*University of Basrah*

*College of pharmacy*

*2022-2023*

# *“Evaluation of the effect of certain disinfectant ( R2) and on bacteria isolated from a delivery wards in Basrah ”*

**This project is submitted to the department of Clinical Laboratory Sciences as a partial fulfilment for graduation in college of pharmacy**

**Done by :**

**Ibrahim Mohammed Mahmood**

**Ameer Qusay Fadhil**

**Nasser Ibrahim Nasser**

**Supervised by :**

**Assistant Prof.Dr.Ayad almakki**

# CONTENTS

## Abstract

Hydrogen peroxide is a widely used antimicrobial chemical. It is used in both liquid and gas form for preservative, disinfection and sterilization applications. Its advantages include its potent and broad spectrum antimicrobial activity, flexibility in use in comparison to other microbiocides. Hydrogen peroxide has been shown to be effective against all forms of microorganisms, including dormant forms with known high resistance such as bacterial spores and protozoal cysts, and also infectious proteins such as prions depending on the specific use of the chemical. However, overall, the effective and safe use of hydrogen peroxide depends on the way it is used, in particular the concentration. In aqueous form it is used in solution with water directly as a preservative, in products as a preservative or on the skin, including in wounds, and on inanimate surfaces.


# Introduction

### 1.1. Bacteria in hospital

Some well-known nosocomial infections include: ventilator-associated pneumonia, Methicillin resistant Staphylococcus aureus, Candida albicans, Acinetobacter baumannii, Clostridium difficile, Tuberculosis, Urinary tract infection, Vancomycin-resistant Enterococcus and Legionnaires' disease. Methicillin-resistant Staphylococcus aureus (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans.

Hospital acquired pneumonia is the second most common nosocomial infection (urinary tract infection is the most common) and accounts for 1520% of the total.

## 1.2. Most common bacteria in delivery ward

The most prevalent Gram-negative bacteria isolated from delivery ward are *Pseudomonas aeruginosa*, an important nosocomial pathogen associated with hospital, *Acinetobacter* species, *E. coli* as well as *Klebsiella.*

While the most prevalent Gram-positive bacteria are *Staphylococcus* species .Some of the microorganisms could not be identified at the species level. These microbiological findings revealed an extremely high percentage of contamination with *Pseudomonas* species, which comprises mainly *Pseudomonas aeruginosa* and other species of *Pseudomonas*, which were not further classified. Overall, there was a predominance of Gram-negative microorganisms.

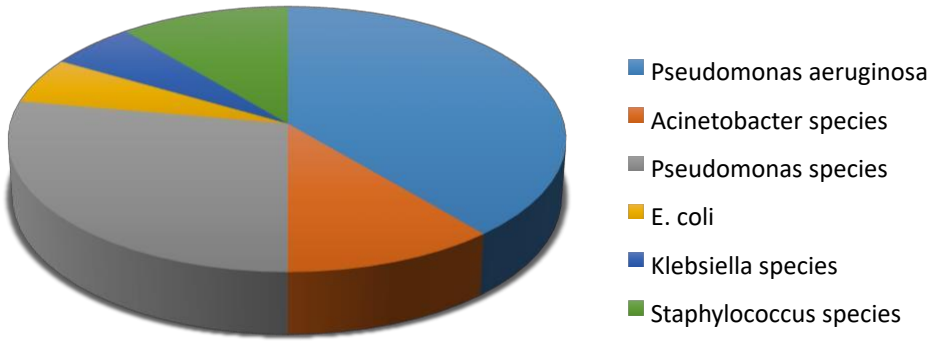



Figure (1) Most common bacteria in delivery ward

## 1.3. Mechanism of bacterial resistance

There are four methods of bacteria to develop resistance against antimicrobial agents: 1) Limiting drug uptake

The structure and functions of the LPS layer in gram negative bacteria provides a barrier to certain types of molecules. This gives those bacteria innate resistance to certain groups of large antimicrobial agents.

Bacteria that lack a cell wall, such as Mycoplasma and related species, are therefore intrinsically resistant to all drugs that target the cell wall including β-lactams and glycopeptides.

2) Modification of drug targets

There are multiple components in the bacterial cell that may be targets of antimicrobial agents; and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. One mechanism of resistance to the β-lactam drugs used almost exclusively by gram positive bacteria is via alterations in the structure and/or number of PBPs (penicillin-binding proteins).

3) Drug inactivation

There are two main ways in which bacteria inactivate drugs; by actual degradation of the drug, or by transfer of a chemical group to the drug. The β-lactamases are a very large group of drug hydrolyzing enzymes.

Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. There are a large number of transferases that have been identified. Acetylation is the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides.

4) Drug efflux

Bacteria possess chromosomally encoded genes for efflux pumps. Some are expressed constitutively, and others are induced or overexpressed under certain environmental stimuli or when a suitable substrate is present.

## 2.1. Definition of disinfectant

A disinfectant is a chemical substance or compound used to inactivate or destroy microorganisms on inert surfaces. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores; it is less effective than sterilization, which is an extreme physical or chemical process that kills all types of life.

## 2.2. Types of disinfectant

1) Air disinfectant

An air disinfectant must be dispersed either as an aerosol or vapor at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

2) Alcohol Disinfectants

Alcohols, usually ethanol or isopropanol, are sometimes used as a disinfectant, but more often as an antiseptic, the distinction being that alcohol tends to be used on living tissue rather than nonliving surfaces. These alcohols are non-corrosive but can be a fire hazard. They also have limited residual activity due to evaporation, which results in brief contact times unless the surface is submerged. They also have a limited activity in the presence of organic material.

Alcohols are most effective when combined with purified water to facilitate diffusion through the cell membrane.

3) Oxidizing Disinfectants

A large number of disinfectants are related to this group. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and hand washes.

## 2.3. Mechanism of disinfectant

- Alcohol

Mechanism of action: Cross-linking, coagulating, and clumping.

Like many disinfectants, alcohols are generally considered to be nonspecific antimicrobials because of their many toxic effects. Alcohols cause cell proteins to clump and lose their function. Specifically, the cell membranes lose their structure and collapse, thereby killing it. The alcohol must be

diluted with water for the optimum effect, as proteins are not denatured as readily with straight alcohol. Alcohol is also effective in inhibiting spore germination by affecting the enzymes necessary for germination. However, once it's removed, spores can recover, so it's not considered a sporicidal.

-Chlorine

Mechanism of action: Oxidizing.

Chlorine is a very common disinfectant used in a wide variety of cleaning solutions and applications because, even in very small amounts, it exhibits fast bactericidal action. Chlorine works by oxidizing proteins, lipids and carbohydrates. Hypochlorous acid, which is a weak acid that forms when chlorine is dissolved in water, has the most effect on the bacterial cell, targeting some key metabolic enzymes and destroying the organism.

-Peroxygen Compounds

Mechanism of action: Oxidizing.

Both hydrogen peroxide and peracetic acid are peroxygen compounds of great importance in infection control because, unlike like most disinfectants, they are unaffected by the addition of organic matter and salts. In addition, the formation of the hydroxyl radical, a highly reactive ion that occurs as peroxygen compounds encounter air, is lethal to many species of bacteria because it is a strong oxidant. Being highly reactive, the hydroxyl radical attacks essential cell components and cell membranes, causing them to collapse. Peroxygen compounds also kill spores by removing proteins from the spore coat, exposing its core to the lethal disinfectant.

-Phenol

Mechanism of action: Cross-linking, coagulating, and clumping.

Phenol and its derivatives exhibit several types of bactericidal action. At higher concentrations, the compounds penetrate and disrupt the cell wall and make the cell proteins fall out of suspension. One of the first things to occur is stopping essential enzymes. The next level in the damage to the bacteria is the loss in the membrane's ability to act as a barrier to physical or chemical attack.

-Quaternary ammonium compounds Work by denaturing the proteins of the bacterial or fungal cell, affecting the metabolic reactions of the cell and causing vital substances to leak out of the cell, causing death.

## Aim

In this research our aim is to compare the effectiveness of hydrogen peroxide with traditionally used ammonia through preparation of serial dilutions of hydrogen peroxide to find minimum inhibitory concentration (MIC) on different types of bacteria.

## Materials and methods

### 1. Materials

#### 1.1. Tools

| No. | Tool Name |
|---|---|
| 1. | Petridish |
| 2. | Loop |
| 3. | Sterilized cotton swabs |
| 4. | Test tube |
| 5. | Micropipette |
| 6. | Ruler |

#### 1.2. Culture media

| No. | Culture media |
|---|---|

| | |
|---|---|
| 1. | Nutrient agar |
| 2. | Mueller-Hinton agar |

## 1.3. Disinfectant

VANISH®

| No. | Ingredient |
|---|---|
| 1. | Hydrogen peroxide |
| 2. | Sodium Carbonate |
| 3. | Sodium Percarbonate |
| 4. | Sodium Sulfate |
| 5. | Sodium Bicarbonate |
| 6. | Sodium Silicate |
| 7. | C12-15 Pareth-9(SAA) |
| 8. | TAED |
| 9. | Water |
| 10. | Protease Enzyme |
| 11. | Disodium Distyrylbiphenyl Disulfonate |
| 12. | Fragrance/Parfum |

## 1.4. Apparatus

| No. | Apparatus name |
|---|---|

| | |
|---|---|
| 1. | Autoclave |
| 2. | Sensitive electric balance |
| 3. | Hot plate |
| 4. | Refrigerator |
| 5. | incubator |
| 6. | Vortex mixer |
| 7. | Densicheck plus |

# Method

## 1.1. Preparation of culture media

We prepared two types of culture media: Nutrient agar for growth of different types of bacteria (gram +ve and gram –ve) and Mueller-Hinton agar for the measurement of zone inhibition of disinfectant.

Media were prepared and sterilized with autoclave at 121C for 15 minutes.

## 1.2. Activation of bacteria

Using the loop, colonies of bacteria were taken and cultured on nutrient agar and incubated for 24 hours at 37C.

## 1.3. Preparation of bacterial suspension

After 24 hours, Using Densicheck plus to measure the number of bacteria in liquid suspension for each sample.

By taking colonies of bacteria by loop and adding 3mL of normal saline to the test tube and measure it. The acceptable readings are in the range of 0.5-0.63 McFarland for gram+ve and gram-ve bacteria.

## 1.4. Preparation of serial dilutions of disinfectant

We prepared serial dilutions from 1:1 to 1:12 by adding 1mL of sterile distilled water to test tube and then adding 1mL of disinfectant (Vanish) for dilution 1:1 ;and then we took 1mL from the latter and add to 1mL of sterile distill water for dilution 1:2; And so on for the other dilutions.

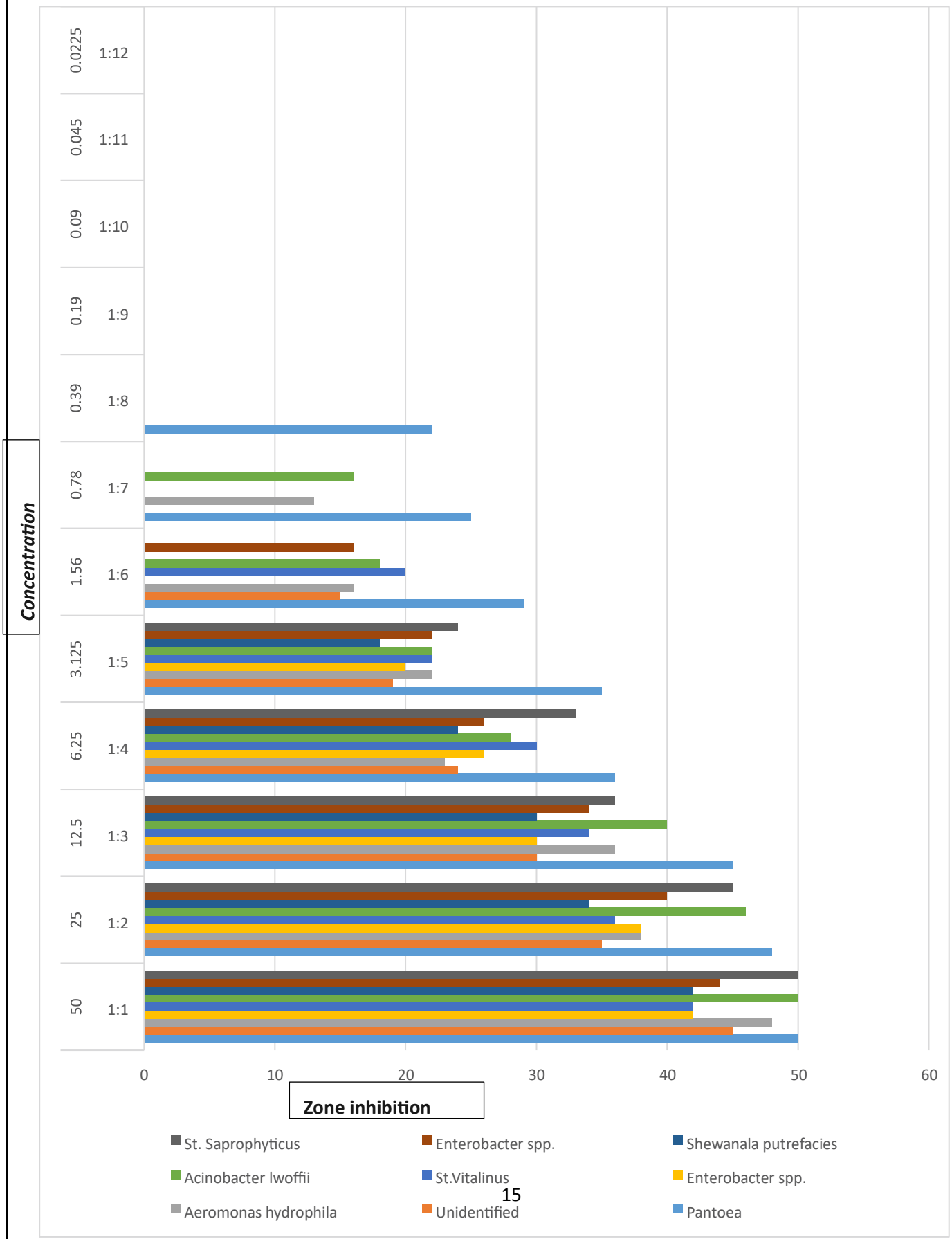
## 1.5. Measure the effectiveness of disinfectant

The culture media (Mueller-Hinton agar) was perforated; 100µL of the bacterial suspension were taken and spread on the plate; and then take 100µL of each dilution of disinfectant and put it inside the holes and let it dry; after that incubate for 24 hours at 37C .

On the next day; the inhibition zone was measured using a ruler for all the dilutions and the appropriate dilution (concentration) is known to inhibit all types of bacteria [lowest concentration to inhibit bacteria].


Figure (2) Zone inhibition measurement using ruler .

# Result


Concentration
0.0225
1:12
0.045
1:11
0.09
1:10
0.19
1:9
0.39
1:8
0.78
1:7
1.56
1:6
3.125
1:5
6.25
1:4
12.5
1:3
25
1:2
50
1:1
0
10
20
30
40
50
60
Zone inhibition
St. Saprophyticus
Enterobacter spp.
Shewanala putrefacies
Acinobacter lwoffii
St.Vitalinus
Enterobacter spp.
Aeromonas hydrophila
Unidentified
Pantoea

# Result

1) At serial dilutions from 1:1 to 1:5(concentration between 50 and 3.125), all bacterial species are inhibited by disinfectant.

Frequency of diversity= (No. of bacteria species present/ total no. of species) * 100%

Frequency of diversity= (0/9) * 100%= 0%

2) At serial dilution of 1:6(concentration =1.56) some species resist inhibition of disinfectant. The species that resist inhibition at this concentration are: St. Saprophyticus, Shewanala putrefacies, Enterobacter spp.

Frequency of diversity = (3/9)*100%= 33.34%

3) At serial dilution of 1:7(concentration = 0.78) some other species are inhibited.

Frequency of diversity= (6/9)* 100% =66.67%

4) At serial dilution of 1:8(concentration=0.39) only Pantoea species are inhibited by disinfectant.

Frequency of diversity= (8/9)*100%= 88.89%

5) At serial dilution of 1:9(concentration= 0.19) none of the bacterial species are inhibited by disinfectant.

Frequency of diversity= (9/9)*100%= 100%

# Discussion

The airborne hydrogen peroxide, either in the form of vapor or dry mist, is known to be an effective method of disinfection of the hospital environment Addition to hydrogen peroxide cleaner disinfectant was effective for disinfection of soft surfaces when applied as a spray with no mechanical wiping. Spray application of this solution could provide an efficient and effective means to disinfect soft surfaces in healthcare settings and it is able to block the formation of biofilms reducing the risk of infections.

The minimum level of effectiveness of hydrogen peroxide is 0.08%; however, if lower concentrations are used, complete destruction of the microorganisms is not guaranteed.

Beside concentration; exposure time play important role in bacterial inhibition by disinfectant.

When testing the concentration of 0.08%, a 100% inhibition percentage was reached for the three established times (After 5, 10 and 15 min). After 10min of exposure at the 0.02% concentration, Staphylococcus aureus does not show an increase in the percentage of inhibition; comparatively, when testing the concentration of 0.04%, an inhibition percentage of 43.75% was obtained, and, at a level of 0.08%, total inhibition of growth was evidenced. After 15 min of contact with the disinfectant, an increase of 100% in the percentage of inhibition was observed with the concentration of 0.04%, in the same way with a level of 0.08%.

The reason why Staphylococcus aureus is not inhibited at concentrations of 0.02% and 0.04% the high-level disinfectant of hydrogen peroxide is because this microorganism produces the extracellular enzyme catalase. This enzyme breaks down the hydrogen peroxide in water and molecular oxygen when this compound is in small amounts in more significant quantities, under experimental conditions Staphylococcus aureus is inhibited by the accumulation of hydrogen peroxide in the medium.

Pseudomonas aeruginosa (one of the most common microorganism in delivery ward) does not show resistance when it is subjected to a concentration of disinfectant based on 2% hydrogen peroxide, which complies with the guidelines established by the commercial house.

This microorganism (Pseudomonas aeruginosa) is sensitive to hydrogen peroxide, an active component of the disinfectant under study because this generates a disturbance of the components of the cell membrane. A disturbance is also generated in chemiosmosis, which is the diffusion of ions across a permeable membrane, causing an alteration in the transport membrane and further causing damage to a cell wall.

Pseudomonas aeruginosa can present resistance by several mechanisms such as the variation in the composition of lipopolysaccharide (LPS) and the content of cations such as magnesium, which produces stable bonds between molecules of LPS and as a complement to this mechanism.

The resistance of Bacillus subtilis to disinfectants is attributed to the fact that the sporulated microorganisms form a barrier to the entry of antimicrobial agents because the membranes that surround the nucleus of the endospore act as an additional factor when limiting the penetration of the chemical agent.

When evaluating a disinfectant against a sporulated microorganism such as Bacillus subtilis, it is necessary to increase the exposure time for many reasons; for example, the spores have a core with a high content of calcium dipicolinate; in addition, the nucleus is partially dehydrated. This characteristic increases the thermo-resistance of the spore, and, at the same time, it confers resistance to chemical substances such as hydrogen peroxide. Also, from the low water content of the spore, the pH of the core cytoplasm contains high levels of specific core proteins termed "small acidsoluble spore proteins" (SASPs). These proteins bind tightly to the DNA in the spore's nucleus and protect it from potential damage from UV radiation, desiccation, and chemical agents.

For each microorganism, the inhibitory effect of the disinfectant is directly influenced by the exposure time; concentration of 3% with a time of 5 min contact for vegetative bacteria and more than 2 h for the sporulated microorganism.

The most significant reduction of microbial growth occurred in the strain of Pseudomonas aeruginosa, which reached a percentage of inhibition of 88% when subjected to a disinfectant concentration of 0.04% with an exposure time of 5 min. These results revealed that Pseudomonas aeruginosa could exhibit inhibition with different concentrations of this disinfectant.

While the smallest reduction was observed with the strain of Staphylococcus aureus, in which, at 10 min of exposure, the population decreases to 44%. On the other hand, the sporulated Bacillus subtilis strain showed a 69% decrease in growth for the first 3 h of exposure to the disinfectant, at a concentration of 0.04%. The reduction of the population on average was 100% for the levels of 0.08%, 1%, and 2%.

Based on the above, we can say that the high-level disinfectant (hydrogen peroxide) is 100% effective when using the concentration recommended by the commercial house (2%) in the shortest time of exposure. Likewise, we can establish that the minimum inhibitory concentration, the lowest level of the disinfectant capable of inhibiting in vitro the visible growth of microorganisms, was 0.08% because, with this value and in the shortest time of contact with the disinfectant evaluated, they achieved satisfactory results.

## Conclusion

1) Based on our result we conclude that Vanish (hydrogen peroxide) can inhibit bacterial growth of varied species even at low concentrations.

2) Pantoea species are the most susceptible species in our research, they were inhibited by disinfectant until 1:8 dilution.

3) Dilution of 1:5(concentration= 3.125) is MIC at which all bacterial species are inhibited by disinfectant.

# Recommendations

## 1.1. Effectiveness

Hydrogen peroxide is more effective than ammonia as disinfectant by killing wide variety of bacterial species.

## 1.2. Toxicity

When it comes to toxicity, Hydrogen peroxide can cause more damage to human than ammonia. Hydrogen peroxide irritates the skin and inhibits wound healing.

The advice about using Vanish has changed that the irritation it causes is not worth the antiseptic effect.

## 1.3. Cost

Regarding cost, Vanish cost more than ammonium compounds.

## 2.1. Selection of appropriate disinfectant

Based on previous parameters we can choose suitable disinfectant accordingly:

Recommended disinfectant is the one with lowest cost and lowest toxicity and highest effectiveness.

Vanish is not recommended where cost and toxicity profile are the main values.

| | **Hydrogen peroxide** | **Ammonia** |
|---|---|---|
| **Effectiveness** |  |  |
| **Toxicity** |  |  |
| **Cost** |  |  |

# References


1. Khan HA, Baig FK, Mehboob R. Nosocomial infections: epidemiology, prevention, control and surveillance. Asian Pac J trop biomed [internet]. Elsevier B.V.; 2017; 7:478–82.
2. WHO. The Burden of Health Care-Associated Infection Worldwide. A Summary; 2010. Geneva; 2016.
3. Mirhoseini SH, Nikaeen M, Shamsizadeh Z. Occurrence of airborne methicillin-resistant Staphylococcus aureus in different hospital wards. *EnvironmentAsia.*
4. W. C. Schumb, C. N. Satterfield and R. L. Wentworth, *Hydrogen Peroxide*, Reinhold Book, New York, 1955.
5. S. S. Block, ' Peroxygen Compounds,' in *Disinfection, Sterilization and Preservation* (Ed. S. S. Block), Lea & Febiger, Philadelphia, 1991, p. 167
6. I. Al-Adham, R. Haddadin and P. Collier, in *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization* 5th ed. (Eds. A. Fraise, J.-Y. Maillard and S. Sattar), Chap. 2, WileyBlackwell, Oxford, 2013, p. 5.

7. G. McDonnell, in *Russell, Hugo and Ayliffe's Principles and Practice of Disinfection, Preservation and Sterilization*, 5th ed. (Eds. A. Fraise, J.-Y. Maillard and S. Sattar), Chap. 10, Wiley-Blackwell, Oxford, 2013, p. 208

8. Rios Castillo AG, Gonzalez Rivas F, Rodriguez Jerez JJ. Bactericidal efficacy of hydrogen peroxide-based disinfectants against gram-positive and gram-negative bacteria on stainless steel surfaces. I Food Sci 2017:82:2351-6.

9. Kohli R, Mittal KL. Developments in surface contamination and cleaning; 2013.

10. Perumal PK, Wand ME, Sutton ]M, Bock LJ. Evaluation of the effectiveness of hydrogen-peroxide-based disinfectants on biofilms formed by gram-negative pathogens. J Hosp Infect 2014;87:227-33.

11. Kanemitsu K. A comparative study of ethylene oxide gas, hydrogen peroxide gas plasma, and low-temperature steam formaldehyde sterilization. Infect Control Hop Epidemiol 2005;26:486-9.