Laboratory Experiment 3

Mariam Zoair

Testing Chemical germicides, disinfectants/ antiseptics and antibiotics using the Disc-Diffusion Assay

Introduction:

In this experiment, we will see the effect of different chemical disinfectants on different types of bacteria. These disinfectants are classified according to the strength of their activity and their effect on other bacteria, as some types of disinfectants contribute to killing bacteria because of their strong effect on them, and other disinfectants have a weak effect on bacteria. The purpose of chemical disinfectants is to kill bacteria and slow down their growth. It can be said that disinfectants are used on living tissues to limit the spread of bacteria between people when people touch surfaces such as faucet handles, door handles, and other things. Therefore, we can say that the purpose of the experiment is to see the effect of different antimicrobial agents on the growth of two different types of bacteria, namely: *Escherichia coli* and *Staphylococcus aureus*.

Material:

- Marker to label plates
- 6 complex agar plates
- Loop
- 2 broth tubes
- Cotton swab
- Sterile paper disc
- Flame
- 2 Nutrient agar that have bacteria on it (*Staphylococcus aureus, Escherichia coli)*
- Tweezers
- Pipette

Staphylococcus aureus:

- *S. aureus:* Iodine, Detergent, Soap, Lysol wipes (my choice)
- *S. aureus*: H2O(water), Hydrogen peroxide, Bleach, Alcohol
- *S. aureus:* Nalidixic acid (NA30), Penicillin (P10), Gentamicin (GM10), Amoxicillin (AM10), Tetracycline (Te 30), Amoxicillin/clavulanic acid (Amc 30).

Escherichia coli:

- *E. coli*: Iodine, Detergent, Soap, urine (my partner choice)
- *E. coli*: H2O(water), Hydrogen peroxide, Bleach, Alcohol
- *E. coli*: Nalidixic acid (NA30), Penicillin (P10), Gentamicin (GM10), Amoxicillin (AM10), Tetracycline (Te 30), Amoxicillin/clavulanic acid (Amc 30).

Procedure:

- We divided the petri-dish, so each one of us took 3 petri-dishes.
- Then, each one of us choiced one of the bacteria to treat with it during the experiment.
- I choiced *S. aureus* bacteria and my partner choiced *E. coli* bacteria.
- We sterilized the loop with the flame, then we used the loop to take the bacteria from the nutrient agar that contained the bacteria, then we put the loop into the broth tube until it gets cloudy.
- Then, we sterilized the loop again.
- We picked up the bacteria from the broth by using the cotton swap, loosening the cap.
- We spread the broth on the petri-dish by using the cotton swap.
- We added the bacteria vertically to all the petri-dish.

- We put the cotton swab back into the paper and dispose of it to make sure the bacteria does not spread on the table and the surface around.
- Then, we used another cotton swab to spread the bacteria horizontally into the petri-dish.
- We swabbed completely the surface of three plates by the bacteria
- We did the same steps for the 2 bacteria.
- We passed the neck of the tube to the flame to sterilize
- We placed a sterile paper disc in each section of the six plates, save the two designated for antibiotics because the sterile papers contain antibodies.
- We put 1 drop of each component on top of the sterile paper.
- I placed a drop of each component (Iodine, Detergent, Soap, Lysol wipes) to the top of each sterile paper in the first inoculation plated with *S. aureus* that was separated into four parts.
- I placed a drop of each component (H2O, Hydrogen peroxide, Bleach, Alcohol) to the top of each sterile paper in the first inoculation plated with *S. aureus* that was separated into four parts.
- We did the same steps with *E. coli* petri-dishes

Results:

Table 1: *Staphylococcus aureus* V.S *Escherichia coli* [H2O(water), Hydrogen peroxide, Bleach, Alcohol]

	Staphylococcus aureus Diameter of zone of inhibition	Interpretation for *Staphylococcus aureus*	*Escherichia coli* Diameter of zone of inhibition	Interpretation for *Escherichia coli*

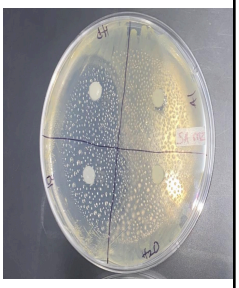
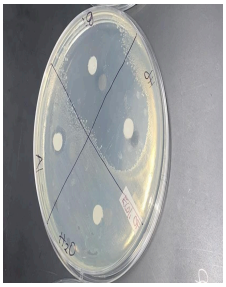
				
H2O (Water)	No zone of inhibition (0 mm)	Resistant	No zone of inhibition (0 mm)	Resistant
Hydrogen peroxide (H2O2)	35 mm	Sensitive	35 mm	Sensitive
Bleach	41 mm	Sensitive	45 mm	Sensitive
Alcohol	8 mm	Sensitive	10 mm	Sensitive

Table 2: *Staphylococcus aureus* V.S *Escherichia coli* (Iodine, Detergent, Soap, Lysol wipes, urine)

	Staphylococcus aureus Diameter of zone of	Interpretation for *Staphylococcus aureus*	*Escherichia coli* Diameter of zone of inhibition	Interpretation for *Escherichia coli*

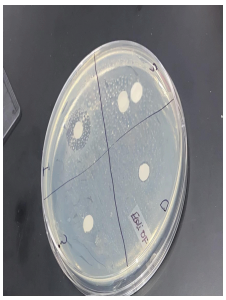
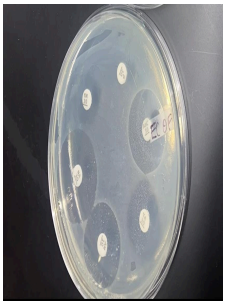
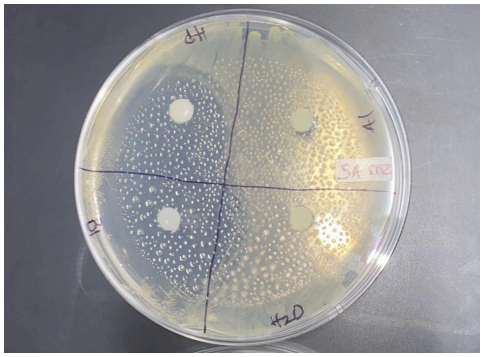
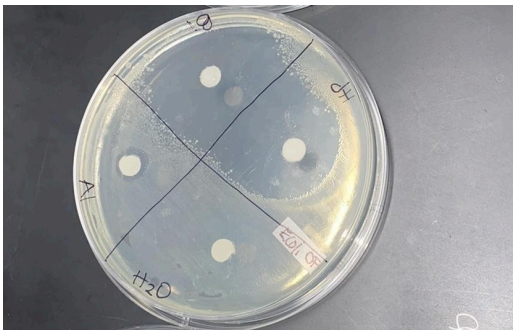
	inhibition			
				
Iodine	17 mm	Sensitive	17 mm	Sensitive
Detergent	7 mm	Sensitive	15 mm	Sensitive
Soap	19 mm	Sensitive	No zone of inhibition (0 mm)	Resistant
Lysol wipes	18 mm	Sensitive	----------	
urine	---------		No zone of inhibition (0 mm)	Resistant

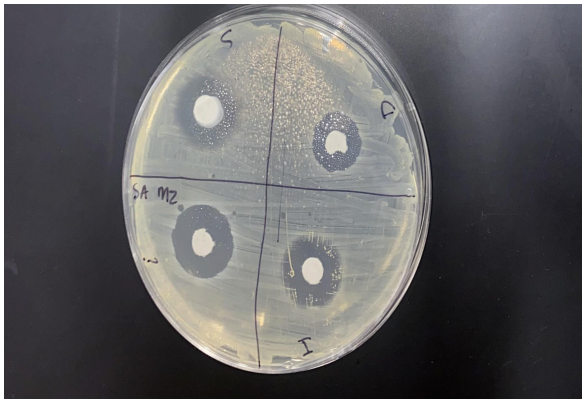
Table3: *Staphylococcus aureus* V.S *Escherichia coli* Nalidixic acid (NA30), Penicillin (P10), Gentamicin (GM10), Amoxicillin (AM10), Tetracycline (Te 30), Amoxicillin/clavulanic acid (Amc 30).

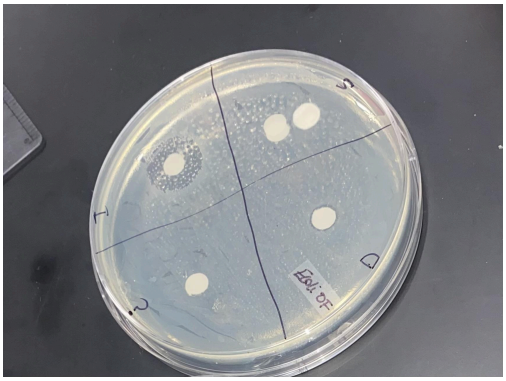
	Staphylococcus aureus Diameter of zone of inhibition	Interpretation for *Staphylococcus aureus*	*Escherichia coli* Diameter of zone of inhibition	Interpretation for *Escherichia coli*
				
Nalidixic acid (NA30)	20 mm	Sensitive	30 mm	Sensitive
Penicillin (P10)	41 mm	Sensitive	No zone of inhibition (0 mm)	Resistant
Gentamicin (GM10)	9 mm	Sensitive	16 mm	Sensitive
Amoxicillin (AM10)	39 mm	Sensitive	23 mm	Sensitive
Tetracycline (Te 30)	32 mm	Sensitive	20 mm	Sensitive

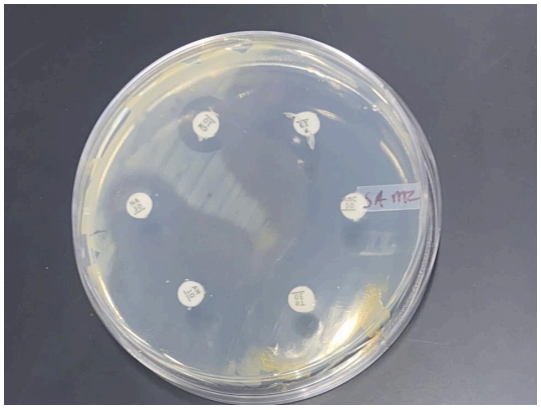
Amoxicillin/clavulanic acid (Amc 30)	39 mm	Sensitive	23 mm	Sensitive

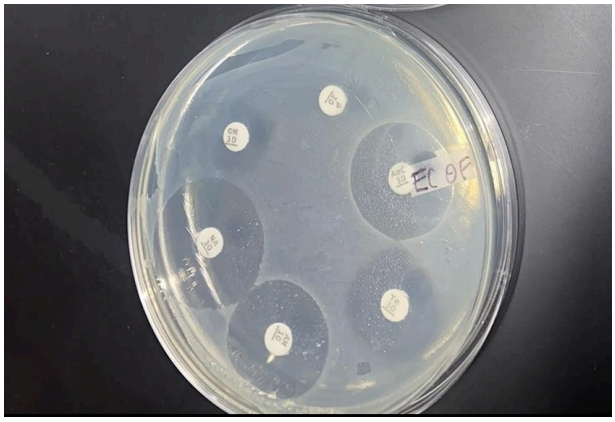












Discussion:

During this experiment, different types of antiseptics were used to observe and follow up their effect on bacteria, namely *Staphylococcus aureus* and *Escherichia coli*, and to measure the area in the event of inhibition. Bacteria classified as gram-positive have less resistance to products than gram-negative bacteria, and as we know that the *E. coli* is a gram-negative but S. aureus is gram-positive, so *E. coli* is more resistant than *S. aureus.* For some inexplicable reason, the findings revealed a comparable diameter of the zone of inhibition. In table 1: We were unable to detect an effect on both bacteria in water. Water is employed as a control agent in the lab because it does not impede bacterial growth. Because the water does not influence bacterial growth, both bacteria have a zone of inhibition of zero; this is also a solid sign that no mistakes were made in the lab.Hydrogen Peroxide had the same impact on the two bacteria as 35 mm, indicating that it is an efficient disinfectant. Bleach has more effect on *E. coli* (45mm) than in the *S. aureus* (41 mm). Alcohol has more effect on *E. coli* (10mm) than in the *S. aureus* (8 mm).

In table 2, We had the same effect of the Iodine on both bacteria as 17mm, indicating that it is an efficient disinfectant. Detergent has more effect on *E. coli* (15mm) than in the *S. aureus* (7 mm). Soaps have more effect on the *S. aureus* (19 mm) than the *E. coli* (0 mm) that make the interpretation for the soap on the *E. coli* resistant and make the interpretation for the soap on the *S. aureus* sensitive. Lysol wipes has a good effect on the *S. aureus* bacteria and it has 18 mm diameter, so it has an efficient disinfectant. We used urine only on the *E. coli* bacteria and it had no effect on the bacteria and it had 0 mm diameter so urine has resistant interpretation.

Table 3 compares the efficacy of several antibiotics against *E. coli* and *S. aureus*. Penicillin was nearly universally used to treat the illness. They used it to treat whatever infection they had

without realizing that certain germs are resistant to it or that it is ineffective. *S. aureus is more* sensitive than the *E. coli* because *E. coli* has no zone of inhibition (0 mm) and this made it more resistant and less sensitive and *S. aureus* was more sensitive because it has 41 mm diameter. From my result, I noticed that the gram negative bacteria is more resistant than the gram positive bacteria to the antibodies. Gram-negative bacteria are more resistant because their cell walls are made up of a thin layer of peptidoglycan surrounded by an outer membrane. So *E. coli* is more resistant than *S. aureus.*

Conclusion:

In the end, In that experiment, we saw different results from the effect of antiseptics and antibiotics on different types of bacteria and how the effectiveness of each form differed from the other. After seeing the result, we can say that the bigger the diameter, the more effective the substance is at restricting bacteria growth. We learned that larger diameter means more sensitive and smaller diameter is lowest sensitive and more resistant. So, pathogens can be destroyed on diverse surfaces in a number of methods, some of which are more effective than others.