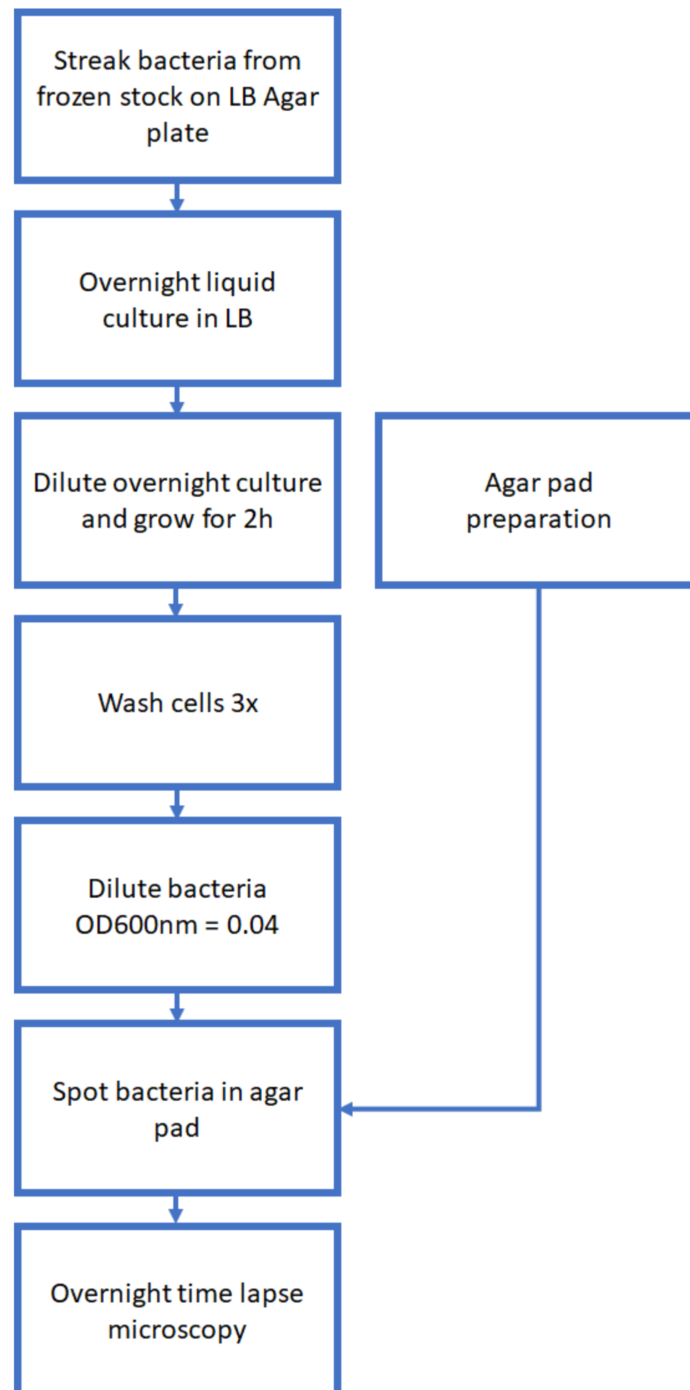
Dynamic Time-Lapse Imaging of Bacteria

Aim:

Evaluate the impact of different antimicrobial treatments on bacterial physiology.

Workflow:

Duration approximation : 3 Day (3 Day for preparation + 1 Day for the measurement)



Materials:

- Bacteria
- Antibiotics at stock solutions

- 50 mL Falcon tubes
- 50 mL Erlenmeyer
- Sterile Loops
- Glass slides
- Glass slide cover slip
- Kimwipes

- Müeller-Hinton Broth (MHB)
- Low melting temperature agarose
- Ethanol
- Water
- Gene Frames

- Micropipette (20 µL and 100 µL)
- Incubator
- Inverted Microscope

Protocol:

Cells and antibiotic dilution series are prepared as previously described for the growth curve except that the bacterial concentration is adjusted to OD 600 nm of 0.035 in order to ensure that single cells with appropriate spacing are spotted on the microscope slide .

Preparation of the Agar Pad :

- Thoroughly clean 2 glass slides per agar pad with ethanol 70% and then with water. Dry with kimwipes. Place the glass slides on top of a new filter paper.
- Carefully peel the plastic cover of the Gene Frame.
- Carefully position the sticky side of the Gene Frame in the middle of the glass slide.
- To prepare the agarose, weight 150 mg of agarose for microscopy and dissolve it thoroughly in 10 mL of Mueller Hinton Broth.
- Heat in the microwave until complete dissolution of the agarose (check every 5-10 sec).
- Filter sterilize the agarose solution into a new falcon tube. The agarose solution can be store at 55°C until use.
- Add the antimicrobial to be tested to the agarose in the appropriate concentration.
- Dispense 500 µL of Mueller Hinton with (or without) antibiotic on the middle of the Gene Frame.
- Carefully place a glass slide on top to make a "sandwich".
- Carefully place the "sandwich" in the fridge (4 C) for 45 min.

Preparation of the microscope sample

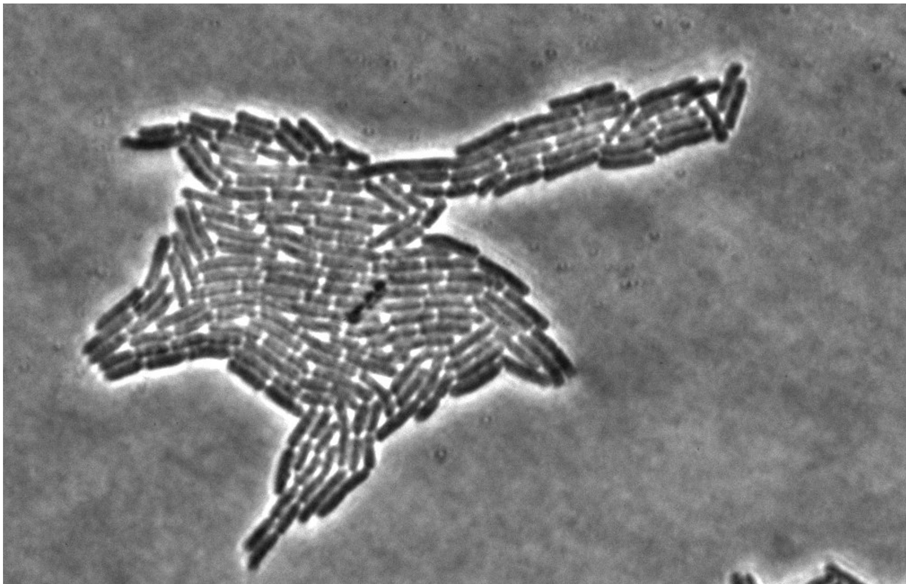
- Carefully slide off the upper glass slide.
- Carefully remove the second and final plastic cover from the Gene Frame to expose the sticky side of the Gene Frame.
- Load 2.5 µl of cell suspension (prepared as previously mentioned) on top of the agarose pad and allow the liquid to disperse by turning the slide up and down.
- Place a clean microscope slide coverslip on top of the Gene Frame and seal it by pressing it around the borders of the Gene Frame.
- Pre-warm the slide preparation for 1 h at 30°C.

Time-lapse fluorescence microscopy

- Pre-warm the environmental chamber of the microscope.
- Initiate the microscopy equipment and place a drop of mineral oil in the 100x objective.
- Invert the glass slide containing the sample so that it faces the objective and place it the microscopy. Monitor the outgrowth of single cells into a microcolony monolayer at 37°C, by acquiring TRANS images every 10 min for 12 h. Representative microscopy images are shown in Fig. 1.

Fig. 1. Representative microscopy images of E. coli untreated and treated with the antibiotic nalidixic acid at the MIC.

E. coli, untreated



E. coli, treated with nalidixic acid at the MIC