Visualisation of the effects of antimicrobial agents on bacterial cells

Charlotte Neely and Amy Ward

Project Supervisor: Dr Clare Miller

Faculty of Science

School of Life Sciences

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Abstract:

The aims and objectives of this project were to view the effects of antimicrobial peptides (AMPs) on bacteria upon the Scanning Electron Microscope (SEM). Also, to work out what the lowest concentration of AMPs is that still has an effect on bacteria using a multipoint inoculator to carry out Minimum Inhibitory Concentration (MIC) tests. This project consisted of two antimicrobial agents, polymyxin B and Nisin. The effects of these were viewed upon two bacteria, *Staphylococcus aureus* and *Escherichia coli*. The methods used in this project consisted of; processing and coating bacteria in order for them to be used upon the SEM, using the SEM and also doing an MIC of the bacteria and AMPs. The results gained proved; the higher the concentration of AMPs, the lower the concentration of bacteria. Also, the lower the dilution of bacteria, the more of an effect the AMPs have on them. Images gained from the SEM are also available in the results. To conclude, polymyxin B does have an effect on *S.aureus* and *E.coli*. The images produced showed some clear images of both organisms. More work on the effects of Nisin on *S.aureus* and *E.coli* can be undertaken in the future along with the effects of Polymyxin B and Nisin on other types of bacteria.

Introduction:

The aims and objectives of this project were to view the effects of antimicrobial peptides (AMP) on bacteria upon the Scanning Electron Microscope (SEM). Also, to work out what the lowest concentration of AMPs is that still has an effect on bacteria using a multipoint inoculator to carry out Minimum Inhibitory Concentration (MIC) tests.

This project consisted of two antimicrobial agents, polymyxin B and Nisin; the effects of these were viewed upon two bacteria, *Staphylococcus aureus* and *Escherichia coli*.

This project was chosen because AMPs are a widely growing advancement within medicine and this type of project is easily able to build upon. Polymyxin B and Nisin were chosen due to the fact that Polymyxin B acts upon Gram negative bacteria and Nisin has an effect on Gram positive bacteria, giving the project a wide range of possible bacteria to work with. This gives reasons for also why *E.coli* and *S.aureus* were chosen due to *E.coli* being Gram negative and *S.aureus* being Gram positive, meaning Polymyxin B would work upon *E.coli* and Nisin would affect *S.aureus (Potapova, 2011)*.

A similar project looked at the effects of polymyxin B upon *E.coli*, viewing the effects of Polymyxin B on the structure of *E.coli*. This project differs as it views an image of the effects using an SEM *(Urakawa, 2010)*.

Method:

MIC Method for dilutions of Polymyxin B:

1. Weigh out 128mg of Polymyxin B into a plastic weighing boat using a fine tipped spatula and empty into a sterile 1ml Eppendorf tube.
2. Add 1ml sterile water using a p100 automatic pipette and dissolve the Polymyxin B into solution. This will be a stock solution.
3. Label this tube 128 mg/ml.
4. Label a further 7 sterile Eppendorf tubes with the following: 64 mg/ml, 32 mg/ml, 16 mg/ml, 8 mg/ml, 4 mg/ml, 2 mg/ml, and 1 mg/ml.
5. Pipette 0.2ml of sterile water into each of these.
6. Again using sterile pipette tips and the p100 automatic pipette, pipette 0.2ml of the 128 mg/ml solution into the 64 mg/ml Eppendorf. This makes a half-fold dilution of the Polymyxin B, with each dilution being half the concentration of the previous.
7. Pipette 0.2ml of the new 64 mg/ml Polymyxin B solution into the 32 mg/ml Eppendorf to complete the 32 mg/ml concentration.
8. Continue the half-fold dilution until the last concentration of 1 mg/ml is reached.
9. These are the stock solutions of Polymyxin for the MIC series. When pipetting to inoculate the agar, use 20µl of each solution into the correct agar (20ml) ready for pour-plating. This makes the concentration in each plate exactly 1, 2, 4, 8, 16, 32, 64, 128 µg/ml respectively.

MIC Method for Nutrient Agar and Mueller Hinton agar upon Polymyxin B and S.aureus:

1. Make up the nutrient agar - 5.6g nutrient agar + 200ml distilled water.
2. Make up Mueller Hinton agar – 7.6g Mueller Hinton agar + 200ml distilled water.
3. Using an automatic 30ml pipette, pour 25ml of agar into a broth bottle. Leaving 8 labelled nutrient agar bottles and 8 labelled Mueller Hinton agar bottles.
4. Put in the autoclave.
5. Once out of the autoclave place in the 55°C incubator so the agar is warm enough to work on the bench with without the agar setting at the bottom of the broth bottle.
6. Culture *Staphylococcus aureus* in a broth culture and incubate overnight at 37°C.

Note: Repeat this again. One set is for the nutrient agar, the other is for the Mueller Hinton agars, label each.

7. Label 12 agar plates – 6 x nutrient agar, 6 x Mueller Hinton agar. 0 (control), 0.2, 0.4, 0.8, 1.6 and 3.2 + name/date/*Staphylococcus aureus*/Polymyxin B

Note: The agar plate labelled 0 (control) consists of agar only – NO Polymyxin B

8. Place the 20µl of each Polymyxin B concentration into the correctly labelled agar bottled solutions.
9. Shake gently making sure the Polymyxin B is mixed in well with the molten agar.
10. Pour the molten agar out of the bottle into the correctly labelled agar plate using full aseptic technique, flaming necks of the broth bottles to ensure no contamination is taking place.
11. Leave on the bench top to solidify – make sure these are totally solidified before turning them over.
12. Leave tilted so condensation from the plate runs down the plate and doesn't have contact with the agar, leaving it dry.

The following day:

13. Serial dilute the *Staphylococcus aureus* 10 fold, producing – 10^{-0} (original culture), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} and control. Using 1ml of the bacteria solution to 9ml of ringer's solution. Again ensure aseptic techniques are followed fully to reduce risk of contamination of the plates.

Note: Label one of the ringers solution "control", this will be ringers solution only – no Polymyxin B solution.

14. Spray the multipoint inoculator with 70% ethanol to ensure it is sterile.

Note: From here on work near the Bunsen burner flame to keep sterility to its maximum outcome.

15. Place the pins into the multipoint inoculator.
16. Pipette 0.5ml of each bacteria concentration into the multipoint inoculator wells; make a map of where each concentration is. Then place the well piece into the multipoint inoculator ensuring the notch is at the top and locked in place.
17. Start the process – placing the agar plates separately into the multipoint inoculator and pressing start on the machine each time which will put each bacterial concentration upon the agar plates.
18. Mark each agar plate as they come off the multipoint inoculator – giving some kind of indication of where the concentrations are on the agar plate.
19. Leave the *Staphylococcus aureus* to dry, once dried incubate overnight (nutrient agar) or leave for 3 hours (Mueller Hinton agar). If no growth after 3 hours, leave in the incubator overnight and compare results the following morning.

MIC Method for Escherichia coli and Staphylococcus aureus upon Polymyxin B inoculated Nutrient Agar:

Day 1

1. Label 36 broth bottles – 0µg/ml (Control), 1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml, 64 µg/ml and 128 µg/ml, in total 9 concentrations – repeat a further 3 times to create tests 1-4. Also include name/date/MIC/*S. aureus 25923 and E.coli TG-1*.
2. Produce nutrient agar in a flask – 11.2g nutrient agar + 400ml water - x2.
3. Boil the first one in a pan.
4. Immediately after boiled, use the automatic pipette to pipette 20ml of the nutrient agar into the labelled broth bottles. Creating 20 bottles with the first 400ml batch, then a further 20 with the second 400ml batch, leaving 4 spare if needed.
5. Leave lids slightly loose for the autoclave.
6. Autoclave.
7. Incubate at 55°C once out of autoclave until needed.
8. Take out Polymyxin B stock's from the fridge for inoculating the agar plates. Make up solutions again following previous method, if solution volumes are diminished.
9. Label up 36 sterile agar plates – label the same as the broth bottles listed in part 1.
10. Pipette 20µg of each Polymyxin B concentration into the correctly labelled broth bottle.
    Note: Draw a small arrow to mark the top of the agar plate when placing on the MIC.
11. Pour plate the correct broth bottle in the corresponding agar plate following full aseptic technique to minimise contamination.
12. Leave on bench flat to set.
13. Once set, tilt plates in a row, agar facing above to avoid any condensation
14. Culture *Staphylococcus aureus* and *Escherichia coli* overnight.

Day 2

15. Serial dilute *Staphylococcus aureus* and *Escherichia coli* 7 fold producing – control (0), 10^{-0} (original culture), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}
16. Spray the multipoint inoculator with ethanol to ensure it is sterile.

Note: From here on work near the Bunsen burner flame to keep sterility to its maximum outcome.

17. Place the pins into the multipoint inoculator.
18. Pipette 0.5ml of each bacteria concentration into the multipoint inoculator wells; make a map of where each concentration is – so you don't forget. Then place the well piece into the multipoint inoculators. Placing the marked arrow on the agar plate to the top of the MIC.

19. Start the process – placing the agar plates separately into the multipoint inoculator and pressing start on the machine each time which will put each bacterial concentration upon the agar plates.
20. Leave the *Staphylococcus aureus* and *Escherichia coli* to dry on the agar plates, once dried incubate at 37°C overnight.

Note: Autoclave the MIC equipment in a large beaker, wrap up in grease proof paper and autoclave again, ready to use for next time.

Day 3

21. View and write down results (remember to turn agar plates over when viewing results to match how they were on MIC).

SEM:

Substances

- Gluteraldehyde (1L)
- Methanol (~150ml)
- Acetone (50ml)
- Sodium Cacodylate (50g)
- Sterile water (~400ml)

Equipment

- Eppendorf tubes
- p100 pipette (100-1000µl)
- p200 pipette (20-200µl)
- SEM stubs
- Fume cupboard
- Bunsen burner
- Weighing scales
- Sterile measuring cylinder (100ml)
- 50ml centrifuge tubes

* Before starting the procedure – a measuring cylinder (100ml) and ~400ml of water needs to be sterilised in the autoclave.

Batch volumes to be made up before starting the procedure **				
Substance	**Percentage needed (%)**	**Substance volume (ml)**	**Sterile water volume (ml)**	**Total volume (ml)**
Gluteraldehyde	3	1.5	48.5	50
Methanol	100	50	0	50
Methanol	70	35	15	50
Methanol	50	25	25	50
Acetone	100	50	0	50
Cacodylate	0.2M	2.1403g	50	0.2M
Cacodylate	0.1M	5ml of 0.2M + 5ml water – for every use		

****** Weigh all the substances out using weighing scales and the sterile measuring cylinder (100ml) inside the fume cupboard. For the Cacodylate (a powdered substance) use a 50ml beaker along with the weighing scales. Then mix the substances in a labelled 50ml centrifuge tube. Refrigerate until needed.

1. Using a p100 pipette, take 1ml of the sample (bacterium) from the cultured bottle and put into an eppendorf tube. Using the aseptic technique.
2. Centrifuge at full speed (130RPMx100) for 5 minutes.
3. Pipette the supernatant out into a waste bucket.

* Note:

- All steps to be carried out in fume cupboard below.
- Also, if the pellet sample dissolves from the Eppendorf tube at any time throughout the procedure, centrifuge again so the pellet returns.

4. Using a p200 pipette, put 200µl of cacodylate buffer in to the eppendorf tube for 3 minutes. Then pipette out into the waste bucket.
5. Pipette 200µl of gluteraldehyde into the eppendorf tube for 30-40 minutes. Then pipette out into the waste bucket.
6. Next, using a p100 pipette, place 400µl of sterile water into the eppendorf tube for 1 minute. Pipette out into a waste bucket and repeat a further 4 times. **5 times in total.**

7. Place 400µl of 50% methanol into the eppendorf tube for 1 minute. Pipette out into a waste bucket and repeat once more. **2 times in total.**
8. Place 400µl of 70% methanol into the eppendorf tube for 1 minute. Pipette out into a waste bucket and repeat once more. **2 times in total.**
9. Place 400µl of 100% methanol into the eppendorf tube for 1 minute. Pipette out into a waste bucket and repeat a further 2 times. **3 times in total.**
10. Place 400µl of acetone into the eppendorf tube for 5 minutes. Pipette out into a waste bucket and repeat once more. **2 times in total.**
11. Using a pipette, apply the pellet of the sample onto the SEM stub immediately after the last coat of acetone has been removed.
12. Leave to air dry.
13. Coat using the sputter coater machine.

Results:

MIC – Agar comparisons, 29-06-11

Mueller Hinton Agar

	Polymyxin B / µg/ml									
Staphylococcus aureus / 1:10		C	1	2	4	8	16	32	64	128
	C	[redacted]	-	-	-	-	-	-	-	-
	10-0	[redacted]	+	+	+	+	+	+	+	+
	10-1	[redacted]	+	+	+	+	+	+	+	+
	10-2	[redacted]	+	+	+	+	+	+	+	+
	10-3	[redacted]	+	+	+	+	+	+	+	+
	10-4	[redacted]	±	±	±	±	±	±	±	±
	10-5	[redacted]	±	±	±	±	±	±	-	-
	10-6	[redacted]	-	-	-	-	-	-	-	-
	10-7	[redacted]	-	-	-	-	±	-	-	-
	10-8	[redacted]	-	-	-	-	-	-	-	-
	10-9	[redacted]	-	-	-	-	-	-	-	-
	10-10	[redacted]	-	-	-	-	-	-	-	-

NOTE: No control for MH agar (NO PB) as ran out of agar.

Nutrient Agar:

	Polymyxin B / µg/ml									
Staphylococcus aureus / 1:10		C	1	2	4	8	16	32	64	128
	C	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	+	+
	10-1	+	+	+	+	+	+	-	±	±
	10-2	+	+	+	+	+	±	-	-	-
	10-3	+	+	+	+	+	±	-	-	-
	10-4	+	±	±	±	±	-	-	-	-
	10-5	±	±	±	±	±	-	-	-	-
	10-6	-	±	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-
	10-8	-	-	-	-	-	-	-	-	-
	10-9	-	-	-	-	-	-	-	-	-
	10-10	-	-	-	-	-	-	-	-	-

MIC Results: S. aureus & E.coli

08.07.11

Test 1

	Polymyxin B / µg/ml									
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

	Polymyxin B / µg/ml									
Staphylococcus aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	±	+	+	+	+	-	-
	10-2	+	+	±	+	+	±	±	-	-
	10-3	+	+	-	±	±	±	±	-	-
	10-4	±	-	-	-	-	±	±	-	-
	10-5	-	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 2

Polymyxin B / μg/ml										
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / μg/ml										
Staphylococcus aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	+	+	±	+	+	+	-	-
	10-3	±	±	±	±	±	±	±	-	-
	10-4	±	-	-	±	-	-	-	-	-
	10-5	-	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 3

Polymyxin B / μg/ml										
E. coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / µg/ml										
Staphylococcus aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	±	-	-
	10-2	+	+	+	+	+	+	±	-	-
	10-3	±	+	±	±	±	±	-	-	-
	10-4	±	±	±	±	±	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 4:

Polymyxin B / µg/ml										
E. coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / µg/ml										
Staphylococcus aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	+	+	+	+	+	-	-	-
	10-3	±	±	±	±	±	-	-	-	-
	10-4	-	-	-	±	±	±	-	-	-
	10-5	-	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

MIC Results

13-07-11

Test 1

		Polymyxin B / µg/ml								
E.coli TG-1 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	±	-	-	-	-	-	-	-	-
	10-5	+	-	-	-	-	-	-	-	-
	10-6	+	-	-	-	-	-	-	-	-
	10-7	+	-	-	-	-	-	-	-	-

		Polymyxin B / µg/ml								
S.aureus 25923 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	+	-	-	-	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	±	+	±	+	+	±	-	-
	10-3	+	±	±	±	+	+	-	-	-
	10-4	+	-	+	-	±	±	-	-	-
	10-5	+	+	+	+	±	±	-	-	-
	10-6	+	+	+	+	-	±	-	-	-
	10-7	+	+	+	+	-	±	-	-	-

Test 2

		Polymyxin B / µg/ml								
E.coli TG-1 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	±	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / µg/ml										
S.aureus 25923 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	+	+	+	+	+	±	-	-
	10-2	+	+	+	+	+	-	-	-	-
	10-3	+	+	+	+	+	+	±	-	-
	10-4	+	+	+	±	±	±	-	-	-
	10-5	±	±	±	±	±	±	-	-	-
	10-6	±	-	±	±	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 3

Polymyxin B / µg/ml										
E.coli TG-1 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	+	-	-	-	-	-	-	-
	10-1	+	-	-	-	-	±	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	±	-	-	-	-	±	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	±	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / µg/ml										
S.aureus 25923 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	+	+	+	+	±	+	-	-
	10-2	+	+	+	+	+	-	+	-	-
	10-3	+	+	+	+	+	-	-	-	-
	10-4	±	+	±	+	±	-	±	-	-
	10-5	±	±	±	+	±	-	-	-	-
	10-6	±	±	±	-	±	-	-	-	-
	10-7	-	-	-	-	±	-	-	-	-

Test 4:

Polymyxin B / µg/ml										
E.coli TG-1 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	+	-	-	-	-	-	-	-
	10-2	+	±	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	±	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / µg/ml										
S.aureus 25923 1:10		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	-	-	-	-	-	-	-	-	-
	10-1	+	+	+	+	+	-	±	-	-
	10-2	+	+	+	+	+	+	±	-	-
	10-3	+	+	+	+	+	+	±	-	-
	10-4	+	+	+	+	±	±	-	-	-
	10-5	+	±	±	±	±	-	-	-	-
	10-6	±	-	±	±	±	-	-	-	-
	10-7	±	-	-	-	-	-	-	-	-

MIC Results: *S. aureus & E.coli*

15.07.11

Test 1

Polymyxin B / µg/ml										
S.aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	-	-	-
	10-1	+	+	+	+	+	+	±	-	-
	10-2	+	+	+	+	+	+	-	-	-
	10-3	+	+	+	+	+	+	-	-	-
	10-4	±	±	±	±	±	±	-	-	-
	10-5	-	-	±	-	±	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / μg/ml										
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	±	±	±	±	-	-	-	-
	10-1	±	±	±	±	±	-	-	-	-
	10-2	-	-	-	-	-	-	-	-	-
	10-3	-	-	-	-	-	-	-	-	-
	10-4	-	-	-	-	-	-	-	-	-
	10-5	-	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 2

Polymyxin B / μg/ml										
S.aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	+	+	+	+	+	±	-	-
	10-3	+	+	+	+	+	±	-	-	-
	10-4	+	±	±	±	±	-	-	-	-
	10-5	±	±	-	±	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / μg/ml										
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	±	±	±	±	-	-	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Test 3

Polymyxin B / μg/ml										
S.aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	+	+	+	+	+	+	-	-
	10-3	+	+	+	+	+	+	+	-	-
	10-4	+	+	±	±	±	±	-	-	-
	10-5	±	±	±	±	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

Polymyxin B / μg/ml										
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	±	±	±	±	-	-	-	-
	10-1	+	-	-	-	-	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

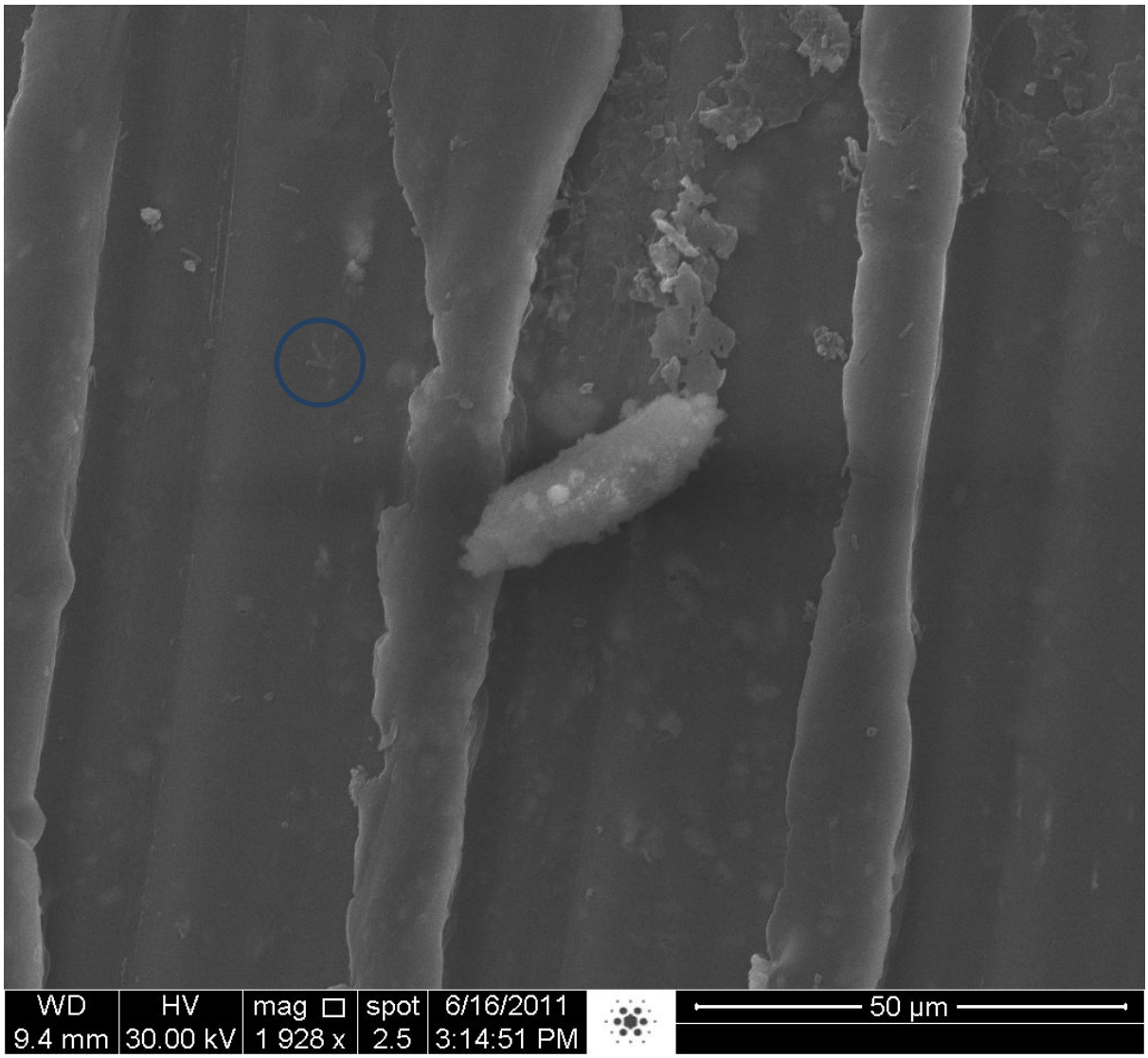
Test 4:

Polymyxin B / μg/ml										
S.aureus 25923		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	+	+	+	+	+	+	-	-
	10-1	+	+	+	+	+	+	+	-	-
	10-2	+	+	+	+	+	±	±	-	-
	10-3	+	+	+	+	+	±	±	-	-
	10-4	+	±	±	±	±	±	±	-	-
	10-5	±	±	-	±	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

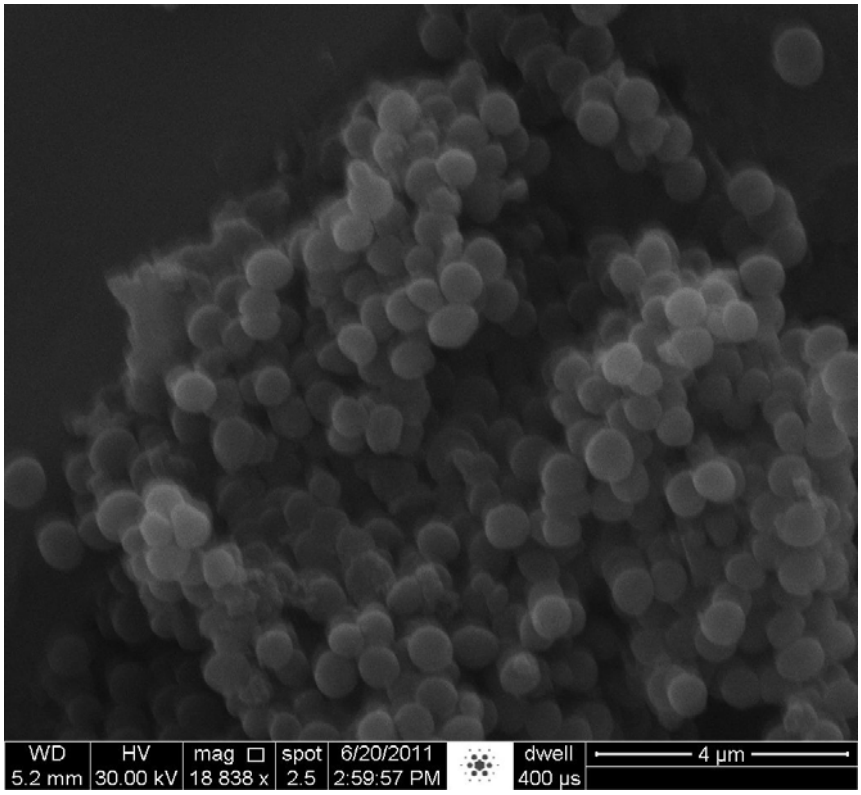
Polymyxin B / µg/ml										
E.coli TG-1		Control	1	2	4	8	16	32	64	128
	Control	-	-	-	-	-	-	-	-	-
	10-0	+	±	±	±	-	-	-	-	-
	10-1	+	±	±	±	±	-	-	-	-
	10-2	+	-	-	-	-	-	-	-	-
	10-3	+	-	-	-	-	-	-	-	-
	10-4	+	-	-	-	-	-	-	-	-
	10-5	±	-	-	-	-	-	-	-	-
	10-6	-	-	-	-	-	-	-	-	-
	10-7	-	-	-	-	-	-	-	-	-

SEM Images:

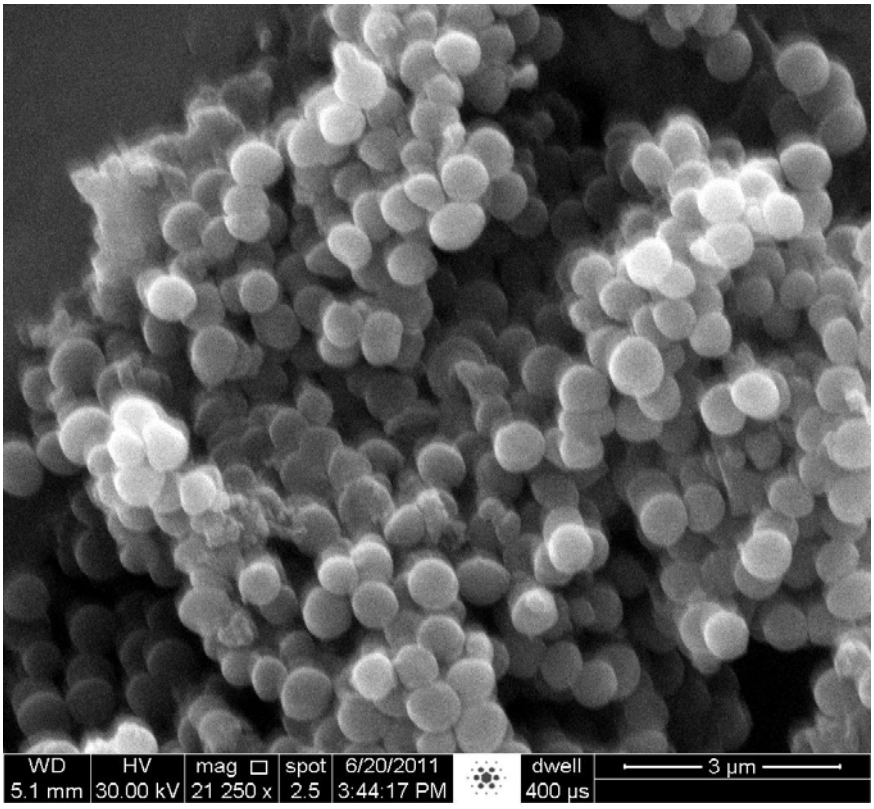
16-06-11 *E. coli TG-1* Practise Image



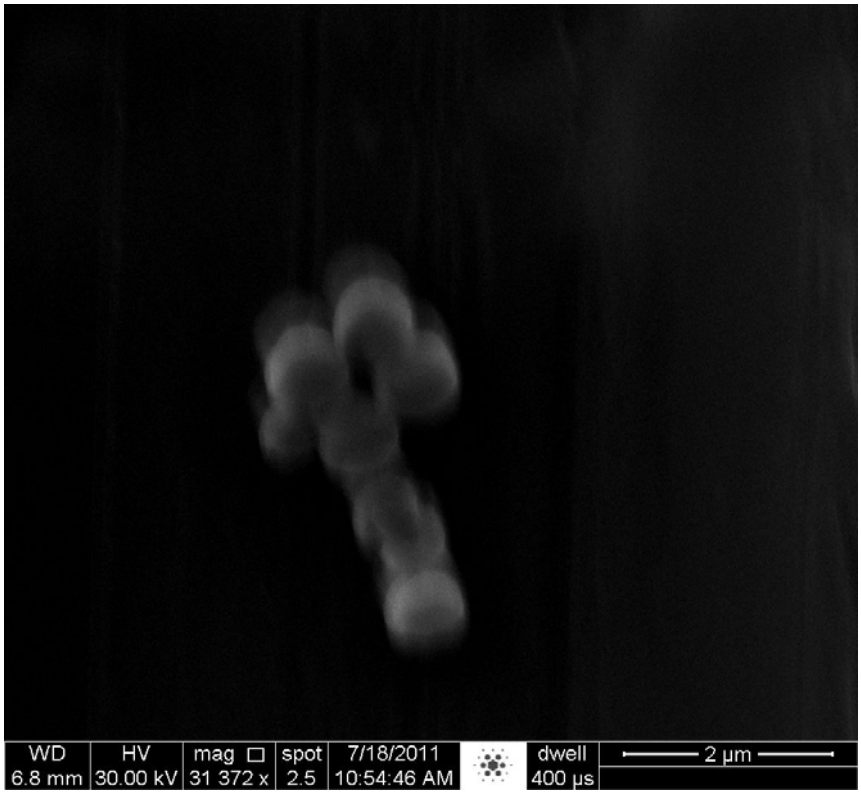
20-06-11 *S. aureus* 25923 Control Image 1



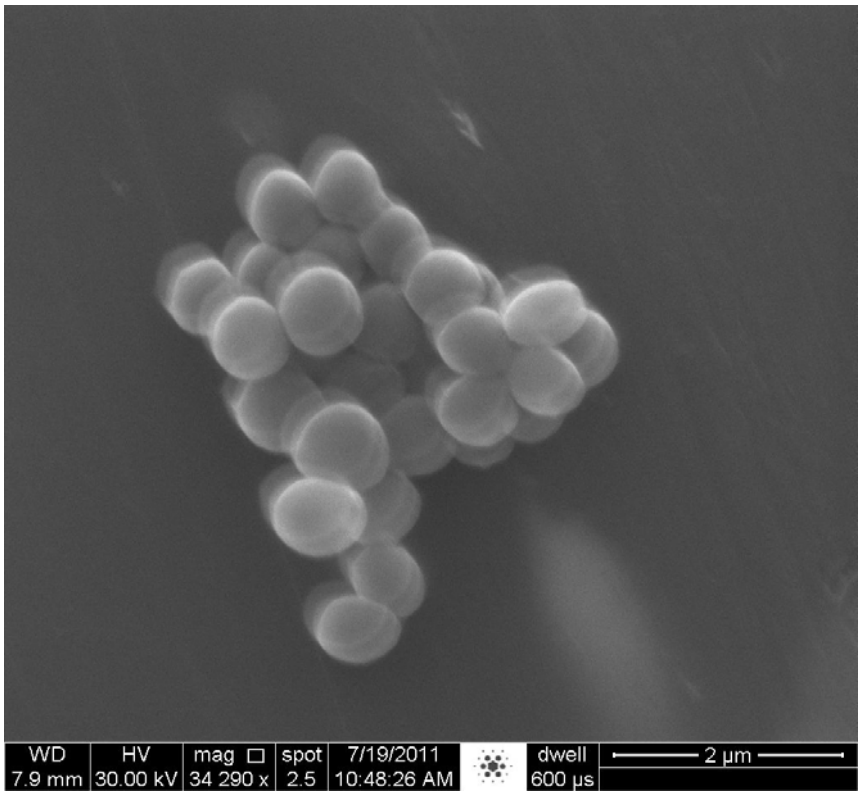
20-06-11 *S. aureus* 25923 Control Image 2



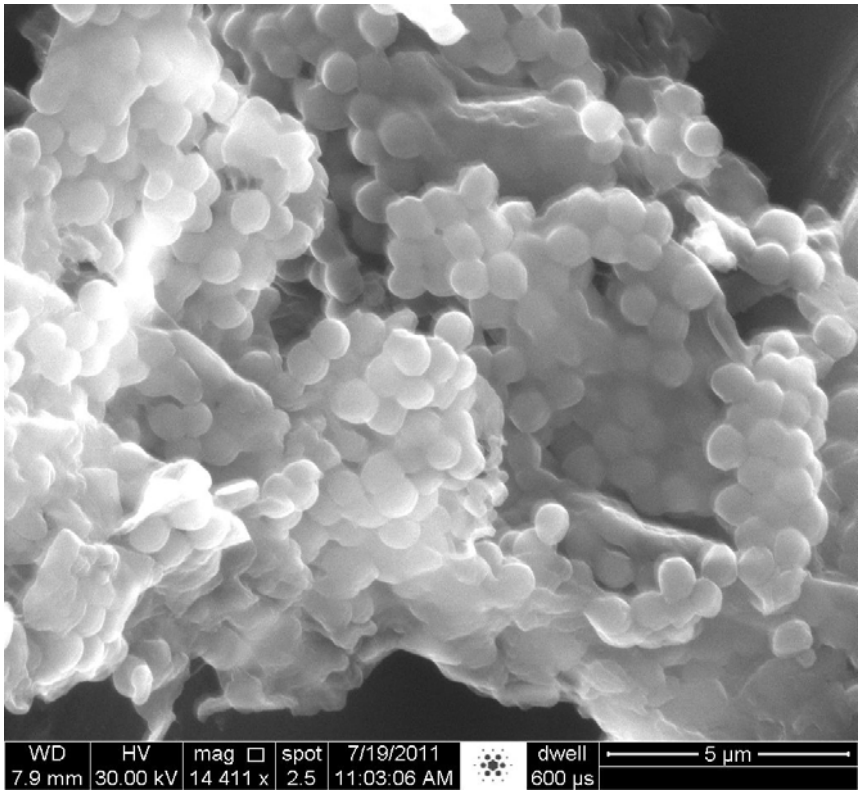
18-07-11 *S. aureus* 25923 PB 2µg/ml MIC Gold Paladium



19-07-11 *S. aureus* 25923 PB 2µg/ml MIC Gold Paladium 001



19-07-11 *S. aureus* 25923 PB 2µg/ml MIC Gold Paladium 002



Discussion:

S. aureus

MIC results showed that the more concentrated the polymyxin B was, the less colonies existed on the sample. Also, the more diluted the *S. aureus* was, the less colonies existed on the sample. 64 and 128µg/ml of Polymyxin B showed no growth at all on any of the results, including the controls. 10^{-6} and 10^{-7} dilutions of *S.aureus* also showed no growth within any of the results.

Upon the control images of the *S.aureus*, we saw that they were grape-like clusters, cocci shaped.

E. coli

MIC results showed that all concentrations of Polymyxin B acted effectively upon the *E. coli* cells throughout all concentrations (1-128µg/ml). Within every duplicate of each MIC run, only the control samples (those agar plates without polymyxin B impregnated into the media), showed growth of cells in colonies. The only growth that wasn't seen was towards the end of the dilutions where there weren't enough cells in the dilution to grow and then be seen in a colony after incubation. Reasons for these results could be as follows: the concentration of the PB was too high, even at a low concentration for the dilutions of the broth cultures to show any growth;

incubation period not long enough to show growth of colonies; broth culture containing 'old cells' instead of newly reproduced *E. coli* cells, therefore lower count of cells within the 10^{-0} broth culture.

Upon the control images of the *E. coli* cells, we saw that there were single rod shaped bacillus cells in groups.

Conclusion:

To conclude, polymyxin B does have an effect on *S.aureus* and *E.coli*. The images produced showed some clear images of both organisms. More work on the effects of Nisin on *S.aureus* and *E.coli* can be undertaken in the future along with the effects of Polymyxin B and Nisin on other types of bacteria.

Acknowledgements:

Many thanks to Mike Shaw for all of the help he gave during the project.

References:

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- Urakawa, H., *et.al.* 2010. Structure-activity relationships of bacterial outer-membrane permeabilizers based on polymyxin B heptapeptides. *Bioorganic & Medical Chemistry Letters,* 20(5), pp.1771-1775.