Detection of antibiotics in urine samples

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Summary

The aim of this study was to investigate the antimicrobial activity of 2 urine samples in raw and treated (filtered) form and to specify the antimicrobial capacity relative to that of Ciprofloxacin. The conclusion of two different experiments is that that the two raw urine samples have antimicrobial activities equal to 0.57 (±0.09) g/L and 0.31 (±0.01) ciprofloxacin, respectively, and that the treated urine samples have no detectable antimicrobial activity.

Materials and Methods

Sample description

Two urine samples were initially received for analysis; one raw urine sample (henceforth denoted A1) and one sample of the same urine having been treated by filtration (henceforth denoted A2). Later, two more samples were received for analysis, one raw urine (henceforth denoted B1) and one sample of the same urine having been treated by filtration (henceforth denoted B2).

The raw urine sample A1 was given to contain Cefotaxim, Meropenem, Bactrim/Eusaprim and Tazocin. The raw urine sample B1 was given to contain Bensylpenicillin, Cefotaxim/Claforan, Cefuroxim/Zinacef, Cloxacillin/Ekvacillin, EryMax/Abboticin, Flagyl/Metronidazol/Elyzol, Rimactan, Bactrim/Eusaprim and Tazpcin.

To concentrate the antibiotics present in samples A1 and A2, these were freeze dried. 500 µl was first removed from each sample for future use. The remaining volumes were freeze dried and reconstituted in 500 µl MilliQ water. The concentrated samples were denoted A1' and A2'.

Pilot experiment

Escherichia coli ATCC 25922 Vitroids were ordered from Sigma Aldrich. To initiate a liquid culture, one vitroid disk of *E. coli* was used to inoculate 5 ml Luria Bertoni (LB) broth in a sterile 15 ml Falcon tube which was then incubated over night at 37 °C. The optical density at 600 nm (OD_{600}) was determined and the culture was diluted with LB to OD_{600} = 0.3.

Aliquots of 100 µl diluted over night *E. coli* culture were plated on three Tryptic Soy Agar plates (TSA, Gamma irradiated environmental settle

plates, 90 mm, from Merk) using a glass spreader. The plates were incubated up side down for 1 h at room temperature.

1 Mast disk containing 5 µg ciprofloxacin (Disk 1) and 4 blank Mast disks (12 x 6.6 mm, Disks 2-5) were placed on the triplicate *E. coli* loaded TSA plates. Prior to being placed on the plates, the blank Mast disks were prepared with; Disk 2) 15 µl sample A1, Disk 3) 15 µl sample A2, Disk 4) 15 µl sample A1' and Disk 5) 15 µl sample A2'. The plates were then incubated at room temperature for 1 h right side up followed by overnight incubation at 37 °C. The diameter of any observed growth inhibition zones around the disks was measured with a caliper. The growth inhibition zone diameter was calculated as the average of five separate measurements of the same inhibition zone.

Final experiment

E. coli cultures were initiated and maintained as described above with the differences being that Tryptic Soy Broth (TSB) was used instead of LB and that in-house prepared Luria Agar plates were used instead of purchased TSA plates. Ciprofloxacin standard solutions were prepared by diluting a stock solution of 0.5 g/L ciprofloxacin in 100 mM sodium acetate buffer (pH = 5) in steps of x2 in the same buffer, yielding a final standard series of 0.5, 0.25, 0.125, 0.0625 and 0.03 g/L ciprofloxacin. Higher antibiotic concentrations were not possible to prepare due to solubility issues. Urine samples A1 and B1 were first diluted 5x in MilliQ-water and then in steps of x2 so that the following dilutions were available for analysis; 5x (sample A1/B1-5, 10x (sample A1/B1-7), 20x (sample A1/B1-9), 40x sample A1/B1-11), 80x (sample A1/B1-13) and 160x (sample A1/B1-15). Urine sample B1 was also used in a 3x dilution with MilliQ-water (B1-3). Urine samples A2 and B2 were used undiluted. 15 µl of all sample- and standard series dilutions were applied to blank Mast disks which were then placed on LA plates on which 100 µl of an over night culture of *E. coli* diluted to OD_{600}=0.4 over night culture had been plated. The layout of the disks on the plates is illustrated in Figures 1 and 2 below. Note that all plates were done in triplicate. The plates were incubated at 37 °C over night after which the diameter of any observed growth inhibition zones around the disks was measured with a caliper. The growth inhibition zone diameter was calculated as the average of five separate measurements of the same inhibition zone.

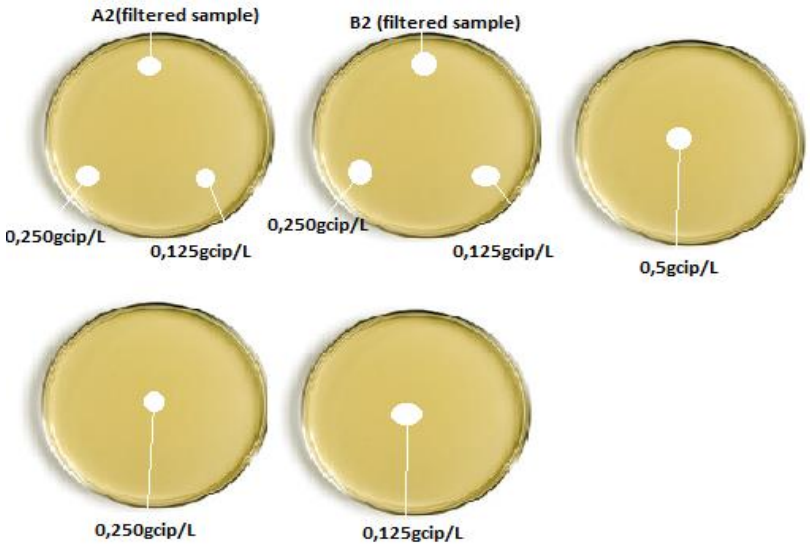


Figure 1. Layout of the plates containing ciprofloxacin reference samples and the undiluted A2 and B2 urine samples.

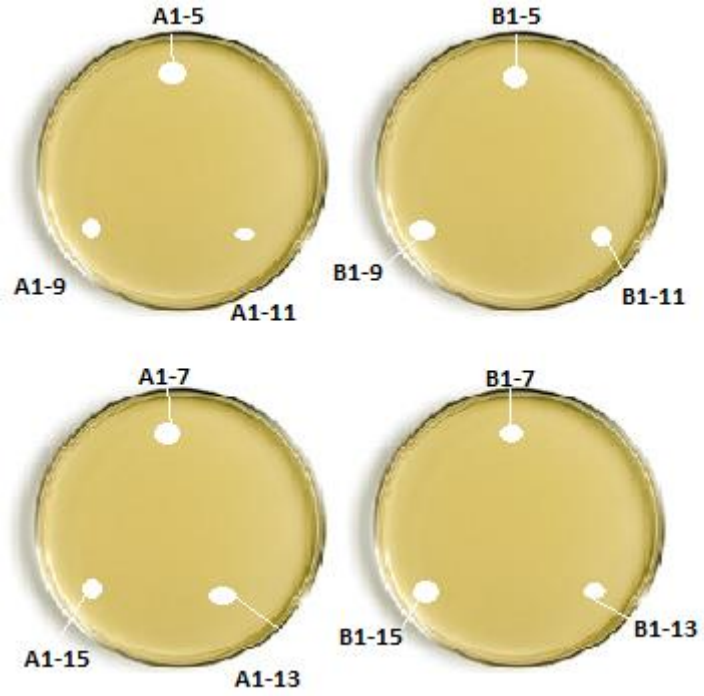


Figure 2. Layout of the plates containing diluted urine samples A1 and B1.

Results

Pilot experiment

Images of the plates can be found in Appendix 1. No growth inhibition zone was identified for samples A2 or A2' which indicates that the

filtration treated urine sample does not contain any detectable levels of antimicrobial agents. The raw urine sample A1 and its' freeze dried counterpart A1' yielded clear growth inhibition zones that were larger than than of the 5 µg ciprofloxacin reference disk. This indicates that the potency of the antimicrobial agents in the A1 sample exceeds the that of a corresponding ciprofloxacin solution at 0.333 g/L. Interestingly, the growth inhibition zone of the freeze dried sample, A1', was smaller than that of the original sample.

Final experiment

Images of the plates can be found in Appendix 2. The results of this extended experiment supports those of the pilot experiment that the filtration treated samples A2 and B2 have no antibiotic activity.

The inhibition zone diameters for reference disks loaded with 15 µl solutions of 0.5, 0.250 and 0.125 g/L ciprofloxacin were determined to be 34.23mm, 31.37mm and 28.52 mm, respectively. The two lower concentrations did not yield detectable growth inhibition zones.

The diameters of A1-5, A1-7, A1-9, A1-11, A1-13 and A1-15 were measured to be 28.65, 25.8, 23.37, 21.17, 18.44 and 16,21 mm, respectively. The diameters for B1-3, B1-5, B1-7, B1-11, B1-13 and B1-15 were measured to 28.46, 22.85, 20.95, 19.73, 16.79, 13.44 and 10.13 mm, respectively. Extensive result tables can be found in Appendix 3.

The growth zone diameters of the ciprofloxacin reference samples were plotted against ciprofloxacin concentration (Figure 3) and the equation of the trend line was used to calculate the equivalent potency of the A1 and B1 urine samples. Unfortunately, only the 5x dilution of sample A1 and the 3x dilution of sample B1 had growth inhibition zones with diameters that were within the range of the standard series. Thus, only these dilutions were used to calculate the corresponding antimicrobial activity of the samples. Compensating for sample dilution, sample A1 has a antimicrobial potency equivalent to a ciprofloxacin solution of 0.57 (±0.09) g/L. The corresponding potency equivalency of sample B1 is 0.31 (±0.01).

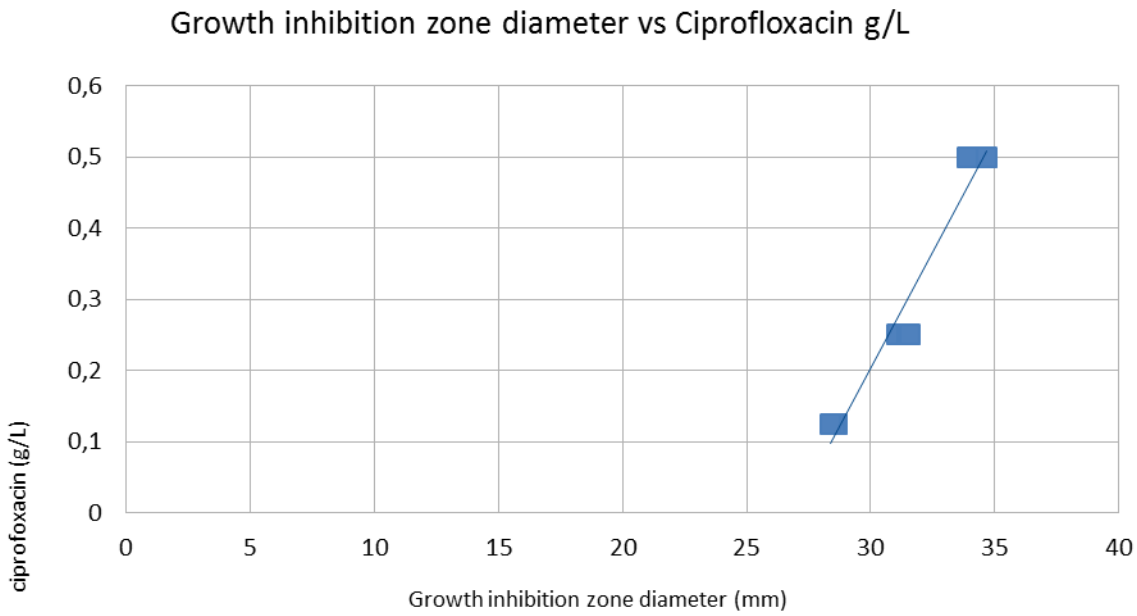


Figure 3. Plot of the growth inhibition zone diameters agains ciprofloxacin concentration of the three reference samples that had antimicrobial activity. Note that n=3 for each concentration.

Conclusion

The experiments done in this study shows that the raw urine samples have antimicrobial activity equivalent to that of a ciprofloxacin solution of 0.3 – 0.6 g/l. The results further show that the filtration treated samples do not exhibit any detectable antimicrobial activity.

Appendix 1

Pilot experiment

Picture 3: Plate A of pilot experiment with clockwise order of samples. The sample order is 5µg Ciprofloxacin (disk with letters on top), sample A1, sample A2, sample A1' and sample A2'.

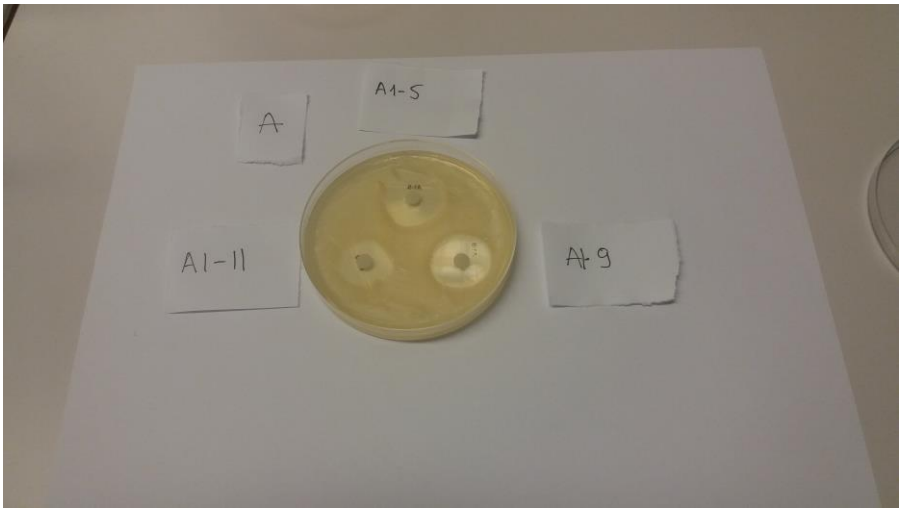
Picture 4: Plate B of pilot experiment with clockwise order of samples. The sample order is 5µg Ciprofloxacin (disk with letters on top), sample A1, sample A2, sample A1' and sample A2'.

Picture 5: Plate C with contrary clockwise order of the samples. The sample order is disk with 5µg Ciprofloxacin Ciprofloxacin (disk with letters on top), sample A1, sample A2, sample A1' and sample A2'.

Appendix 2

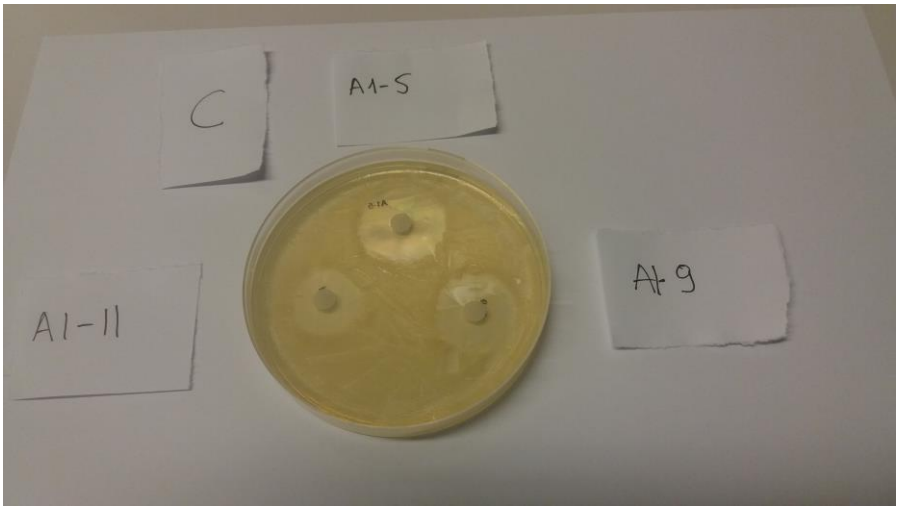
Final experiment

Sample plates

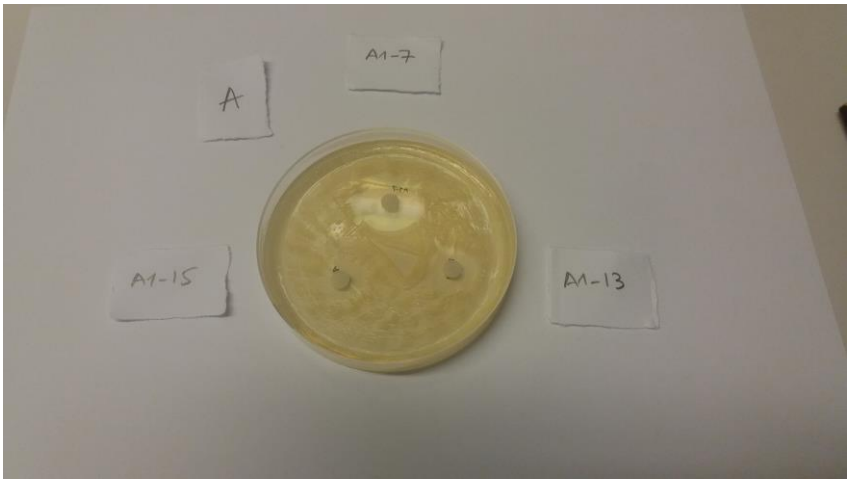


Picture 6: Plate A that contains 3 disks with A1-5, A1-9 and A1-11

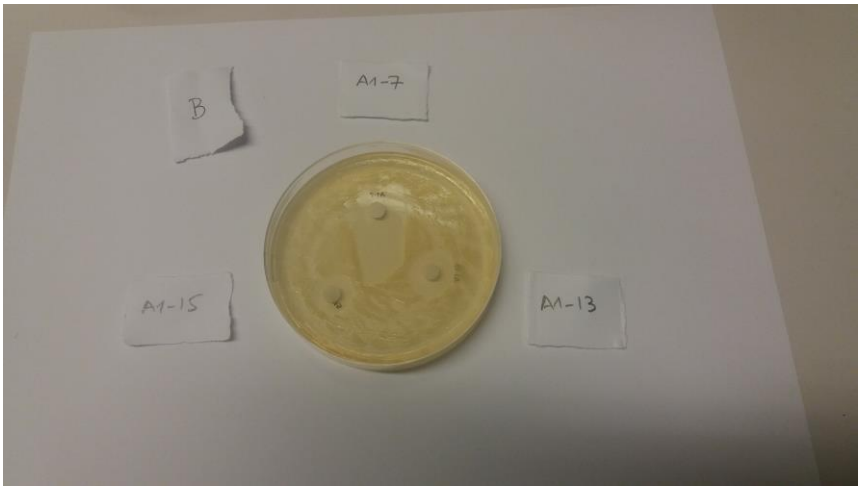
Picture 7: Plate B that contains 3 disks with A1-5, A1-9 and A1-11



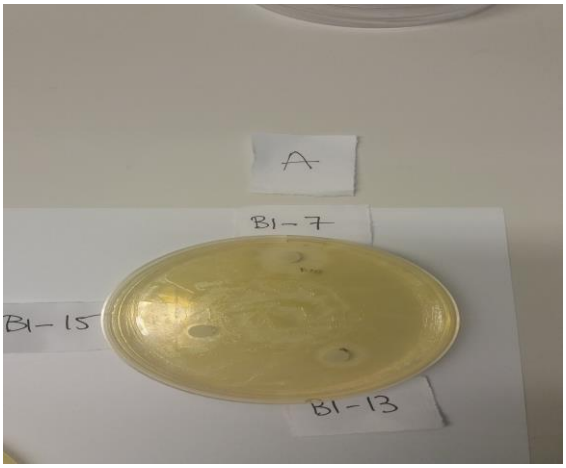
Picture 8: Plate C that contains 3 disks with A1-5, A1-9 and A1-11



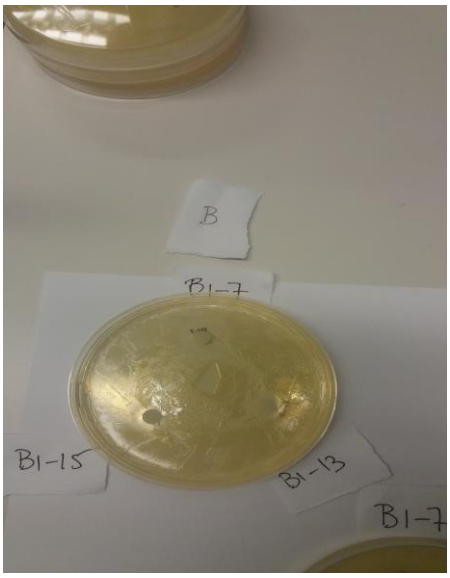
Picture 9: Plate A that contains 3 disks with A1-7, A1-13 and A1-15



Picture 10: Plate B that contains 3 disks with A1-7, A1-13 and A1-15.

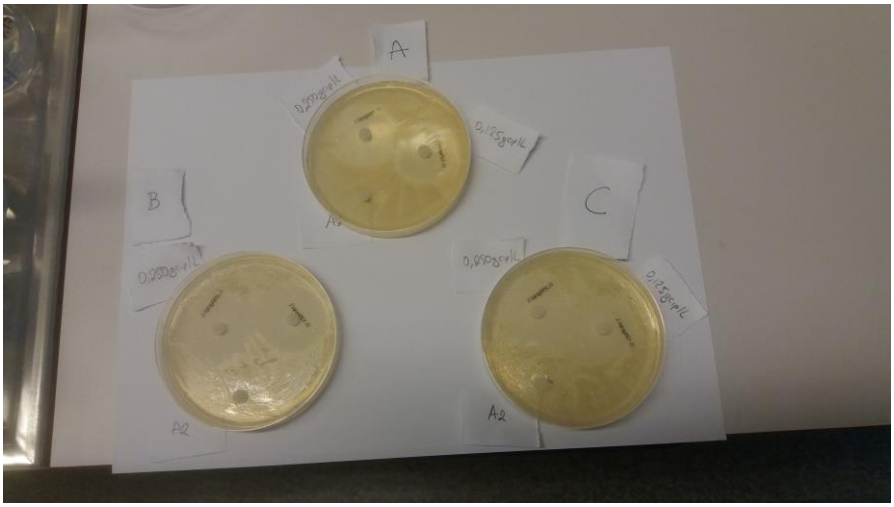


Picture 14: Plate A that contains 3 disks with B1-7, B1-13, B1-15.

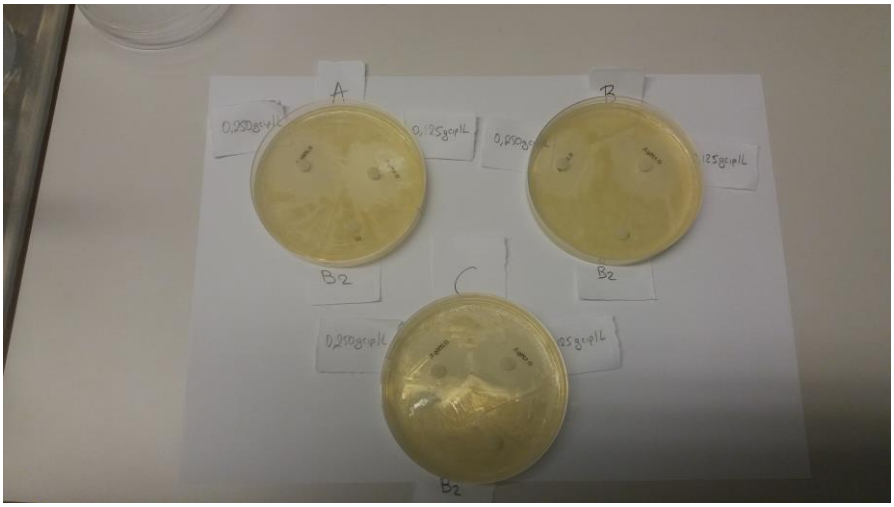


Picture 15: Plate B that contains 3 disks with B1-7, B1-13 and B1-15.

Picture 16: Plate C that contains 3 disks with B1-7, B1-13 and B1-15.

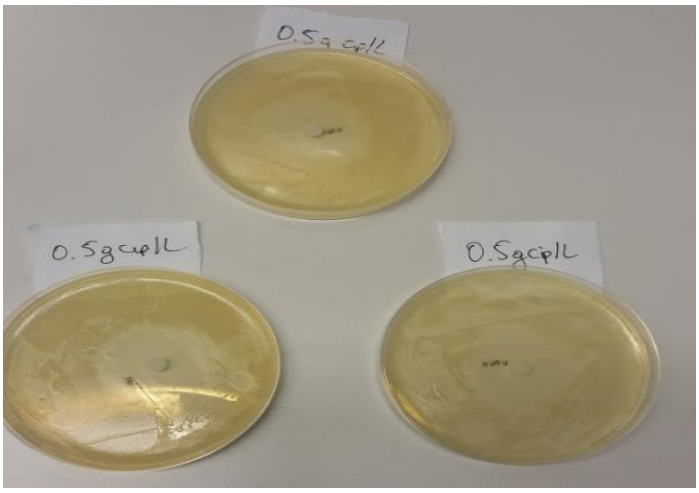


Picture 17: Plate A,B and C that contains 3 disks with 0,250g cip/L, 0,125g cip/L and sample A2.

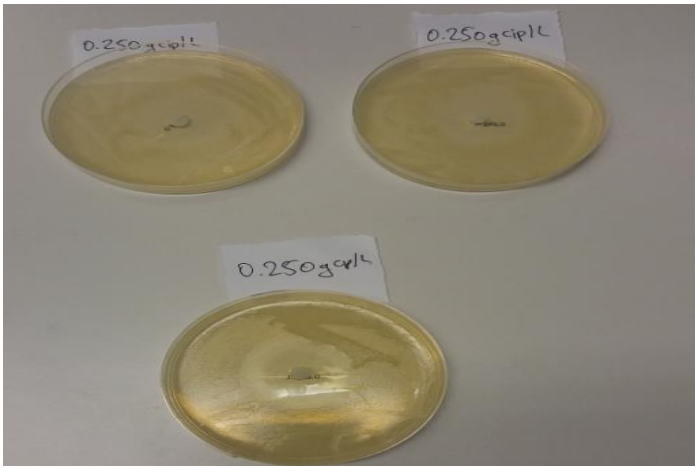


Picture 18: Plate A,B and C that contains 3 disks with 0,250g cip/L, 0,125g cip/L and sample B2.

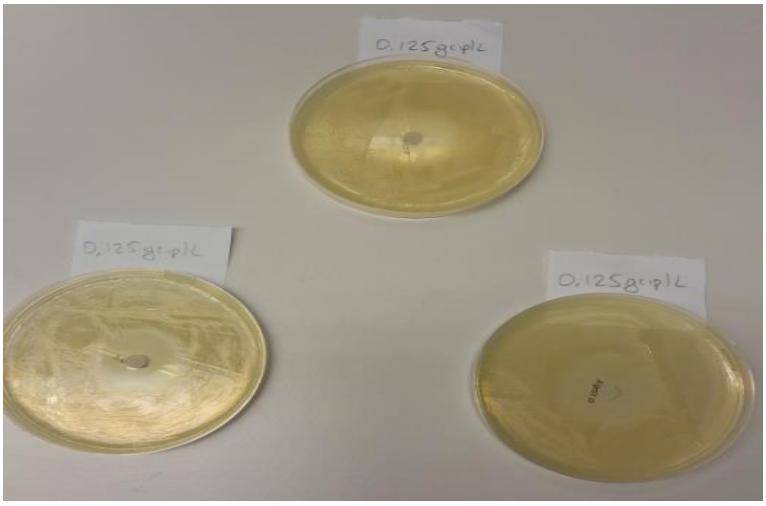
Reference plates



Picture 19: Reference plates A,B and C that contain disk with Ciprofloxacin concentration 0,5g/L.



Picture 20: Reference plates A,B and C that contain disk with Ciprofloxacin concentration 0,250g/L.



Picture 21: Reference plates A,B and C that contain disk with Ciprofloxacin concentration 0,125g/L. **Appendix 2**

Appendix 3

Sample A1

A1-5

Plate A

	Diameter (mm)
1	29,15
2	28,9
3	28,65
4	28,7
5	29,3

Plate B

	Diameter (mm)
1	28,75
2	28,5
3	28,4
4	28,65
5	28,9

Plate C

	Diameter (mm)
1	29
2	28,2
3	28,3

4	28,4
5	28,38

A1-7

Plate A

	Diameter (mm)
1	23
2	25,55
3	25,6
4	25,65
5	26,2

Plate B

	Diameter (mm)
1	-
2	-
3	-
4	-
5	-

Plate C

	Diameter (mm)
1	26,6

2	24,9
3	25,2
4	26,35
5	25

A1-9

Plate A

	Diameter (mm)
1	23
2	25
3	23,5
4	23,15
5	23

Plate B

	Diameter (mm)
1	23,05
2	23,4
3	23,7
4	23,25
5	23,6

Plate C

	Diameter (mm)
1	23
2	23,65
3	23,85
4	24,15
5	23

A1-11

Plate A

	Diameter (mm)
1	20,7
2	21,2
3	20,55
4	21,1
5	21,35

Plate B

	Diameter (mm)
1	21,2
2	20,85
3	21,3
4	21,25

5	21,3

Plate C

	Diameter (mm)
1	21,25
2	21,3
3	21,4
4	21,5
5	21,3

A1-13

Plate A

	Diameter (mm)
1	18,1
2	18,3
3	17,95
4	18,2
5	18,15

Plate B

	Diameter (mm)
1	19,6

2	18,1
3	19,3
4	18,5
5	18,7

Plate C

	Diameter (mm)
1	18,7
2	17,9
3	18,2
4	18,6
5	18,4

A1-15

Plate A

	Diameter (mm)
1	16,5
2	15,85
3	16
4	16,35
5	15,9

Plate B

	Diameter (mm)
1	16,1
2	16,2
3	16,25
4	16,2
5	16,3

Plate C

	Diameter (mm)
1	16,4
2	16,1
3	16,35
4	16,35
5	16,3

Sample B1

B1-3

Plate A

	Diameter (mm)
1	28,3
2	28,7

3	28,4
4	28,5
5	28,6

Plate B

	Diameter (mm)
1	28
2	28,65
3	28,7
4	28,4
5	28,55

Plate C

	Diameter (mm)
1	28,4
2	28,3
3	28,3
4	28,45
5	28,7

B1-5

Plate A

	Diameter (mm)

1	22,7
2	23,3
3	23
4	23,4
5	23,45

Plate B

	Diameter (mm)
1	23,1
2	22,7
3	22,5
4	22,8
5	23

Plate C

	Diameter (mm)
1	22,45
2	22,6
3	22,4
4	23,4
5	23

B1-7

Plate A

	Diameter (mm)
1	21
2	21,3
3	21,5
4	20,5
5	20,45

Plate B

	Diameter (mm)
1	20,75
2	19,8
3	20,5
4	20
5	20,5

Plate C

	Diameter (mm)
1	19,8
2	20,5
3	20,5
4	20,7
5	20,65

B1-9

Plate A

	Diameter (mm)
1	20,1
2	19,75
3	20
4	19,45
5	19,8

Plate B

	Diameter (mm)
1	20,75
2	19
3	20,35
4	20
5	19,6

Plate C

	Diameter (mm)
1	20,5
2	20
3	19,6

4	20,4
5	19,3

B1-11

Plate A

	Diameter (mm)
1	17
2	16
3	16,3
4	16,7
5	17

Plate B

	Diameter (mm)
1	16,6
2	17
3	17,5
4	16,6
5	16,6

Plate C

	Diameter (mm)

1	17,2
2	16,9
3	16,7
4	17,1
5	16,7

B1-13

Plate A

	Diameter (mm)
1	13,7
2	13,3
3	13,8
4	13,7
5	13,6

Plate B

	Diameter (mm)
1	14
2	12,9
3	13,2
4	13
5	13,6

Plate C

	Diameter (mm)
1	13,4
2	13,3
3	13
4	13,6
5	13,5

B1-15

Plate A

	Diameter (mm)
1	10,75
2	11
3	10,4
4	10,4
5	10,65

Plate B

	Diameter (mm)
1	10,15
2	10,3
3	10,35

4	10,4
5	10,25

Plate C

	Diameter (mm)
1	9,7
2	8,9
3	9,5
4	10
5	9,5