INTRODUCTION

Samples must be collected from their correct infection site with minimal contamination. The human body is covered in bacteria that normally cohabit in symbiosis and do no harm, but these are just as likely to be cultured as other organisms. Because of this reason, it is important to use a stereo sample container or swab to only collect the sample from the site of infection and not contaminated it with other parts of the body. When studying urine samples, the samples can be easily contaminate by any residues during micturition. Cleaning the area to remove potential contaminants before taking the sample can improve the chances to find more effectively the infective agent.

It is crucial to use proper aseptic techniques. It is also important to be aware of the type of sample that is used for the diagnosis and identification of bacteria. A contaminated sample or one obtained without the proper care and not within the proper time, could make the results inaccurate and not functional.

Food poisoning is an illness that occurs when contaminated food is eaten. This happens because pathogens such as bacteria, grow on the foodstuff when it is not handled or stored correctly. The aim of culturing the sample is the growth of the causative agents that could be causing the infection. With a selected media, plate-based methods are used for multiple applications such as bacterial culture growth and bacteria can be quickly identified. ELISA or the Gram Stain method are two examples.

The disc-diffusion method is a test used in bacteriology to identify growing pathogens like bacteria and fungi. In a plate with a culture medium, the small discs of a standard filter paper are bathed in certain antibiotics. Visible areas of bacteria can be seen after incubation determining the degree of sensitivity. The diameter of the zone of inhibition of growth reports the antimicrobial potency of the compounds (Horváth, Bencsik, Ács and Kocsis, 2016). It is influenced by several conditions which can be changed to get different results. The test used for fungi and bacteria sensitivity is very similar. The two main differences are the inoculated mycelial fungi in the center of the plate which contains antibiotics and that antibiotics can be in solution form.

1. QUANTITATIVE BACTERIOLOGY IN DIAGNOSIS OF URINARY TRACT INFECTIONS

First part of the experiment

A supposed infected sample was studied to confirm the presence of bacteria. A bacteria count was conducted to determine if the urine sample was truly infected. Following the steps from the LSC203 Medical Microbiology Exercises 2021 – 2022 handbook (Spiers, Andrew, 2021), these were the results obtained.

Firstly, the MacConkey plate's cell count number was noted on the laboratory notebook. The plate with the dilution 10^{-3} grew 6 colonies of *E. Faecalis*. The dilution 10^{-4} had 48 colonies of *P. Vulgaris*. And lastly, 4 colonies of *E. Faecalis* and 1 colony of *E. Coli* could be observed in the plate with the dilution 10^{-5}.

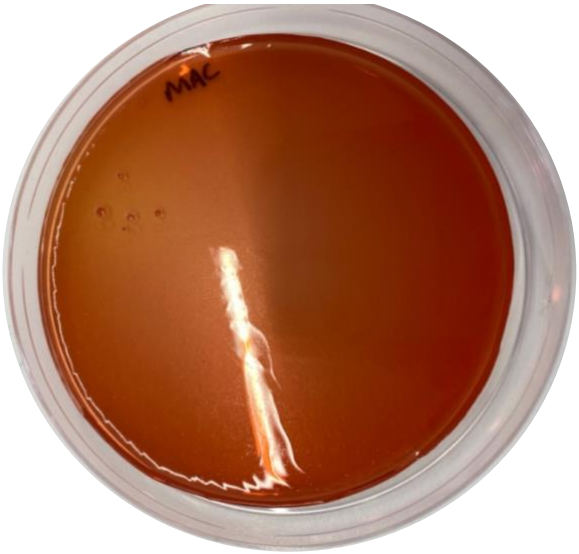


Figure 1. MacConkey plate containing the dilution 10^{-5} of the supposed infected sample. The sample was diluted. Using a micropipette five 20 µl drops were dispensed onto the plate. After some time to let the inocula be absorbed onto the plate, it was incubated in the inverted position overnight at 37°C. This photo was taken a week after the procedure was done, as it was the first opportunity to do so.

After, the cell count number of the MacConkey plates without salt was recorded. The plate with the dilution 10^{-3} grew 8 colonies of *E. faecalis*. The dilution 10^{-4} had 56 colonies of *P. Vulgaris*. And lastly, 9 colonies of *E. Faecalis* could be observed in the plate with the dilution 10^{-5}.

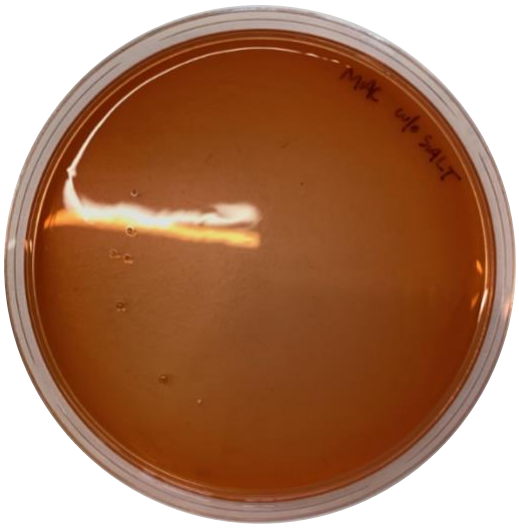


Figure 2. MacConkey without salt plate containing the dilution 10^{-3} of the supposed infected sample. The sample was diluted. Using a micropipette, five 20 µl drops were dispensed onto the plate. After some time to let the inocula be absorbed onto the plate, it was incubated in the inverted position overnight at 37°C. This photo was taken a week after the procedure was done, as it was the first opportunity to do so.

The colonies forming units (CFU) per ml estimate the number of bacteria that are in the sample. The visible colonies in the plate are multiplied by the dilution factor to provide a CFU/ml result.

Only one colony of bacteria *E.Coli* was observed in the plates, thus the sample was barely infected with this bacteria.

The bacteria *E.Feacalis* presented the highest number of bacteria in the MacConkey plates without salt with the dilution 10^{-5}. The number of bacteria in the sample was obtained by using the colonies forming units per ml equation. CFU/ml= Colonies x Plate diluted (ml) x Dil.

$9x\frac{1000\mu l}{100\ \mu l} \times 10^5 = 9x10^6\ CFU/ml$

Considering that all the steps were followed correctly with the proper care, infected urines possess counts of 10^5 or more bacteria per milliliter. This sample presented $9x10^6\ CFU/ml$ of *E.Feacalis*. This number of bacteria is sufficient to be worried, thus the sample was infected with *E.Feacalis.*

The bacteria *P. Vulgaris* presented the highest number of bacteria in the MacConkey plates without salt with the dilution 10^{-4}. The number of bacteria in the sample was obtained by using the colonies forming units per ml equation. CFU/ml= Colonies x Plate diluted (ml) x Dil

CFU/ml= Colonies x Plate diluted (ml) x Dil

$56x\frac{1000\mu l}{100\ \mu l} \times 10^4 = 5.6x10^6\ CFU/ml$

Considering that all the steps were followed correctly with the proper care, infected urines possess counts of 10^5 or more bacteria per milliliter. The result obtained was $5.6x10^6\ CFU/ml$. This number of bacteria is sufficient to be worried, thus the sample was infected with *P. Vulgaris*.

Second part of the experiment

A Gram stain test was conducted to confirm the number of bacteria in the original sample under the microscope.

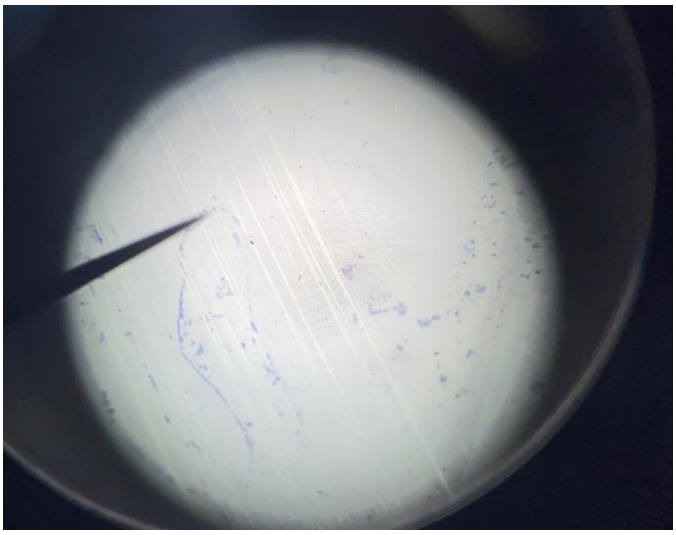


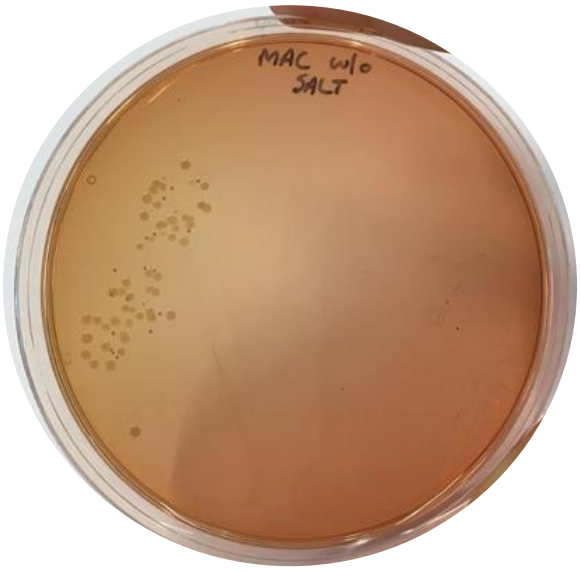
Figure 3. The gram reaction of the stained simple. A heat-fixed bacterial film was covered with crystal violet solution, iodine solution, alcohol (95% ethanol), and safrain solution for 60 seconds. The film was rinsed with water between every step. After allowing it to dry, this picture was taken.

The identification of bacteria is possible by studying the cellular morphology of each stained sample. A crystal violet stain can be observed in figure 3. *E. Coli* and *P.vulagris* are Gram-negative bacteria, which means that they do not retain violet stain, therefore they are not present in the sample. *E. faecalis* is a gram-positive bacterium, and the sample stayed purple. For this reason, we can conclude that the only bacteria present in the sample was *E. faecalis.*

2. SELECTION AND IDENTIFICATION OF FOOD POISONING BACTERIA

The selection and identification of bacteria were made by using chromogenic media. The presence of dyes and certain chemicals in it allowed the colonies to develop with colour tones. In the experiment, sample B is inoculated in the mannitol-salt agar and ChromID_CPS Elite chromogenic media to see if it was contaminated by bacteria. Following the steps from the LSC203 Medical Microbiology Exercises 2021 – 2022 handbook (Spiers, Andrew, 2021), these were the results obtained.

For the dilution 10^{-2}, the MacConkey plate grew 45 colonies of *E. Vulgaris* and 14 *E.Coli*; and the MSA plate had no colonies. The results obtained with the dilution 10^{-3} were 45 colonies of *E. Vulgaris* and 20 *S. Aureus* in the MacConkey without salt plate and again 0 bacteria in



the MSA plate.

Figure 4. MacConkey without salt plate containing the dilution 10^{-3} of the supposed infected sample. Sample B was diluted and 100 µl were inoculated using the spread plating method

onto the plate. Afterwards, it was incubated for 24 h at 37ºC. Then the technicians stored the plate at 4ºC until the next laboratory session, which was six days after, and this photo was taken. 45 colonies of *E. Vulgaris* and 20 colonies of *S. Aureus* can be observed in the figure.

In conclusion, sample B was infected with primary *E. Vulgaris. E. coli* and *S. Aureus* were present in the sample as well.

Biochemical profiling

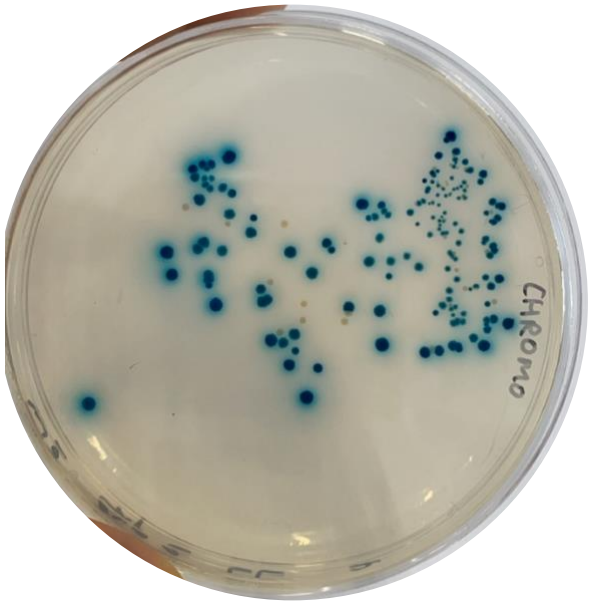


Figure 5. A Mannitol-salt agar and ChromID.CSP Elite agar were inoculated with sample B and 160 colonies of *E. Faecalis* and 29 colonies of *S. Aureus* can be seen in the plate.

A single colony of the Chromo plate was inoculated into a 5ml ringer solution and used to fill the string API 20E. This is a method used for the identification of bacteria based on biochemical tests.

Figure 6. API 20-E string after inoculating each well with the bacterial solution. LDC, ODC, ADH, H2S, and URE were filled halfway because the rest was filled with sterile mineral oil. The strip was incubated overnight at 37ºC. The picture was taken the day after the incubation.

These were the results obtained.

Table 1: API20E results. The bacterial solution tubes from 1 to 20 were classified according to their nature, positive or negative. The score was added to the positive wells and the sum of the scored triad made one of the 7-digital code.

Triad	I			II			III			IV			V			VI			VII		
Tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Oxidase
Reaction	-	-	-	-	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	+	+
Point	0	0	0	0	2	4	1	2	4	0	0	4	1	0	4	1	2	4	0	2	4
Add	0			6			7			4			5			7			6		
7-digital code	0674576																				

The identification of bacteria is possible due to the 7-digital code. This number 0674576 can be read in the API catalog. This is performed using the database (V4.0) with the analytical profile index (bioMérieux, 2002).

3. ANTIBIOTIC SENSITIVITY ASSAYS

My results

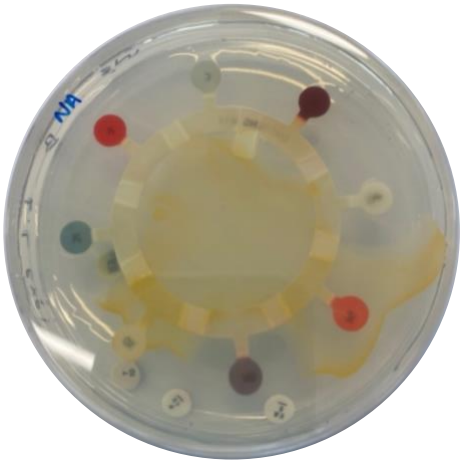


Figure 7. M14 ring. Bacteria solutions were spread in the bacterial plate. The antibiotics used were Ampicillin (AP), Cephalothin (KF), Colistin Sulphate (CO), Gentamicin (GM), Streptomycin (S), Sulphatriad (ST), Tetracycline (T), Cotrimoxazole (TS).

The only two results obtained were the diameter of the zone of inhibition growth of Streptomycin, which was 22 mm, and Tetracycline, which removed all the bacteria in a diameter of 13 mm. The inoculation of the bacterial plates with the M14 ring and the antibiotics was executed in a bad way due to the lack of knowledge and experience. This led

to the erroneous outcome that can be seen in figure 7 and the rest of the antibiotics couldn't be measured.

Figure 8. M14 ring. Using the spread plate technique, the plate was inoculated with the solutions provided. The fungi antibiotics used were Nystatin (NS), Miconazole (MCL), Amphotericin B (AMB), Econazole (ECN), and Flucytosine (FY).

As can be seen in figure 8, none of the fungi antibiotics worked. This was probably because the experiment was not carried properly, and some mistakes led to this outcome.

Class results

The class data set was revised, and the outliers removed. The data were eliminated because they were numerically distant from the rest of the data. This could indicate faulty data, erroneous procedures, or unusual circumstances. For this reason, the data points that fall outside the lower inner fence (LIF=Q_1-(1.5xIQR)) and upper inner fence (UIF=Q_3+(1.5xIQR)), where Q1 =lower quartile, IQR= interquartile range, and Q3= upper quartile, were removed. The data that presented experimental or multiple errors and incorrect data types were not used for the graphics. See Appendix A to observe the data that was used.

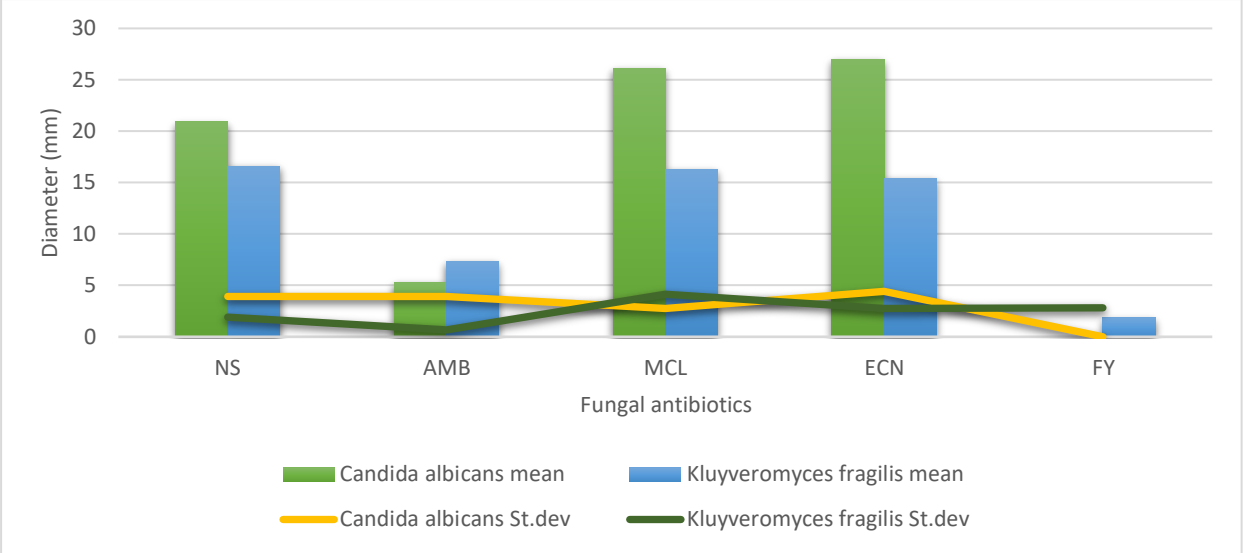


Figure 9. Fungi class result. Showing here is the mean and standard deviation of the set of data, which is the diameter of the zone of inhibition of growth, measured in millimetres. The fungi antibiotics used were Nystatin (NS), Miconazole (MCL), Amphotericin B (AMB), Econazole (ECN) and Flucytosine (FY). In this graphic, the mean of the data results of the fungi *Candida albicans* and *Kluyveromyces fragilis* is being compared to observe which antibiotic is effective against them. The standard deviation indicates how closely the data values in the dataset cluster around the mean.

The chart illustrates the diameter of the zone of inhibition of growth of each antibiotic reacting to *Candida albicans* and *Kluyveromyces fragilis.* It shows that the antibiotics Amphotericin B and Flucytosine presented significant small diameter measurements, therefore they do not work against these bacteria. On the contrary, Miconazole and Econazole produced an effect on *Candida albicans*, and that is why the zone of inhibition is bigger. The antibiotics Miconazole and Econazole had a medium effect in the *Kluyveromyces fragilis*. The same situation happened with Nystatin for both bacteria, this could denote a small antibiotic resistance. In conclusion, the antibiotics Amphotericin B and Flucytosine did not work, and the rest had a diameter superior to 15 mm. This could be because these two antibiotics are used together for some treatments and these bacteria could have developed resistance against them already (P. H. J. van der Voort, 2008).

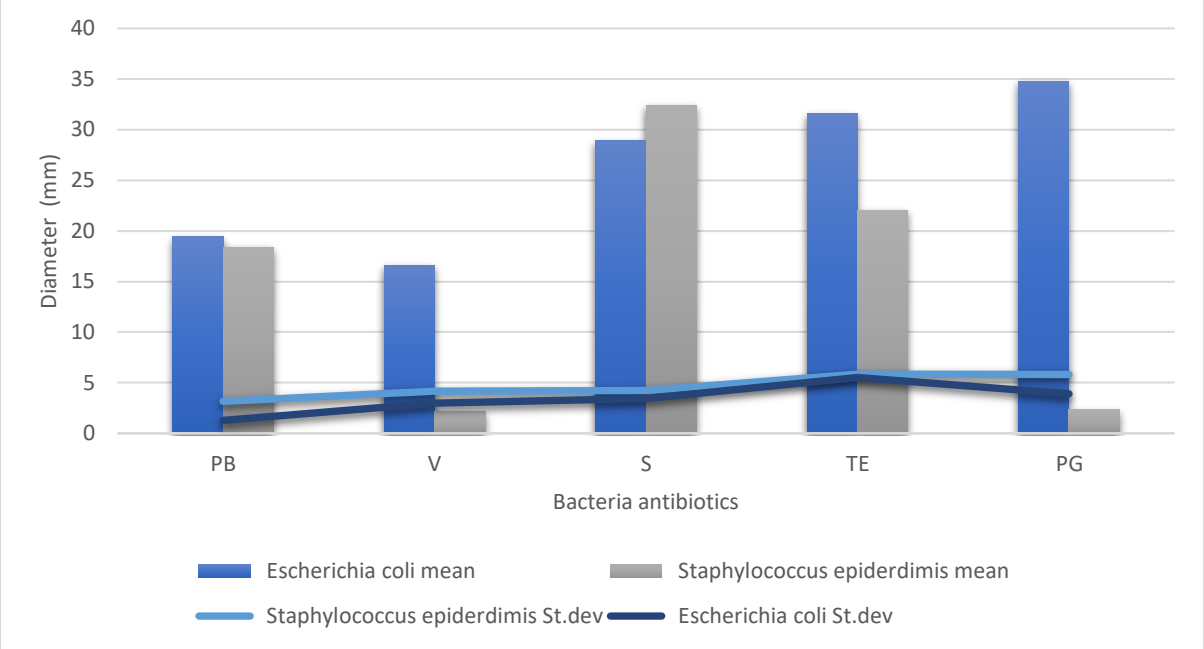


Figure 10. Individual disc class result. Showing here is the mean and standard deviation of the set of data. The data is the diameter of the zone of inhibition of growth, measured in millimetres. The bacteria antibiotics used were Polymixin B (PB), Vancomycin (V), Streptomycin (S), Tetracycline (TE) and Penicillin G (PG). In this graphic, the mean of the data results of the bacteria *Escherichia coli* and *Staphylococcus epidermis* are being compared to observe which antibiotic is effective against them. The standard deviation indicates how closely the data values in the dataset cluster around the mean.

The graphic shows the diameter of the zone of inhibition of growth of each antibiotic reacting to *Escherichia coli* and *Staphylococcus epidermis*. It could be noticed that the antibiotics Streptomycin and Tetracycline presented a significant number in the diameter data, therefore they worked on the bacteria tested. On the other hand, *Staphylococcus epidermis* showed substantial-high resistance to Vancomycin and penicillin G, as the data was dramatically low. In general, this bacterium did not react as much as *Escherichia coli* to the antibiotics, except for Streptomycin. The reason for it could be the nature of the bacteria, *Escherichia coli* is a gram-negative anaerobic bacteria and *Staphylococcus epidermis* gram-positive.

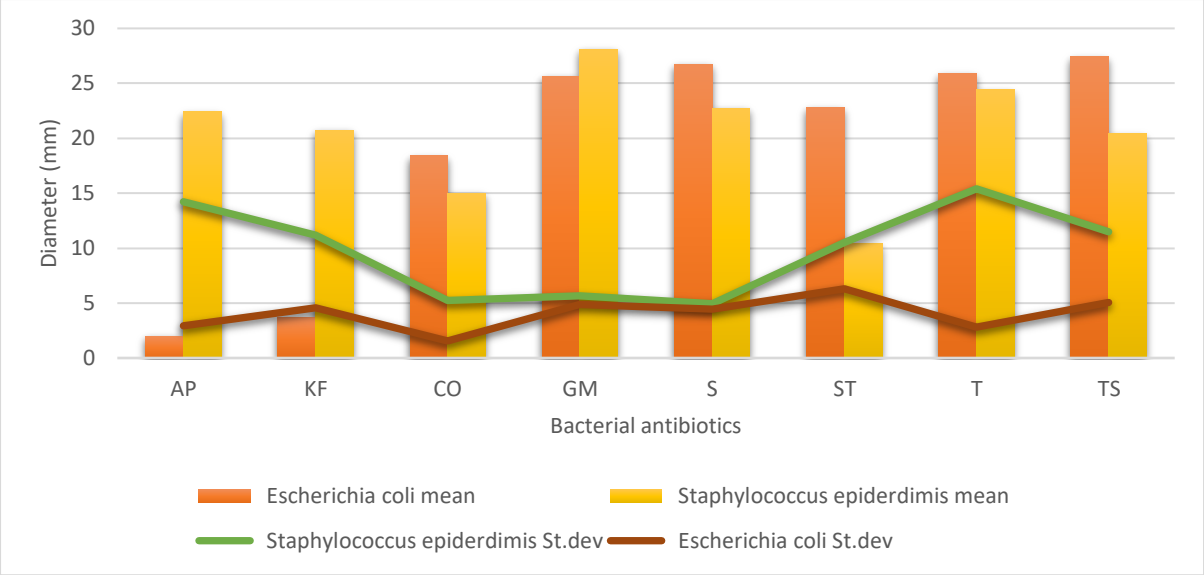


Figure 11. M14 Ring class result. Showing in the graph is the mean and standard deviation of the set of data. The data is the diameter of the zone of inhibition of growth, measured in millimetres. The bacteria antibiotics used were Ampicillin (AP), Cephalothin (KF), Colistin Sulphate (CO), Gentamicin (GM), Streptomycin (S), Sulphatriad (ST), Tetracycline (T), Cotrimoxazole (TS). The mean of the data results of the bacteria *Escherichia coli* and *Staphylococcus epidermis* are being compared to observe which antibiotic is effective against them. The standard deviation indicates how closely the data values in the dataset cluster around the mean.

The figure represents the diameter of the zone of inhibition of growth of each antibiotic reacting to *Escherichia coli* and *Staphylococcus epidermis*. It is interesting to note that the antibiotics Gentamicin, Streptomycin, Tetracycline, and Cotrimoxazole worked very well against both bacteria. *Escherichia coli* presented an enormous resistance against the antibiotics Ampicillin and Cephalothin. On the contrary, *Staphylococcus epidermis* presented a wide diameter measurement, indicating the effectiveness of the antibiotics. This bacterium was slightly resistant against Sulphatriad. Overall, the antibiotics worked against these two bacteria.

4. Conclusion

Firstly, the sample from the first experiment was infected with *E. faecalis.* This was confirmed by the microscopic examination of cells from an infected sample. The identification and counting of colonies indicated that the sample was also infected with *P. Vulgaris.*

Secondly, sample B from the second experiment was infected with primary *E. Vulgaris. E. coli* and *S. Aureus* were present in the sample as well. The API 20 E 7-digit code was 0674576, which can be read in the API catalogue for the identification of bacteria.

Lastly, the antibiotics most effective against *Candida albicans* and *Kluyveromyces* were Miconazole, Econazole, and Nystatin. The ones that worked against *Escherichia coli* and *Staphylococcus epidermis* in the M14 ring were Gentamicin, Streptomycin, Tetracycline, and Cotrimoxazole. Streptomycin and Tetracycline worked effectively against these two bacteria in the individual discs.

5. SELF-REFLECTION

As an international student, going to the laboratory was a double challenge. I didn't have any experience in it from my high school back in Spain, so my lack of knowledge of the technical words and experience in the laboratory made me feel anxious and a bit scared. The coronavirus measures did not allow us to gain the expected knowledge from our first year and all the experience we had was through the website Labster. My solution was to read the lab protocol the day before, making sure I understood every word, and to do a bit of research about the topic.

I was enjoying a lot the content in the module so even if I was a bit nervous every Monday morning, I was looking forward to going to campus and learning with my classmates. I believe that real growth comes from finding yourself comfortable in uncomfortable and unfamiliar situations. After the first session, I felt despair and overwhelmed due to my inexperience. I want to carry a career in the research area and the laboratory is an essential part of it. This cross my mind because I was lost and I made several mistakes, but as the days went by, I realized that confidence comes from experience. This module allowed me to discover more about microbiology and the on-campus sessions made me see that this could be a career path that I would enjoy. I am glad that I had this opportunity to see this and to develop my skills to feel more secure and confident in the laboratory.

APPENDIX A

Table 2: *Staphylococcus epiderdimis* class data.

Replicate	M14 Mast Ring								Individual Discs				
	AP	**KF**	**CO**	**GM**	**S**	**ST**	**T**	**TS**	**PB**	**V**	**S**	**TE**	**PG**
1									21	21	30	>30	>30
2			6	32	22								
3	30	20	16	33	32	30							
4			15		20	0							
5	14	16	12	30	16	8	47	28	15	15	30	46	38
6		22	18	22	25	9	25	24	23	20	34		40
7	6	6	6			6	6	6		6			21
8	24	20	24	26	26	26	26	20	24	11	28		34
9	25	30	18	22	26	21	23	23	23	19	31	30	28
10	21	31	14						21	19	35	25	31
11	40	26	17	20	14	0	30	30	20	17	35	31	35
12	42	36	13	29	22	15	38	32	18.5	17	24.5	32	38.5
13			14	29	24	0			15	19	24	32	36
14						0							
15	0	0	22	38	23	10	0	0	16	16	25	28	42
17									19	20	26	31	35
18									17	16	24	29	39

Table 3: *Escherichia coli* class data.

Replicate	M14 Mast Ring								Individual Discs				
	AP	KF	CO	GM	S	ST	T	TS	PB	V	S	TE	PG
1									17	0	35	19	0
2									17	0	35	19	0
3	0	0		13	25		22	16					
4	0	0	17	27	30	20	27	29					
5	6	6	18	22	18	21	24		18			15	
6	6	6	16	25	30	15	25	29		6	34	18	6
7										6	30		12
8	6	6	18	28	30	25	22	22	19	6	32	20	6
9	6	6	20	22	20	9	28	24	19	6	34	16	6
10	6	6	16	25	28	18	27	28	16	6.2	30	19	
11	0	0	18	27	25	26	30	35	18	0	29	24	0
12	0	14	17				30	32	18		39	28	0
13	0	0	20	21	21	21	21	21	18	0	25		0
14	0	0	19	30	30	29	28	30	19	0	30	20	0
15	0	0	19	30	30	29	28	30	19	0	30	20	
16	0	11	20	30	30	31	26	30	19	0	35	28	0
17	0	0	21	30	25	27	25	26	21	0	35	28	0
18	0	0	19	29	32	26	25	31	20	0	33	34	0

Table 4: Fungal species class data.

Replicate	*Candida albicans*					*Kluyveromyces fragilis*				
	NS	**AMB**	**MCL**	**ECN**	**FY**	**NS**	**AMB**	**MCL**	**ECN**	**FY**
1	20	7	27	25	0					
2	15	7	25	24	0					
3	17	8	23	23	0					
4	17	8	27	18	0					
5	24	7		35	0					
6						17	7	13	15	0
7						13	6	16	13	0
8	21	8					7	20	15	6
9	17	8	27	25		19	8	19	15	6
10	20	8	25	20		17	8	13	13	6
11	24	8	33	30		18	7	17	21	5
12	12	7	24			18	7	17	13	6
13	22	7	31	36		17	7	19	12.5	6
14	30	0	25	32	0	17	7	25	15	0
15	20	0	27	27	0	17	7	9	17	0
16	23	4	24	26	0	17	7	14	16	0
17	25	9	26	30	0	15	7	18	17	0
18	25	9	26	30	0	15	7	18	17	0
19	24		25		0	18		15	11	0
20	21.5	7	26.5	26	0	16	8	16	18	0
21	20	8	25	26	0	17	8	15	15	0
22	27	0	30		0	15		14	16	0
23	17	12	20	24	0		8			7
24	18	0	23	22	0	12	7	13	11	0
25	22	0	28	29	0	20	9			0
26	22	0	28	29	0	19				0
27	18	0	24	28	0	17	8	25	21	0
28	22	0	28	29	0	15	7	10	16	0