability results from factors that facilitate equipment, product and drugs contamination in hospitals **(Tortora et al., 2013)**, it can colonize a range of humid environmental surfaces and is commonly associated with hospital infections. According to literature (**Murray, Rosenthal, & Pfaller, 2006)**, infections caused by this microorganism include respiratory tract infections in patients with cystic fibrosis or chronic granulomatous disease; urinary tract infection; urinary tract infection in patients using catheters; and sepsis, particularly in patients with contaminated

intravascular catheters. Except for pulmonary infection, in general, *B. cepacia* has a relatively low virulence level, and infections with this microorganism generally do not result in death**(J. A. Caetano et al., 2011)**.

Pseudomonas spp. are straight or slightly curved gram- negative bacilli, which are mobile through polar flagella; they are omnipresent organisms, easily found throughout the hospital environment in humid reservoirs, including food, cut flowers, sinks, toilets, floor cleaning mops, equipment, particularly for respiratory treatment, and even in disinfectant solutions. The large-scale environmental distribution of Pseudomonas is guaranteed by its simple requirement for growth. They also have different structural factors and toxins that stimulate their virulence potential, making them resistant to the most commonly used antibiotics. *Pseudomonas aeruginosa* is the most common clinically significant species, causing various infections, as it is typically resistant to most antibiotics. Another species found in the study was *Pseudomonas putida*, little associated with infections in human beings **(Tortora et al., 2013)**.

Klebsiella pneumoniae can cause primary lobar pneumonia, which frequently involves the necrotic destruction of alveolar spaces, formation of cavities and production of bloody sputum. These bacteria also cause infections in wounds, soft tissues and the urinary tract **(Murray et al., 2006)**.Another gram- negative bacillus that was found, from the *Enterobacteriaceae* family, was *Enterobactercloacae*. Infections caused by microorganisms from the *Enterobacter* genre are rare in immunocompetent patients, but common in neonates and immunocompromised patients. The main problem with this bacteria group is resistance to multiple antibiotics **(Murray et al., 2006)**

In the same study on liquid soap dispensers in a hospital environment demonstrated, out of 28 dispensers, 19 (68%) tested positively for one or more bacterial species. The isolated bacteria were: A. baumannii, *P. aeruginosa*, *Staphylococcus spp*., *Enterobacter cloacae*, *K. pneumoniae*, *Methicillin-resistant Staphylococcus aureus*, *Candida albicans*, and *Bacillus* species. Dispensers in that study were plastic, rectangular and wall-mounted, with a button for soap dispensing. Moreover, they were cleaned weekly. The same study observed that a significant num- ber of soap residues remained close to the distribution hole and in the slits around the dispenser button **(J. A. Caetano et al., 2011)**.

In old study conducted in Japan in the early 1990s by Amemiya and Taguchi (1992) revealed significant contamination in soap from public restrooms. They found that as many as 4 x 107 bacteria per mL could be recovered from liquid hand soaps and that 71% contained 1,000 or more bacteria per mL. after 19 years in United States study the 541 Liquid soap samples were collected from public restrooms in five cities (Boston; Atlanta; Columbus, Ohio; Los Angeles; and Dallas), consisting of 428 from sink areas and 113 from showers. The percentage of samples that contained Heterotrophic plate counts, numbers above 500 CFU/mL was 24.8%, averaging 3.0 x 10^6 CFU/mL and ranging from 590 to 1.3 x 10^7 CFU/mL. Total coliform bacteria were detected in 15.9% of the samples, averaging 3.9 x 10^6 CFU/mL and ranging from <10 CFU/mL to 6.5 x 10^7 CFU/mL. The different species of Gram-negative bacteria that was isolated. Species of *Klebsiella* occurred most frequently, followed by *Enterobacter*, *Serratia*, and *Pseudomonas*. No *S. aureus* was detected in any of the liquid soap samples analyzed. Office restrooms had the highest percentage of contamination with heterotrophic bacteria (47.5%) and coliform bacteria (35.0%) and restrooms in retail stores had the least (15.3% for heterotrophic and 10.6% for coliform bacteria). The rates of contamination of soap were similar among all five metropolitan areas **(M. Chattman et al., 2011)**.

2.9.2 Gram-negative bacteria

All of the organisms detected in the soap samples were Gram-negative bacteria. This is most likely because of the presence of sodium lauryl sulfate (SLS) in the soap, which inhibits Gram-positive bacterial growth. In fact, SLS is used to inhibit Gram-positive growth in selective media such as mEndo agar **(Laboratories, 1998)**. All of the organisms that were identified in study were Gram-negative opportunistic pathogens. The opportunistic pathogens most commonly found in liquid hand soap included *Pseudomonas* spp., *Serratia marcescens,* and *Klebsiella pneumoniae*. These bacteria are also among the most prevalent organisms that cause opportunistic infections. These were also the same species of bacteria that Amemiya and Taguchi (1992) isolated most often in liquid soap in Japan. While largely associated with infections in compromised patients (immunocomprimised, burn patients, post-surgical) they are capable of

causing infections of wounds (cuts to the skin), folliculitis, and urinary tract infections **(Kallman, Lundberg, Wretlind, & Ortqvist, 2006)**. It has also been reported that bacteria present in the liquid soap remain on the hands after use **(Sartor et al., 2000)**. Found that after hands washing with an *S. marcesens* contaminated liquid hand soap pump, the hands of health care workers were 54 times more likely to be contaminated with *S. marcescens*. Infections and outbreaks resulting from the use of contaminated soap or disinfection products are not uncommon in health care settings. Several different types of soaps contaminated with *S. marcescens* have caused a variety of infections including bacteremia, conjunctivitis, meningitis, and joint infections. Species of *Pseudomonas* and *Burkholderia* found in soap have been linked to various outbreaks in hospitals and infections including skin ulcers, bacteremia, and urinary tract infections **(Dolan, 2006)**. Hand lotion contaminated with *P. aeruginosa* was implicated as the vector resulting in hand transfer of the organism to infected infants **(Becks & Lorenzoni, 1995)**.

In same year the University of Arizona published a study about using the contaminated Bulk-Soap-Refillable dispensers after several outbreaks linked to the use of contaminated soap in health care settings have been reported **(Buffet-Bataillon, 2009; D. J. Weber, W. A. Rutala, & E. E. Sickbert-Bennett, 2007)**, and study conducted in Japan, examined bacterial contamination of hand washing soaps obtained from restrooms of various public use facilities. The authors found 17 different species of bacteria, many of which were opportunistic pathogens, including *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter* species, and *Pseudomonas* species **(Amemiya & Taguchi, 1992)**, and other studies conducted in the United States demonstrated that 25% of bulk-soap-refillable dispensers in public restrooms were excessively contaminated **(M. Chattman et al., 2011)**. Bacterial loads averaged more than 10^6 CFU/ml of soap, and 16% of the samples contained coliform bacteria. Interestingly, of the 15 different species isolated in this study, 7 were identical to those found in the Japanese study, including both *K.pneumoniae* and *S.marcescens*.Both *S. marcescens* and *K. pneumoniae* are opportunistic pathogens known to transmit via the hands **(G. Reybrouck, 1983)**

University of Arizona study conducted in three experiments: the first soap experimentally contaminated with either *K. pneumoniae* (5.85 log10 CFU/ml) or S. marcescens (3.72 log10 CFU/ml) followed by a 30-s rinse. Neither test organism was recovered from the hands of subjects prior to washing hands or from the subjects that washed with uncontaminated control soap. In contrast, for *K. pneumoniae*, a mean of 2.74 log10 CFU/hand was recovered from subjects after washing with *K. pneumonia* contaminated soap, and for *S. marcescens*, a mean of 3.60 log10 CFU/hand was recovered from subjects after washing with *S. marcescens* contaminated soap. Interestingly, more bacteria were recovered from hands washed with *S. marcescens* contaminated soap than from those washed with *K. pneumonia* contaminated soap ($P < 0.0001$), even though the level of *K. pneumoniae* contamination was 100-fold higher. In a second experiment, subjects performed a 10-s hand wash with 1.5 ml of liquid soap experimentally contaminated with either a high level of *S. marcescens* (7.51log10 CFU/ml) or with a low level of *S. marcescens* (4.51 log10 CFU/ml) followed by a 10-s rinse. It is known that when soap that is not contaminated is used for hand washing, it is more effective at removing transient bacteria when greater volumes of soap and longer wash times are used **(Fuls et al., 2008)**. Therefore, the second controlled study was conducted under conditions chosen to be more representative of the hand washing behaviors typically observed **(Garbutt, Simmons, Patrick, & Miller, 2007)**. The mean numbers of *S. marcescens* cells recovered after washing with high-and low-level-contaminated soap were 5.28 log10 CFU and 1.70 log10 CFU per hand, respectively ($P < 0.0001$). The number of bacteria transferred to an agar surface after washing were 2.23 log10 CFU and 0.30 log10 CFU per hand for the high- and low-level- contaminated soap, respectively ($P = 0.001$). The third experiment study was conducted with students and staff to assess the levels of Gram-negative bacteria remaining on or transferred from hands after washing with contaminated soap from these dispensers or with uncontaminated control soaps. Prior to washing with contaminated bulk soap, uncontaminated bulk soap, and uncontaminated soap from sealed refills, the mean numbers of bacteria recovered from hands of subjects were 1.17, 0.99, and 1.67 log10 CFU per hand, respectively. The mean number of bacteria recovered from the hands after hand washing with the contaminated soap (2.59 log10 CFU per hand) was significantly higher than the pre-hand- washing value ($P < 0.0001$).

Gram-negative bacteria were de- tected in 97% (60/62) of hands tested after washing with bulk soap compared to 52% (32/62) before washing. Incontrast, the mean number of bacteria recovered from hands after washing with uncontaminated bulk soap (0.82 log10 CFU per hand) was reduced compared to the prewashing numbers. When hands were washed with uncontaminated soap from the new replacement sealed-system dispensers, the mean numbers of bacteria recovered from hands after washing (1.37 log10 CFU perhand) were also reduced compared to the prewashing numbers and were statistically lower than those recovered from hands washed with contaminated soap ($P < 0.0001$). The mean number of Gram-negative bacteria recovered from the hands after washing with contaminated soap was significantly higher for students (2.82 log10 CFU per hand) than that for staff (2.22 log10 CFU per hand; $P = 0.008$) **(Zapka et al., 2011)**.

The most common transient microorganisms include gram negative coliforms and *Staphylococcus aureus*. Hand washing with plain soap is effective in removing most transient microorganisms **(Katz, 2004)**. The mechanical action of washing and rinsing removes most of the transient microorganism present **(Noskin, Stosor, Cooper, & Peterson, 1995)**. Health care workers wash their hands in two ways: (a) the social hand wash, which is the cleaning of hands with plain, non-medicated bar or liquid soap and water for removal of dirt, soil, and various organic substances; (b) the hygienic or antiseptic hand wash, which is the cleaning of hands with antimicrobial or medicated soap and water. Most antimicrobial soaps contain a single active agent and are usually available as liquid preparations. Appropriate hand washing results in a reduced incidence of both nosocomial and community infections **(Kampf & Kramer, 2004)**. Much studies have been written and debated regarding the use of bar versus liquid skin cleansers in relation to infection control **(Boyce & Pittet, 2002)**. And in the study by university of Baghdad – Iraq - show that among 50 swabs of bar soaps, 30 (60%) swabs were found colonized. A total of 44 microorganisms were isolated. *Pseudomonas aeruginosa* (41%) was the most frequent isolated bacteria followed by *Escherichia coli* (13.6%) and *Acinetobacter baumanii* (11.4%).From liquid soaps, 6 microorganisms were detected at only 7 tips (15.9%) of the total 44 containers. This includes 4 (66.6%) *P. aeruginosa*, one (16.6%) *Proteus penneri* and one (16.6%) *Flavimonas oryzihabitans*. Comparison of the rates of bacterial colonization between

bar soaps and liquid soaps: Bar soaps were found more colonized than the liquid soaps significantly ($p<0.05$). *P. aeruginosa* was the most frequent isolate in both two group whereas isolation rate was significantly higher ($p<0.05$) in bar soaps but not in the liquid soaps ($p>0.05$) as statistically.

2.9.3 Bar or liquid Soap

The most common hand-cleaning agents are bar soap and liquid soap in disposable plastic containers. When in use, bar soaps are frequently misused because they are typically stored in contact with moisture and remain moist for long periods of time. It is usually kept in a container, on or next to a wash basin. More often than not, it resides in surface water. The resulting jelly mass is unsightly, difficult to use effectively. This supplies an environment which provides the perfect opportunity for bacteria and organisms to grow. Most bars of soap in communal areas are used by a number of different people. This means that one bar of soap can be in direct contact with skin bacteria from more than one person, and may harbour live pathogenic bacteria **(E. L. Larson, Eke, Wilder, & Laughon, 1987)**. Cross infection can and does occur under these circumstances **(McBride, 1984)**. When using a bar of soap, the CDC (Centre for Disease Control) recommends placement on a drainable rack between uses **(Boyce & Pittet, 2002)**. Soap racks that promote drainage of all water from the bar should be installed. In addition, there should be easy access to replacements when soap is lost, dropped, melted, or consumed. Small soap bars were also recommended that can be changed and used in preference to larger bars that are more likely to melt or become colonized with bacteria **(Nix, 2000)**. Liquid soap on the other hand is much better to use. Liquid soap is dispensed straight from a plastic container. It has not been exposed to skin bacteria or other contaminants. As a result, cross contamination is not likely to occur, providing a more cleaning and more hygienic alternative **(McBride, 1984)**. McBride et al reported that bar soaps were found to have higher bacterial cultures after use than liquid soaps **(McBride, 1984)**. In another study, Kabara and Brady obtained samples from bar and liquid soaps from 26 public bathrooms which were investigated. Liquid soaps were found to be negative for bacteria, while 100% of the 84 samples obtained from bar soaps yielded positive cultures **(Kabara & Brady, 1984)**. In an epidemiological study, the researchers isolated several strains of Pseudomonas from

45 of 353 environmental samples used by multiple providers (13%) and found that the 5 most common strains were frequently found on patients. They also affirmed that the hands are a major vehicle for the transfer of Pseudomonas bacteria and implicated bar soap in its spread **(Bruun, McGarrity, Blakemore, & Coriell, 1976)**. Other groups of researchers have found that bacteria survive on soap bars in continuous use in public lavatories, even when cultured 48 hours following their last use **(E. L. Larson et al., 1987)**. The role of the soap dishes in infection control has also been studied by Sarmad M.H. Zeiny from Iraq he found that dishes were found wet, and surfaces of soaps were generally covered by squashy mass and bars were found heavily contaminated (%88). This study revealed quite lower contamination rate in liquid soaps compared with bar soaps, although they didn't include suggested antibacterial agents for hand antisepsis such as triclorasan or chlorhexidine **(S. M. H. Zeiny, 2009)**. However, liquid soaps would be expected to be sterile. So, there should be problems with the handling. Honestly, in this study any strict procedures had not been followed in the wards for the how often liquid dispensers should be cleaned, disinfected or exchanged. After the results were obtained, procedures were described for handling and usage of liquid soaps and dispensers immediately **(S. M. H. Zeiny, 2009)**.

2.9.4 Strain of bacteria capable of metabolizing anionic detergent

Sodium dodecyl sulfate (SDS) is an anionic detergent widely used in the manufacturing of a number of household and industrially useful products **(Karsa, 1999)**. Like any other chemical, SDS is discharged in water bodies like ponds and rivers **(Singer & Tjeerdema, 1993)**. Studies have revealed that SDS is toxic to aquatic animals such as fish, microbes like yeasts and bacteria **(Venkatesh Chaturvedi & Kumar, 2010b)**, and also to mammals **(Venkatesh Chaturvedi & Kumar, 2010a)** So, bioremediation of this detergent was realized to be an effective method to reduce its toxicity in environments **(Zeng et al., 2007)** There have been a number of reports of isolation of SDS-degrading bacteria from different parts of the world **(Shukor, Husin, Rahman, Shamaan, & Syed, 2009)** It has been reported that members of the family Pseudomonas are capable of degrading SDS and utilizing it as a carbon source **(Ellis, Hales, Ur-Rehman, & White, 2002)** though bacterial strains other than Pseudomonas have also been reported from different parts of the word **(Shukor et al.,**

2009). The pathway of SDS degradation is also well documented **(Thomas & White, 1989)**. The pathway is initiated with the enzyme alkyl sulfates which cleaves sulfate group of SDS forming 1-dodecanol (C12 alcohol), which is subsequently oxidized to 1-dodecanoic acid. Finally, 1-dodecanoic acid enters into β- oxidation pathway and is utilized as carbon source **(Thomas & White, 1989)**. Most if not all commercially available soaps contain some type of preservative to inhibit microbial growth. It would thus appear that degradation of the preservatives over time is the likely explanation for the occurrence of the bacteria. Future research will be aimed at assessing the public health risk associated with this problem, to determine the factors that result in contamination, and to determine the best methods to reduce the problem. Possible solutions might include better maintenance of the dispensers (cleaning, replacement of soap), use of preservatives more resistant to degradation, or the use of disposable sealed soap refills **(M. Chattman et al., 2011)**.

Chapter 3
Methodology

3.1 Source and Number of Sample

The study was conducted on seven general governmental hospitals in Gaza strip, Palestine (Table 3.1). The sample collection program lasted from April 2015 to July 2015. A total of 338 samples were collected (233 Antiseptics and Disinfectants and 105 Soap)

Table (3.1): General governmental hospitals in the Gaza Strip

Hospital name	Location	Number of samples	
		Soap	Antiseptics
Kamal Adwan	Jabalia	15	31
Beit Hanon	Beit Hanon	13	20
Al-Shifa	Gaza	22	41
Al-Aqsa	Deir El balah	12	26
Nasser	Khanyounis	20	55
European Gaza	Khanyounis	15	46
Abu yousef Al Najjar	Rafah	8	18
Total		105	233

3.2 Sample Collection

Antiseptics and/or disinfectants and samples of soaps were collected from various points in each of the seven hospitals in sterile cup. Each sample (50 - 100 ml) was aseptically transferred to a sterile plastic cups, identified and labeled with the necessary data by identification card and transported to the laboratory in an ice box within 2-4 hours of collection and were examined in the same day.

3.3 Media and Reagents

3.3.1 Media

All media used from HiMedia (India) were prepared and sterilized according to manufacturer's recommendations

- Baird Parker Agar (BPA)
- MacConkey agar (MAC)
- Nutrient agar
- Plate count agar

- Violet Red Bile Agar (VRBA)
- Mueller Hinton Agar (MHA)
- Rose Bengal Agar (RBA)
- Cetrimide Agar (CA)
- Mannitol Egg Yolk Polymyxin Agar (MYP) (Oxoid)

3.3.2 Reagents and Identification Systems

- Analytical profile index (API) 20E (BioMerieux, France)
- Catalase reagent (3% H_2O_2) (HiMedia, India)
- Coagulase test (HiMedia, India)
- Gram stain kit (HiMedia, India)
- Oxidase test (Oxoid, UK)
- Potassium iodide
- Sodium thiosulfate
- Starch solution

3.4 Equipment, Glassware and Disposables

- Autoclave (Tuttnauer, USA)
- Automatic pipettors and associated sterile pipette tips capable of delivering up to 10 ml and 1 ml volumes
- Balance (weights; 2000g capacity, sensitivity of 0.1 g) (Sartorius, Germany)
- Colony counter (Anderman, England)
- Incubators (Memert, Germany)
- Light Microscope (Zeiss, Germany)
- Refrigerator (Sanyo, USA)
- Vortex mixer
- Top pan balance capable of weighing to 0.1 g Cups (sterile, 100 ml) (Firatmed, Turkey)
- Flasks (sterile)

- Inoculating loops
- L-shaped glass rods (sterile)
- Needles and Syringes (1.5 and 10 ml)
- Petri dishes (90x15) (Firatmed, Turkey)
- Pipettes (sterile total delivery) 10 ml and 1 ml graduated in 0.1 ml volumes (optional)
- Spreaders (sterile, disposable)

3.5 Quality of Soap and Efficacy of Antiseptics and Disinfectants

Soap samples were tested for their microbiological quality while antiseptics and/or disinfectants samples were tested for their microbiological quality and for their chemical content.

3.5.1 Microbiological Quality of Soap

Using separate sterile pipettes, decimal dilutions of 10^{-2}, 10^{-3}, and 10^{-4} were prepared, and more as appropriate, of soap by transferring 10 ml of previously diluted sample to 90 ml of diluents. All dilutions were shaken 25 times in 3 cm arc. One ml of each dilution was pipetted into separate, duplicate, and appropriately marked Petri dishes containing different types of media as shown in table 3.2. Dilution bottle was re-shaken 25 times in 3 cm arc if it stands more than 3 min before it is pipette into Petri dish. 0.1 ml of each dilution was transferred aseptically into the nutrient agar surface. The diluted samples are distributed onto the surface of the specified plate by a sterile L-shaped glass rod (spreader). The plates were incubated at 35^{o}C for 24-48 h. Colonies were counted and the total aerobic microorganisms was calculated per gram **(Andrews, 2001)**.

Table (3.2): Plated media and their purpose

Media	**Microorganism growth**
Plate count agar	*For total aerobic bacterial count*
MacConkey agar	selective isolation and differentiation of *Gram negative bacteria*
Nutrient agar	*General media*
Baird Parker Agar	Staphylococci
Violet Red Bile Agar	*E. aerogenes, E. coli, Salmonella spp.*
Dichloran Medium Base with Rose Bengal	Fungi-(yeasts and molds count)
Cetrimide Agar	Selective isolation of *Pseudomonas aeruginosa*
Mannitol Egg Yolk Polymyxin Agar	*Bacillus cereus*

3.5.2 Microbiological Efficacy of Antiseptics and Disinfectants

3.5.2.1 Microorganisms used

All antiseptics and disinfectant samples were tested for their microbiological efficacy against three types of microorganisms those were *E. coli, P. aeruginosa and S. aureus* (Clinical isolates from Al-Shifa hospital) were plated on Mueller Hinton Agar and incubated in incubator for 24 hours of 37 ^{o}C.

3.5.2.2 Stainless steel cylinder method

It depends upon the diffusion of the Antiseptics and/or disinfectants from vertical steel cylinders placed on the surface of inoculated agar medium. This procedure produces zones of inhibition around the cylinder containing the tested Antiseptics and/or disinfectants solution depending upon their types and concentration (Figure 3.1). This method is commonly employed in the assay of pharmaceutical substance. For assay, use of petri plates with 20 X 100 mm dimension and stainless steel cylinders with the outside diameter 8 mm, inside diameter 6 mm and length 10 mm is recommended. Were one cylinder was used per plate. The cylinder was placed on inoculated plates. **(Souza-Filho et al., 2008)** The incubation the plates was done for 24 hours of 37 ^{o}C.

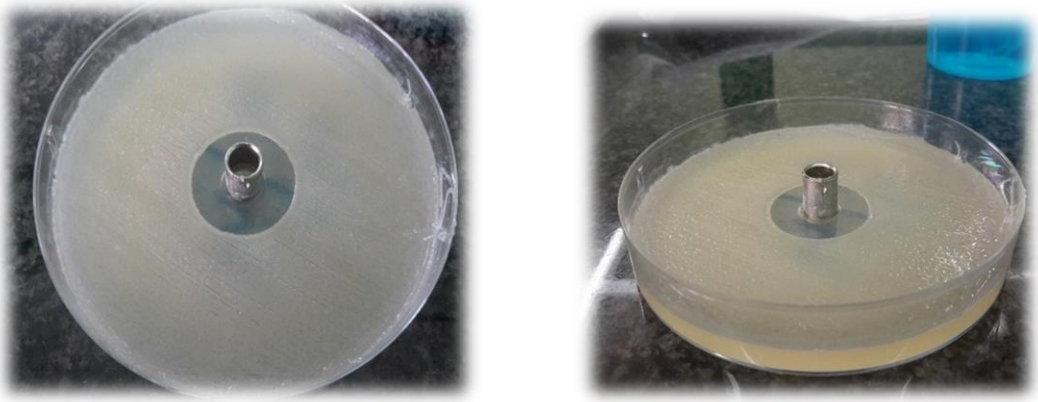


Fig (3.1): Zone of inhibition around the stainless steel cylinders as a result of the antimicrobial action of Antiseptic and/or disinfectant

3.5.3 Chemical Efficacy of Antiseptics and Disinfectants

3.5.3.1 pH measurements

Readings of pH were taken using Bante210 Benchtop pH Meter after calibrating with the standard solution supplied by NICE Chemicals Pvt. Ltd., Kochi, Kerala (pH = 7). The samples were coded before the analysis of the pH. Procedures were divided so that one person was involved in coding, then another in mixing and the last person in the measurement of pH, so that the person involved in measurement of pH does not know the identity of the sample being tested. Soap sample weighing 150 mg was mixed in 15 ml distilled water without producing much lather. It was kept undisturbed for 24 h for maximum dissolution of soap. Then the pH of each sample was measured. Table 3.3 shows the pH normal range of soaps and selected disinfectants **(Li, Chen, & Zhang, 2017)**.

Table (3.3): Normal range of pH of soap and antiseptics

Substance	Range of pH
Soap	5.4 – 5.9
Povidone Iodine	1.5 – 6.5
Chlorhexidine Gluconate	3 – 7.5
Cetrimide	3 – 7.5

3.5.3.2 Concentration of Hypochlorous acid

All hypochorous acid samples were titrated with potassium iodide. All primary solutions were prepared by the Food Chemistry Department at the Public Health

Laboratories (Ministry of Health). The titration process was done by filling a 50 ml burette with the standardized sodium thiosulfate solution. Sodium thiosulfate from the burette was added to the hypochlorous acid solution sample in a conical flask until it changed color from brown to a straw-yellow. A couple drops of 1% starch solution was added to the conical flask, the solution then turned to deep blue, after that sodium thiosulfate from burette was added drop-wise to the conical flask until the solution changed from deep blue to colorless. At this point, the burette value was read to calculate the hypochlorous acid concentration **(Eryilmaz & Palabiyik, 2013) .**

$$\boldsymbol{Conc.\%} = \left(\frac{\boldsymbol{mass\ of\ NaOCl}}{\boldsymbol{mass\ of\ sample\ solution}}\right) \times \mathbf{100}$$

3.5.3.3 Concentration of Ethyl Alcohol

Distillation Method

Alcohol extraction method was used (Extraction of alcohol from the sample and collection to measure the percentage of alcohol) this was done by a vacuum distillation system (Heidolph Laborota, model 4000ˢ Germany) (figure 3.2). 20 ml of alcohol sample were transferred to evaporator flask then system is turned on. The value of alcohol was calculated on collecting flask. The effecting concentration range of alcohol is between (69.5 – 70.4) % **(Cartwright)** .

Fig (3.2): Vacuum distillation system

3.5.3.4 Concentration of Povidone Iodine

Calculation the concentration of povidone iodine were done by titration with sodium thiosulphite and all the primary solution were prepared by Drug Chemistry Department at the Public Health Lab. The effective concentration range of Povidone Iodine is between (85 - 120) % **(Cartwright)**. 10 ml of sample was transferred to 250 ml flask and 10 ml of 0.1 M HCl and sufficient distilled water were added to produce 150-ml to fill 50 ml burette with 0.02 M sodium thiosulfate and titrated with sample. The end point was determined by removing the color of povidone iodine **(Cartwright)** .

3.5.3.5 Concentration of Chlorhexidine Gluconate

This test was done by spectrophotometer at 254 nm in the Water Chemistry Department at the Public Health Lab. The effective concentration range of Chlorhexidine Glucanate is between (90-110) %. **(Cartwright)**

3.5.3.6 Concentration of Cetrimide: The effective concentration range of Cetrimide is between (90 - 110) % **(Cartwright)**. This test was not done because the required reagents for the assay were not available in public health lab.

3.6 Data Analysis: All data obtained from the sample analysis were tabulated using Microsoft Excel and then uploaded to SPSS v. 13 (Statistical Package for Social Sciences). Chi square test was used for assessing the statistical significance of the data, and p-values of ≤ 0.05 were considered significant.

Chapter 4
Results

The data presented in this chapter is a summary of the raw data and the results of statistical analysis of microbiological investigations of soap and antiseptics / disinfectants samples collected from seven general governmental sample in Gaza strip. The aims of conducting analysis on soap samples were to evaluate their microbiological and chemical quality.

4.1 Distribution of the Samples

In this study, 342 samples (237 hospitals antiseptics/disinfectants and 105 hospitals soap) were collected and analyzed during the period of from April 2015 to June 2016. The investigation includes several microbiological and chemical parameters. Among the microbiological parameters is the total plate count (TPC), total coliform , *E. coli*, *S. aureus*, *Bacillus* spp., *Pseudomonas* spp., *Enterococcus, mold* and *yeas,* and chemical parameters such as pH and concentration.

All samples were collected from governmental hospitals in the Gaza strip as shown in table (4.1). Sample are distributed as follows; 9.7% for Beit Hanoun hospital in north zone, 13.5% for Kamal Adwan hospital in Jabalia zone, 18.4% for Al-Shifa hospital in Gaza zone, 11.1% for Al-Aqsa hospital in middle zone, 21.9% for Nasser hospital in west khan Younis zone, 17.8% for European Gaza in east khan Younis and Rafah zone and 7.6% for Abu Yousef Al Najjar hospital in west Rafah area. Meanwhile, the table shows the percentage of the soap and Antiseptics/Disinfectants (Anti/Dis) samples as 12.4% and 8.4% in Bait Hanoun, 14.3% and 13.1% in Kamal Adwan, 21% and 17.3% in Al-Shifa, 11.4% and 11% in Al-Aqsa, 19.1% and 23.2% in Nasser, 14.3% and 19.4% in European Gaza and 7.6% and 7.6% in Abu Yousef Al Najjar respectively.

Table (4.1): Distribution of tested samples according to Hospitals

Hospitals	N	Type of samples				%
		Soap		Anti/Dis		
		N	%	N	%	
Beit Hanoun	**33**	13	12.4	20	8.4	**9.7**
Kamal Adwan	**46**	15	14.3	30	13.1	**13.5**
Al-Shifa	**63**	22	21	40	17.3	**18.4**
Al-Aqsa	**38**	12	11.4	26	11	**11.1**
Nasser	**75**	20	19.1	54	23.2	**21.9**
European Gaza	**61**	15	14.3	45	19.4	**17.8**
Abu Yousef Al Najjar	**26**	8	7.6	18	7.6	**7.6**
Total	**338**	**105**	**100**	**233**	**100**	**100**

Anti= Antiseptics, Dis= Disinfectants

4.2 Microbiological quality of Soaps

The microbiological result was used to judge the quality of the tested samples by comparing them to the standards (those complied are judged as Passed and those which did not comply are judged as Failed. all of which are illustrated in table 4.2

Table (4.2): The microbiological results of the pass or fail in tests in each hospital

Hospitals	N	Soap samples				%
		Pass		Fail		
		N	%	N	%	
Beit Hanoun	**13**	11	84.7	2	15.4	**12.4**
Kamal Adwan	**15**	1	6.7	14	93.3	**14.3**
Al-Shifa	**22**	18	81.9	4	18.2	**21**
Al-Aqsa	**12**	3	25	9	75	**11.4**
Nasser	**20**	18	90	2	10	**19.1**
European Gaza	**15**	15	100	0	0	**14.3**
Abu Yousef Al Najjar	**8**	7	87.5	1	12.5	**7.6**
Total	**105**	**73**	**69.5**	**32**	**30.5**	**100**

The microbiological tests conducted on soap samples included Total Bacterial Count (T.B.C), *S. aureus, E. coli, coliform, Bacillus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Mold* and *yeast.* The results showed that the percentage of contaminated samples of both bacteria and fungi was 18/105 (17.1%) and total percentage value was 32/105 (30.5%), the most common contaminant was coliform 13/105 (12.4%), *Pseudomonas* spp. 12/105 (11.4%) and *Bacillus* spp. 6/105 (5.7%). The contamination with yeast 11/105 (10.5%) was more than the mold 7/105 (6.7%). All of which are presented in **table 4.3**.

Table (4.3): Microbiological results for soap samples

Hospitals	**parameters**												
	Total number of sample	**Contamination Sample**	**Bacteria**	**T.B.C**	***S. aureus***	***E. coli***	***Coliform***	***Bacillus* spp**	***Pseudomonas* spp**	***Enterococcus* spp**	***mold***	***yeast***	**Fungi**
Beit Hanoun	**13**	**2**	**2**	2	0	0	0	2	2	0	0	0	**0**
Kamal Adwan	**15**	**14**	**5**	14	4	5	13	4	0	1	0	11	**11**
Al-Shifa	**22**	**4**	**0**	0	0	0	0	0	0	0	4	0	**4**
Al-Aqsa	**12**	**9**	**9**	9	0	0	0	0	9	0	2	0	**2**
Nasser	**20**	**2**	**1**	0	1	0	0	0	0	0	1	0	**1**
European Gaza	**15**	**0**	**0**	0	0	0	0	0	0	0	0	0	**0**
Abu Yousef Al Najjar	**8**	**1**	**1**	1	0	0	0	0	1	0	0	0	**0**
All	**105**	**32**	**18**	26	5	5	13	6	12	1	7	11	**18**
Percentage		**30.5**	**17.1**	**24.8**	**4.8**	**4.8**	**12.4**	**5.7**	**11.4**	**1.0**	**6.7**	**10.5**	**17.1**

The pH test results showed that the average of pH of soap samples in hospitals indicate the highest passing value as 18/20 (90%) in Nasser Hospital, the lowest passing value as 0/15 (0%) in European Gaza hospital, and the total passing value as 43/105 (41%), and the total failing value as 62/105 (59.1), all of which are clarified in **table 4.4.**

Table(4.4): The percentage of pass and fail results of soap by pH in each hospital

Hospitals	**N**	**pH test**				**Total**
		Pass		**Fail**		
		N	**%**	**N**	**%**	%
Beit Hanoun	**13**	2	15.4	11	84.6	**12.9**
Kamal Adwan	**15**	3	20	12	80	**14.3**
Al-Shifa	**22**	3	13.6	19	86.4	**21**
Al-Aqsa	**12**	10	83.3	2	16.7	**11.4**
Nasser	**20**	18	90	2	10	**19.1**
European Gaza	**15**	0	0	15	100	**14.3**
Abu Yousef Al Najjar	**8**	7	87.5	1	12.5	**7.6**
Total	**105**	**43**	**41**	**62**	**59**	**100**

Soap samples which passed in both tests (microbiological and pH) is shown in table 4.5. The total percentage of passed (in both tests) is 28/105 (26.7%). The total percentage of samples that failed one test (microbiological or pH) was 61/105(58.1%), while the total percentage of samples failed in both tests was 16/105(15.2%). The highest percentage of passed samples was 7/8(87.5%) in Abu Yousef Al Najjar hospital. As seen in the table, Beit Hanoun Hospital and European Gaza Hospital had the lowest total percentage of passing results as 0/13 (0%); 0/15 (0%).

Table (4.5): The percentage of pass results in microbiological and pH tests in each hospital

Hospitals	N	Total					
		Pass		Fail one		Failed both	
		N	%	N	%	N	%
Beit Hanoun	**13**	0	0	13	100	0	0
Kamal Adwan	**15**	1	6.7	2	13.3	12	80
Al-Shifa	**22**	2	9.1	17	77.3	3	13.7
Al-Aqsa	**12**	1	8.3	11	91.7	0	0
Nasser	**20**	17	85	2	10	1	5
European Gaza	**15**	0	0	15	100	0	0
Abu Yousef Al Najjar	**8**	7	87.5	2	25	0	0
Total	**105**	**28**	**26.7**	**61**	**58.1**	**16**	**15.2**

4.3 Antiseptics/Disinfectants Tests

The five most common chemical compound used as Antiseptics/Disinfectants in Palestinian authority hospitals in Gaza-Palestine is Povidone Iodine (with different brand names; BETADINE 10%, BETAVIDINE 10%, BETODINE 10%, IODOCARE 10%, POTEDENE 10%, POVIOFIX 10%, SOMEDINE 10%, IODIFLOR 7.5% and POVDINE 7.5%), **Alcohol** compound (Alcohol and Ethyl Alcohol), **Chlorhexidine (CHX)** with different brand names (Cetrimide BP and SEPTAL, SAVIOR, SUNDEN and CAR FIRST AID) and **Hypochlorous acid** locally manufactured known as (**Chlorine**). (Table 4.6)

Table (4.6): Number and percentage of Antiseptics/Disinfectants collected from all hospitals

Antiseptics/Disinfectants	Frequency	Percentage
Povidone Iodine	85	.359
Alcohol	60	.253
Chlorhexidine	59	24.9
Chlorine	29	12.2
Total	**233**	**100**

The initial test that was used to detect the efficacy of Antiseptics/Disinfectants is the clear zone from stainless steel tube test against three microorganisms (*S. aureus, P. aeruginosa, and E. coli*). Table 4.7 showed percentage of zone of inhibition for tested microorganisms (*S. aureus, P. aeruginosa,* and *E. coli*) in each hospital. As noticed, the average of zone of inhibition is 28.2 mm for *S. aureus*, 20.8 mm for *P. aeruginosa*, and 20.7mm for *E. coli*.

Table (4.7): Average of zone of inhibition in mm. for *S. aureus, P. aeruginosa,* and *E. coli* in each hospital in Gaza strip

HOSPITAL	*S. aureus*	*P. aeruginosa*	*E. coli*
Beit Hanoun	25.7	12.9	8.8
Kamal Adwan	28.9	22.6	24.9
Al-Shifa	30.1	21.3	14.6
Al-Aqsa	13.7	8	11.7
Nasser	31.1	27.3	25.2
European Gaza	33.3	25.9	28.8
Abu Yousef Al Najjar	34.6	27.3	30.9
Average	**28.2**	**20.8**	**20.7**

The average of zone of inhibition formed from tested Povidone Iodine is clarified in table 4.8 that shows the Average of zone of inhibition as 25.8 mm, 16.1 mm, and 16.8 mm, for *S. aureus, P. aeruginosa, and E. coli* respectively, and the least mean of zone of inhibition is *S. aureus*: 2.4 mm, *P. aeruginosa*: 0.0 mm and *E. coli*: 7.90 mm in Al-Aqsa Hospital.

Table (4.8): Average of zone of inhibition in mm. and Povidone Iodine from hospitals

HOSPITAL	Number of PI Samples	Mean of clear zone		
		S. aureus	***P. aeruginosa***	***E. coli***
Beit Hanoun	7	24.6	8.1	2.1
Kamal Adwan	10	27.5	14.2	18.9
Al-Shifa	15	32.0	20.4	18.1
Al-Aqsa	10	2.4	0.0	7.9
Nasser	20	30.3	20.1	19.8
European Gaza	18	31.8	24.4	17.6
Abu Yousef Al Najjar	5	32.0	25.6	32.8
Average	**85**	**25.8**	**16.1**	**16.8**

The Average of zone of inhibition formed from tested Alcohol samples is shown in table 4.9 Results showed that the average of zone of inhibition in all hospitals as *S. aureus*: 16.7 mm, *P. aeruginosa*: 13.1 mm and *E. coli*: 9.7 mm. The highest S zone of inhibition is 26.3 mm in Abu Yousef Al Najjar Hospital, and the lowest *S. aureus, P. aeruginosa, and E. coli* zone of inhibition is 3.3 mm, 0.0 mm and 0.0 mm respectively in Al-Aqsa Hospital. The European Gaza Hospital has the highest *P. aeruginosa* and *E. coli* zone of inhibition equal to 22.0 mm.

Table (4.9): Average of zone of inhibition in mm. for Alcohol from hospitals

HOSPITAL	Number of Alcohol Samples	Mean of clear zone		
		S. aureus	***P. aeruginosa***	***E. coli***
Beit Hanoun	5	13.6	6.0	0.0
Kamal Adwan	8	4.9	11.3	9.3
Al-Shifa	12	22.8	16.9	0.0
Al-Aqsa	6	3.3	0.0	0.0
Nasser	14	20.2	14.2	15.4
European Gaza	8	25.6	22.0	22.0
Abu Yousef Al Najjar	7	26.3	21.1	21.0
Average	**60**	**16.7**	**13.1**	**9.7**

The Average of zone of inhibition formed from tested (CHX) samples is clarified in table 4.10, showing the average of mean of zone of inhibition for *S. aureus, P. aeruginosa, and E. coli* as 35.2 mm, 25.7 mm and 24.9 mm respectively. Also, the highest mean of zone of inhibition of *S. aureus* was 45.6 mm in Beit Hanoun Hospital, the highest mean of zone of inhibition for *P. aeruginosa* was 34.6 mm in Nasser Hospital, and the highest mean of zone of inhibition for *E. coli* was 33.5 mm in Abu Yousef Al Najjar Hospital. Finally, the lowest mean of zone of inhibition for the three tested bacteria was in Kamal Adwan Hospital.

Table (4.10): Average of zone of inhibition for CHX from hospitals

HOSPITAL	Number of Chlorhexidine Samples	Mean of clear zone		
		S. aureus	***P. aeruginosa***	***E. coli***
Beit Hanoun	5	45.6	23.0	23.1
Kamal Adwan	5	26.9	17.0	16.9
Al-Shifa	12	34.4	31.2	26.6
Al-Aqsa	6	34.1	19.1	19.6
Nasser	12	27.2	34.6	27.3
European Gaza	15	38.0	28.8	27.5
Abu Yousef Al Najjar	4	40.5	26.3	33.5
Average	**59**	**35.2**	**25.7**	**24.9**

CHX= Chlorhexidine

The average of mean of zone of inhibition formed from tested locally manufactured with brand name as Chlorine is shown in table 4.11, indicating the average mean of zone of inhibition as 36.7 mm, 35.4 mm and 39 mm for *S. aureus, P. aeruginosa, and E. coli*, the highest average of mean of zone of inhibition as 58ml and 56ml for *S. aureus* and *P. aeruginosa* in Abu Yousef Al Najjar Hospital and the highest average of mean of zone of inhibition for *E. coli* as 70mm for European Gaza Hospital, while the lowest average of mean of clear zone for three tested bacteria was in Al-shifa Hospital.

Table (4.11): Average zone of inhibition in mm. and Chlorine from hospitals

HOSPITAL	Number of Chlorine Samples	Mean of clear zone		
		S. aureus	*P. aeruginosa*	*E. coli*
Beit Hanoun	3	24.7	20.7	17.0
Kamal Adwan	8	42.1	39.5	41.9
Al-Shifa	3	13.0	11.7	13.3
Al-Aqsa	2	37.5	30.5	35.5
Nasser	10	41.7	43.6	39.3
European Gaza	1	40.0	46.0	70.0
Abu Yousef Al Najjar	2	58.0	56.0	56.0
Average	**29**	**36.7**	**35.4**	**39.0**

In this study, the chemical parameters were tested as concentration and pH to indicate the efficacy of Anti/Dis samples. The normal ranges of concentration and pH were shown in table 4.12 which shows the normal range acceptable to test content % and pH for the sample of chemicals that have been studied.

Table (4.12): Normal ranges of concentration and pH for Anti/Dis

Antiseptics/Disinfectants	Concentration %	pH
Povidone Iodine	85 - 120	1.5 - 6.5
Alcohol	69.5 - 70.4	ND*
Chlorhexidine	90 - 110	3 - 7.5
Chlorine	5 - 0.5	ND*

ND: Not determined

As seen in table 4.13, the total percentage passing samples by concentration is 140/233 (60.09%) and the percentage of failing sample in all hospitals is 93/233 (39.91%), and the highest percentage of passing results is 79.66% for CHX, and lowing percentage of passing results is 48.28% for Chlorine.

Table (4.13): Percentage of pass and fail of Anti/Dis by concentration

Antiseptics/Disinfectants	N	Concentration			
		Pass		fail	
		N	%	N	%
Povidone Iodine	**85**	43	50.6	42	49.4
Alcohol	**60**	36	60	24	40
Chlorhexidine	**59**	47	79.7	12	20.3
Chlorine	**29**	14	48.3	15	51.7
Total	**233**	**140**	**60.1**	**93**	**39.9**

The first chemical parameter is pH, the percentage of passing and falling of the samples is shown in table 4.14, which shows that total percentage of passing results is 141/144 (97.9%), and that the total percentage of falling results is 3/144 (2.1%). The table also shows only Povidone Iodine and CHX result, because pH value could not be tested for Alcohol and Chlorine.

Table (4.14): percentage of pass and fall of Anti/Dis by pH

Antiseptics/Disinfectants	N	pH			
		Pass		fail	
		N	%	N	%
Povidone Iodine	**85**	84	98.8	1	1.2
Chlorhexidine	**59**	57	96.6	2	3.4
Total	**144**	141	97.9	3	2.1

The differences between hospitals in percentage of passing and falling values by concentration were clarified in table 4.15, showing that the highest percentage of passing results is 21/26 (80.8%) in Al-Aqsa Hospital and the lowest percentage of passing results is 18/40 (45%) in Al-shifa Hospital

Table (4.15): Percentage of pass and fall of Anti/Dis in hospital by concentration

Hospital	N	Concentration				%
		Pass		fail		
		N	%	N	%	
Beit Hanoun	**20**	15	75	5	25	**8.6**
Kamal Adwan	**30**	20	66.7	10	33.3	**12.9**
Al-Shifa	**40**	18	45	22	55	**17.2**
Al-Aqsa	**26**	21	80.8	5	19.2	**11.2**
Nasser	**54**	32	59.3	22	40.7	**23.2**
European Gaza	**45**	22	48.9	23	51.1	**19.3**
Abu Yousef Al Najjar	**18**	12	66.7	6	33.3	**7.7**
Total	**233**	**140**	**60.1**	**93**	**39.9**	**100**

The most common Antiseptics used in hospitals is Povidone Iodine, the percentages of passing and failing results by concentration are clarified in table 4.16, with the total percentage of passing results were 43/85 (50.6%), and the total percentage of falling results were 42/85 (49.4%). The highest percentage of passing results is 9/10 (90%) in Al-Aqsa Hospital and none of the samples pass in Al-Shifa Hospital (0.0%).

Table (4.16): Percentage of pass and fall of Povidone Iodine by concentration in hospitals

Hospital	N	Povidone Iodine				%
		Pass		fail		
		N	%	N	%	
Beit Hanoun	**7**	4	57.1	3	42.9	**8.2**
Kamal Adwan	**10**	6	60	4	40	**11.8**
Al-Shifa	**15**	0	0	15	100	**17.6**
Al-Aqsa	**10**	9	90	1	10	**11.8**
Nasser	**20**	16	80	4	20	**23.5**
European Gaza	**18**	4	22.2	14	77.8	**21.2**
Abu Yousef Al Najjar	**5**	4	80	1	20	**5.9**
Total	**85**	**43**	**50.6**	**42**	**49.4**	**100**

The second common antiseptic used in hospitals is Alcohol, the total percentage of passing results is 36/60 (60%), and the total percentage of failing results is 24/60 (40%). All samples from Beit Hanoun have a passing percentage of (100%), and the lowest percentage of passing results is 3/14 (21.4%) in Nasser Hospital, as clarified in table 4.17

Table (4.17): Percentage of pass and fall of Alcohol by concentration in hospitals

Hospital	N	Alcohol				%
		Pass		fail		
		N	%	N	%	
Beit Hanoun	**5**	5	100	0	0	**8.3**
Kamal Adwan	**8**	5	62.5	3	37.5	**13.3**
Al-Shifa	**12**	11	91.7	1	8.3	**20.0**
Al-Aqsa	**6**	4	66.7	2	33.3	**10.0**
Nasser	**14**	3	21.4	11	78.6	**23.3**
European Gaza	**8**	4	50	4	50	**13.3**
Abu Yousef Al Najjar	**7**	4	57.1	3	42.9	**11.7**
Total	**60**	**36**	**60**	**24**	**40**	**100.0**

The third common antiseptic used in hospitals is CHX, it has the highest total percentage of passing results 47/59 (79.7%) and the total percentage of failing results is 12/59 (20.3%). The highest percentage of passing results is 4/4 (100%) in Abu Yousef Al Najjar Hospital, and the lowest percentage of passing results is 2/4 (50%), in Kamal Adwan hospital, all results were clarified in table 4.18

Table (4.18): Percentage of pass and fail of CHX by concentration in hospitals

Hospital	N	Chlorhexidine				%
		Pass		fall		
		N	%	N	%	
Beit Hanoun	**5**	4	80	1	20	**8.5**
Kamal Adwan	**4**	2	50	2	50	**6.8**
Al-Shifa	**10**	7	70	3	30	**16.9**
Al-Aqsa	**8**	7	87.5	1	12.5	**13.6**
Nasser	**10**	9	90	1	10	**16.9**
European Gaza	**18**	14	77.8	4	22.2	**30.5**
Abu Yousef Al Najjar	**4**	4	100	0	0	**6.8**
Total	**59**	**47**	**79.7**	**12**	**20.3**	**100**

The forth common and locally manufactured disinfectants is Chlorine. The total percentage of passing results is 14/29(48.3%), and total percentage of falling results is 15/29(51.7%). The highest percentage of passing results is 7/8(87.5%), and in three hospitals shown no passing results this hospital were Al Shefa, European Gaza, and Abu Yousef Al Najjar hospital. That shown in table 4.19.

Table (4.19): Percentage of pass and fall of Chlorine by concentration in hospitals

Hospital	N	Chlorine				%
		Pass		fall		
		N	%	N	%	
Beit Hanoun	**3**	2	66.7	1	33.3	**10.3**
Kamal Adwan	**8**	7	87.5	1	12.5	**27.6**
Al-Shifa	**3**	0	0	3	100	**10.3**
Al-Aqsa	**2**	1	50	1	50	**6.9**
Nasser	**10**	4	40	6	60	**34.5**
European Gaza	**1**	0	0	1	100	**3.4**
Abu Yousef Al Najjar	**2**	0	0	2	100	**6.9**
Total	**29**	**14**	**48.3**	**15**	**51.7**	**100**

4.4 The Relationship between Concentration of Anti/Dis and *S. aureus, P. aeruginosa, and E. coli*.

We use Pearson correlation coefficient to test the relationship between (Conc. % and *S. aureus, P. aeruginosa, and E. coli*) and the results in table (4.20) which shows that the correlation coefficient is equal to -0.061 and p-value is equal 0.387 for *S. aureus* which is greater than 0.05, this means that there is **no relationship** between concentration and *S. aureus*, at significance level $\alpha \leq 0.05$. And shows that the correlation coefficient equals -0.126 and p-value equals 0.094 for *P. aeruginosa* which is greater than 0.05, which means that there is **no relationship** between concentration and *P. aeruginosa* at significance level $\alpha \leq 0.05$. And shows that the correlation coefficient equals -0.188 and p-value equals 0.015 for *E. coli* which is less than 0.05, this means that there is **a relationship** between concentration and *E. coli* at significance level $\alpha \leq 0.05$.

Table (4.20): The relationship between concentration of Anti/Dis and *S. aureus, P. aeruginosa, and E. coli*

Statistic	***S. aureus***	***P. aeruginosa***	***E. coli***
Pearson Correlation	-0.061	-0.126	-0.188
P-value	0.387	0.094	0.015

* Correlation is significant at the 0.05 level (2-tailed).

4.5 The relationship between pH and *S. aureus, P. aeruginosa,* and *E. coli*.

We use Pearson correlation coefficient to test the relationship between pH and *S. aureus, P. aeruginosa, and E. coli*, and the results in table 4.21 show that the correlation coefficient equals 0.272 and p-value equals 0.001 for *S. aureus* which is less than 0.05, this t mean there is a relationship between pH and *S. aureus* at significance level $\alpha \leq 0.05$. And shows that the correlation coefficient equal 0.262 and p-value equal 0.004 for *P. aeruginosa* which is less than 0.05 that mean there is a relationship between pH and *P. aeruginosa* at significance level $\alpha \leq 0.05$. And shows that that the correlation coefficient equal -0.188 and p-value equal 0.015 for *E. coli*

which is greater than 0.05, that means that there is no relationship between pH and *E. coli* at significance level $\alpha \leq 0.05$.

Table (4.21): The relationship between pH and *S. aureus, P. aeruginosa, and E. coli*

Statistic	***S. aureus***	***P. aeruginosa***	***E. coli***
Pearson Correlation	0.272	0.262	0.138
P-value	0.001	0.004	0.131

* Correlation is significant at the 0.05 level (2-tailed).

4.6 The percentage of total passed for all types of samples in all tests at each hospital

The total number of all samples type collected from the seven hospital was 338 (soap 105 and Anti/Dis 233). Table 4.22 illustrated the percentage of passed samples in both tests at all hospitals as 166/338 (49.1%) only.

All samples were categorized by researcher into Soap and Antiseptics/Disinfectants distributed according to hospitals as: Beit Hanoun; (13/105; 12.4%) and (20/233; 8.4%) and Kamal Adwan; (15/105; 14.3%) and (31/233; 13.1%) and Al Shifa; (22/105; 21%) and (41/233; 17.3%) and Al Aqsa; (12/105; 11.4%) and (26/233; 11%) and Nasser; (20/105; 19.1%) and (55/233; 23.2%) and European Gaza; (15/105; 14.3%) and (46/233; 19.4%) and Abu Yousef Al Najjar; (8/105; 7.6%) and (18/233; 7.6%) respectively.

Table (4.22): The number and percentage of samples that passed in both type of detergent (Soap, Anti/Dis) on each hospital

Hospitals	Total Number of Samples	Number and Percentage of Passed samples				Total Pass	
		Soap samples		Antiseptics/Disinfectants			
		No	%	No	%	No	%
Beit Hanoun	33	0/13	0.0	15/20	75	15	45.5
Kamal Adwan	45	1/15	6.7	20/30	66.7	21	46.7
Al-Shifa	62	2/22	9.1	18/40	45	20	32.3
Al-Aqsa	38	1/12	8.3	21/26	80.8	22	57.9
Nasser	74	17/20	85	32/54	59.3	49	66.2
European Gaza	60	0/15	0.0	22/45	48.9	22	36.7
Abu Yousef Al Najjar	26	6/8	75	11/18	66.7	17	65.4
Total	**338**	**27/105**	**25.7**	**139/233**	**59.7**	**166**	**49.1**

Chapter 5
DISCUSSION

In this study we evaluated the microbiological (bacteria and fungi) quality of antiseptics and soaps samples, measured the efficacy of antiseptics/disinfectants, and determined the chemical concentration of antiseptics, in seven general hospitals in Gaza-Palestine, There is no published data on this issue. Therefore, results obtained in the present study could not be compared with any previous local data.

Samples (338 samples divided to 105 Soap samples and 233 Antiseptics/Disinfectants samples) were collected from seven general governmental hospitals in Gaza strip, distributed over the five governorates in Gaza strip: North (Beit Hanoun and Kamal Adwan hospital), Gaza (Al Shifa hospital), Midzone (Al Aqsa hospital), Khan Younis (Nasser and European Gaza hospital), Rafah zone (Abu Yousef Al Najjar). All of them, are operated by the ministry of health of the Palestinian Authority. Only liquid soap were examined in this study (which is the available form) and Antiseptics/Disinfectants including Alcohol, Povidone Iodine, Chlorhexdine and Chlorine.

The microbiological parameters which were examined for soap samples in this study are Total Bacterial Count (T.B.C), *Staphylococcus aureus, Escherichia coli,* coliform, *Bacillus* spp*, Pseudomonas* spp*, Enterococcus* and Mold and yeast. While Anti/Dis samples were evaluated for their antibacterial activities against *S. aureus, Pseudomonas* spp.*, and E. coli*. In addition, the pH of soap and Anti/Dis was also determined. The chemical concentration of Ani/Dis was determined chemically.

5.1 The percentage of soap and Anti/Dis based on various microbiological and chemical tests in seven hospitals

The axioms anyone think that any material used in hospitals must be completely sterile, because it directly affect human's life and public health, espically the material used for that purpose as soap, antiseptics and disinfectants. This study showed different results. The total soap percentage that complied with the standards in the largest and most popular seven governmental general hospitals in Gaza strip – Palestine was only 27/105 (25.7%). And this is lower than those found in many studies as in Iraq (41%)

(S. M. Zeiny, 2009), in Brazil (44.7%) **(Joselany Afio Caetano, Lima, Miranda, Serufo, & Ponte, 2011)**, and in USA (75.2%) **(M. Chattman, S. Gerba, & C. Maxwell, 2011)**. The most common cause of contamination was *Pseudomonas* spp as shown in an Iraqi study **(S. M. Zeiny, 2009)** and in Brazilian study **(Joselany Afio Caetano et al., 2011)** but USA study show the *Klebsiella* spp was the most frequent organisms instead of *Pseudomonas* spp **(Marisa Chattman et al., 2011)**. The total Anti/Dis percentage of passed results in those hospitals was 100% with regard to the microbiological quality. This is unlike the study in Republic of Trinidad and Tobago hospitals shown 169/180 (93.9%) the 11 contaminated samples contamination by *Pseudomonas* spp **(Gajadhar, Lara, Sealy, & Adesiyun, 2003)** and unlike another study in Gondar university hospital in Ethiopia which showed 83/86 (96.5%) and the 3 contaminated samples with *Klebsiella* spp **(Deress, Girma, Birhan, Biadgo, & Alemu, 2014)**.

The average of zone of inhibition for *S. aureus, P. aeruginosa*, and *E. coli* were 29 mm, 22.1 mm, 21.7 mm respectively. This is in accordance with many studies that showed the gram-negative bacteria tend to be more resistant than gram-positive organisms as staphylococci **(Billeter, Levy, Chomel, & Breitschwerdt, 2008)**. An in Indian study showed the *E. coli* as the most resistant organisms followed by *P. aeroginosa*. While, *S. aureus* was the least resistant organisms **(Bhat, Prajna, Menezez, & Shetty, 2011)**.

5.2 Distribution of tested samples according to hospital

Highest percentage of collected samples was from Nasser 21.9%, followed by the largest hospital "Al-Shifa hospital" with percentage of 18.4% because some departments in Al-Shifa did not possess some types of Antiseptics/Disinfectants.

5.3 The percentage of pass and fail results of soap in hospitals

There are two parameter (microbiological test, pH test) used to judge sample compliance. For any sample to pass it should pass in both tests, and the results by hospital were:

5.3.1 Beit Hanoun: total number of soap sample was 13, no one pass in both tests, and all sample fail on one of test (2 in micro, 11 in pH). That mean the percentage of fail was 100%. The most common microbes found in the two contaminated sample were *Bacillus* spp and *Pseudomonas* spp. It is of a significance finding that all 11 sample that pass in the microbiological test failed in pH test which means that the pH has extreme pH has prevented bacteria from growing or surviving. It is also worth noting that all tested soap samples are locally manufactured under no supervision.

5.3.2 Kamal Adwan: Out of the 15 soap samples, only one sample passed both test. Two sampled failed in the microbiological test. Coliform, yeast, *E. coli*, *S. aureus*, *Enterococcus* spp were recovered from contaminated samples. The total percentage of failure was 93.3% that was highest value among all hospitals. All tested soap samples are locally manufactured under no supervision using refillable bottles.

5.3.3 Al-Shifa: 22 soap samples were collected. Only two from them passed both test. 17 samples failed in one test (1 in microbiology test, 16 in pH test), and three samples failed in both tests. Molds contaminated four of the failed samples. The total percentage of failure was 91% (the third highest value between hospitals). The same explanation as in the above section applies.

5.3.4 Al-Aqsa: 12 soap samples were collected, only one sample passed both tests, and other 11 samples failed in one test (9 in microbiological test, 2 in pH test). Sample passing in pH test failed in microbiological test. Total percentage of pass was 91.7% (the second highest failure value among hospitals).

5.3.5 Nasser: from the 20 soap samples collected, 17 samples passed both tests that is highest pass value, tow sample fail in one test (1 in microbiological test, 1 in pH

test), and only one sample was failed in both test, two sample fail in microbiological test contaminated with mold and *S. aureus*.

5.3.6 European Gaza: all 15 collected samples failed in one test (pH test). Microbiologically, all samples did not show any growth for the obvious reason of having extreme pH values which interfere with microbial growth.

5.3.7 Abu Yousef Al Najjar: 6 out 8 of the collected sample passed both test and other two sample failed in one test. One of two failed in microbiological test by contamination with *Pseudomonas* spp and other one failed in pH test. Unsupervised production and storage of local soap may be the reason for this failure.

5.4 Soap

The total failing value as 32/105 (30.5%), these results are lower than the Brazilian study (55.9%) (Joselany Afio Caetano et al., 2011), and higher than the Iraqi study (15.9%) (S. Zeiny, 2009),and USA study (24.8%) (Chattman & Maxwell, 2011). And the most microorganisms cause contamination are coliform (12.4%), then *Pseudomonas* spp (11.4%), and yeast (10.5%). Unlike Brazilian study the most microorganisms cause contamination for fifty-nine liquid soap are *Burkholderia cepacia* (4.6%), *Pseudomonas* spp (4%) and *Klebsiella* (1%)(Joselany Afio Caetano et al., 2011). But in the Iraqi study are (66.6%) *Pseudomonas*, (16.6%) *Proteus, and* (16.6%) *Flavimonas(S. Zeiny, 2009).* And in USA study the most frequency of detection are *Klebsiella oxytoca* (28.6%), *Klebsiella pneumonia* (27.6%), and *Enterobacter aerogenes* (12.4%)(Chattman & Maxwell, 2011). And the total failed value by pH is (59.1%). I did not found any study test pH of soap in hospitals that's May because in Gaza hospitals used local manufacture soap, without any scientific basis and used highly amounts of high concentration acid or high concentration base to bass in microbiological test. That was very clear from form of some sample of liquid soap.

5.5 Antiseptics and Disinfectants

5.5.1 Microbiological test

All antiseptics and disinfectants (Anti/Dis) samples (233) collected has passed the microbiological test, unlike that reportedly in USA study particularly in North Carolina which reported many type of bacteria contaminating several types of Anti/Dis. *Bacillus cereus* and *Burkholderia cepacia* were found to contaminate Alcohols. *Pseudomonas* spp., *Flavobacterium* sp., *Ralstonia pickettii, Serratia marcescens* and *Achromobacter xylosoxidans* were detected in Chlorhexidine, *Burkholderia cepacia* and *Pseudomonas aeruginosa* contaminated Povidone iodine **(David J Weber, William A Rutala, & Emily E Sickbert-Bennett, 2007)**.

The average of zone of inhibition for *S. aureus, P. aeruginosa*, and *E. coli* were 29 mm, 22.1 mm, 21.7 mm respectively this is as in many studies show the gram-negative bacteria tend to be more resistant than gram-positive organisms as staphylococci **(Billeter et al., 2008)**, and as in Indian studies shown the *E.coli* was most resistant organisms then *P.aeroginosa*. And *S.aureus* was less resistant organisms **(Bhat et al., 2011)**.

5.5.2 Concentration and pH tests

The total percentage failure by concentration is 39.9%. The highest failure was in Chlorine (51.7%). Iit is locally manufactured with primitive means and the dilutions is usually made without scientific basis. No previous studies testing the concentration of Anti/Dis because all Anti/Dis used in original bottles comes with mostly effective dilution. unlike the situation where, many Anti/Dis were diluted and are not in their original bottles.

The percentage failure by concentration in Povidone Iodine is (49.4%), it is second to locally manufactured Chlorine. The main cause is believed to be the dilution, and in Alcohol is (40%) this is may be because alcohol is highly volatile substance and poor handling and storage for long time, and maybe because of poor dilution. The lowest percentage failed value is CHX (20.3%). The only possible explanation for this is that CHX comes in small bottles, thus, lowering risks of poor handling.

The percentage of failing by pH for Povidone Iodine is (1.2%) and for CHX is (3.4%). No previous studies about pH of Anti/Dis in hospital were found in the literature. The low results compared with the failure due to concentration test maybe because the dilution by distilled water had no effect in pH.

5.6 Relationship between the Concentration and pH of Anti/Dis and zone of inhibition of *S. aureus, P. aeruginosa,* and *E. coli*.

There is asignificant relationship between the concentration of Anti/Dis and zone of inhibition of *E. coli* when P-value is lower 0.05. The concentration effect is clearly demonstrated on *E. coli* than *S. aureus* and *P. aeruginosa*. On the contrary, there is significantly relationship between pH of Anti/Dis and zone of inhibition of *S. aureus* and *P. aeruginosa* with P-value is lower 0.05. The pH effect is obvious on *S. aureus* and *P. aeruginosa* than *E. coli*. Both concentration and pH test is important to control the infection elements in hospital.

Chapter 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study is the first in the Gaza strip to evaluate the microbiological (bacteria and fungi) quality of soap and antiseptics/disinfectants and measure microbiologically and chemically the efficacy antiseptic/disinfectants. Therefore, results obtained in the current study could not be compared with any local data. The followings could be concluded from the results of this study:

1) More than half sample collected from biggest central hospital in Gaza stripe was failed in tests, the results shows 172 sample from 338 was failed with percentage 50.9%.
2) The percentage of fail in Antiseptics/Disinfectants samples were 40.3%, and in soap samples were 74.3%.
3) The results shows by hospitals:
    - Beit Hanoun: total failing percentage = 54.5%, and failed in Antiseptics/Disinfectants = 25%, and all soap samples were failed.
    - Kamal Adwan: total failing percentage = 53.3%, and failed in Antiseptics/Disinfectants = 33.3%, and failed in soap = 93.3%.
    - Al-Shifa: total failing percentage = 67.7%, and failed in Antiseptics/Disinfectants = 55%, and failed in soap = 90.9%.
    - Al-Aqsa: total failing percentage = 42.1%, and failed in Antiseptics/Disinfectants = 19.2%, and failed in soap = 91.7%.
    - Nasser: total failing percentage = 33.8%, and failed in Antiseptics/Disinfectants = 40.7%, and failed in soap = 15%.
    - European Gaza: total failing percentage = 63.3%, and failed in Antiseptics/Disinfectants = 14.4%, and all soap samples were failed.
    - Abu Yousef Al Najjar: total failing percentage = 34.6%, and failed in Antiseptics/Disinfectants = 33.3%, and failed in soap = 25%.
4) The most three microbiological parameter cause contamination of soaps were coliform (12.4%), *Pseudomonas* spp. (11.4) and yeast (10.5%).
5) Total fail percentage in both tests (microbiological and pH, concentration) was 73.3%.

6) Highest failed percentage of Antiseptics/Disinfectant concentration test was 55% in Al-Shifa, then 51.7% in European Gaza, and 40.7% in Nasser. Which biggest three hospital in Gaza strip.
7) The total failed percentage by Antiseptics/Disinfectants:
    - Povidone Iodine: all Povidone Iodine samples were collected from Al-Shifa hospital (biggest hospital in Gaza strip) was failed, and 77.8% from European Gaza was failed, and 42.9%from Beit Hanoun was failed. And 49.4 % as total failed result.
    - Alcohol: 78.6% of sample that collected from Nasser hospital was failed, 50% was failed in European Gaza, and 42.9% was failed in Abu Yousef Al Najjar with total failed result 40%.
    - Chlorhexidine: 50% of Kamal Adwan samples was failed, 30% of Al-Shifa, and 22.2% of European Gaza was failed. With total failed 20.3%.
    - Chlorine: All sample of Al-Shifa, European Gaza, and Abu Yousef Al Najjar was completely failed, and 60% of Nasser hospital, and 50% of Al Aqsa hospital Sample was failed with total percentage 51.7%.
8) There was strong relationship between zone of inhibition and concentration for *E. coli* and no relationship for *S. aureus,* and *P. aeruginosa*.
9) There was strong relationship between zone of inhibition and pH for *S. aureus*, and *P. aeruginosa* and no relationship for *E. coli*.

6.2 Recommendations

In light of the results and the above-mentioned conclusions, the following recommendations may be valuable in reducing the health risks of using soap and Antiseptics/Disinfectants in hospitals:

1) The continuous monitoring of all types of soaps and antiseptics/disinfectants that are used in hospitals is required as an urgent need.
2) Locally manufactured soaps and disinfectants should be regulated and monitored especially small-scale manufacturers.
3) The dilution and storage of antiseptics/disinfectants policy should be established and monitored in hospitals.

4) Make use of the services of the public health laboratory, which is capable of performing the required tests.
5) Utilize both microbiological and chemical tests for detecting the quality/efficacy of soaps and antiseptics/disinfectants.
6) Collect samples from all departments in hospitals and not from stores or suppliers only.
7) Use the originals antiseptics/disinfectants bottles of antiseptics and never dilute the material unless indicated by the manufacturer.
8) Review the infection control policies to ensure that any materials used in the process is of a suitable quality.
9) It is recommended that there should be more studies on this subject in Gaza.

Referencs

Akinsanya, J. A. (1993). Introduction to Sterilization, Disinfection and Infection Control. *Journal of Advanced Nursing, 18*(2), 334-334.

Alberts, B., Wilson, J. H., & Hunt, T. (2008). *Molecular biology of the cell* (5th ed.). New York: Garland Science.

Amemiya, K., & Taguchi, F. (1992). Survey of bacterial contamination of hand washing liquids. *journal Antibacterial Antifungal Agents, 20*, 459-463.

Andersen, B. M. (2010). European Centre for Disease Prevention and Control swine flu guidelines: 'cough hygienically' into your sleeve? *J Hosp Infect, 75*(1), 73-74. doi: 10.1016/j.jhin.2010.01.010

Andrews, W., Hammack, T., Maturin, L., Peeler, J., Hitchins, A., Feng, P., Watkins, W., Rippey, S., Chandler, L. and Hammack, T. (2001). Bacteriological analytical manual. *Center for Food Safety and Applied Nutrition*.

Ascenzi, J. M. (1995). *Handbook of Disinfectants and Antiseptics*: Taylor & Francis.

Barrette Jr, W. C., Hannum, D. M., Wheeler, W. D., & Hurst, J. K. (1989). General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry, 28*(23), 9172-9178.

Becks, V. E., & Lorenzoni, N. M. (1995). Pseudomonas aeruginosa outbreak in a neonatal intensive care unit: a possible link to contaminated hand lotion. *Am J Infect Control, 23*(6), 396-398.

Bennett, J. V., Jarvis, W. R., & Brachman, P. S. (2007). *Bennett & Brachman's hospital infections* (5th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins.

Bhat, R., Prajna, P., Menezez, V. P., & Shetty, P. (2011). Antimicrobial activities of soap and detergents. *Advances in Bioresearch, 2*(2), 52-62.

Billeter, S. A., Levy, M. G., Chomel, B. B., & Breitschwerdt, E. B. (2008). Vector transmission of Bartonella species with emphasis on the potential for tick transmission. *Med Vet Entomol, 22*(1), 1-15. doi: 10.1111/j.1365-2915.2008.00713.x

Block, S. S. (1991). Disinfection, Sterilization, and Preservation (January 1991 ed., pp. 1162): Lippincott Williams & Wilkins.

Block, S. S. (2001a). *Disinfection, sterilization, and preservation* (5th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Block, S. S. (2001b). *Disinfection, sterilization, and preservation*: Lippincott Williams & Wilkins.

Bloomfield, S. F. (2002). Significance of biocide usage and antimicrobial resistance in domiciliary environments. *J Appl Microbiol, 92*(31), 144S-157S.

Bobichon, H., & Bouchet, P. (1987). Action of chlorhexidine on buddingCandida albicans: Scanning and transmission electron microscopic study. *Mycopathologia, 100*(1), 27-35.

Bohnet, M. (2003). *Ullmann's encyclopedia of industrial chemistry* (6th, completely rev. ed.). Weinheim: Wiley-VCH.

Boyce, J. M., & Pittet, D. (2002). Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Infect Control Hosp Epidemiol, 23*(S12), S3-S40.

Bradley, C., & Fraise, A. (1996). Heat and chemical resistance of enterococci. *Journal of Hospital Infection, 34*(3), 191-196.

Brul, S., & Coote, P. (1999). Preservative agents in foods: mode of action and microbial resistance mechanisms. *International journal of food microbiology, 50*(1), 1-17.

Bruun, F. N., McGarrity, G. J., Blakemore, W. S., & Coriell, L. L. (1976). Epidemiology of Pseudomonas aeruginosa infections: determination by pyocin typing. *J Clin Microbiol, 3*(3), 264-271.

Buffet-Bataillon, S. R., V. Betremieux, P. Beuchee, A. Bauer, M. Pladys, P. Le Gall, E. Cormier, M. Jolivet-Gougeon, A. (2009). Outbreak of Serratia marcescens in a neonatal intensive care unit: contaminated unmedicated liquid soap and risk factors. *Hospital Infection, 72*(1), 17-22. doi: 10.1016/j.jhin.2009.01.010

Bush, L. W., Benson, L. M., & White, J. H. (1986). Pig skin as test substrate for evaluating topical antimicrobial activity. *J Clin Microbiol, 24*(3), 343-348.

Caetano, J. A., Lima, M. A., Di Ciero Miranda, M., Serufo, J. C., & Ponte, P. R. (2011). Identification of bacterial contamination in liquid soap for hospital use. *Rev Esc Enferm USP, 45*(1), 153-160.

Caetano, J. A., Lima, M. A., Miranda, M. D. C., Serufo, J. C., & Ponte, P. R. L. (2011). Identification of bacterial contamination in liquid soap for hospital use. *Revista da Escola de Enfermagem da USP, 45*(1), 153-160.

Camper, A. K., & McFETERS, G. A. (1979). Chlorine injury and the enumeration of waterborne coliform bacteria. *Applied and Environmental Microbiology, 37*(3), 633-641.

Campos, R. K., Andrade, K. R., Ferreira, P. C., Bonjardim, C. A., La Scola, B., Kroon, E. G., & Abrahao, J. S. (2012). Virucidal activity of chemical biocides against mimivirus, a putative pneumonia agent. *J Clin Virol, 55*(4), 323-328. doi: 10.1016/j.jcv.2012.08.009

Cartwright, A. C. *The British pharmacopoeia, 1864 to 2014 : medicines, international standards, and the state*.

Cavitch, S. M. (1995). *The natural soap book : making herbal and vegetable-based soaps*. Pownal, Vt.: Storey Communcations.

Chattman, M., Gerba, S., & Maxwell, C. (2011). Occurrence of heterotrophic and coliform bacteria in liquid hand soaps from bulk refillable dispensers in public facilities. *J Environ Health, 73*(7), 26-29.

Chattman, M., Gerba, S. L., & Maxwell, C. P. (2011). Occurrence of heterotrophic and coliform bacteria in liquid hand soaps from bulk refillable dispensers in public facilities. *J Environ Health, 73*(7), 26-29.

Chattman, M., & Maxwell, S. L. (2011). Occurrence of heterotrophic and coliform bacteria in liquid hand soaps from bulk refillable dispensers in public facilities. *J Environ Health, 73*(7), 26.

Chaturvedi, V., & Kumar, A. (2010a). Bacterial utilization of sodium dodecyl sulfate. *Int J Appl Biol Pharmaceut Tech, 3*, 1126-1131.

Chaturvedi, V., & Kumar, A. (2010b). TOXICITY OF SODIUM DODECYL SULFATE IN FISHES AND ANIMALS. A REVIEW. *Int J Appl Biol Pharmaceut Tech, 2*, 630-633.

Chaturvedi, V., & Kumar, A. (2011). Isolation of a strain of Pseudomonas putida capable of metabolizing anionic detergent sodium dodecyl sulfate (SDS). *Iran J Microbiol, 3*(1), 47-53.

Chiang, C. F., Sung, F. C., Chang, F. H., & Tsai, C. T. (2006). Hospital waste generation during an outbreak of severe acute respiratory syndrome in Taiwan. *Hospital, 27*(5), 519-000522.

Chlorine, I. (1995). Chlorine and Iodine Formulations. *Handbook of Disinfectants and Antiseptics*, 133.

Chopra, I., Linton, A., Hugo, W., & Russell, A. (1987). Microbial resistance to veterinary disinfectants and antiseptics. *Disinfection in veterinary and farm animal practice. Oxford: Blackwell Scientific Publications Ltd*, 43-65.

Collins, C. H., Allwood, M., Bloomfield, S. F., & Fox, A. (1981). *Disinfectants, their use and evaluation of effectiveness*: Academic Press.

Control, A. f. P. i. I., & Epidemiology. (1996). *APIC Infection Control and Applied Epidemiology: Principles and Practice*: Mosby.

Cordier, J.-L. (2004). Microbiological criteria: Purpose and limitations. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 95*(1), 28-31.

Davies, A. (1973). The mode of action of chlorhexidine. *Journal of Periodontal research, 8*(s12), 68-75.

De Nobel, J. G., Klis, F. M., Priem, J., Munnik, T., & Van Den Ende, H. (1990). The glucanase-soluble mannoproteins limit cell wall porosity in Saccharomyces cerevisiae. *Yeast, 6*(6), 491-499.

Denyer, S. P., & Stewart, G. (1998). Mechanisms of action of disinfectants. *International Biodeterioration & Biodegradation, 41*(3), 261-268.

Deress, T., Girma, M., Birhan, W., Biadgo, B., & Alemu, A. (2014). Isolation of Bacteria from Commonly Used Antiseptic and Disinfectant Solutions in Gondar University Hospital. North West Ethiopia. *American Journal of Nursing Research, 2*(3), 44-49.

Dodd, C. E., Sharman, R. L., Bloomfield, S. F., Booth, I. R., & Stewart, G. S. (1997). Inimical processes: bacterial self-destruction and sub-lethal injury. *Trends in Food Science & Technology, 8*(7), 238-241.

Dolan, S. A. E., T. James, J. F. (2006). Bubbles to Wubbles: an investigation involving the contamination of soap bubble products at a pediatric hospital. *J Spec Pediatr Nurs, 11*(3), 189-195. doi: 10.1111/j.1744-6155.2006.00065

Dukan, S., & Touati, D. (1996). Hypochlorous acid stress in Escherichia coli: resistance, DNA damage, and comparison with hydrogen peroxide stress. *J Bacteriol, 178*(21), 6145-6150.

Ehrenkranz, N. J., Bolyard, E. A., Wiener, M., & Cleary, T. J. (1980). Antibiotic-sensitive Serratia marcescens infections complicating cardiopulmonary operations: contaminated disinfectant as a reservoir. *Lancet, 2*(8207), 1289-1292.

El Moug, T., Rogers, D., Furr, J., El-Falaha, B., & Russell, A. (1986). Antiseptic-induced changes in the cell surface of a chlorhexidine-sensitive and a chlorhexidine-resistant strain of Providencia stuartii. *Journal of Antimicrobial Chemotherapy, 16*(6), 685-689.

Ellis, A. J., Hales, S. G., Ur-Rehman, N. G., & White, G. F. (2002). Novel alkylsulfatases required for biodegradation of the branched primary alkyl sulfate surfactant 2-butyloctyl sulfate. *Appl Environ Microbiol, 68*(1), 31-36.

Eryilmaz, M., & Palabiyik, I. M. (2013). Hypochlorous acid-analytical methods and antimicrobial activity. *Tropical Journal of Pharmaceutical Research, 12*(1), 123-126.

Favero, M. S. (2002). Products containing biocides: perceptions and realities. *J Appl Microbiol, 92 Suppl*(31), 72S-77S.
Filipe, R. (2010). The European Centre for Disease Prevention and Control launches call for external experts. *Euro Surveill, 15*(45).
Fraise, A., Maillard, J.-Y., & Sattar, S. (2012). *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization*: John Wiley & Sons.
Fuls, J. L., Rodgers, N. D., Fischler, G. E., Howard, J. M., Patel, M., Weidner, P. L., & Duran, M. H. (2008). Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Appl Environ Microbiol, 74*(12), 3739-3744. doi: 10.1128/AEM.02405-07
Gajadhar, T., Lara, A., Sealy, P., & Adesiyun, A. A. (2003). Microbial contamination of disinfectants and antiseptics in four major hospitals in Trinidad. *Revista Panamericana de Salud Pública, 14*(3), 193-199.
Garbutt, C., Simmons, G., Patrick, D., & Miller, T. (2007). The public hand hygiene practices of New Zealanders: a national survey. *N Z Med J, 120*(1265), U2810.
Gerberding, J. L. (1998). Nosocomial transmission of opportunistic infections. *Infect Control Hosp Epidemiol, 19*(8), 574-577.
Gilbert, P., McBain, A., & Rickard, A. (2003). Formation of microbial biofilm in hygienic situations: a problem of control. *International Biodeterioration & Biodegradation, 51*(4), 245-248.
Griffiths, P. A., Babb, J. R., Bradley, C. R., & Fraise, A. P. (1997). Glutaraldehyde-resistant Mycobacterium chelonae from endoscope washer disinfectors. *J Appl Microbiol, 82*(4), 519-526.
Grossgebauer, K. (1970). Virus disinfection. *Disinfection. Marcel Dekker, Inc., New York, NY*, 103-148.
Guralnik, D. B. (1980). *Webster's New world dictionary of the American language* (2d college ed.). Cleveland, Ohio: W. Collins.
Hageman, H., & Havinga, E. (2006). uptake of 14c-chlorhexidine diacetate to escherichia coli and pseudomonas aeruginosa and its release by azolectin. *FEMS Microbiology Letters*.
Health Canada, L. C. f. D. C. D. o. N., & Occupational, I. (2012). Routine practices and additional precautions: preventing the transmission of infection in health care. *Can Commun Dis Rep, 25 Suppl 4*, 1-126.
Hiom, S., Furr, J., Russell, A., & Hann, A. (1995). The possible role of yeast cell walls in modifying cellular response to chlorhexidine diacetate. *Cytobios, 86*(345), 123-135.
Hiom, S., Hann, A., Furr, J., & Russell, A. (1995). X-ray microanalysis of chlorhexidine-treated cells of Saccharomyces cerevisiae. *Letters in applied microbiology, 20*(6), 353-356.
Hiom, S. J., Furr, J., Russell, A., & Dickinson, J. (1992a). Effects of chlorhexidine diacetate and cetylpyridinium chloride on whole cells and protoplasts of Saccharomyces cerevisiae. *Microbios, 74*(299), 111-120.
Hiom, S. J., Furr, J., Russell, A., & Dickinson, J. (1992b). Effects of chlorhexidine diacetate on Candida albicans, C. glabrata and Saccharomyces cerevisiae. *Journal of Applied Bacteriology, 72*(4), 335-340.

Holah, J. (2003). CEN/TC 216: its role in producing current and future European disinfectant testing standards. *International Biodeterioration & Biodegradation, 51*(4), 239-243.

Holah, J. T., Taylor, J. H., Dawson, D. J., & Hall, K. E. (2002). Biocide use in the food industry and the disinfectant resistance of persistent strains of Listeria monocytogenes and Escherichia coli. *Symp Ser Soc Appl Microbiol*(31), 111S-120S.

Hugo, W. B. (1971). *Inhibition and destruction of the microbial cell*: Academic Press.

Hume, E. B., Flanagan, J., Masoudi, S., Zhu, H., Cole, N., & Willcox, M. D. (2009). Soft contact lens disinfection solution efficacy: clinical Fusarium isolates vs. ATCC 36031. *Optom Vis Sci, 86*(5), 415-419. doi: 10.1097/OPX.0b013e31819fa239

Ioannou, C. J., Hanlon, G. W., & Denyer, S. P. (2007). Action of disinfectant quaternary ammonium compounds against Staphylococcus aureus. *Antimicrobial agents and chemotherapy, 51*(1), 296-306.

Johnston, M., Lambert, R., Hanlon, G., & Denyer, S. (2002). A rapid method for assessing the suitability of quenching agents for individual biocides as well as combinations. *Journal of applied microbiology, 92*(4), 784-789.

Kabara, J. J., & Brady, M. B. (1984). Contamination of bar soaps under "in-use" conditions. *J Environ Pathol Toxicol Oncol, 5*(4-5), 1-14.

Kallman, O., Lundberg, C., Wretlind, B., & Ortqvist, A. (2006). Gram-negative bacteria from patients seeking medical advice in Stockholm after the tsunami catastrophe. *Scand J Infect Dis, 38*(6-7), 448-450. doi: 10.1080/00365540500452499

Kampf, G., & Kramer, A. (2004). Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clin Microbiol Rev, 17*(4), 863-893, table of contents. doi: 10.1128/CMR.17.4.863-893.2004

Karsa, D. R. (1999). *Industrial applications of surfactants IV*: Elsevier.

Katz, J. D. (2004). Hand washing and hand disinfection: more than your mother taught you. *Anesthesiol Clin North America, 22*(3), 457-471, vi. doi: 10.1016/j.atc.2004.04.002

Khunkitti, W., Avery, S., Lloyd, D., Furr, J., & Russell, A. (1997). Effects of biocides on Acanthamoeba castellanii as measured by flow cytometry and plaque assay. *Journal of Antimicrobial Chemotherapy, 40*(2), 227-233.

Khunkitti, W., Lloyd, D., Furr, J., & Russell, A. (1998). Acanthamoeba castellanii: growth, encystment, excystment and biocide susceptibility. *Journal of Infection, 36*(1), 43-48.

Kulikovsky, A., Pankratz, H., & Sadoff, H. (1975). Ultrastructural and chemical changes in spores of Bacillus cereus after action of disinfectants. *Journal of Applied Bacteriology, 38*(1), 39-46.

Laboratories, D. (1998). *Difco Manual* (2nd ed.): Difco Laboratories.

Lambert, P. A. (2004). Mechanisms of action of biocides. *Russell, Hugo and Ayliffe's principles and practice of disinfection, preservation and sterilization*, 139-153.

Larson, E., & Morton, H. (1991). Alcohols. *Disinfection, sterilization and preservation. 4th ed. Philadelphia: Lea & Febiger*, 191-203.

Larson, E. L., Eke, P. I., Wilder, M. P., & Laughon, B. E. (1987). Quantity of soap as a variable in handwashing. *Infect Control, 8*(9), 371-375.
Lelieveld, H., Mostert, M., & Holah, J. (2005). *Handbook of hygiene control in the food industry*: Elsevier.
Levy, S. B. (2001). Antibacterial household products: cause for concern. *Emerg Infect Dis, 7*(3 Suppl), 512-515. doi: 10.3201/eid0707.010705
Li, F., Chen, M., & Zhang, W. (2017). Effect of Binary/Ternary Fatty Acids Ratio and Glycerin on the Phase Behaviors of Soap Solutions. *Journal of Surfactants and Detergents, 20*(2), 425-434.
Lloyd, D., Turner, N., Khunkitti, W., Hann, A., Furr, J., & Russell, A. (2001). Encystation in Acanthamoeba castellanii: Development of Biocide Resistance1. *Journal of Eukaryotic Microbiology, 48*(1), 11-16.
Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., & Stahl, D. A. (2012). *Brock biology of microorganisms* (13th ed.). San Francisco: Benjamin Cummings.
Maillard, J.-Y., Beggs, T., Day, M., Hudson, R., & Russell, A. (1994). Effect of biocides on MS2 and K coliphages. *Applied and Environmental Microbiology, 60*(6), 2205-2206.
Maillard, J. Y. (2002). Bacterial target sites for biocide action. *Journal of applied microbiology, 92*(s1), 16S-27S.
Maillard, J. Y. (2004). Viricidal activity of biocides. *Principles and practice of disinfection, preservation and sterilization, 4*, 272-323.
Maillard, J. Y., Beggs, T., Day, M., Hudson, R., & Russell, A. (1995). Effects of biocides on the transduction of Pseudomonas aeruginosa PAO by F116 bacteriophage. *Letters in applied microbiology, 21*(4), 215-218.
Masri, N. M., Hanbali, L. B., Kamar, A. H., Kanafani, L. M. S., Hanbali, M. B., & Haddad, J. J. (2013). The Immunomodulatory, Antimicrobial and Bactericidal Efficacy of Commonly Used Commercial Household Disinfectants, Sterilizers and Antiseptics: Putative Anti-Inflammatory Infection Control Mechanisms and Comparative Biochemical Analysis of the Microbial Growth of Gram-Positive Bacteria. *American Journal of Medical and Biological Research, 1*(4), 103-133.
Mbithi, J. N., Springthorpe, V. S., & Sattar, S. A. (1990). Chemical disinfection of hepatitis A virus on environmental surfaces. *Applied and Environmental Microbiology, 56*(11), 3601-3604.
McBride, M. E. (1984). Microbial flora of in-use soap products. *Appl Environ Microbiol, 48*(2), 338-341.
McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical microbiology reviews, 12*(1), 147-179.
McDonnell, G., & Russell, A. D. (2001). Antiseptics and disinfectants: activity, action, and resistance. *Clinical microbiology reviews, 14*(1), 227.
McNaught, A. D., Wilkinson, A., & International Union of Pure and Applied Chemistry. (1997). *Compendium of chemical terminology : IUPAC recommendations* (2nd ed.). Oxford England ; Malden, MA, USA: Blackwell Science.
Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2006). *Microbiología médica*: Elsevier.

Nix, D. H. (2000). Factors to consider when selecting skin cleansing products. *J Wound Ostomy Continence Nurs, 27*(5), 260-268. doi: 10.1067/mjw.2000.107876
Noskin, G. A., Stosor, V., Cooper, I., & Peterson, L. R. (1995). Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. *Infect Control Hosp Epidemiol, 16*(10), 577-581.
Oxford University Press. (*AskOxford.com Oxford dictionaries* Retrieved from http://www.askoxford.com/?view=uk
Park, J. B., & Park, N.-H. (1989). Effect of chlorhexidine on the in vitro and in vivo herpes simplex virus infection. *Oral surgery, oral medicine, oral pathology, 67*(2), 149-153.
Percival, S., Bowler, P., & Russell, D. (2005). Bacterial resistance to silver in wound care. *Journal of Hospital Infection, 60*(1), 1-7.
Poole, K. (2002). Mechanisms of bacterial biocide and antibiotic resistance. *Journal of applied microbiology, 92*(s1), 55S-64S.
Prescott, L. M., Harley, J. P., & Klein, D. A. (2005). *Microbiology*: McGraw-Hill Higher Education.
Price, P., B. (1939). Ethyl alcohol as a germicide. *Archives of Surgery, 38*(3), 528-542.
Prince, H. N., Prince, D. L., & Prince, R. N. (1991). Principles of viral control and transmission. *Disinfection, sterilization, and preservation, 4th ed. Philadelphia: Lea & Febiger*, 411-444.
Prüss, A., Giroult, E., & Rushbrook, P. (2014). *Safe management of wastes from health-care activities*: World Health Organization.
Reybrouck, G. (1983). Role of the hands in the spread of nosocomial infections. 1. *J Hosp Infect, 4*(2), 103-110.
Reybrouck, G. (1998). The testing of disinfectants. *International Biodeterioration & Biodegradation, 41*(3), 269-272.
Rodgers, F., Hufton, P., Kurzawska, E., Molloy, C., & Morgan, S. (1985). Morphological response of human rotavirus to ultra-violet radiation, heat and disinfectants. *Journal of medical microbiology, 20*(1), 123-130.
Russell, A. (1991). Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. *Journal of Applied Bacteriology, 71*(3), 191-201.
Russell, A. (2004). Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon. *Journal of Hospital Infection, 57*(2), 97-104.
Russell, A., & Day, M. (1995). Antibiotic and biocide resistance in bacteria. *Microbios, 85*(342), 45-65.
Russell, A., & Hugo, W. (1988). Perturbation of homeostatic mechanisms in bacteria by pharmaceuticals. *Homeostatic mechanisms in microorganisms. Bath University Press, Bath, England*, 206-219.
Russell, A., Jones, B. D., & Milburn, P. (1985). Reversal of the inhibition of bacterial spore germination and outgrowth by antibacterial agents. *International journal of pharmaceutics, 25*(1), 105-112.
Russell, A. D. (1982). The destruction of bacterial spores.
Russell, A. D. (2002). Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol, 92 Suppl*, 121S-135S.

Russell, A. D. (2004). Factors influencing the efficacy of antimicrobial agents. *Russell, Hugo and Ayliffe's principles and practice of disinfection, preservation and sterilization*, 98-127.
Russell, A. D., & Day, M. J. (1993). Antibacterial activity of chlorhexidine. *J Hosp Infect, 25*(4), 229-238.
Russell, A. D., & McDonnell, G. (2000). Concentration: a major factor in studying biocidal action. *J Hosp Infect, 44*(1), 1-3. doi: 10.1053/jhin.1999.0654
Rutala, W. A. (1996). APIC guideline for selection and use of disinfectants. *American journal of infection control, 24*(4), 313-342.
Rutala, W. A., & Weber, D. J. (1999). Infection control: the role of disinfection and sterilization. *J Hosp Infect, 43 Suppl*, S43-55.
Sartor, C., Jacomo, V., Duvivier, C., Tissot-Dupont, H., Sambuc, R., & Drancourt, M. (2000). Nosocomial Serratia marcescens infections associated with extrinsic contamination of a liquid nonmedicated soap. *Infect Control Hosp Epidemiol, 21*(3), 196-199. doi: 10.1086/501743
Sauerbrei, A., Schacke, M., Gluck, B., Bust, U., Rabenau, H. F., & Wutzler, P. (2012). Does limited virucidal activity of biocides include duck hepatitis B virucidal action? *BMC Infect Dis, 12*, 276. doi: 10.1186/1471-2334-12-276
Selvaraju, S. B., Khan, I. U., & Yadav, J. S. (2005). Biocidal activity of formaldehyde and nonformaldehyde biocides toward Mycobacterium immunogenum and Pseudomonas fluorescens in pure and mixed suspensions in synthetic metalworking fluid and saline. *Applied and Environmental Microbiology, 71*(1), 542-546.
Setlow, P. (2006). Spores of Bacillus subtilis: their resistance to and killing by radiation, heat and chemicals. *Journal of applied microbiology, 101*(3), 514-525.
Shaker, L., Dancer, B., Russell, A., & Furr, J. (1988). Emergence and development of chlorhexidine resistance during sporulation of Bacillus subtilis 168. *FEMS Microbiology Letters, 51*(1), 73-76.
Shaker, L., Furr, J., & Russell, A. (1988). Mechanism of resistance of Bacillus subtilis spores to chlorhexidine. *Journal of Applied Bacteriology, 64*(6), 531-539.
Shaker, L., Russell, A., & Furr, J. (1986). Aspects of the action of chlorhexidine on bacterial spores. *International journal of pharmaceutics, 34*(1), 51-56.
Sharp, D., & Leong, J. (1980). Inactivation of poliovirus I (Brunhilde) single particles by chlorine in water. *Applied and Environmental Microbiology, 40*(2), 381-385.
Shukor, M. Y., Husin, W. S., Rahman, M. F., Shamaan, N. A., & Syed, M. A. (2009). Isolation and characterization of an SDS-degrading Klebsiella oxytoca. *J Environ Biol, 30*(1), 129-134.
Singer, M. M., & Tjeerdema, R. S. (1993). Fate and effects of the surfactant sodium dodecyl sulfate. *Rev Environ Contam Toxicol, 133*, 95-149.
Solveig Langsrud, Maan Singh Sidhu, Even Heir, & Holck, A. L. (2003). Bacterial disinfectant resistance-a challenge for the food industry. *International Biodeterioration & Biodegradation, 51*(4), 283–290. doi: 10.1016/S0964-8305(03)00039-8
Souza-Filho, F. J. d., Soares, A. d. J., Vianna, M. E., Zaia, A. A., Ferraz, C. C. R., & Gomes, B. P. F. d. A. (2008). Antimicrobial effect and pH of chlorhexidine

gel and calcium hydroxide alone and associated with other materials. *Brazilian dental journal, 19*(1), 28-33.
Springthorpe, V. S., Grenier, J. L., Lloyd-Evans, N., & Sattar, S. A. (1986). Chemical disinfection of human rotaviruses: efficacy of commercially-available products in suspension tests. *Epidemiology and Infection, 97*(1), 139-161.
Springthorpe, V. S., & Sattar, S. A. (1990). Chemical disinfection of virus-contaminated surfaces. *Critical Reviews in Environmental Science and Technology, 20*(3), 169-229.
Steinhauer, K. (2010). Antimicrobial efficacy and systematic use of disinfectants. *Current Research, Technology Ans Education Topics In Applied Microbiology Ans Microbial Biotecnology*, 369-376.
Stevenson, A. (2010). *Oxford dictionary of English*: Oxford University Press.
Talaro, K. P., & Chess, B. (2012). *Foundations in microbiology* (8th ed.). New York: McGraw-Hill.
Talaro, K. P., & Rhoads, T. L. (2012). *Foundations in Microbiology* (8th ed.).
Taylor, J. H., Rogers, S. J., & Holah, J. T. (1999). A comparison of the bactericidal efficacy of 18 disinfectants used in the food industry against Escherichia coli O157:H7 and Pseudomonas aeruginosa at 10 and 20 degrees C. *J Appl Microbiol, 87*(5), 718-725.
Thomas, O. R., & White, G. F. (1989). Metabolic pathway for the biodegradation of sodium dodecyl sulfate by Pseudomonas sp. C12B. *Biotechnol Appl Biochem, 11*(3), 318-327.
Tortora, G. J., Funke, B. R., & Case, C. L. (2013). *Microbiology : an introduction* (11th ed.). Boston: Pearson.
Trujillo, R., & Laible, N. (1970). Reversible inhibition of spore germination by alcohols. *Appl Microbiol, 20*(4), 620-623.
Wadhwa, V., Kabra, S., Khaki, P., Gur, R., Bhalla, P., Rai, S., . . . Gautam, V. K. (2007). Outbreak of burn wound infections by Salmonella enterica serovar Menston and the role of disinfectant testing in finding the cause of spread. *J Hosp Infect, 65*(2), 180-181. doi: 10.1016/j.jhin.2006.10.001
Wallen, R. D. (2002). Disinfection, Sterilization, and Preservation. *Biomedical Instrumentation & Technology, 36*(2), 141-141.
Weber, D. J., Rutala, W. A., & Sickbert-Bennett, E. E. (2007). Outbreaks associated with contaminated antiseptics and disinfectants. *Antimicrob Agents Chemother, 51*(12), 4217-4224. doi: 10.1128/AAC.00138-07
Weber, D. J., Rutala, W. A., & Sickbert-Bennett, E. E. (2007). Outbreaks associated with contaminated antiseptics and disinfectants. *Antimicrobial agents and chemotherapy, 51*(12), 4217-4224.
Wijesinghe, L., & Weerasinghe, T. (2012). A Study on the Bactericidal Efficiency of Selected Chemical Disinfectants and Antiseptics. *OUSL Journal, 6*, 44-58.
Yasuda-Yasaki, Y., Namiki-Kanie, S., & Hachisuka, Y. (1978). Inhibition of Bacillus subtilis spore germination by various hydrophobic compounds: demonstration of hydrophobic character of the L-alanine receptor site. *J Bacteriol, 136*(2), 484-490.
Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. *Pharmacological reviews, 55*(1), 27-55.

Yves Chartier, Jorge Emmanuel, Ute Pieper, Annette Prüss, Philip Rushbrook, Ruth Stringer, . . . Zghondi., R. (2013). *Safe management of wastes from health-care activities* (2nd ed.): World Health Organization.

Yves Chartier, Jorge Emmanuel, Ute Pieper, Annette Prüss, Philip Rushbrook, Ruth Stringer, . . . Zghondi., R. (2014). *Safe management of wastes from health-care activities* (2nd ed.): World Health Organization.

Zapka, C. A., Campbell, E. J., Maxwell, S. L., Gerba, C. P., Dolan, M. J., Arbogast, J. W., & Macinga, D. R. (2011). Bacterial hand contamination and transfer after use of contaminated bulk-soap-refillable dispensers. *Appl Environ Microbiol, 77*(9), 2898-2904. doi: 10.1128/AEM.02632-10

Zeiny, S. (2009). Isolation of some microorganisms from bar soaps and liquid soaps in hospital environments. *Iraqi J Pharm Sci, 18*(1), 28-32.

Zeiny, S. M. (2009). Isolation of Some Microorganisms from Bar Soaps and Liquid Soaps in Hospital Environments. *Iraqi Journal of Pharmaceutical Sciences, 18*(1), 28-32.

Zeiny, S. M. H. (2009). Isolation of Some Microorganisms from Bar Soaps and Liquid Soaps in Hospital Environments. *Iraqi Journal of Pharmaceutical Sciences, 18*(1), 28-32.

Zeng, G., Fu, H., Zhong, H., Yuan, X., Fu, M., Wang, W., & Huang, G. (2007). Co-degradation with glucose of four surfactants, CTAB, Triton X-100, SDS and Rhamnolipid, in liquid culture media and compost matrix. *Biodegradation, 18*(3), 303-310. doi: 10.1007/s10532-006-9064-8