
**Biology and Biochemistry Department**

**BIOL243**

**Microbiology lab**

**Sec 3**

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**Title**

# Chemical effects on bacterial growth

## Objective:

The purpose of this study is to see how different antibiotics and chemicals affect the development of the bacteria Enterococcus faecalis and Staphylococcus aureus. For some antibiotics, determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) is also necessary.

## Introduction:

The presence of the biocide[1] helps us to control the growth of microorganisms and by means of these chemical agents that cause inhibit the growth of microbes (e.g. bacteriostatic, fungistatic) or cause the killing of these microorganisms (e.g. Bactericidal). (1)

We use many terms in order to be able to control microorganisms, for example, sterilization is a chemical or physical process that is used to kill all living organisms, as a complete sterilization of these organisms, as well as viruses and Endospores (Used on inanimate objects). While disinfection is comparable, products or biocides are often applied to inanimate objects or surfaces. Disinfectants may be sporostatic, although they are not always sporicidal. An Antisepsis is a chemical agent by which living tissues are protected from microorganisms by preventing their growth or killing them and these microorganisms are destroyed so that they are no longer present in or on the tissues. Then the antibiotic is a chemical agent, either natural or synthetic, that selectively kills or inhibits bacteria or other microbes at a low concentration. And the Internally chemotherapy chemicals are used to kill or prevent the development of bacteria within host tissues. (2)(1)(3)(4)

Antibiotics come in all shapes and sizes, and they all have an impact on our health. Antimicrobial activity is affected by a variety of parameters, including the size of the inoculum, bacterial condition, antibiotic concentrations, and temperature. Many factors influence the efficiency of antibiotic treatment, particularly three: the antibiotic itself, the patient's body system and the target pathogens. (7)

## The Materials:

*** Effect of disinfectants and antiseptics on bacterial growth:**

1. *E. coli* and *S. aureus* stock cultures.
2. Nutrient Broth Adar Plates.
3. Sterile cotton swabs.
4. Generic disinfectants: Dettol, Ethanol, Listerine.

***Effect of chemotherapeutic agents on bacterial growth:**

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1. *E. coli* and *S. aureus* stock cultures.
2. McFarlane standard[2].
3. 150 mm Muller-Hinton agar plates.
4. Antibiotic discs.

***Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):**

1. Antibiotic Stock solution 1000 µg/ml.
2. Sterile 96 wells plates.
3. Stock cultures of *E. Coli* and *S. aureus.*
4. Nutrient agar plates.
5. Multichannel micropipette.
6. Sterile pipette tips.

## Methods:

*** Effect of disinfectants and antiseptics on bacterial growth:**

- To sanitize the loop, the Bunsen burner was lighted.

- A tiny amount of a stock culture was extracted from a sterile inoculation loop, and *E. coli* was removed from the loop's end.

- After immersing the inoculation ring in the nutrient broth-agar cultures, the solution was shaken multiple times to ensure homogeneity solution.

- A sterile cotton swab was dipped in the broth, and then wiped in several directions in such a way that the entire Mueller-Hinton agar plate was evenly inoculated to ensure that it was completely inoculated.

- The tweezers were sterilized by immersing their tip in 95% ethanol and then gently passed over a flame and cooled under autoclaving conditions.

- Using sterile tweezers tablet (from previously submerged tablets) of each disinfectant briefly to get rid of excess liquid and then place in its designated position on the plate.

- *S. aureus* bacteria were tested using the same approach. After that, the two plates were incubated at 37°C.

***Effect of chemotherapeutic agents on bacterial growth:**

- A standard turbidity inoculum was prepared for the test bacteria so that a certain density of bacteria spread on the plate.

- Then a 150 mm diameter Mueller-Hinton agar plate was inoculated with the standard inoculum to completely cover the agar surface with bacteria.

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- Then standard tablets containing an antibiotic were placed on the plate.

-The plate was then incubated at 37 °C for 24 hours.

- Then the diameter of any areas caused by damping was measured in millimeters (mm).

- It was then determined if the bacteria were intermediate, sensitive, intermediate, or resistant to each antimicrobial agent.

## Results:

**Table1:** The diameter of the zone of inhibition around each antimicrobial disc in both *S. aureus* and *E. coli* plates

| Disinfectant | S. aureus | E.coli |
|---|---|---|
| | Zone of inhibition diameter (mm) | |
| **$H_2O$ (control)** | 6 mm | 6 mm |
| **Dettol** | 22 mm | 14 mm |
| **70 % ethanol** | 15 mm | 12 mm |
| **95% ethanol** | 10 mm | 8 mm |
| **Listerine** | 11 mm | 9 mm |

**Table2:** The diameter of the zone of inhibition around each antibiotic disc in both *S. aureus* and *E. coli*

| Antibiotic & its concentration (µg) | S. aureus | E.coli |
|---|---|---|
| | Zone of inhibition diameter (mm) | |
| **Penicillin (P, 10 )** | 35 mm | 6 mm |
| **Azithromycin (AZM, 15)** | 38 mm | 20 mm |
| **Ampicillin (AMP, 20)** | 34 mm | 12 mm |

## Discussion:

Two types of bacteria have been used: *Escherichia coli* and *Staphylococcus aureus*.

The *Escherichia coli* is a gram negative while the *Staphylococcus aureus* is a gram positive. Therefore, it is expected and inevitable that the resistance to *Escherichia coli* is higher than that of *Staphylococcus aureus*, and one of the reasons is due to the two membrane (outer membrane and Peptidoglycan) the

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peptidoglycan is thin that are present in Gram negative, in contrast to Gram positive in which there is only one layer of peptidoglycan and she's thick**.**

Water is the control, so it's 6mm in *Escherichia coli* and *Staphylococcus aureus* for I.Z.

Ethanol has a cidal (cidal → agents kill – Static → agents inhibit growth) impact on bacteria by dissolving their plasma membrane and denatureing their proteins. In the S. aureus plate, it was obvious that 70% ethanol had stronger antimicrobial activity action than 95% ethanol. This is due to the fact that the greater the concentration of ethanol in the water (with an adequate concentration of ethanol itself), the greater its destructive effect. This is because water reduces the rate of evaporation. However, ethanol has a greater effect on gram positive bacteria than gram negative bacteria, because the gram negative has an outer membrane that must be destroyed before the inner plasma membrane can be broken while gram positive don't have outer membrane. (5)

*And of course ethanol is a volatile substance, and the higher its concentration, the faster its volatilization, and therefore the less impact it has.

Both Gram-positive and Gram-negative bacteria are killed and inhibited by LISTERINE Disinfectant. (6)

The OH groups of the chloroxylenol molecule connect to certain proteins on the bacterial cell membrane in Dettol, disrupting membrane function and allowing the bacterial cell's contents to flow out, resulting in death. (8)

Medical microbiologists have established the MIC and MBC as crucial levels of an antibiotic that may be relied on in medication development since uncalculated big dosages of antibiotics are not healthy for human usage. The lowest dose of an antimicrobial agent that would impede apparent growth of a bacterium after overnight incubation is known as the Minimum Inhibitory Concentration (MIC). After a culture has been isolated, it can be assessed using broth dilution procedures, in which equal quantities of bacteria are cultivated in wells of liquid media containing progressively decreasing amounts of the medication. The last well containing clear (not turbid) media would be the MIC.

The Minimum Bactericidal Concentration (MBC), on the other hand, specifies the lowest concentration at which an antimicrobial agent will kill a bacterium. It's an add-on to the MIC exam. The liquid media well containing the microbe and antibiotic MIC, as well as the preceding two wells, are plated onto agar dishes once the MIC is determined.

## Conclusion:

To conclusion, many substances chemical have the potential to harm microbial development by either killing or inhibiting microbes. Our investigation, however, revealed that the antibacterial activity of each chemical is regulated by a number

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of factors, including the chemical agent's concentration, as well as the kind and nature of the bacterium.

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