ANTIMICROBIAL ACTIVITY OF LUGOL SOLUTION AGAINST MICROORGANISMS ISOLATED FROM CLINICAL SPECIMENS

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I certify that in my opinion the thesis submitted by Mustafa Ali MAQBOL titled “THE ANTIMICROBIAL ACTIVITY OF LUGOL SOLUTION AGAINST MICROORGANISMS ISOLATED FROM CLINICAL SPECIMENS” is fully adequate in scope and in quality as a thesis for the degree of Master of Science.

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This thesis is accepted by the examining committee with a unanimous vote in the Department of Medıcal Mıcrobıology as a master's thesis. 16/05/2023

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Mustafa Ali MAQBOL

ABSTRACT

M. Sc. Thesis

ANTIMICROBIAL ACTIVITY OF LUGOL SOLUTION AGAINST MICROORGANISMS ISOLATED FROM CLINICAL SPECIMENS

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Both gram-positive and gram-negative bacteria benefit from iodine's antibacterial capabilities. However, bacterial infections are becoming increasingly difficult to treat as microorganisms develop resistance to traditional therapies, necessitating the progress of novel antibiotics. Developing new antibiotics represents a time-consuming process. As a result, the current study aims to assess the effectiveness of Lugol's solution as an antibacterial agent against antibiotic-resistant gram-negative and gram-positive bacteria obtained from clinical samples.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Lugol's solution are measured after completing antibiotic sensitivity tests on 100 bacterial isolates that were resistant to 70% or more of the tested antibiotics.These bacterial isolates were named strains with known antibiotic sensitivity and were used in the study.

It was prepared that six serial dilutions of 2% Lugol's solution (32µl, 64µl, 128µl, 256µl, 512µl, 1024µl) and tested them on standard bacterial strains (Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus). It was documented that the results of susceptibility, which measures the inhibition of bacterial growth. It was found that a concentration of 32µl of Lugol's solution did not inhibit any bacterial growth, while a concentration of 64µl inhibited 33.33% of the bacterial growth. At a concentration of 128µl, the inhibition rate increased to 66.66%, and at 256µl, the solution inhibited 100% of bacterial growth. Concentrations of 512µl and 1024µl also inhibited 100% of bacterial growth.

It was also tested that 2% Lugol's solution on the 100 isolated bacterial specimens and found that the inhibition of bacterial growth increased steadily with increasing Lugol dilution. Specifically, a concentration of 32µl inhibited 9% of bacterial growth, and a concentration of 64µl inhibited 28% of bacterial growth. Concentrations of 128µl, 256µl, 512µl, and 1024µl inhibited 44%, 82%, 92%, and 96% of bacterial growth, respectively.

Overall, gram-positive bacteria were more susceptible to Lugol's iodine solution than gram-negative bacteria. These results suggest that Lugol's solution may be useful in the treatment of bacterial infections, especially those caused by antibiotic-resistant strains, as an alternative to conventional antibiotics.

Key Words : Gram negative, Gram positive, Bacteria, Lugol solution, Antibiotic resistant.

Science Code : 10105.07

ÖZET

Yüksek Lisans Tezi

KLİNİK ÖRNEKLERDEN İZOLE EDİLEN MİKROORGANİZMALARA KARŞI LUGOL SOLÜSYONUNUN ANTİMİKROBİYAL AKTİVİTESİ

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İyot, hem gram-pozitif hem de gram-negatif mikroorganizmalar üzerinde antibakteriyel etkiye sahiptir. Bununla birlikte, mikroorganizmaların geleneksel tedavilere karşı direnç geliştirmesi ve yeni antibiyotiklerin geliştirilmesini gerektirmesi nedeniyle bakteriyel enfeksiyonların tedavisi giderek daha zor hale gelmektedir. Yeni antibiyotik geliştirmek zaman alıcı bir süreçtir. Bu nedenle bu çalışma, klinik örneklerden izole edilen antibiyotiğe dirençli gram negatif ve gram pozitif bakterilere karşı antibakteriyel ajan olarak Lugol solüsyonunun etkinliğini değerlendirmeyi amaçlamıştır.

Lugol solüsyonunun minimum inhibitör konsantrasyonunu (MIC) ve minimum bakterisit konsantrasyonunu (MBC) belirlemek için, antibiyotik duyarlılık testlerinden sonra test edilen antibiyotiklerin %70 veya daha fazlasına dirençli olan 100 bakteri

izolatında duyarlılık testleri gerçekleştirdik. Bu bakteri izolatları antibiyotik duyarlılığı bilinen suşlar olarak adlandırıldı ve çalışmada kullanıldı.

%2 Lugol solüsyonunun (32ul, 64ul, 128ul, 256ul, 512ul, 1024ul) altı seri dilüsyonu hazırlandı ve standart bakteri suşları (Escherichia coli, Pseudomonas aeruginosa ve Staphylococcus aureus) üzerinde test edildi. Bakteri üremesinin inhibisyonunu ölçen duyarlılık sonuçlarının belgelendiği belgelenmiştir. 32ul Lugol solüsyonu konsantrasyonunun herhangi bir bakteri üremesini engellemediği, 64ul konsantrasyonunun ise bakteri üremesini %33.33 inhibe ettiği bulundu. 128 ul'lik bir konsantrasyonda inhibisyon oranı %66.66'ya yükseldi ve 256 ul'de solüsyon bakteri gelişimini %100 inhibe etti. 512ul ve 1024ul konsantrasyonları da bakteri üremesini %100 engelledi.

Ayrıca %2 Lugol solüsyonunun izole edilmiş 100 bakteri örneği üzerinde test edildiği ve artan Lugol dilüsyonu ile bakteri üremesinin inhibisyonunun istikrarlı bir şekilde arttığı bulundu. Spesifik olarak, 32 ul'lik bir konsantrasyon, bakteri üremesini %9 oranında inhibe etti ve 64 ul'lik bir konsantrasyon, bakteri büyümesini %28 oranında inhibe etti. 128ul, 256ul, 512ul ve 1024ul'lik konsantrasyonlar, sırasıyla %44, %82, %92 ve %96 bakteri üremesini inhibe etti.

Genel olarak, Lugol'ün iyot solüsyonunun gram pozitif bakterilere karşı gram negatif bakterilerden daha etkili olduğunu gözlemledik. Bu bulgular, Lugol solüsyonunun, özellikle antibiyotiğe dirençli suşların neden olduğu bakteriyel enfeksiyonların tedavisinde geleneksel antibiyotiklere faydalı bir alternatif olabileceğini düşündürmektedir.

Anahtar Kelimeler : Gram negatif, Gram pozitif, Bakteri, Lugol solüsyonu, Antibiyotik dirençli.

Bilim Kodu **:** 10105.07

DEDICATION

To the light of my eyes the light of my path and the joy of my life, to the fountain of goodness and tenderness, the harbor of roses and safety, My mother then my mother, then my mother. To the one who honored me by bearing his name may Allah protect my father.

Inspire me like to improve and lighten my ways, and try his best to keep me moving ahead, stand firm, and keep fighting. My wonderful father is the source of my strength and courage, as well as my pride.

Whose prayers and words were the companion of brilliance and excellence. To the support , the arm and the arm, my brothers Zaid, Hussein, and Sister Mina, I dedicate to you devotion with love, honor and dignity.

My valuable Supervisor, who contributed to my professional life with his valuable knowledge, Prof. Dr. Hasan SOLMAZ, I like to thank him. Laboratory studies and who assisted me with my writing, shared valuable information with me.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C : degrees Celsius

g : gram

kg : kilogram

L : litre

mg : miligram

ml : mililitre

µg : mikrogram

µl : mikrolitre

PART 1

INTRODUCTION

1.1. ANTIBIOTIC RESISTANCE

As bacteria develop defences against drugs, antibiotic resistance occurs [1]. Any microbe, including infection-resistant fungi, antiviral resistance in viruses, antibiotic resistance in bacteria, and antiprotozoal resistance in protozoa, can cause resistance. Superbugs are occasionally used to refer to microorganisms that are classified as extensively drug-resistant (XDR) or thoroughly drug-resistant (CDR) [2]. Antimicrobial resistance is a natural occurrence, albeit frequently caused by poor antibiotic use and sickness treatment [3,4]. Antimicrobial resistance, which refers to microorganisms that acquire antibiotic resistance, is an essential component of AMR [1]. Horizontal transfer of resistance across species and genetic mutation are both potential causes of drug-resistant microorganisms [5].

Every year, millions die due to clinical disorders connected to AMR. Resistant may develop spontaneously as a result of random mutations. However, the selection of mutations that might render antibiotics ineffective appears to be favored by prolonged antibiotic use [6]. Treatment of infections caused by bacteria and viruses that have developed resistance requires either more powerful antibiotics or the use of potentially harmful alternatives. Specific procedures may be more expensive than others. Multidrug-resistant (MDR) bacteria resist a broad spectrum of drugs [3]. [4]. One strategy to prevent overusing antibiotics, resulting in antibiotic resistance, is to take drugs precisely as directed [7]. [8]. When possible, narrow-spectrum antibiotics are better than broad-spectrum antibiotics because they kill the targeted organisms more effectively, have fewer side effects, and slow the growth of antibiotic resistance [9]. [10]. Home users of these drugs require training in their correct administration. Standard sanitation and hygiene practises, including as handwashing and patient-to-

patient disinfection, help minimise the spread of drug-resistant diseases. Also, they should encourage patients, visitors, and family members to adhere to these guidelines [11]. Drug-resistant bacteria are becoming more widespread as a result of increased antibiotic use as well as the transmission of resistant strains between species [7]. The release of improperly treated pharmaceutical industry effluents has also been linked to increased resistance, particularly in countries where bulk medications are manufactured [12].

As a result of antibiotics' enhanced selection pressure on bacterial populations, which kills more susceptible germs, the proportion of resistant bacteria that continue to multiply grows. Resistant bacteria can grow faster than susceptible ones, even at relatively low antibiotic doses [13]. As antibiotic resistance increases, alternative medicines are becoming more important. Although there has been a demand for novel antibiotic medications, this is becoming less common [14].

Owing to the rising prescribing and administration of antibiotic drugs in poorer nations, antimicrobial resistance is spreading worldwide [15]. The World Health Organization defines antimicrobial resistance as bacteria's resistance to antimicrobial medications that previously treated germ-related disorders [1]. Antibiotics cannot cause a person to develop resistance. The pathogen is resistant, not the afflicted human or other species [16]. Drug resistance can emerge in different sorts of bacteria. Antibiotic, antifungal, antiviral, and antiparasitic resistance exist [3]. [4].

A subcategory of resistant pathogens is antibiotic resistance. Microbiologically based resistance, which arises from altered or inherited genes and renders bacteria resistant to the antibiotic's associated resistance mechanism, is by far the most frequent kind of resistance. The clinical and microbiological subcategories of this more focused resistance are broken down even more so that it can be linked to harmful bacteria. Many treatments fail due to the survival of germs that are normally susceptible to a medicine but have developed resistance after being exposed to the drug's effects. Bacteria can conjugate, transduce, or convert to convey the genetic trigger for resistance in both instances of acquired resistance. Due to this, new bacterial infections strongly linked to the initial pathogen may resist the treatment [17]. Antibiotic usage

is a major contributor to antimicrobial resistance. As a result, either those bacteria that are inherently resistant to antimicrobials become significantly more common than those that are easily destroyed with therapy, or microorganisms develop resistance to the drugs used to treat them [18]. Antibiotic-resistant microorganisms have emerged in part because of the widespread overuse of antibiotics [19].

Antibiotic resistance can develop over time simply because bacteria are exposed to antibiotics. Biological evolution explains how species that can survive, reproduce, and adapt to their environment will persist [20]. So, in the environment, the types of bacteria that can withstand extended exposure to certain antimicrobial chemicals will naturally predominate, whereas those lacking this resistance would go extinct [19]. Some of the most recent antibiotic resistances appeared naturally, long before antibiotics were introduced or therapeutically used by humans. For instance, methicillin resistance has been related to a disease that affects hedgehogs; this may be owing to the infection's co-evolutionary adaptation to hedgehogs carrying a dermatophyte that produces antibiotics [21].

Antimicrobial treatment will eventually lose effectiveness against most infections as most illnesses and bacteria that are currently common develop resistance. Antibacterial drugs are being utilized more often, which aids in accelerating this natural process [22]. Consumer self-medication, that is refered to as "the use of medications on one's own initiative or on the advice of another person, who is not a competent medical expert," is one of the key elements affecting the evolution of antimicrobial resistance. [23]. Patients utilize excessive or unnecessary amounts of antimicrobials due to seeking inaccurate medical advice from friends, family members, and the media to heal their own diseases. When they require more money to see a doctor or in impoverished nations where the economy needs more doctors, many people turn to this as a last resort.

Governments in these underdeveloped nations have resorted to legalizing the sale of OTC drugs to enable people to access antimicrobials without seeking a doctor or paying for a visit [24]. Several antimicrobials are overused due to the ease with which they may be acquired without a doctor's prescription, leading to the development of

resistant bacteria strains. India is a good example of a country facing these problems. In Punjab, 73% of the population took care of both long-term and short-term health problems by themselves [23]. The fundamental issue with self-medication is that most individuals are unaware of the negative effects of antibiotic resistance or how self-medication or self-diagnosis may contribute to it.

Researchers analysed 3,537 articles that were published in Europe, Asia, and North America to ascertain how much the general public knows and believes about the problem. Antibiotic resistance is a significant kind of antimicrobial resistance. Although 88% of the 55,225 survey participants felt antibiotic resistance was connected to a physical change in the body, only 70% had ever heard of it [23]. Antimicrobial resistance is more likely to spread since so many people may self-medicate with antibiotics, and the majority are uninformed about what it is. Healthcare professionals' clinical negligence is another element in the rise of antibiotic resistance. According to CDC investigations, up to 50% of the patients tested had incorrect information about the necessity of antibiotic treatment, the medication used, and the length of therapy. Another investigation conducted at a prestigious French hospital's intensive care unit found that between 30% to 60% of antibiotic prescriptions were unjustified [25]. These inappropriate antimicrobial medication applications encourage bacteria to undergo genetic alterations that lead to resistance [26].

The COVID-19 epidemic's over use of antibiotics may make this issue with world health worse [27]. [28]. Moreover, pandemics may strain some healthcare systems to the point that antibiotic-resistant infections develop [29]. Improved hand sanitation, lessened travel abroad, and less elective medical procedures, however, might decrease the selection and diffusion of AMR pathogens in the short term, claims a study. [30]. Disinfectants such various alcohol-based antibacterial soaps and antiseptic agent washes may potentially enhance antimicrobial resistance [31]. The researchers have shown that everyday disinfectant usage is associated with mutations that promote antibiotic resistance [32].

As a result of pharmaceutical manufacturing facilities, hospitals, and clinics' untreated effluents, microbes in the environment might be exposed to antibiotics and acquire resistance [33]. Improper drug disposal, among other things, and other factors:

1.1.1. Manufacture of Food

The issue of resistant bacteria influences the nutritional business, particularly the animals used in food production. Livestock is given antibiotics as a growth promoter and a prophylactic step to lessen the chance of sickness. As a result, bacterial strains that can cause sickness and even death are introduced into people's food. Even though this technique results in higher yields and meat products, it seriously threatens the emergence of antibiotic resistance [34]. World Health Organization Expert Panel on Embedded Observation of Drug Resistance strongly recommended limiting the use of therapeutically effective antimicrobials in cattle notwithstanding the lack of evidence linking their use to the development of antimicrobial resistance. The Expert Panel also recommended that such antimicrobials be made illegal to promote growth and prevent disease [35]. According to an investigation published by the National Academy of Sciences that documented the use of antibiotics in cattle worldwide, by 2030, the 228 countries studied will have consumed 67% more antibiotics overall. In several countries, including South Africa, Brazil, Russia, India, and China, a 99% rise is predicted [22]. Antibiotic usage in cattle is prohibited in several nations, including the United States, Canada, China, and Japan. Individual antibiotic resistance may decrease as a result of these restrictions [35].

1.1.2. Pollutants

Crops are frequently defended against insects and plant pests by herbicides. Anti-microbial herbicides can occasionally protect crops against various diseases, including bacteria, viruses, fungi, algae, and protozoa. Due to the extensive use of several pesticides to boost agricultural yield, many of these bacteria have evolved a resistance to these anti-microbial substances. The EPA has registered and released around 4000 anti-microbial pesticides, showing their widespread use [36]. Because crops use 90% of all pesticides, it is estimated that 0.3 g of herbicides are used for every single meal

consumed. Most of these supplies serve to limit the distribution of disease and, hopefully, shield the general populace from harm. Despite widespread usage, fewer than 0.1% of antimicrobial drugs are estimated to reach their targeted targets. An herbicide still in use accounts for about 99% of all chemical pollutants [37]. These antimicrobial substances can move via the soil, the air, and the water, interacting with new bacteria and triggering the emergence of herbicide tolerance in those microorganisms.

1.1.3. Water, Sanitary and Hygienic

The wash infrastructure must be improved to combat antibiotic resistance (AMR). This will help to prevent infectious infections. The "Interagency Coordination Group on Antimicrobial Resistance" published a paper in 2018 that claimed that the transmission of illnesses through polluted water increases the need for antibiotic therapy by significantly burdening people with gastrointestinal illness [38]. This is a major issue in developing countries, where an increase in infectious diseases due to inadequate sanitation is one of the main factors driving demand for antibiotics [39]. A dangerous feedback loop where the need for antimicrobials increases as their efficacy decreases has been established by rising antibiotic use and the prevalence of infectious diseases [39].

The effective use of wash infrastructure can reduce the frequency of diarrhea cases that require antibiotic treatment by 47 to 72 percent [39], depending on the type of intervention and how effectively it works. If infrastructure could reduce diarrheal illness, antibiotic-treated diarrhoea cases would decline significantly. This is expected to impact anywhere from 5 million individuals in Brazil and as many as 590 million in India by 2030 [39]. Rising consumption and resistance are closely correlated, which implies that reducing resistance will immediately decrease AMR's rapid spread. By 2030, everyone will have access to sanitary facilities, according to Sustainable Development Goal Number 6. The frequency of resistant bacteria was decreased in hospital staff members who washed their hands more often [40]. AMR may be primarily reduced by upgrading healthcare institutions' water supply and sanitation infrastructure [38]. There is still much room for development. According to WHO and

UNICEF data from 2015, 38% of healthcare facilities worldwide lack access to water, 17% lack toilets, and 35% lack both water and soap or alcohol-based hand rub for handwashing [41].

1.1.4. Treatment of Heavy Sewage

Antimicrobial product makers must use industrial wastewater treatment methods to improve how their wastewater is treated. This will cut down on the amount of residues that get into the environment [38].

1.1.5. Mechanisms and Organisms

Drug inactivation or modification: β-lactamases are produced in some penicillin-G resistant bacteria to deactivate penicillin G. Transferase enzymes, which add functional groups to drugs, can also chemically change them; major resistance mechanisms to aminoglycosides include acetylation, phosphorylation, and adenylation. Acetylation, the most common approach, has an effect on a variety of medications [42, 43]. Similarly, with MRSA and other penicillin-resistant bacteria that modify PBP, the penicillin-binding site, the target or binding site, is altered. Ribosomal protection proteins are another defensive mechanism seen in many bacterial species. These proteins shield the bacterial cell against medicines that interfere with ribosomes. The conformation of ribosomes in a bacterial cell is changed when ribosomal protection proteins attach to them. Antibiotics are prevented from adhering to ribosomes and interrupting protein synthesis, enabling the ribosomes to continue generating the proteins required by the cell [44].

Changes in metabolism: For example, unlike mammalian cells, certain sulfonamide-resistant bacteria use preformed folic acid rather than PABA, a crucial precursor for producing folic acid and nucleic acids in sulfonamide-resistant bacteria [45].

Decreased drug concentration: Either increasing the active efflux (pump-out) of drugs across the cell surface or decreasing their permeability is one method to improve the efficacy of drugs [46]. Several bacterial species contain pumps within their cellular

membranes that transport antibiotics outside the cell before they cause damage. Antibiotics with a specific substrate linked to them tend to be more effective [47]. In the same way as fluoroquinolone resistance [48].

A heat shock protein that is present in *Listeria monocytogenes* and is comparable to HflX in other bacteria was able to release the ribosome when the ribosome was postponed by medications like lincomycin and erythromycin. The drug's ribosome is freed, enabling further translation, which results in resistant bacteria [49].

1.2. AIMS AND GOALS

Due to the progression of tolerance of bacteria to existing antibiotics, difficulties are encountered in the therapy of bacterially-based infectious illnesses. As a result, new antibacterial drugs need to be researched and developed. Developing antibiotics is both a time and consuming process. The current investigation is an attempt to analyse the efficacy of Lugol's solution as an antibacterial agent. This research is set:

- To examine if Lugol's Solution has antibacterial effects on pathogenic bacterial isolates of Gram-positive and Gram-negative types.
- After determining the resistance of the isolates obtained from clinical samples to the antibiotics in use, determining the effectiveness of Lugol's solution on these isolates and comparing them Given the findings of the antibiotic susceptibility test.
- Determining the utilized Lugol's solution's minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC).

PART 2

REVIEW OF LITERATURE

2.1. LUGOL SOLUTION

Each substances used to control and reduce germs are referred to as "disinfectants," which are often split into (Antiseptics) and (Disinfectants). When administered to live tissues or to non-living objects like untreated surfaces, both kinds are used to eliminate or halt the development of bacteria. When administered to the skin or other living tissues, antiseptics kill bacteria and prevent their development. When it comes to "disinfectants," they are chemicals that are present, influence the organisms, and are only used on inert materials like beds and grounds [50].

Rising worries about potential contamination and infection dangers in materials are the cause of the current situation. Human use of disinfectants has expanded in the food and general consumer sectors as a consequence of their broad use in limiting illness procedures and the prevention of hospital infections [51, 52, 53]. Antiseptics and antibiotics are different in a some ways, including different locations they can affect, antiseptics work on several places in addition to restricting their use externally, unlike antibiotics, which have fixed and specific cellular targets in the microbe ([Fig. 2.1.A].). [54] . The following important sites are potential locations for the use of antiseptics on germ cells (as an example of microorganisms):

2.1.1. The Outer Membrane of the Germ Cell Wall

Gram-negative bacteria's germ cells are shielded from their surroundings by a membrane made mostly of lipopolysaccharides, which are thought to perform a significant function in preserving structural integrity. This membrane is critical to the

cell's survival. When a disinfectant comes into touch with the membrane, one or more of the following outcomes are likely to occur:

- Polar disinfectant particles dissolve and enter via the fat phase.
- The membrane is traversed by additional molecules thanks to specific transporting mechanisms.
- By interacting with particular places, the remaining molecules can affect the membrane's ability to regulate itself.

2.1.2. Germ Cell Wall

The bacterial cell wall is one of its crucial components because it gives the bacteria strength and enables them to resist outside influences. Gram-positive bacteria, in contrast to Gram-negative bacteria, have a far more permeable cell wall. Examples of disinfectants that impact the cell wall include sodium hypochlorite and phenols.

2.1.3. Cytoplasmic Membrane

The following methods allow the antiseptic substance to pass through the cytoplasmic membrane:

- Initial passive diffusion (nonspecific and slow).
- Effective transfer (qualitative and permits the disinfectant to accumulate in the bacteria following transmission or interaction with transmembrane proteins).

Alcohol and iodine are two disinfectants that have an impact on the cytoplasmic membrane.

2.1.4. Cytoplasm and Nucleus

Some cleaning agents work by altering my protein. When the cytoplasm dries out, coagulation develops. Similar to how formaldehyde causes proteins to denaturate, it likewise affects nucleic acids. Alkylation of bacterial nucleic acids and cytoplasm.

2.1.5. Bacterial Spores

Since the presence of Acid Dipicolinic Acid makes bacteria more resistant to the effects of disinfectants. The cell stability and tolerance to outside influences were improved in those spores (Fig. 2.1.B). The spores can be impacted and their stability disrupted by an efficient disinfectant that generates potent oxidizing products (such as water or oxygen and chlorine) [55].

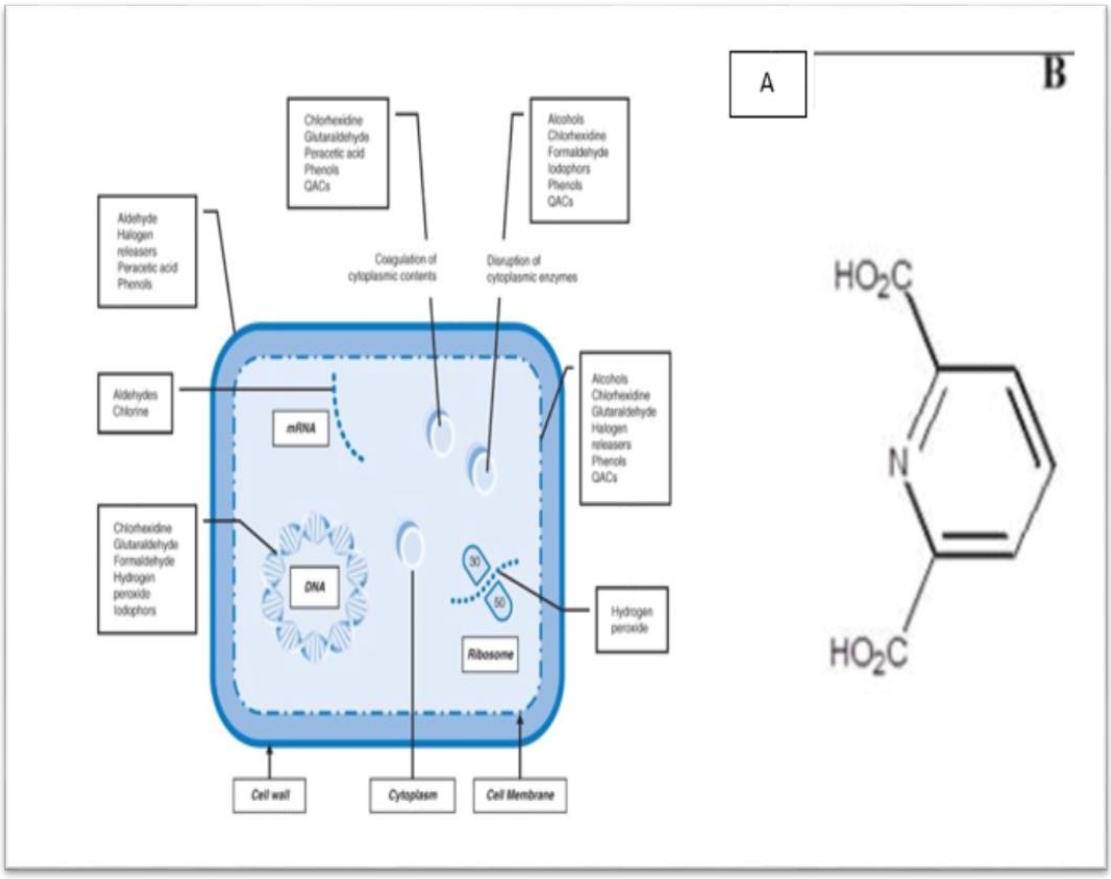


Figure 2.1. A (Some sites of action of antiseptics on the germ cell "Fanning S.Altered tolerance to biocides, 2011", B (acid formula) Dipicolinic Acid. "Stève Olugu Voundi, Maximilienne , Ascension Nyegue, Blaise Pascal Bougnom, François-Xavier Etoa 2017"

2.2. IODINE COMPOUNDS

Iodine-releasing among all halogenated disinfectants, iodine-releasing disinfectants had a significant place. The antiseptic properties of these compounds are mostly due to the oxidative molecular element iodine. Iodine's significance as an antiseptic agent

inspired scientists to carry out numerous investigations and research on it, allowing for the production of several disinfectants with iodine content.

2.2.1. General Introduction to The Chemistry of Iodine

The word "iodine" comes from the Greek word "iodes," which also denotes the color violet and signifies "violet". Iodine is found in its powder as a glossy, crystalline solid with a dark violet tint that dissolves at 113.5°C and vaporizes at 184.4°C to produce fumes that range in color from violet to pink. We can tell that elemental iodine may ascend into vapors immediately from the solid state by looking at the color of the lids on its containers or the environment it is kept in (Figure 2.2). Iodine often produces a dark brown hue in polar storage whereas it produces violet in nonpolar stores [56,57].

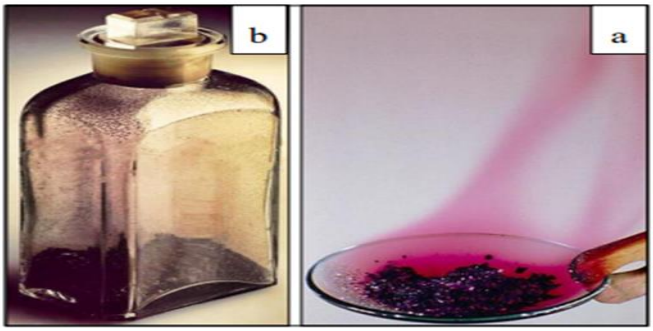


Figure 2.2. Iodine; a (by heating, b) at room temperature, “Cooper, R. A. 2007” ,” European Pharmacopoeia volume 2002”.

Iodine's hydrophobic characteristics explain why it dissolves poorly in aqueous solutions, degrading Organic solutions: Heptane is highly soluble in organic solutions including ethanol, ether, and chloroform, but its solubility in water is relatively low (L/g 33.0 at 25°C). Iodine combines with iodine ions to create iodine ions, increasing its solubility when these ions (like potassium iodide KI) are present. I_3^-, (iodide-Tri) (highly soluble in water) [56, 57]. Iodine's solutions are unstable. And that depends on the water, Which might engage in a number of chemical reactions depending on the changing circumstances. Within its aqueous solutions, a sizable variety of possible iodine formulations have been discovered (Fig 2.3) [58] .

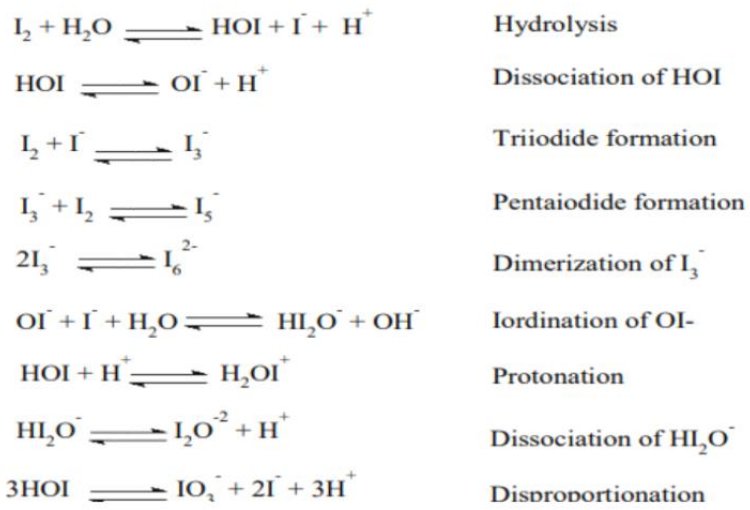


Figure 2.3. Possible reactions of iodine in its aqueous solution. “Gottardi, W. (2001)”.

We note in the previous equations that the aqueous solution of iodine contains more than ten formulas (IO_3^- ,H_2OI^+,OI^-, I_2O^-, H2OI $^-$,OI $^-$,HOI, I_6^{-2}, I_2, I_3^-, I_5^-) only three of them have lethal activity for microorganisms (HOI, H_2OI^+) (most effective and type) (I_2) [56,59].

The form that iodine takes in its aqueous solutions is significantly influenced by the medium's pH since an acidic medium encourages molecular iodine (I_2) to replace other processes and take over as the dominant form at low pH levels. Inactive formulations such as (OI^-, IO_3^-,)) (Where an increase in the synthesis of the iodine ion is seen (IO_3^-) (at pH > 7) to become inactive) are produced when the stability of iodine is negatively impacted by moderate to alkaline medium at these values [60]. The solution is unstable. Iodine in its aqueous solution reacts in various ways depending on the pH level. It is shown in Figure (4).

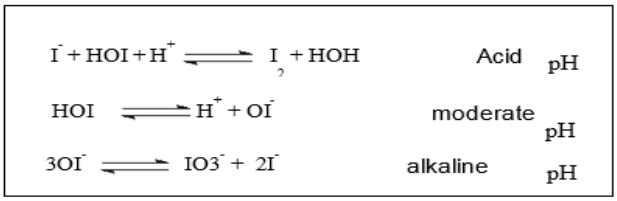
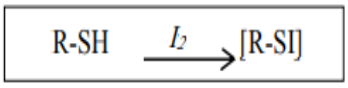


Figure 2.4. Reactions of iodine in its aqueous solution at different pH. “Brittain, H. G. (1998)”.

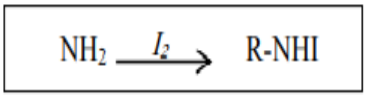
2.2.2. The Mechanism of Action of Iodine

Iodine's capacity to rapidly penetrate an organism's cell wall and attach to proteins is a crucial component in the occurrence of these effects, even if the precise mechanism underlying these effects is not yet fully understood [61, 62]. Iodine can have each of the following effects to exert its antimicrobial effect:

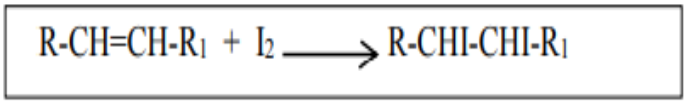
Iodine binding to proteins causes many forms of denaturation due to the oxidation of links (H-S). Because sulfur contains amino acids like cysteine that prevent the double-bridge attachment of protein chains, the production of bacterial proteins is hampered.



The iodine molecule stops OH groups from forming hydrogen bonds (due to its interaction with groups). (H-N) can occur in the amino acids lysine, histidine, and arginine as well as when it reacts with the phenolic group tyrosine to form mono- and di-iodine derivatives. Iodine atoms cause the bond network to break down. The presence of hydrogens, which are essential to the stability and structure of the resultant proteins.



Iodine interacts with lipid membrane's unsaturated fatty acids, causing shifts in the physical and chemical characteristics of the membrane that result in holes being formed in the germ cell membrane and the subsequent loss of the membrane's constituent parts.



Due to its actions on the walls, membranes, and cytoplasm of microorganisms, iodine has a broad lethal range that includes bacteria. This results in the immediate death of microorganisms upon exposure to iodine. As well as being effective against Gram-positive and Gram-negative bacteria, fungi, viruses, and protozoa, iodine has a wide range of antimicrobial activity [64,63,62,60].

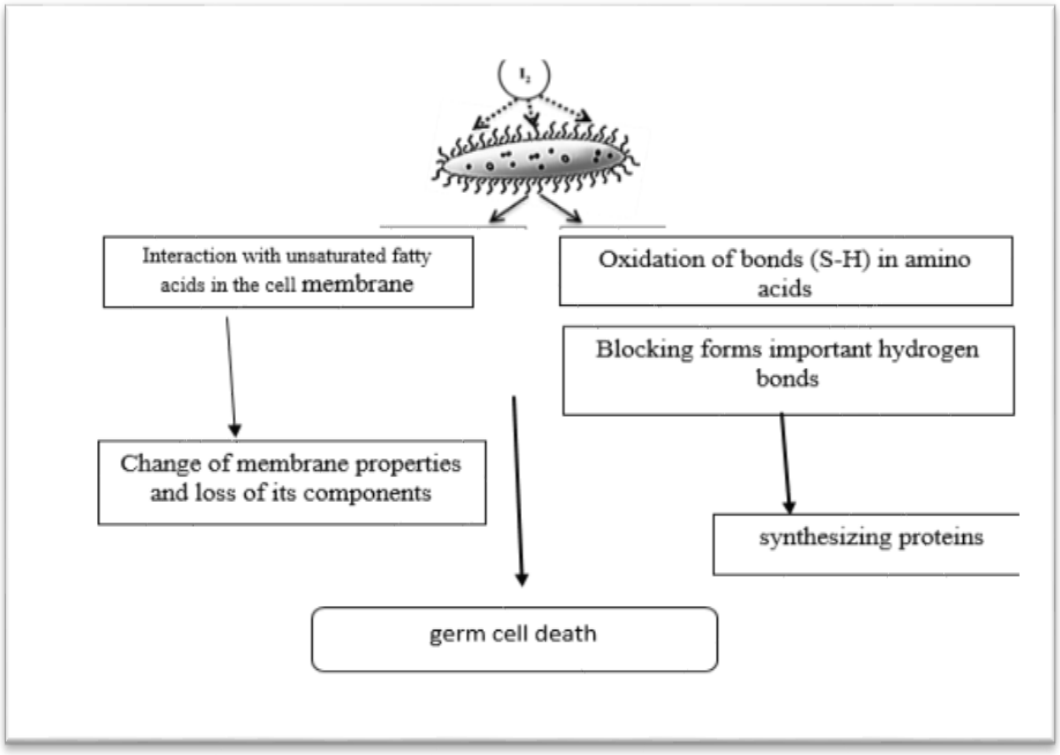


Figure 2.5. The effect of iodine on the germ cell, "(Brittain, H. G. (1998)), (Gordon, J. 1993), (Schreier, H; Erdos, G; Reimer, K; Konig, B; Konig, W; Fleischer, W. 1997), (Reimer, K; Schreier, H; Erdos, G; Konig, B; Konig, W; Fleischer, W. 1998)".

2.2.3. Historical Development of Iodine Disinfectants

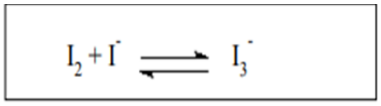
Theophrastus, an Aristotelian follower, discovered in the fourth century BC that sea plants high in iodine may be used to treat sunburn discomfort [65]. During Napoleon's expedition in Egypt between 1798 and 1801 AD, extracts obtained from iodine-rich plants, including those from this substance, were used to treat wounded troops [66].

Iodine's ability to destroy microorganisms was first discovered in 1880 AD by the scientist Davaine Casimir [67], and surgeons soon started using it to wash the skin

before an operation. Aqueous solutions have been employed in surgical procedures since the middle of the nineteenth century [68].

The use of aqueous iodine solutions as disinfectants has been connected to a number of problems, including low water solubility and stability. High local damage (skin irritation) and little chemical reactions [59]. It has been tried to combine elemental iodine with potassium iodide salts (KI), as in aqueous iodine solutions, in order to address these limitations.

A 2% concentration of Lugol solution is used, which involves the addition of ionic iodine (I^-) to increase its solubility by creating the tertiary iodine moiety (I_3^-). This creates a soluble form of iodine that follows the following equation:



Later, alcoholic iodine solutions (also known as iodine tinctures) were developed in order to more effectively solve the solubility issue. Iodine tinctures also employ potassium iodide to regulate homeostasis (I_3^-) (but it also addressed the problem of solubility by making use of a percentage of about the iodine triiod), the alcohol content of ethanol can reach up to 70% (v/w or w/w). The approach provides a better solubility of iodine and a bigger concentration of it , by preparing these solutions so that they contain 2-7% (of iodine (I_2)) with a lower concentration of potassium iodide than Lugol's solutions [69].

Later (in the early 1950s) and with the intention of solving all iodine difficulties, the so-called "Iodophors," which are polymers or large complex organic polymers containing iodine, developed. These polymers have been used to create a number of designs, the most well-known of which are these are some of the patterns:

- Polyoxamer Iodophors
- Cationic Surfactant Iodophors

- Nonionic Surfactant Iodophor
- I-PVP, sometimes referred to as Povidone-Iodine or Iodophors Polyvinylpyrrolidone.

  Later, iodoine Cadexomer, a different kind of iodine transporter, also emerged. Why has iodine been used as the most popular approach to address iodine issues since that time, (aqueous and alcoholic), when compared to earlier generations, the alterations made to this generation of iodine disinfectants did not result in a loss of antiseptic effectiveness [70, 71] among the most popular (Iodine-Cadexomer, Iodine-Povidone) use [72].

2.2.4. Cadexomer -Iodine

An iodine carrier that is created when dextrin and epichlorhydrin combine when exchanging ion groups and iodine are present. It is a helical-shaped polymer of modified starch that is soluble in water and contains 9.0% iodine when measured by dry weight. The formula for the Cadexomer compound Iodine is shown in Figure (2.6) [73,74].

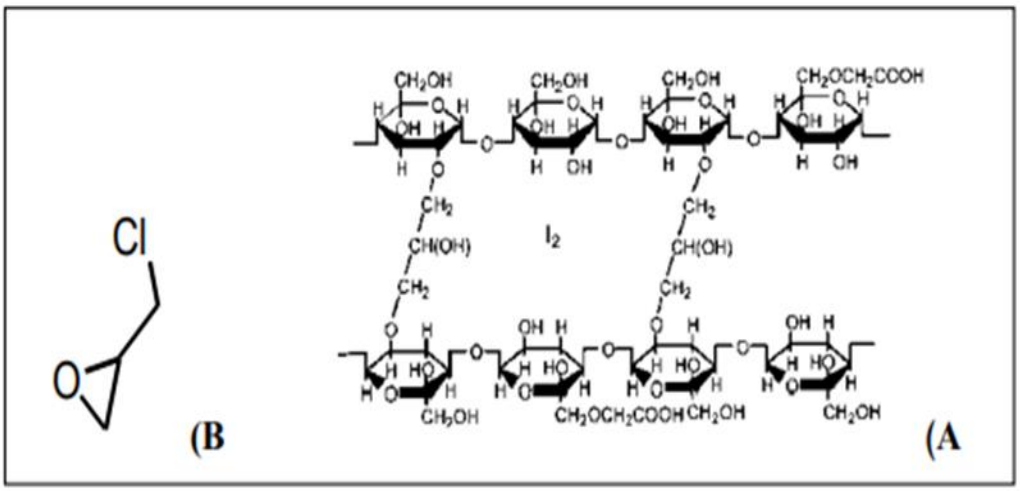


Figure 2.6. A (formula of the Iodine Cadexomer complex "Yasuhiro Noda, Kiori Fujii, S. Fujii,2009", B (formula of epichlorhydrin).

Due to its affinity for water, this compound appears as tiny spherical beads that may absorb liquid. When a wound exudates, it is already there. After that, it starts to enlarge and develop a gelatinous structure. This results in a gradual release of the iodine

molecule from the beads as their pores expand. In addition, the beads can treat wounds by removing material like dirt and bacteria [75,76,77]. And, on the other, they aid to hasten wound healing by promoting epidermal regeneration, and its downfall [78].

2.2.5. Povidone Iodine (Pvp-I)

The term "povidone-iodine complexes" refers to one of the classes of iodine carriers that is the most often employed in medicine. It is based on polymer units (PVP), which interact with iodine molecules at a certain frequency (typically 1:20) (figure 7). Many pharmaceutical products, such as lotions, sprays, ointments, creams, wound dressings, etc, contain povidone-iodine [79, 80].

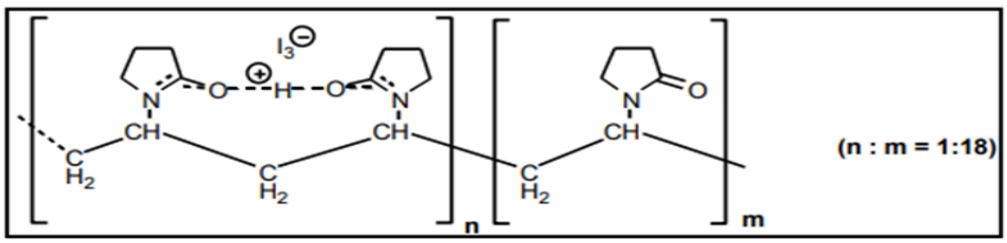


Figure 2.7. Formula of the povidone-iodine complex. “Michael W. Stewart 2022”

2.2.6. Povidone Iodine's Ability to Kill Bacteria

2.2.6.1. Spectrum effect of Povidone Iodine

The broad-spectrum disinfectant halogen derivatives destroys microorganisms [81]. [Table 1]. Compares the spectra of two widely used antiseptics, chlorhexidine (of the biguanides group) and ethyl alcohol, with those of a group of halogen derivatives called povidone-iodine (the algal group) [82,83 ,84,85].

Table 2.1. The deadly spectrum of some commonly used disinfectants.

Antimicrobial	Bacteria			Spores	Fungi	Viruses
	Gram +	Gram -	Actinobacteria			
Halogenated compound						
Povidone iodine 10%	BC +++	BC +++	BC ++	SC ++	FC +++	VC ++
Biguanides						
Chlorhexidine	BC +++	BC +++	NA	NA	FC ++	VC +
Alcohol						
Ethanol 70%	BC +	BC +	BC +	NA	FC +	VC +
Activity : Low active +, Moderate ++, High active +++						
(Bactericidal) :BC, (Fungicidal) ;FC ,(Virucidal) ;VC, NA ;(No activity), SC ;(Sporocidal)						

"(Yasuda, T; Yoshimura, S; Katsuno, Y., 1993),(Russell, AD; Day, MJ. 1993),(Elbaze, P; Ortonne, JP. 1989),(Stickler, DJ; Thomas, B. 1980)".

Studies have shown that the iodine molecule, which is a key component of povidone iodine, has antibacterial properties. Bacteria, fungus, and protozoa are all included in the high and broad spectrum. With longer exposure times [86,87, 68]. Iodine is also effective against spores and a variety of viruses, including (influenza virus) strains and the (human immunodeficiency virus) [88, 89]. Iodine's efficacy against viruses varies depending on whether or not they are encapsulated, with encapsulated viruses being more vulnerable due to the hydrophobic iodine molecule's propensity to connect with the fatty component of the viral envelope [90]. Research has demonstrated that iodine is very efficient against isolates of epidemiologically significant pathogens from wound tissues, including pathogens. As a result, the spectrum of deadly iodine encompasses both germs (G +,G -). Research has shown [91, 92, 93]. The antibiotic-resistant bacteria that iodine helps eliminate include methicillin-resistant Staphylococcus aureus (MRSA) and Mycobacterium tuberculosis. Moreover, it works well against *Pseudomonas aeruginosa* and *Escherichia coli* [82, 94].

- The Speed of the Disinfectant Effect

The type of bacteria and their capacity for resistance influence how quickly povidone-iodine takes action. For instance, the ideal povidone-iodine contact duration differs according on the strains *of Escherichia coli* and *Staphylococcus aureus* (15-80 seconds) and (between 15-120 seconds) (Table 2). [95,96]. After being applied for 30

to 60 seconds , povidone-iodine typically demonstrated high lethal effectiveness against the majority of Common bacterial species.

Table 2.2. The contact time required to kill some germs and fungi. “Kumar, S; Babu, R; Reddy, J; Uttam, A. 2011”.

Type (Number of strain)	Solicitation time (Sec)
Proteus (41)	15-120
Staphylococcus (36)	15-80
Pseudomonas (36)	15 – 900
Streptococcus (25)	15-30
Escherichia (23)	30-120
Salmonella (9)	15-60
Candida (8)	10-120
Serratia (6)	60-120
Spores-*Baccillus*; *Clostridium* (6)	2-5 Hours
Trichomonas (5)	30-60
Enterobacter (4)	60
Klebsiella (4)	60
Clostridium (4)	30-60
Shigella (3)	60
Corynebacterium (3)	60
Diplococcus (3)	60
Mycobacterium (3)	60-120
Bacillus (3)	10-30
Sarcina (2)	60
Trichophyton (2)	60
Aspergillus (2)	30
Mima (1)	60
Herella (1)	60
Edwardsiella (1)	60
Citrobacter (1)	60
Providencia (1)	60
Acinetobacter (1)	10
Epidermophyton (1)	60
Microsporum (1)	60
Pencillium (1)	30
Nocardia (1)	60

2.2.7. The Chemistry of Povidone –Iodine

Before we begin with the details of the chemistry of povidone-iodine, it is necessary to distinguish and define the following important terms:

Available Iodine; refers to iodine that has been titrated with a sodium thiosulfate standard solution. When reported as a ratio (v/w) and determined in proportion to dry weight, it typically varies between 12-9% in pharmaceutical formulations, such as in povidone-iodine skin treatments [97].

Ion Iodide; Iodine I moiety (I^-) Contributing to the formation of the tri-iodine complex (I_3^-).

Tri-iodide (I_3^-); It the impact of the interaction of molecular iodine (I_2) with the iodine monomer.

Total Iodine; It is the titrated sum of iodine with sodium thiosulfate (iodine) (available) and the mono-electron iodine.

Free Iodine; It is a component of the simple iodine with polyvinylpyrrolidone that is readily accessible. Iodine is another name for the substance that directly causes povidone [95]. Iodine's antiseptic effects. In the dialysis test, the membrane is extractable by heptane from an aqueous solution of povidone-iodine or trans-iodine [98]. As we will see later, a spectrophotometer may be used to measure the quantity of free iodine, as can chemical techniques like oxidation-reducer [99, 100]. Iodine is thought of scientists developed methods for figuring out Al-concentration Hur's in PVP-I solutions because it is primarily responsible for the antibacterial effectiveness of these solutions.

2.2.8. Povidone –Iodine Complex Behavior in Aqueous Solutions

The chemistry of iodine becomes more complex with the presence of iodine carriers capable of complexing its molecules, as it is assumed that these carriers that have

functional groups (containing oxygen in carbonyl groups) interact with iodine to form complexes (donor - acceptor), where iodine forms the acceptor part.

Molecular iodine can be prepared as a type of iodine carrier using Polyvinylpyrrolidone (PVP). Iodine also has several formulations when combined with the PVP polymer in water. Two of these formulations are highlighted in the following equations:

$$PVP\text{-}HI_3 \rightleftharpoons PVP + H^+ + I_3^-$$

$$PVP\text{-}I_2 \rightleftharpoons PVP + I_2$$

Iodine's association with the polymer molecule povidone-iodine imparts several characteristics to it. The product's excellent solubility (at room temperature) resolves the issue of skin pigmentation caused by iodine in water and traditional iodine solutions [101, 60]. Moreover, these formulations reduce the smell of iodine solutions. This is due to the fact that it does not create iodine vapor pressure, and the resulting complex is less toxic and irritating since iodine is released gradually rather than in large volumes as with conventional iodine solutions. Povidone-iodine in aqueous solution is a polymer product that has a fixed number of iodine units, where iodine and oxygen share a molecule (HI_3), due to its complicated construction including a polymer. As shown in Figure (2.8), the iodine molecule can bond directly with the polymer, while the carbonyl group can form hydrogen bonds with hydrogen atoms [60,101] .

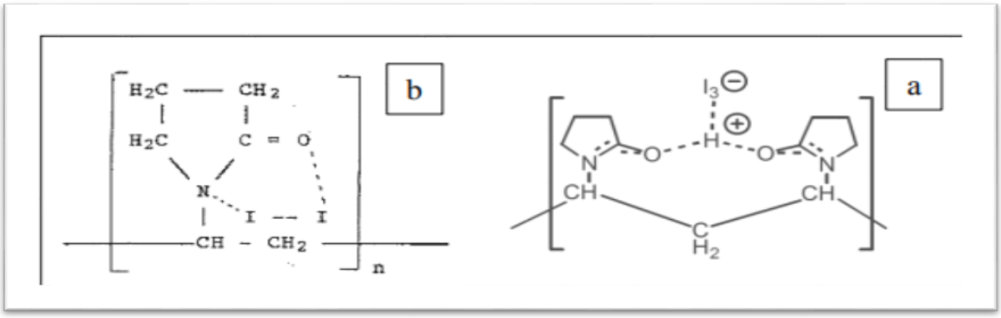


Figure 2.8. Structure of I - PVP by complex iodine type D.N.Makhayeva,G.S.Irmukhametova&V.V. Khutoryanskiy,2020".

2.2.9. Factors Affecting the Effectiveness Of Povidone –Iodine

Povidone-efficacy several factors affect iodine's effectiveness as a disinfectant, such as the concentration of the disinfectant, the duration of exposure, and the presence of organic and inorganic contaminants [52, 102].

2.2.9.1. Organic and Inorganic Pollutants

Serum, blood, pus, and faeces are all examples of organic elements that might impede a disinfectant's physical or chemical capacity to do its job. Chemical interaction is the most common type, which results in the formation of a complex disinfectant that is either ineffective or less effective. Disinfectants containing chlorine and iodine are particularly susceptible to this type of interaction with the mentioned contaminants. On the other hand, as physical barriers, organic contaminants can shield bacteria from the antiseptic agent's action [104, 103]. Studies suggest that inorganic contaminants like salt crystals can help protect life by allowing microorganisms to penetrate the crystals and shield them from purification methods [105, 106].

2.2.9.2. Physical and Chemical Factors

Temperature and pH are the two most crucial physical and chemical parameters that have a general impact on the efficacy of disinfectants.

Effect of Heat

Temperature increases often boost most disinfectant's efficacy, which might lead to a sudden surge in the antibacterial ingredient dissolves under heat, decreasing its potency [107]. In a study on the effect of changing the temperature of a solution I-PVP (2%) revealed its antiseptic effectiveness, researcher Leung came to the conclusion that increasing the temperature from 25°C to 32°C was not followed by a change in the temperature. In the antibacterial efficacy of the solution. In order to improve patient comfort during interactions without the use of local anesthetics, the study advised using I-PVP solution after elevating its temperature to 32°C [108].

The efficiency of povidone-iodine, however, was found to be correlated with water temperature (at Stability of other variables), according to a study by the scientist Heiner on the potential use of 2% iodine-povidone solution for water disinfection. After being exposed to I-PVP, the water's bacterial load dropped. A definite decrease at a temperature of 30°C compared to two degrees temperature (20 and 10°C) was seen with a specific concentration and for a certain amount of time [109]. The stability of the disinfectant solution at elevated temperatures was not the subject of either study, although a different investigation showed that the molecular iodine level decreased when the storage temperature was elevated to 52°C for 14 days [98].

The Effect of pH: The efficiency of different disinfectants is affected by pH differently; for example, some disinfectants, including quaternary ammonium compounds, glutaraldehyde, and hexamidine, become more effective as pH rises, whereas other antiseptics become less effective (such as phenols, hypochlorite salts and iodine disinfectants) [110]. Studies on povidone-iodine solutions have revealed that this antiseptic is more effective in a medium. The ideal pH range (3-6) .[95], which ranges from acidic to mild (7-5.2), [High pH values are above the limit 20]. This is connected to a decline in povidone-efficacy. Iodine's this decline is brought about by a drop in the amount of free iodine, which gives the solution its antibacterial properties. Dispense povidone-iodine solutions (as seen in).

Other iodine disinfectants are ineffective to weak in alkaline medium. As shown in Table (3) effect of the pH of an aqueous solutions of iodine's on the percentage of its various formulas [110, 59].

Table 2.3. The effect of the pH of the aqueous solution of iodine on the percentage of its different formulas. “Russell, AD. (2004)”.

Iodine %			PH
OI^-	HOI	I_2	
-	1.2	98.8	3.0
-	6.3	93.7	4.5
-	30.5	69.3	6.0
0.1	83.5	16.4	7.5
2.2	97.0	0.7	9

The previous table highlights the significance of modifying the pH of the povidone-iodine solution within the range (3-6), where it takes iodine is within this range, with a maximum of formula (I_2), with a disinfecting action, coupled with a rise in pH value. Iodine stability and disinfection solution efficacy have decreased in the aforementioned solution. Recommendation from multinational corporations to achieve the best antiseptic efficacy and the tolerance of live tissues to the applied solutions, povidone-iodine solutions with a pH of around 5 (and not more than 6) should be prepared in general [111].

2.2.9.3. Concentration of Disinfectant and Duration of Exposure

Scientist Rackur's research revealed that polymeric agglomerations in povidone-iodine complex solutions can trap free iodine, which undergoes a process called extension. As the solution's iodine concentration rises, the molecules separate, reducing their interaction with the polymeric groups and allowing more free molecular iodine to enter. This leads to a gradual increase in the amount of free molecular iodine, starting at 10% and reaching its maximum value at a ratio of 1/100, which corresponds to a concentration of 1.0%. However, the free iodine content in povidone-iodine complex solutions decreases with increasing dilation, which is different from other iodine formulations that behave normally during all phases of extension (as illustrated in Figure 2.9). Nonetheless, the povidone-iodine complex behavior becomes normal again in extensions greater than 1/100 [60,113,114] .

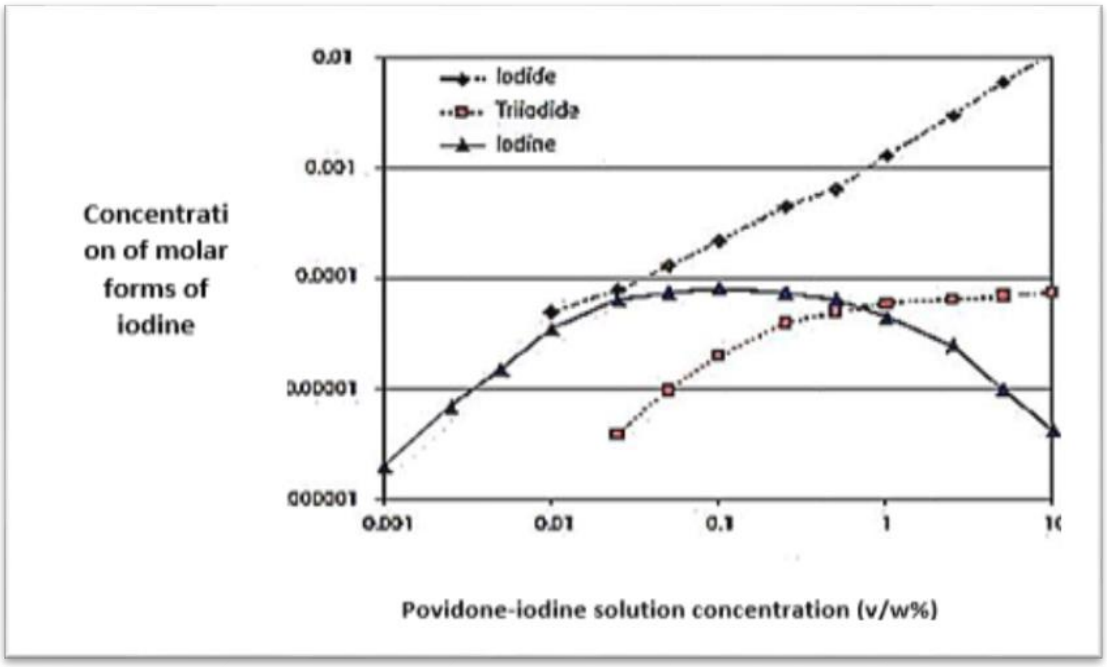


Figure 2.9. The correlation between the concentration of iodine with various formulas and the concentration of povidone-iodine solution (64). “Rackur, H. 1985”.

Berkelman and Haley's findings [115,116] are consistent with previous research. Two separate in vitro investigations have demonstrated that povidone-iodine solutions with a ratio of up to 1/100 can eradicate all bacterial colonies in a shorter amount of time than the original (10%) solution. The lethal efficacy of povidone-iodine is closely associated with the rise in free iodine concentrations, as indicated by the combination of test findings. Figure 10 shows the relationship between uncomplicated iodine concentration and deadly effectiveness after 15 seconds of exposure to I-PVP solution with varying concentrations [113] .

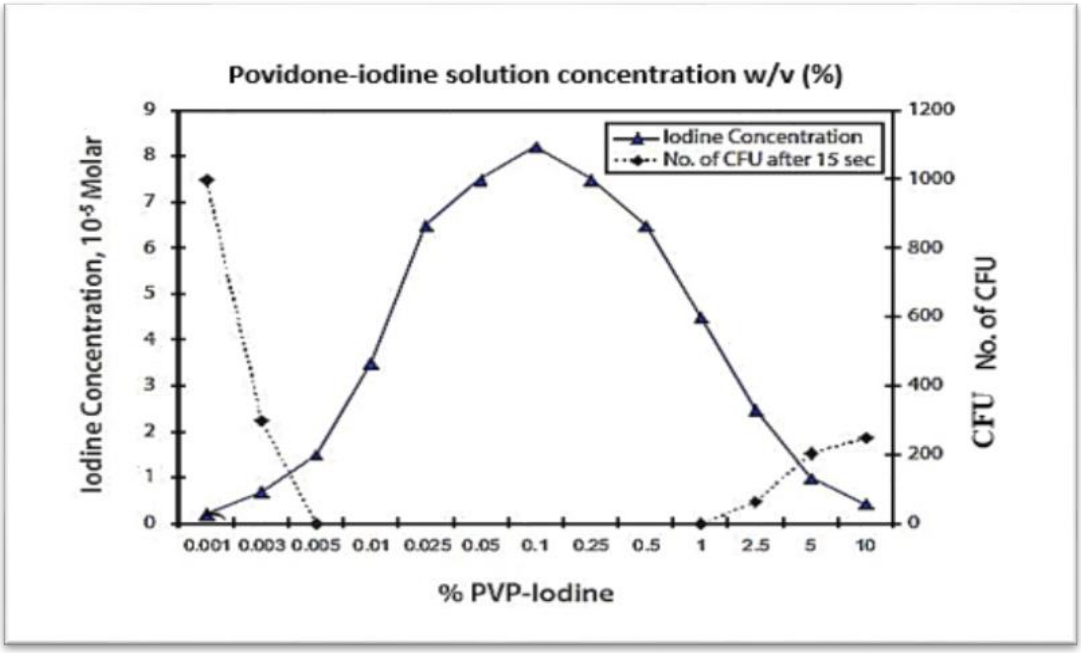


Figure 2.10. Relationship between uncomplicated iodine concentration and lethal efficacy after 15 seconds of exposure to a solution [64]. Different concentrations of PVP-I. “Rackur, H. 1985”.

The findings of in vivo investigations on organic matter differ from those of in vitro tests on glass. Ferguson, for example, tested the efficiency of a 5% povidone-iodine solution to a 1% diluted solution for disinfection before cataract surgery and discovered that the 5% solution was more bactericidal. Similarly, Bing also compared the effectiveness of three concentrations of povidone solution (10%, 5%, and 1%) for lowering pregnancy and bacteria in the conjunctiva when combined with topical iodine and levofloxacin (3.0%) before cataract surgery, and found that the 10% solution performed better than the other two options. The duration of exposure and contact with the disinfectant can also affect the time of exposure to a disinfectant, as reaching the necessary disinfectant effectiveness is crucial for lowering infection rates in healthcare facilities like hospitals. The pH of the solutions, the quantity of free iodine, and the rate of bactericidal activity against Staphylococcus aureus and Escherichia coli were all measured using the disc diffusion technique. It is essential to ensure the stability of the control sample throughout the duration of validity for these preparations. It was discovered that a high level of free iodine increases the rate and inhibition diameter of bactericidal activity.

2.2.10. General Information

Discovered iodine carriers (I_2 iodophors) to study has suggested iodine-related issues, including insoluble in water, inadequate. Its alcohol solutions are stable, less unpleasant, and just as effective as iodine while also being safer. The iodine's binding Instead of using high iodine concentrations, another chemical minimizes toxicity. Iodine-carrier formulations are frequently used in all medical specialties to cleanse the skin. Surgical techniques that are invasive. Povidone-iodine, often known as I-PVP, is one of the most well-known of these preparations. It contains iodine in a combination with polyvinyl acetate.

In addition to killing Gram-positive and Gram-negative bacteria, spores, fungus, protozoa, and viruses, povidone-iodine possesses many other antiseptic qualities. The non-titrated part associated with polyvinylpyrrolidone with iodine free is known as sodium thiosulfate and contains the titrated iodine molecules. The amount of free iodine is between.The effectiveness of the antiseptic is immediately connected to the antiseptic solution. The percentage of action of povidone solutions varies depending on the For several factors, such as the type of bacteria studied, its resistance on the one hand, and the quality of the solution preparation.

On the other hand, Iodine Antiseptic and free iodine concentration. Furthermore, the presence of organic and inorganic contaminants, pH, the presence of some excipients that may interact with iodine and lower its concentration, and a variety of other variables all influence the preparation of a stable disinfectant solution. One of the most popular forms of disinfectants used in hospitals, for home usage, and for public health purposes is povidone-iodine. Cleaning of mucous membranes and wounds. Numerous forms Povidone-iodine is available in pharmaceutical grades as well as aqueous and alcoholic solutions, lotions, gels, ointments, creams, and sprays. One of the most important factors that must be studied to know the effect of povidone iodine, by measuring and monitoring several important variables over a period of time.

Several factors can be considered when evaluating the impact of povidone-iodine as an antiseptic agent, including:

- pH suitability: The pH of the antiseptic solution should be appropriate for topical application to living tissue and for achieving maximum effectiveness.
- Volumetric techniques can be used to assess the concentration of free iodine responsible for the antibacterial action.
- In vitro bactericidal activity test: The effectiveness of the antiseptic can be evaluated using methods such as the disc diffusion and tube extension techniques, which measure the severity and speed of bacterial killing.
- Stability study: The shelf life of povidone-iodine preparations can be assessed by conducting a stability study to evaluate their efficacy over time.

Overall, these factors can be used to determine the validity or effectiveness of povidone-iodine as an antiseptic agent

2.2.11. Classification of Disinfectants According to Their Effectiveness Into Three Levels (High-Medium-Low)

2.2.11.1. Sterilizers or Disinfectants with High Capacity

Hospital disinfectants are referred to as disinfectants that possess a strong ability to eliminate three distinct types of microorganisms, namely *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Staphylococcus aureus*, among other bacteria. This type of disinfectant is capable of spore activity and can effectively eradicate the aforementioned bacteria when subjected to glutar for at least 20 minutes, or when treated with 2% aldehyde or a water oxygen solution ranging from 30% to 6% [119].

2.2.11.2. Medium Grade Disinfectants

They are disinfectants that can stop the growth of or kill small non-enveloped viruses, most fungi, and bacteria. It is important to note that medium-class disinfectants, such as each of povidone iodine, alcohol, and oxygenated water at a concentration of 6-3% [119]., frequently have a limited effect on spores.

2.2.11.3. Low Grade Disinfectants

Narrow spectrum compounds are categorized as low grade disinfectants. Examples of disinfectants in this category include quaternary ammonium salts, phenols, and detergents (which work well as surface cleansers). Frequently able to eradicate most bacteria, certain fungi, and enveloped viruses without harming coated non-viruses and tuberculosis bacilli [119].

2.2.12. What is the Mechanism of Povidone-Iodine

Similar to iodine, povidone-iodine works by inflicting irreparable cell damage through a similar process. The following can be used to sum up how it works (Figure 11) .[120]:

- It interacts enzymes in the cell wall's respiratory chain.
- It interacts with the cell wall's amino acids.
- It breaks down the protein's essential tertiary structure.
- Which results in the germ cell being harmed and dying.

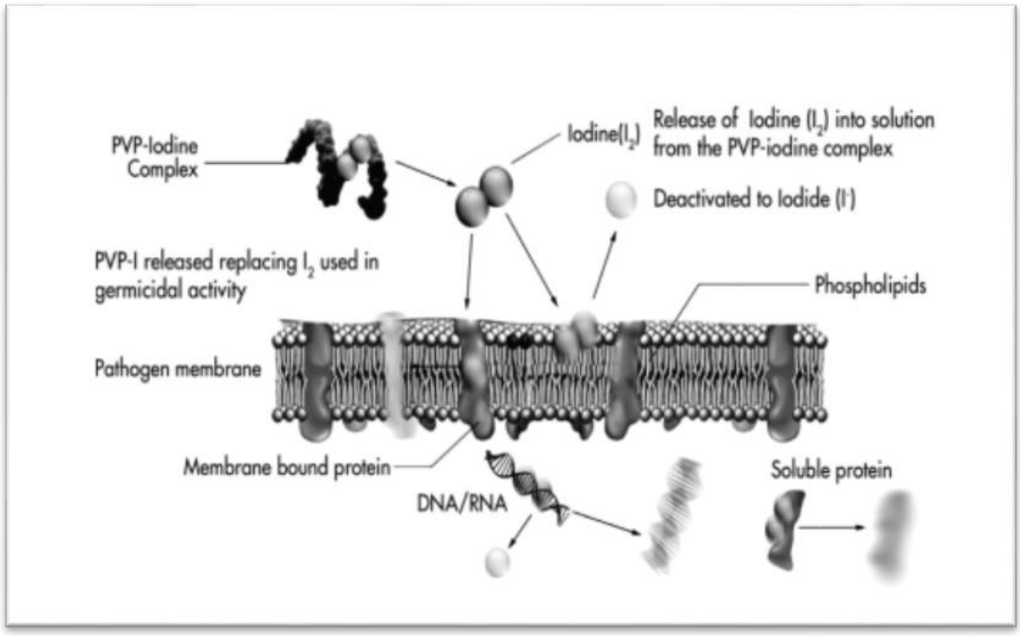


Figure 2.11. Povidone-iodine mechanism of action. "Paul Lorenz Bigliardi, Syed Abdul Latiff Alsagoff Hossam Yehia El-Kafrawi, Martin Anthony Villa, 2017"

2.2.13. Povidone-Iodine Applications

- Skin sanitizer
- Surgeon's hand sanitizer
- Wound antiseptic
- Antiseptic in case of minor injury
- Burn treatment
- Ulcer treatment
- Female antiseptic
- Antiseptic for teeth and mouth
- Veterinary antiseptic

2.2.14. Povidone-Iodine Contraindications

Hypothyroid patients, those with known or suspected iodine sensitivity, expectant mothers, nursing infants, and newborns all need medical care before receiving iodine preparations. Hypothyroid patients, those with known or suspected iodine sensitivity, expectant mothers, nursing infants, and newborns all need medical care before receiving iodine preparations. Until six months of age. I-PVP usage for a long time may result in mild hyperactivity. Long-term use for those with impaired thyroid function is not advised. However, some studies observed thyroid function throughout I-PVP clinical trials. No modifications were made [122,121]. To prevent toxicity or the potential danger of problems associated to the glands. When treating open wounds that need time-consuming therapy or in children with severe burns, thyroid and iodine preparations should be used cautiously. In patients with chronic kidney disease, iodine preparations should also be avoided [64].

2.2.15. Previous Studies on Povidone-Iodine Preparations

To ensure that infectious microorganisms are not transmitted to patients, sanitation and sterilization are required. The primary sterilising agents employed in healthcare institutions are physical (steam under pressure, dry heat, ethylene oxide gas) or aqueous substances. Sterilization is the technique of eradicating or killing all

microorganism life [107]. The method that destroys a lot of organisms is described as antiseptic and disinfectant. Except for bacterial spores, all or any harmful germs. Things are typically disinfected in healthcare environments using liquid chemicals or moist pasteurization. Each element that influences efficacy, the efficacy of this mechanism may be diminished or eliminated by disinfection [107]. Cleaning is the process of mechanically removing visible filth (such as organic and inorganic elements) using water, detergents, and enzymatic substances. Prior to disinfection and sterilization, cleaning is crucial and vital because organic and inorganic debris might interfere with the disinfection process and reduce its efficacy [107]. In health-care programmes, a variety of disinfectants are utilised, either singly or in combination (for example, peroxyhydrogen and peracetic acid). Alcohol, chlorine and its derivatives, formaldehyde, glutaraldehyde, hydrogen peroxide, povidone-iodine, peracetic acid, phenol, and quaternary ammonium compounds are all disinfectants. These unique products of these compounds are used in commercial formulae. It must be approved by either the Food and Drug Administration (FDA) or the Environmental Protection Agency (EPA). Typically, a product is created with a particular function in mind and is intended for that function alone. Users must carefully study the instruction manual to choose the appropriate product for their intended application and verify that it is utilised properly [107].

Povidone-Iodine: Health experts have traditionally utilized iodine fluids or tinctures primarily as an antiseptic for the skin or tissues. Iodine and a soluble substance or carrier are combined to form iodophors, which act as an ongoing iodine repository. The aqueous solution contains trace levels of free iodine. The most well-known and extensively used povidone iodine is a polyvinylpyrrolidone-iodine combination. This product and other iodophors preserve iodine's bactericidal activity while being uncontaminated and reasonably devoid of toxicity and irritation.

Povidone-iodine uses: Povidone-iodine is used as an antiseptic for the skin, surgical instruments, burns, wounds, and as an oral and feminine antiseptic. In addition to its past applications, povidone-iodine has been used to clean medical tools like thermometers, endoscopes, and hydrotherapy tanks. Due to concentration differences, povidone-iodine is not acceptable for use as a disinfectant on hard surfaces.

Previous Studies: Numerous investigations have been done to assess the efficacy of povidone-iodine formulations, examining their Bacteriostatic spectrum, contrasting them with other disinfectants, determining their speed of action, and examining their response to extension. These research used the widely used, adaptable, broad-spectrum, and low-bacterial-resistance povidone-iodine. It's affordable and simple to use [123, 124, 125, 126, 127].

Antiseptics including povidone-iodine, octenidine *dihydrochloride*, and ethacridine lactate were tested for their ability to inhibit biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus aureus* using a unique biofilm-oriented antiseptics assay [130].

In a recent investigation, the research team led by Bing Li compared the effectiveness of different concentrations (1%, 5%, and 10%) of povidone-iodine in reducing bacterial load in the conjunctiva before cataract surgery when used in conjunction with topical levofloxacin (3.0%). The study included 271 people who were separated into three groups and given varied quantities of povidone-iodine before swabbing to detect bacterial burden. The results illustrated that the 10% concentration of povidone-iodine was more efficient than the lower values in lowering bacterial burden. The study also found a positive correlation between the increase in free iodine content and the increase in antibacterial activity [128].

Vorherr H and colleagues conducted a study on non-pregnant women to examine the effects of povidone-iodine vaginal washing (Betadine) on serum thyroxine, total iodine, iodine protein-bound, inorganic iodine, and iodine levels. The study found that serum iodine levels increased significantly after just 15 minutes of application and continued to rise considerably at 30, 45, and 60 minutes after washing. During a brief period of observation, total serum iodine and inorganic iodine levels increased up to fivefold and 15 fold, correspondingly, while thyroxine levels remained unchanged. However, the excessive iodine load caused by povidone-iodine could harm the thyroid gland of the fetus and newborns, leading to a goiter caused by iodine-induced hyperthyroidism. Therefore, povidone-iodine should not be used to treat vaginitis in

pregnant women, and caution should be exercised in frequent use to avoid potential harm [129].

2.3. ANTIBIOTIC RESISTANCE

The development of antibiotics in the middle of the twentieth century revolutionised the way that bacterial infections were handled medically. Many lethal infections became treatable, and antimicrobial drugs (such as antibiotics and their counterparts for viruses, fungi, and parasites) have helped millions of people. Antibiotics are now necessary for treating bacterial infections as well as providing preventative treatment for high-risk patients undergoing operations such as organ transplants, cancer chemotherapy, and prenatal care. Neverthless, the rapid emergence and spread of bacteria resistant to antibiotics has put these medical advancements in grave danger (source: www.earto.eu).

In 1943, the widespread use of penicillin greatly reduced bacterial infections, as well as accompanying disease and mortality. However, bacteria resistant to penicillin began to emerge after only four years. Antibiotics of various sorts were developed in response by pharmaceutical corporations. Antibiotics, after being widely used for more than 50 years, are no longer as effective as they once were due to the evolution of resistance against practically all major bacterial illnesses worldwide (Johnson, 2006). Moreover, even though pharmaceutical firms have developed many new antibiotics in the past three decades, microorganisms have become more resistant to these drugs. Bacteria frequently have the genetic potential to develop and disseminate resistance to medications that are therapeutically beneficial [144].

PART 3

MATERIALS & METHOD

3.1. PROCUREMENT OF BACTERIAL STRAINS

In this study, 100 bacteria were tested, which were collected from patients in various departments of Karabük Training and Research Hospital between April 6th, 2022 and June 22nd, 2022. These specimens included urine, stool, nose, throat, endotracheal aspirate, blood, sputum, wound, abscess, and pleura samples, and were tested using BD Phoenix Device cultivations in the Microbiology Laboratory of Karabük Training and Research Hospital. Gram-negative and gram-positive bacteria resistant to the majority of the antibiotics listed and evaluated in the microbiology laboratory were passaged and delivered to the Department of Medical Microbiology research laboratory at Karabük University Faculty of Medicine. The bacteria produced in a single colony were used in the antibacterial effect tests of lugol solution. It was found that the isolated bacteria were: *"Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus cohnii ssp cohnii, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Enterococcus faecium, Acinetobacter baumannii, Streptococcus pyogenes, Pseudomonas aeruginosa, Morganella morganii, Corynebacterium striatum, Corynebacterium amycolatum, Providencia rettgeri, Cedecea davisae, Achromobacter species, Myroides odoratimimus, Saccharomyces cerevisiae, Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei."*

3.2. LUGOL SOLUTION

The Medical Microbiology lab of the Faculty of Medicine at Karabuk University developed the 2% Lugol solution used in the studies. The volume was increased to 100

ml by dissolving 2 grams of iodine and 4 grams of potassium iodide in a little amount of distilled water.

3.3. CULTURE MEDIA AND CHEMICAL

Muller Hinton Broth (MHB) (Biolife) and Muller Hinton Agar (MHA) (Biolife) were used in the testing procedure to determine the effect of 2% Lugol solution on microorganisms isolated from clinical samples. The preparation of the media was carried out as recommended by the manufacturer (Biolife).

3.4. ANTIBIOTICS DRUGS

Antibiotic susceptibilities of bacterial strains obtained from patient samples were also tested in the Microbiology Laboratory of Karabuk Training and Research Hospital. For this purpose, Vancomycin, Ceftriaxone, Ceftazidime, Tetracyclines, Ciprofloxacin, Trimethoprim/Sulfamthoxazole, Amikacin, Tigecycline, Gentamicin, Ertapenem, imipenem, Meropenem, Ampicillin, Levofloxacin, Moxifloxacin, Tobramycin, Colistin, Teicoplanin, Cefazolin, Cefixime, Cefoxitin, Streptomycin, Nitrofurantoin, Amoxicillin-Clavulanate, Piperacillin Tazobactam, Fosfomycin, Cefuroxime, Cefepime, Ampicillin Sulbactam, Daptomycin, Oxacillin, Clindomycin, Erythromycin, Fusidic acid, Linezolid, PencillinG, and Rifampicin were used.

3.5. METHODS

In order to make 100 ml of Lugol Solution (2%), 2 grams of iodine and 4 grams of potassium iodide must be dissolved in a little amount of distilled water. Bacterial strains were isolated from clinical samples sent for diagnosis to the Microbiology Diagnostic Laboratory of Karabük University Education and Research Hospital. Gram-positive and Gram-negative bacterial isolates resistant to 70% of the antibiotics used in susceptibility testing were identified and single colonies were obtained by passage to Mueller Hinton Agar (MHA). These single colonies were then separately passaged to Mueller Hinton Broth (MHB).

For the test culture, MHB was injected with MHA colonies and incubated overnight at 37 degrees Celsius. The culture was then adjusted to McFarland 0.5 using MHB for the preparation of test dilutions and antibiotic susceptibility tests. Muller Hinton Broth (MHB) and Muller Hinton Agar (MHA) were used in the preparation of test dilutions and antibiotic susceptibility tests.

In order to conduct the Lugol sensitivity test, 1 ml of the culture that had been adjusted to McFarland 0.5 was poured into each of seven separate tubes. Then, 32 µl of 2% Lugol solution was added to tube 1, 64 µl to tube 2, 128 µl to tube 3, 256 µl to tube 4, 512 µl to tube 5, and 1024 µl to tube 6. Tube 7 was used as a control and no Lugol solution was added. Overnight, the containers were incubated at 37°C in an incubator. Following incubation, samples from all tubes, including the control tube, were streaked onto MHA plates. After incubating the plates at 37°C, growth was identified in the tube samples.

The sensitivity of Lugol's solution was first tested in standard bacteria (*Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213)) and after it was found to be effective. Both Gram-positive and Gram-negative bacteria seen in clinical samples were used in the tests.

3.6. STATISTICAL ANALIYSIS

Statistical Packages for the Social Sciences (SPSS) (2016) was used to analyse the impact of variables on the study's variables. In this study, the Chi-square test was employed to find a considerable difference between percentages (0.05 and 0.01 probability).

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

χ^2: Chi-square , Σ: Summation , O: Observed No. , E: Expected No.

Note: 0.05 *Significant ($P \leq 0.05$).

0.01 **Highly Significant ($P \leq 0.01$). NS insignificant.

PART 4

RESULT

We conducted six sequential dilutions of 2% lugol solution (32µl, 64µl, 128µl, 256µl, 512µl, 1024µl) and evaluated their effectiveness on standard bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*), that points out to both Gram-positive and Gram-negative bacteria and are known to be among the most infectious pathogenic bacteria in humans. We recorded the susceptibility results, which assess the inhibition of bacterial growth. We discovered that a concentration of 32µl of 2% lugol solution did not inhibit bacterial growth, while a concentration of 64µl inhibited 33.33% of bacterial growth. The inhibition rate increased to 66.66% at a concentration of 128µl, and the solution completely inhibited bacterial growth at 256µl. Concentrations of 512µl and 1024µl also entirely inhibited bacterial growth.

We also tested 2% lugol solution on 100 isolated bacterial samples and observed that the inhibition of bacterial growth steadily increased as Lugol's dilution increased. Specifically, a concentration of 32µl inhibited 9% of bacterial growth, while a concentration of 64µl inhibited 28% of bacterial growth. Concentrations of 128µl, 256µl, 512µl, and 1024µl inhibited 44%, 82%, 92%, and 96% of bacterial growth, respectively.

We have observed the following antibiotic resistance patterns in various strains of Staphylococcus bacteria. *Staphylococcus epidermidis*; resistant to oxacillin, clindamycin, erythromycin, fusidic acid, ciprofloxacin, levofloxacin, moxifloxacin, but susceptible to lugol solution, *Staphylococcus haemolyticus*; resistant to gentamicin, oxacillin, trimethoprim/sulfamethoxazole, erythromycin, fusidic acid, ciprofloxacin, levofloxacin, moxifloxacin and susceptible to lugol solution, *Staphylococcus hominis*; resistant to gentamicin, trimethoprim/sulfamethoxazole, clindamycin, erythromycin, fusidic acid, ciprofloxacin, levofloxacin, moxifloxacin,

tetracycline, cefoxitin and susceptible to lugol solution, *Staphylococcus aureus*; resistant to amikacin, oxacillin, trimethoprim/sulfamethoxazole, erythromycin, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline, penicillin G and susceptible to lugol solution, *Staphylococcus cohnii ssp cohnii*; resistant to amikacin, gentamicin, oxacillin, trimethoprim/sulfamethoxazole, clindamycin, erythromycin, fusidic acid, ciprofloxacin, levofloxacin, moxifloxacin, fosfomycin and susceptible to lugol solution. Sensitivity to lugol solution was detected in all *Staphylococcus* species, no resistance was detected.

We observed that *Escherichia coli* exhibited resistance to multiple antibiotics including trimethoprim/sulfamethoxazole, tobramycin, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, and amoxicillin-clavulanate. Additionally, it displayed resistance to lugol solution at concentrations of 32µL, 64µL, and 128µL. This resistance pattern was consistent in various combinations of antibiotics and lugol solution concentrations tested.

We observed multiple strains of *Klebsiella pneumoniae* exhibiting antibiotic resistance. The first strain showed resistance to trimethoprim/sulfamethoxazole, tobramycin, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, and fosfomycin. Additionally, it demonstrated resistance to lugol solution at concentrations of 32µL and 64µL. The second strain of *Klebsiella pneumoniae* was resistant to amikacin, gentamicin, ertapenem, imipenem, meropenem, cefazolin, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, cefepime, ampicillin-sulbactam. It also exhibited resistance to lugol solution at concentrations of 32µL, 64µL, and 128µL. Furthermore, another strain of *Klebsiella pneumoniae* displayed resistance to gentamicin, trimethoprim/sulfamethoxazole, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam. It was also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL. The fourth strain of *Klebsiella pneumoniae* showed resistance to amikacin, gentamicin, ertapenem, imipenem, meropenem, cefazolin, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, cefepime, ampicillin-sulbactam. It exhibited resistance to lugol solution at a concentration of 32µL. The fifth strain of

Klebsiella pneumoniae demonstrated resistance to gentamicin, ciprofloxacin, tobramycin, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate. Additionally, it displayed resistance to lugol solution at concentrations of 32µL, 64µL, and 128µL. The sixth strain of *Klebsiella pneumoniae* was resistant to trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, tobramycin, ertapenem, imipenem, meropenem, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam. Moreover, it showed resistance to lugol solution at concentrations of 32µL, 64µL, and 128µL. The seventh strain of *Klebsiella pneumoniae* exhibited resistance to trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, cefazolin, cefixime, ceftazidime, ceftriaxone, ampicillin, fosfomycin. It also displayed resistance to lugol solution at concentrations of 32µL, 64µL, and 128µ.

Based on our observations, we have identified the following resistance patterns in *Enterococcus faecium*. We observed *Enterococcus faecium* exhibiting the following resistances; antibiotic resistance; gentamicin, ciprofloxacin, levofloxacin, ampicillin, amoxicillin-clavulanate, streptomycin and lugol solution resistance; 32µL, 32µL and 64µL.

We observed the following resistance profiles in *Acinetobacter baumannii* strains; antibiotic resistance; amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, imipenem, meropenem and lugol solution resistance; 32µL, 64µL, 128µL. We also observed the following resistance patterns in *Acinetobacter baumannii/calco.cplx*: antibiotic resistance; amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, imipenem, meropenem and lugol solution resistance; 32µL, 64µL. In addition, we observed the following resistance profiles in *Acinetobacter baumannii*: antibiotic resistance; ciprofloxacin, levofloxacin, imipenem, meropenem and lugol solution resistance: 32µL, 64µL, 128µL, 256µL, 512µL. Furthermore, *Acinetobacter baumannii* displayed the following resistance characteristics: antibiotic resistance; amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, imipenem, meropenem and lugol solution resistance; 32µL, 64µL, 128µL. Moreover, *Acinetobacter baumannii* exhibited the following resistance patterns: antibiotic resistance; amikacin,

gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, imipenem, meropenem and lugol solution resistance: 32µL, 64µL, 128µL, 256µL. Lastly, *Acinetobacter baumannii* strains demonstrated the following resistance features: antibiotic resistance; amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, imipenem, meropenem, colistin and lugol solution resistance; 32µL, 64µL, 128µL.

Based on our observations, we have identified the following resistance patterns in *Candida* species. We observed the following resistance profiles in *Candida glabrata*: antibiotic resistance; oxacillin, clindamycin, erythromycin, fusidic acid, ciprofloxacin, levofloxacin, moxifloxacin and lugol solution resistance: 32µL, 64µL. Additionally, we observed the following resistance pattern in *Candida albicans*: antibiotic resistance; none and lugol solution resistance: 32µL, 64µL. Moreover, *Candida tropicalis* exhibited the following resistance profile: antibiotic resistance; none and lugol solution resistance: 32µL, 64µL. We also observed the following resistance characteristics in *Candida krusei*: antibiotic resistance; none and lugol solution resistance; 32µL. Furthermore, *Candida Spp*. (unspecified Candida species) displayed the following resistance pattern: antibiotic resistance; none and lugol solution resistance; 32µL and *Candida tropicalis* demonstrated the following resistance profile: antibiotic resistance; none and lugol solution resistance: 32µL, 64µL, 128µL.

We observed that *Morgarella mongarii* is resistant to the following antibiotics; gentamicin, ciprofloxacin, levofloxacin, imipenem, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, and colistin. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL.

We observed that *Corynebacterium striatum* is resistant to the following antibiotics; clindamycin, ciprofloxacin, rifampin, and penicillin G. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL. Additionally, *Corynebacterium amycalatum* is resistant to clindamycin, ciprofloxacin, tetracycline, rifampin, and penicillin G. It is also resistant to lugol solution at a concentration of 32µL.

We observed that *Streptococcus pyogenes* is resistant to the following antibiotics; daptomycin, vancomycin, clindamycin, erythromycin, levofloxacin, moxifloxacin, tetracycline, and penicillin G. However, it does not show resistance to lugol solution.

We observed that *Pseudomonas aeruginosa* is resistant to the following antibiotics; amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, ertapenem, imipenem, meropenem, cefazolin, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, cefepime, and ampicillin-sulbactam. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL. Additionally, there is another strain of *Pseudomonas aeruginosa* that is resistant to the same antibiotics except for trimethoprim/sulfamethoxazole. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL.

We observed that *Saccharomyces cerevisiae* shows no resistance to antibiotics and lugol solution. Additionally, there is another strain of *Saccharomyces cerevisiae* that is resistant to antibiotics but not lugol solution at a concentration of 32µL.

We observed that *Cedecea davisae* is resistant to the following antibiotics: amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, tobramycin, cefixime, ceftazidime, ceftriaxone, and fosfomycin. It is also resistant to lugol solution at concentrations of 32µL, 64µL, 128µL, and 256µL.

We observed that *Providencia rettgeri* is resistant to the following antibiotics: amikacin, gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, tobramycin, ertapenem, imipenem, meropenem, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, and fosfomycin. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL.

We observed that *Achromobacter* species is resistant to the following antibiotics: amikacin, ciprofloxacin, levofloxacin, ceftazidime, piperacillin-tazobactam, and cefepime. It is also resistant to lugol solution at a concentration of 32µL.

We observed that there is one strain of *Providencia rettgeri* that is resistant to the same antibiotics and lugol solution concentrations as mentioned in the previous observation. Additionally, there is another strain of *Providencia rettgeri* that is resistant to gentamicin, trimethoprim/sulfamethoxazole, ciprofloxacin, levofloxacin, tobramycin, ertapenem, imipenem, meropenem, cefixime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, and fosfomycin. It is also resistant to lugol solution at concentrations of 32µL, 64µL, and 128µL.

We observed that *Myroides odoratus/odoratimimus* is resistant to the following antibiotics: amikacin, ciprofloxacin, levofloxacin, tobramycin, imipenem, meropenem, ceftazidime, and piperacillin-tazobactam. It is resistant to lugol solution at a concentration of 32µL. Additionally, there is another strain of *Myroides odoratus/odoratimimus* that is resistant to the same antibiotics except for cefepime. It is also resistant to lugol solution at concentrations of 32µL and 64µL.

The effectiveness of Lugol's iodine solution against Gram-positive bacteria has been demonstrated. We noted that the percentage of susceptible yeast to lugol solution was 70.82%, while the percentage of susceptible Gram-positive bacteria to lugol solution was 76.76%, and the percentage of susceptible Gram-negative bacteria was 46.42%.

Table 4.1. Standard Strain

	%2 Lugol's Solution µL(mgr)					
Standard Strain	32 (1,6192)	64 (3,2384)	128 (6,4768)	256 (12,9536)	512 (25,9072)	1024 (51,8144)
Escherichia coli (ATCC25922)*	R	R	S	S	S	S
Pseudomonas aeruginosa (ATCC 27853)*	R	R	R	S	S	S
Staphylococcus aureus (ATCC 29213)*	R	S	S	S	S	S
R: No. (%) S: No. (%)	3 (100%) 0 (0.0%)	2 (66.67%) 1 (33.33%)	1 (33.33%) 2 (66.67%)	0 (0.00%) 3 (100%)	0 (0.00%) 3 (100%)	0 (0.00%) 3 (100%)
Chi-Square (χ^2) P-value	11.38 ** 0.0001	7.91 ** 0.0075	7.91 ** 0.0075	11.38 ** 0.0001	11.38 ** 0.0001	11.38 ** 0.0001
** ($P \leq 0.01$).						

*6 hours of culture (after adjustment to McFarland 0.5) 5µl of Standard Strain added to each dilution (R): There is growth,(Resistance) not susceptible, (S): No growth, susceptible.

Table 4.2. Clinical samples

No	Hospital Protocol No	Bacteria isolated	Gram stain feature	%2 Lugol's Solution Effect µL(mgr)						
				32 (1,6192)	64 (3,2384)	128 (6,4768)	256 (12,9536)	512 (25,9072)	1024 (51,8144)	P-value
1	55749362	*Staphylococcus epidermidis*	Positive	S	S	S	S	S	S	0.0001 **
2	55761227	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
3	55756442	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
4	55755243	*Staphylococcus haemolyticus*	Positive	R	S	S	S	S	S	0.0001 **
5	55749029	*Klebsiella pneumoniae*	Negative	R	R	S	S	S	S	0.0074 **
6	55751394	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
7	55744478	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
8	55749240	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
9	55753724	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
10	55751244	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
11	55761202	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
12	55761625	*Enterococcus faecium*	Positive	R	S	S	S	S	S	0.0001 **
13	55756645	*Staphylococcus hominis*	Positive	S	S	S	S	S	S	0.0001 **
14	55770099	*Staphylococcus haemolyticus*	Positive	R	S	S	S	S	S	0.0001 **
15	55776810	*Escherichia coli*	Negative	R	R	S	S	S	S	0.0074 **
16	55776807	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
17	55763189	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
18	55771134	*Staphylococcus hominis*	Positive	S	S	S	S	S	S	0.0001 **
19	55769562	*Acinetobacter baumannii*	Negative	R	R	R	S	S	S	1.00 NS
20	55776198	*Staphylococcus haemolyticus*	Positive	R	R	R	S	S	S	1.00 NS
21	55787180	*Klebsiella pneumoniae*	Negative	R	R	S	S	S	S	0.0074 **
22	55803235	*Staphylococcus haemolyticus*	Positive	R	S	S	S	S	S	0.0001 **
23	55767230	*Staphylococcus epidermidis*	Positive	R	S	S	S	S	S	0.0001 **
24	55766308	*Staphylococcus epidermidis*	Positive	S	S	S	S	S	S	0.0001 **
25	55764501	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
R: No. (%) S: No. (%)				21 (84%) 4 (16%)	16 (64%) 9 (36%)	13 (52%) 12(48%)	0 (0%) 25 (100%)	0 (0%) 25 (100%)	0 (0%) 25 (100%)	--
Chi-Square (χ^2) P-value				14.38 ** 0.0001	9.83 ** 0.0072	0.793 NS 0.662	14.92 ** 0.0001	14.92 ** 0.0001	14.92 ** 0.0001	--
** (P≤0.01), NS: Non-Significant.										

Table 4.3. Clinical samples

No	Hospital Protocol No	Bacteria isolated	Gram stain feature	%2 Lugol's Solution Effect µL(mgr)						
				32 (1,6192)	64 (3,2384)	128 (6,4768)	256 (12,9536)	512 (25,9072)	1024 (51,8144)	P-value
26	55754854	*Candida glabrata*	yeast	R	R	S	S	S	S	0.0074 **
27	55790644	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
28	55754717	*Candida albicans*	yeast	R	R	S	S	S	S	0.0074 **
29	55780219	*Morgarella Mongarii*	Negative	R	R	R	S	S	S	1.00 NS
30	55785242	*Escherichia coli*	Negative	R	R	R	R	R	S	0.0001 **
31	55775847	*Corynebacterium Striatum*	Positive	R	R	R	S	S	S	1.00 NS
32	55784461	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
33	55784555	*Streptococcus pyogenes*	Positive	S	S	S	S	S	S	0.0001 **
34	55774393	*Staphylococcus epidermidis*	Positive	R	R	S	S	S	S	0.0074 **
35	55771930	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
36	55776313	*Acinetobacter baumannii/calco.cplx*	Negative	R	R	S	S	S	S	0.0074 **
37	55790630	*Staphylococcus haemolyticus*	Positive	S	S	S	S	S	S	0.0001 **
38	55802360	*Pseudomonas aeruginosa*	Negative	R	R	R	S	S	S	1.00 NS
39	55797736	*Klebsiella pneumoniae*	Negative	R	R	S	S	S	S	0.0074 **
40	55797274	*Staphylococcus epidermidis*	Positive	S	S	S	S	S	S	0.0001 **
41	55801261	*Candida albicans*	yeast	R	R	S	S	S	S	0.0074 **
42	55803201	*Saccharomyces cerevisiae*	yeast	S	S	S	S	S	S	0.0001 **
43	55778170	*Pseudomonas aeruginosa*	Negative	R	R	R	S	S	S	1.00 NS
44	55795672	*Staphylococcus aureus*	Positive	R	S	S	S	S	S	1.00 NS
45	55795735	*Candida tropicalis*	yeast	R	R	S	S	S	S	0.0074 **
46	55778631	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
47	55794117	*Staphylococcus haemolyticus*	Positive	S	S	S	S	S	S	0.0001 **
48	55796032	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
49	55797589	*Staphylococcus epidermidis*	Positive	R	R	R	S	S	S	1.00 NS
50	55814665	*Klebsiella pneumoniae*	Negative	R	R	R	R	S	S	0.0074 **
R: No. (%) S: No. (%)				20 (80%) 5 (20%)	19 (76%) 6 (24%)	12 (48%) 13 (52%)	2 (8%) 23 (92%)	1 (4%) 24 (96%)	0 (0%) 25 (100%)	--
Chi-Square (χ^2) P-value				13.75 ** 0.0001	12.08 ** 0.0001	0.793 NS 0.662	14.62 ** 0.0001	15.07 ** 0.0001	14.92 ** 0.0001	--
** (P≤0.01), NS: Non-Significant.										

Table 4.4. Clinical samples

No	Hospital Protocol No	Bacteria isolated	Gram stain feature	%2 Lugol's Solution Effect µL(mgr)						P-value
				32 (1,6192)	64 3,2384)	128 (6,4768)	256 (12,9536)	512 (25,9072)	1024 (51,8144)	
51	55820036	*Candida albicans*	yeast	R	R	S	S	S	S	0.0074 **
52	55814780	*Staphylococcus haemolyticus*	Positive	R	S	S	S	S	S	0.0001 **
53	55821589	*Klebsiella pneumoniae*	Negative	R	R	R	R	S	S	0.0074 **
54	55815365	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
55	55815472	*Klebsiella pneumoniae*	Negative	R	R	R	R	S	S	0.0074 **
56	55803823	*Staphylococcus epidermidis*	Positive	R	R	S	S	S	S	0.0074 **
57	55822131	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
58	55809606	*Staphylococcus hominis*	Positive	R	S	S	S	S	S	0.0001 **
59	55810339	*Candida tropicalis*	yeast	R	R	R	S	S	S	1.00 NS
60	55802733	*Acinetobacter baumannii*	Negative	R	R	R	R	R	S	0.0001 **
61	55802831	*Staphylococcus haemolyticus*	Positive	R	S	S	S	S	S	0.0001 **
62	55810913	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
63	55804484	*Escherichia coli*	Negative	R	R	R	S	S	S	1.00 NS
64	55777707	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
65	55807760	*Staphylococcus hominis*	Positive	R	S	S	S	S	S	0.0001 **
66	55810986	*Candida krusei*	yeast	R	S	S	S	S	S	0.0001 **
67	55810837	*Staphylococcus haemolyticus*	Positive	R	R	R	S	S	S	1.00 NS
68	55791416	*Cedecea davisae*	Negative	R	R	R	R	S	S	0.0074 **
69	55795247	*Providencia rettgeri*	Negative	R	R	R	S	S	S	1.00 NS
70	55838871	*Corynebacterium amycalatum*	Positive	R	S	S	S	S	S	0.0001 **
71	55826403	*Staphylococcus epidermidis*	Positive	R	S	S	S	S	S	0.0001 **
72	55827489	*Candida Spp.*	yeast	R	S	S	S	S	S	0.0001 **
73	55816824	*Klebsiella pneumoniae*	Negative	R	R	R	S	S	S	1.00 NS
74	55826962	*Enterococcus faecium*	Positive	R	R	S	S	S	S	0.0074 **
75	55827631	*Staphylococcus epidermidis*	Positive	R	S	S	S	S	S	0.0001 **
R: No. (%) S: No. (%)				25 (100%) 0 (0%)	16 (64%) 9 (36%)	13 (52%) 12 (48%)	4 (16%) 21 (84%)	1 (4%) 24 (96%)	0 (0%) 25 (100%)	--
Chi-Square (χ^2) P-value				14.92 ** 0.0001	9.83 ** 0.0072	0.793 NS 0.662	14.38 ** 0.0001	15.07 ** 0.0001	14.92 ** 0.0001	--
** (P≤0.01), NS: Non-Significant.										

Table 4.5. Clinical samples

No	Hospital Protocol No	Bacteria isolated	Gram stain feature	%2 Lugol's Solution Effect μL(mgr)						P- value
				32 (1,6192)	64 (3,2384)	128 (6,4768)	256 (12,9536)	512 (25,9072)	1024 (51,8144)	
76	55819720	Achromobacter Species	Negative	R	S	S	S	S	S	0.0001 **
77	55831958	Klebsiella pneumoniae	Negative	R	R	R	S	S	S	1.00 NS
78	55820489	Providencia rettgeri	Negative	R	R	R	S	S	S	1.00 NS
79	55820753	Myroides odoratus/odoratimimus	Negative	R	S	S	S	S	S	0.0001 **
80	55835027	Saccharomyces cerevisiae	yeast	R	S	S	S	S	S	0.0001 **
81	55834043	Candida albicans	yeast	R	R	R	S	S	S	1.00 NS
82	55844170	Acinetobacter baumannii	Negative	R	R	R	S	S	S	1.00 NS
83	55836061	Escherichia coli	Negative	R	R	R	R	R	R	0.0001 **
84	55836864	Candida tropicalis	yeast	R	R	S	S	S	S	0.0074 **
85	55830475	Providencia rettgeri	Negative	R	R	R	S	S	S	1.00 NS
86	55829092	Myroides odoratus/odoratimimus	Negative	R	R	S	S	S	S	0.0074 **
87	55835740	Staphylococcus epidermidis	Positive	R	R	R	R	S	S	0.0074 **
88	55922517	Staphylococcus Cohnii ssp cohnii	Positive	R	S	S	S	S	S	0.0001 **
89	55944121	Klebsiella pneumoniae	Negative	R	R	R	R	S	S	0.0074 **
90	55939256	Staphylococcus hominis	Positive	R	R	R	R	R	R	0.0001 **
91	55841084	Klebsiella pneumoniae	Negative	R	R	R	R	R	R	0.0001 **
92	55942894	Staphylococcus hominis	Positive	R	R	R	R	S	S	0.0074 **
93	55933350	Klebsiella pneumoniae	Negative	R	R	R	R	S	S	0.0074 **
94	55933947	Klebsiella pneumoniae	Negative	R	R	R	R	R	S	0.0001 **
95	55938961	Escherichia coli	Negative	R	R	R	R	R	S	0.0001 **
96	55927448	Acinetobacter baumannii	Negative	R	R	R	R	S	S	0.0074 **
97	55939257	Staphylococcus epidermidis	Positive	R	R	S	S	S	S	0.0074 **
98	55935752	Staphylococcus epidermidis	Positive	R	R	R	R	S	S	0.0074 **
99	55941527	Klebsiella pneumoniae	Negative	R	R	R	R	R	R	0.0001 **
100	55851757	Acinetobacter baumannii	Negative	R	R	R	S	S	S	1.00 NS
R: No. (%) S: No. (%)				25(100%) 0 (0%)	21 (84%) 4 (16%)	18 (72%) 7 (28%)	12 (48%) 13 (52%)	6 (24%) 19 (76%)	4 (16%) 21 (84%)	--
Chi-Square (χ^2) P-value				14.92 ** 0.0001	14.38 ** 0.0001	12.89 ** 14.38 **	0.793 NS 0.662	9.83 ** 0.0072	14.38 ** 0.0001	--
** (P≤0.01), NS: Non-Significant.										

Table 4.6. Result of Antibiotic template

NO	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	P-vaue
1	S	S	R	S	S	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1.00 NS
2	S	S	.	.	R	.	.	.	.	.	.	.	.	.	.	R	S	S	S	R	R	R	R	R	R	S	S	.	.	.	.	.	.	.	.	.	.	0.892 NS
3	S	S	.	.	R	.	.	.	.	.	.	.	.	.	.	S	R	S	S	S	R	R	R	R	R	S	S	.	.	.	.	.	.	.	.	.	.	0.892 NS
4	S	R	R	S	R	S	S	S	R	R	S	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	0.892 NS
5	S	S	.	.	R	.	.	.	.	.	.	.	.	.	.	R	S	S	S	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0276 *
6	S	S	.	.	.	.	.	.	.	.	.	.	.	.	.	R	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	.	.	.	.	.	.	0.659 NS
7	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	.	.	.	.	.	.	0.0001 **
8	S	S	.	.	S	.	.	.	.	.	.	R	R	.	.	S	.	S	.	R	R	R	R	R	.	S	S	.	.	.	.	S	.	.	.	.	.	0.935 NS
9	.	.	.	.	R	.	.	.	.	.	.	R	.	.	.	S	S	S	S	R	R	R	R	R	R	S	S	.	.	.	.	.	S	.	.	.	.	0.935 NS
10	S	R	.	.	R	.	.	.	.	.	.	S	S	.	.	S	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	.	.	.	.	.	.	0.933 NS
11	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	.	.	.	.	.	.	**0.0001 ****
12	.	R	.	.	.	S	S	.	.	.	S	R	R	.	.	.	.	.	.	.	.	.	.	R	R	.	.	.	.	.	.	.	.	R	.	.	.	0.038 *
13	S	R	.	S	R	S	S	R	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.0085 **
14	S	R	.	S	R	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.029 *
15	S	S	.	.	S	.	.	.	.	.	.	R	R	.	.	S	.	S	.	R	R	R	R	R	.	S	S	.	.	.	.	S	.	.	.	.	.	0.921 NS
16	S	S	.	.	R	.	.	.	.	.	.	S	S	.	.	S	.	S	.	R	R	.	R	R	R	R	S	.	.	.	.	S	.	.	.	.	.	0.935 NS
17	S	S	.	.	R	.	.	.	.	.	.	S	S	.	.	S	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	S	S	.	.	.	.	0.072 NS
18	.	.	R	S	R	S	S	S	R	R	S	R	R	S	R	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	R	.	.	0.5023 NS
19	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	**0.0001 ****
20	S	R	.	S	S	S	S	R	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.050 *
21	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	S	.	.	.	.	.	.	**0.0001 ****
22	S	R	R	S	S	R	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	R	.	.	0.047 *
23	R	R	R	S	R	S	S	R	R	R	S	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	**0.0063 ****
24	R	R	R	S	.	.	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**0.0046 ****
25	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	.	S	.	R	R	.	R	R	R	.	S	.	.	.	.	S	S	.	.	.	.	0.062 NS
R: % **S:** %	24 % 64 %	52 % 40 %	24 % 0%	0 % 36 %	56 % 20 %	4 % 32 %	0 % 40 %	28 % 8%	36 % 0%	36 % 0%	0% 40 %	60 % 12 %	56 % 12 %	32 % 4%	12 % 16 %	16 % 28 %	16 % 24 %	16 % 44 %	16 % 28 %	52 % 4%	44 % 0%	48 % 0%	56 % 0%	60 % 0%	52 % 0%	32 % 20 %	12 % 48 %	12 % 0%	12 % 0%	12 % 0%	8% 8%	0% 25 %	0% 12 %	4% 0%	25 % 0%	-	-	--
Chi. (χ^2)	9.4 **	11.4 **	9.6 **	13.5 **	10.4 **	9.8 **	10.6 **	8.9 **	13.5 **	13.5 **	10.6**	12.4**	11.0**	9.8 **	1.2 NS	2.9 NS	1.9 NS	8.7 **	2.9 NS	11.4**	15.3**	16.3**	17.4**	17.9**	15.6**	2.7 NS	9.7 **	4.6 *	4.6 *	4.6 N	0 NS	8.9 NS	4.6 NS	0.8 NS	8.9 NS	NS	NS	--

*** (P≤0.05), ** (P≤0.01).**

Antibiotics; 1.Amikacin 2. Gentamicin 3. Oxacillin, 4. Daptomycin, 5. Trimethoprim/Sulfamethoxazole, 6. Teicoplnin, 7. Vancomycin, 8. Clindamycin, 9. Erythromycin, 10. Fusidic acid, 11. Linezolid, 12. Ciprofloxacin, 13. Levofloxacin, 14. Moxifloxacin, 15. Tetracycline, 16. Tobramycin, 17. Ertapenem, 18. imipenem, 19.meropenem, 20. Cefazolin, 21. Cefixime, 22. Ceftazidime, 23. Ceftriaxone, 24. Ampicillin, 25. amoxicillin-clavulanate, 26. piperacillin-tazobactam, 27. Fosfomycin, 28.cefuroxime, 29.cefepime ,30. ampicillin-sulbactam, 31.Colistin, 32. Tigecycline, 33. Nitrofurantoin, 34. Streptomycin, 35. Cefoxitin, 36.Rifampin, 37.Penicillin G

Table 4.7. Result of Antibiotic template

NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	P-value
26	S	S	R	S	S	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.793 NS
27	S	R	.	.	S	.	.	.	.	.	.	R	S	.	.	R	S	S	.	R	R	R	R	R	R	.	S	.	.	.	.	.	.	.	.	.	.	0.061 NS
28	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
29	.	R	.	.	S	.	.	.	.	.	.	R	R	.	.	.	S	R	S	.	.	S	S	R	R	S	.	.	S	R	R	.	.	.	.	.	.	0.793 NS
30	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	S	S	R	R	R	R	R	R	R	R	S	R	.	.	.	.	R	R	.	.	.	.	0.0001 **
31	.	.	.	.	.	.	S	R	.	.	S	R	.	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	R	0.802 NS
32	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	R	R	R	R	R	R	R	S	.	.	.	.	.	.	.	.	.	.	0.0001 **
33	.	S	.	R	S	S	R	R	R	.	S	.	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	0.0037 **
34	R	R	R	S	S	S	S	R	R	R	S	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
35	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	S	.	S	.	R	R	R	R	R	S	S	R	.	.	.	.	.	.	.	.	.	.	0.061 NS
36	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	0.0001 **
37	S	S	R	S	S	S	S	R	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	1.00 NS
38	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	S	S	.	.	.	.	.	0.0001 **
39	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	S	.	.	.	.	.	0.0001 **
40	S	R	R	S	S	R	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.061 NS
41	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
42	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
43	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	S	S	.	.	.	.	.	0.0001 **
44	R	S	R	S	R	S	S	S	R	S	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	R	0.894 NS
45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
46	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	.	.	.	.	.	.	.	0.0001 **
47	S	R	R	S	R	S	S	S	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	0.061 NS
48	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	.	.	.	.	.	.	0.0271 *
49	S	R	R	R	R	R	S	R	S	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	0.0338 *
50	S	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
R: % **S:** %	24% 48%	44% 36%	28% 0%	8% 24%	52% 28%	8% 24%	4% 32%	28% 8%	28% 4%	24% 4%	0% 36%	80% 0NS	76% 4%	32% 0%	16% 16%	16% 8%	20% 20%	32% 16%	28% 12%	40% 0%	24% 0%	40% 4%	40% 4%	44% 0%	40% 4%	28% 12%	28% 12%	16% 0%	16% 4%	20% 0%	12% 12%	4% 12%	4% 0%	-	4% 0%	4% 0%	12% 0%	--
Chi. (χ^2)	8.8**	2.1 NS	9.7**	5.2*	7.6**	5.2*	9.1**	7.8**	8.7**	7.3%	9.1**	13.8**	12.4**	8.9**	0 NS	2.3 NS	0 NS	5.2*	5.2*	10.2**	8.1**	10.4**	10.4**	12.4**	10.4**	5.2*	5.2*	5.1*	4.76*	6.8**	1.0 NS	2.7 NS	0.61N S		0.61N S	0.61N S	4.7*	--
*** (P≤0.05), ** (P≤0.01).**																																						

Table 4.8. Result of Antibiotic template

NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	P-value
51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
52	S	R	.	S	R	S	S	.	.	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.703 NS
53	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	S	S	S	S	R	R	R	R	R	R	S	S	.	.	.	.	.	.	.	.	.	.	0.690 NS
54	S	R	.	.	S	.	.	.	.	.	.	R	R	.	.	.	S	S	S	R	.	R	R	R	R	R	.	R	R	R	S	.	.	.	.	.	.	0.0027 **
55	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
56	S	R	R	S	S	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.849 NS
57	S	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	S	S	.	.	.	.	S	S	.	.	.	.	0.382 NS
58	S	S	R	S	S	S	S	S	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	R	.	.	0.905 NS
59	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
60	S	S	.	.	S	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	0.00 NS
61	.	R	.	S	S	S	S	.	.	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	R	.	.	0.271 NS
62	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	S	.	.	.	.	.	0.0001 **
63	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	S	S	.	.	.	.	R	S	.	.	.	.	0.319 NS
64	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	S	.	.	.	.	.	0.0001 **
65	S	R	.	S	R	S	S	S	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	.	.	.	.	R	.	.	0.0057 **
66	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
67	S	R	.	S	R	R	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.0061 **
68	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	.	R	R	R	S	S	S	R	.	.	.	.	.	.	.	.	.	.	0.0393 *
69	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	.	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
70	.	.	.	.	.	.	S	R	.	.	S	R	.	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	R	0.0438 *
71	.	S	R	S	R	S	S	.	.	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.00 NS
72	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
73	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	S	S	R	.	R	R	R	R	R	.	R	R	R	R	S	.	.	.	.	.	0.0001 **
74	.	R	.	.	.	S	S	.	.	.	**S**	R	R	.	.	.	.	.	.	.	.	.	.	R	R	.	.	.	.	.	.	.	.	R	.	.	.	0.0093 **
75	R	R	R	S	R	S	S	R	R	R	**S**	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0028 **
R: %	28%	60%	16%	0%	56%	4%	0%	16%	20%	32%	**0%**	84%	80%	32%	16%	20%	20%	20%	20%	32%	24%	40%	40%	40%	40%	24%	20%	16%	16%	16%	12%	4%	0%	4%	20%	4%	4%	--
S: %	36%	20%	0%	28%	20%	32%	40%	8%	0%	0%	**40%**	0%	0%	0%	20%	4%	20%	24%	24%	0%	0%	0%	0%	4%	4%	16%	16%	0%	0%	0%	8%	12%	8%	0%	0%	0%	0%	
Chi. (χ^2)	2.1NS	10.4**	5.2*	8.7**	9.6**	8.9**	10.2**	2.3NS	7.6**	9.3**	**10.2****	14.1**	13.8**	9.3**	5.3*	5.3*	0NS	0.79NS	0.79NS	9.3**	7.9**	12.7**	12.7**	11.5**	11.5**	2.3NS	0.76NS	5.2NS	5.2*	5.2*	0.76NS	2.7NS	2.3NS	0.75NS	7.9**	0.75NS	0.75NS	--
*** (P≤0.05), ** (P≤0.01).**																																						

Table 4.9. Result of Antibiotic template

NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	P-value
76	R	.	.	.	.	.	.	.	.	.	.	R	R	.	.	.	.	S	S	.	.	R	.	.	.	R	.	.	R	.	S	.	.	.	.	.	.	0.0082 **
77	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	R	R	R	R	.	R	R	R	R	R	.	R	R	R	R	.	.	.	.	.	.	0.0001 **
78	R	R	.	.	S	.	.	.	.	.	.	R	R	.	.	R	R	R	R	.	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
79	R	.	.	.	.	.	.	.	.	.	.	R	R	.	.	R	.	R	R	.	.	R	.	.	.	R	.	.	.	.	.	.	.	.	.	.	.	0.0001 **
80	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
81	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
82	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	0.0001 **
83	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	S	S	.	.	.	.	0.217 NS
84	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
85	S	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	.	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
86	R	.	.	.	.	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	R	.	.	.	R	.	.	R	.	R	.	.	.	.	.	.	0.0001 **
87	S	R	R	S	R	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	R	.	.	.	R	.	.	.	.	.	.	0.038 *
88	R	R	R	S	R	S	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	S	.	.	R	.	.	.	.	.	.	.	.	.	S	0.0076 **
89	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	.	S	S	S	R	.	R	R	R	R	R	.	R	R	R	S	.	.	.	.	.	.	0.0031 **
90	S	R	.	S	R	.	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.0067 **
91	S	S	.	.	R	.	.	.	.	.	.	R	S	.	.	R	S	S	S	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0078 **
92	S	R	.	S	R	.	S	R	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	0.0071 **
93	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
94	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	R	R	R	R	R	R	R	R	.	.	.	.	.	.	.	.	.	.	0.0001 **
95	R	R	.	.	S	.	.	.	.	.	.	R	R	.	.	R	S	S	S	R	R	R	R	R	R	R	S	.	.	.	.	S	S	.	.	.	.	0.033 *
96	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	.	0.0001 **
97	S	R	R	S	R	S	S	R	R	R	S	R	R	R	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.0063 **
98	S	R	R	S	R	.	S	R	R	R	S	R	R	R	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.038 8
99	S	S	.	.	R	.	.	.	.	.	.	R	R	.	.	R	R	R	R	R	R	R	R	R	R	R	S	.	.	.	.	.	.	.	.	.	.	0.0007 **
100	R	R	.	.	R	.	.	.	.	.	.	R	R	.	.	.	.	R	R	.	.	.	.	.	.	.	.	.	.	.	R	S	.	.	.	.	.	0.0001 **
R: % S: %	44% 44%	56% 20%	16% 0%	0% 24%	68% 8%	0% 12%	0% 24%	24% 0%	24% 0%	24% 0%	0% 24%	88% 0%	84% 4%	24% 0%	8% 16%	36% 0%	24% 16%	44% 20%	44% 16%	32% 0%	32% 0%	52% 0%	40% 0%	40% 4%	40% 0%	52% 0%	28% 12%	8% 0%	16% 0%	8% 0%	16% 16%	0% 12%	0% 8%	-	8% 0%	-	0% 4%	--
Chi. (χ^2)	0 NS	9.1**	5.3*	8.7**	12.5**	4.7*	8.7**	8.7**	8.7**	8.7**	8.7**	14.1**	13.7**	8.7**	2.3 NS	9.7**	2.3 NS	8.6**	8.9**	9.3**	9.3**	12.8**	11.3**	10.5**	11.3**	12.8**	5.3*	5.3 NS	5.3*	2.3 NS	0 NS	4.7*	0.76NS		2.3 NS		0.76NS	--
*** (P≤0.05), ** (P≤0.01).**																																						

PART 5

DISCUSSION & CONCLUSION

The lugol solution contains free iodine (I2). It is directly in charge of the Antiseptic povidone-antiseptic iodine's effectiveness. Iodine's antibacterial method of action is unknown. However, this is believed to be linked to the fact that it can rapidly permeate the cell wall of microbes [145]. By interfering with hydrogen bonding and changing membrane structure, Boothman (2009) found that povidone-iodine affects the structure and function of enzymes and cell proteins in microbial cells and bacterial cell activity. He claims that the combination of these several mechanisms of action ensures the swift elimination of germs and prevents the development of bacterial resistance. He also noted that iodine's microbicidal action is due to a few direct toxic effects on the cell wall rather than particular molecular pathways (like antibiotics), hence iodine-resistant strains are rare [146]. Iodine is effective because it disrupts the bacterial biofilm's structural integrity. The glycocalyx of mucopolysaccharides that forms the biofilm is slimy and protective. According to recent studies, sustained-release iodine is more effective than silver or polyhexamethylene biguanide (PHMB) at breaking down biofilms [147]. It is crucial to confirm that these solutions' concentrations match those set forth by the Pharmacopoeia American USA. The Susceptible of inhibition bacterial growth and the rate of the bactericidal activity increased together with the level of free iodine in the investigated formulations. The relationship between Lugol Dilution And Susceptible (inhibition bacterial growth) increasing steadily, that is, the higher lugol dilution, the greater the inhibition.

Our research has shown that the Lugol solution exhibited a 76.76% efficacy against gram-positive bacteria, 70.82% efficacy against yeast, and 46.42% efficacy against gram-negative bacteria. Additionally, our results show that gram-positive bacteria were more inhibited by the lugol iodine solution than gram-negative bacteria were.

The samples with the numbers 83, 90, 91, and 99 showed resistance to lugol dilution over the course of our analysis. As part of this research, we used a laboratory-developed formulation of 2% iodine as an antibacterial agent, which is a simple antimicrobial solution to prepare. Iodine's antimicrobial properties have been exhaustively examined over the years, and this study focused on its antibacterial properties. However, the iodine-based antibacterial solution used in this study had a peculiar odor and a yellowish tint, making it unsuitable for use. Further research is necessary to develop a more acceptable iodine compound that has a pleasant smell and color, and could be used as an effective antibacterial. Our findings suggest that lugol solution could potentially serve as a supplement to topical antibiotics for treating illnesses. This could reduce the need for frequent topical antibiotic application.

Povidone-iodine (PVP-I) is a highly effective broad-spectrum antibacterial, according to extensive in vitro studies. It was effective against typical bacterial wound isolates as well as antibiotic-resistant strains. Lacey and Catto (1993) discovered that after 10 seconds of exposure to PVP-I, more than 99% of methicillin-resistant *Staphylococcus aureus* (MRSA) cells were killed. Cadexomer iodine decreased MRSA and total bacteria in partial-thickness swine wounds compared to the control and vehicle groups, according to Mertz et al. (1999).

Grønseth et al. (2017), found that 1.0% and 0.1% lugol solution effectively removed *S.aureus* from the biofilm and may be an alternative to traditional topical antibiotics in diseases such otitis media, pharyngitis, and wounds where *S.aureus* biofilm is suspected. Rahman et al (2019) suggested that 1% iodine provides better results against infection than commercially available hand sanitizers, and that lugol can be an effective alternative to hand washing to provide asepsis for healthcare professionals in emergency outreach programs and in water-scarce areas.

Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, three of the most common varieties of Gram-positive and Gram-negative bacteria, were used in our tests. These bacteria are among the common pathogens responsible for causing infections in humans. Using the serial dilution method to assess the antimicrobial activity of the povidone solution, we observed different levels of inhibition rates that

were directly proportional to the concentration of iodine. *Staphylococcus aureus* exhibited the highest sensitivity to the solution, with a greater percentage of inhibition, followed by *Pseudomonas aeruginosa*, and finally *Escherichia coli* with the least response.

Bacterial diseases are a big problem for world health because they cause a lot of illness and death. Because of this, it is very interesting to find new antibiotic medicines to treat bacterial illnesses. The current investigation fundamentally aims to assess the solution's potential antibacterial action in inhibiting the growth of hazardous germs and to see whether it may improve the efficacy of any existing drugs.

Povidone-iodine (PVP-I) solutions exert their bactericidal effect when the free iodine (I_2) is present. To ensure their effectiveness, it is crucial to maintain their concentration within the standards established by the USP.

The objective of the current experiment was to identify the active antibacterial ingredient in various samples of povidone-iodine solutions. These solutions are critical in lowering the number of infections in hospitals and other healthcare institutions. The research involved adjusting the pH of the solutions, identifying the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) on bacterial specimens, and measuring the amount of free iodine present in the solutions.

We observed an increase in the antibacterial activity of the solutions in correlation with the high level of free iodine. This finding highlights the importance of maintaining the appropriate concentration of PVP-I solutions to ensure their efficacy in preventing infections.

5.1. CONCLUSION

To ensure the efficacy of commercial povidone-iodine solutions, it is crucial to monitor factors such as pH, temperature, and free iodine concentration over time. PVP-I has been thoroughly studied in vitro and in vivo and shown to be a safe and effective wound antiseptic that can penetrate biofilms without generating bacterial resistance. However, concerns about allergies and cytotoxicity have been raised based on results from animal studies, and more well-formed clinical trials on human subjects are required to further investigate its effectiveness and safety.

In this study, the focus was on monitoring the povidone-iodine antiseptic prepared in the university laboratory, and several important variables were measured and monitored during its validity period, including pH, free iodine content, antibacterial activity, and stability. The difference in previous results may be due to the release of unstable iodine (I_2) from the PVP-I complex, or to the dilution reducing the auxiliary role of iodine in contacting bacterial cells.

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CURRICULUM VITAE

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