Clarified STAR Protocol: Antimicrobial susceptibility testing to evaluate minimum inhibitory concentration values of clinically relevant antibiotics

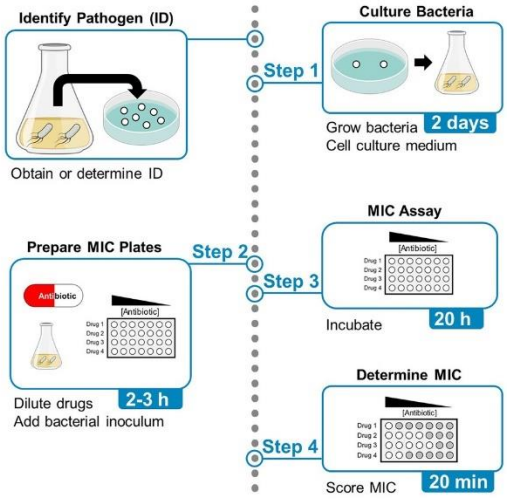


Figure 1. MIC Assay Flow Through

Before you begin:

I. Determine the optimal MIC testing range for your pathogen using the EUCAST MIC distribution repository (https://mic.eucast.org/search/).
   i. Select a continuous range of ten 2-fold serial dilutions that would encompass the clinical breakpoints used to categorize the pathogens as susceptible, intermediate, or resistant.
   ii. Calculate 2x the highest antibiotic concentration based on this range for the maximum concentration for your serial dilutions.

II. Prepare your stock solutions and culture media in the correct solvents (see the list on the fridge for reference). Store at 4°C for short term storage or -20°C for long term storage.
   i. Sterile LB (with/without antibiotics) ~ 15-50 mL
   ii. LB + 2x highest antibiotic concentration ~ 2-5 mL

Protocol:

Timing: 2 Days

1. Streak out culture of choice for isolation on an LB + Antibiotic plate and grow at 37°C for 18 hrs.
2. Inoculate 3 mL sterile LB without antibiotics with a single colony from your isolation plate.
   a. If you have a strain that has been selected for using antibiotics, continue to include it in your culture medium.
   b. Grow overnight for 18 hours at 37°C and 220 rpm.

Timing: 2-3 hours

3. Using your prepared LB + 2x antibiotic stock, add 100 µL of your stock to wells A_1-H_1 of a clear 96 well plate.
4. Add 50 µL of LB, or appropriate media, to each well in columns 2-12.
   a. Add an additional 50 µL of LB to column 12 for your negative control (media only).

Serial Dilutions:

5. Transfer 50 µL from column 1 into column 2, pipetting up and down 3 times to mix.
    a. Repeat the transfer process in columns 3-10.
    b. Discard the additional 50 µL of culture from column 10.

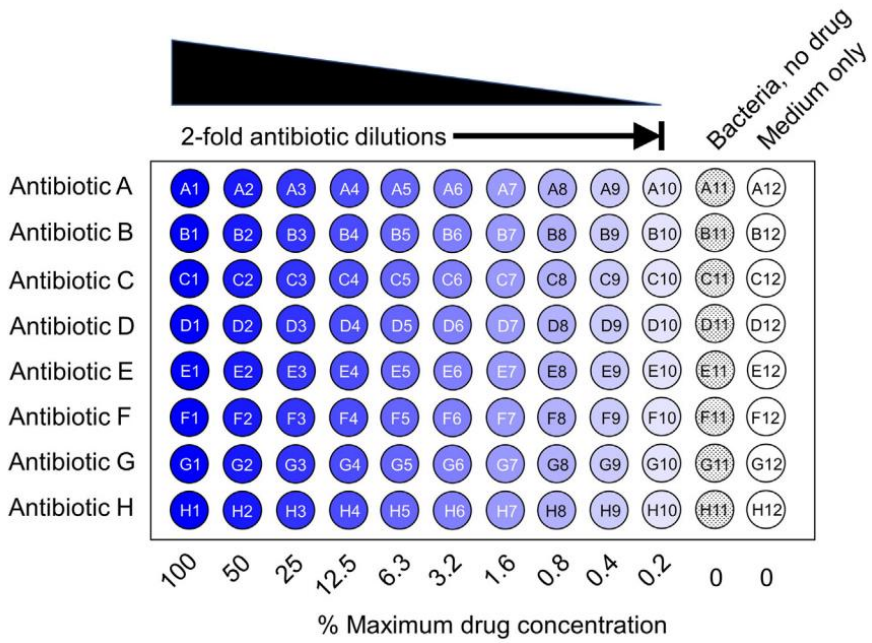


Figure 2. Serial Dilutions of Antibiotics for MIC Assay

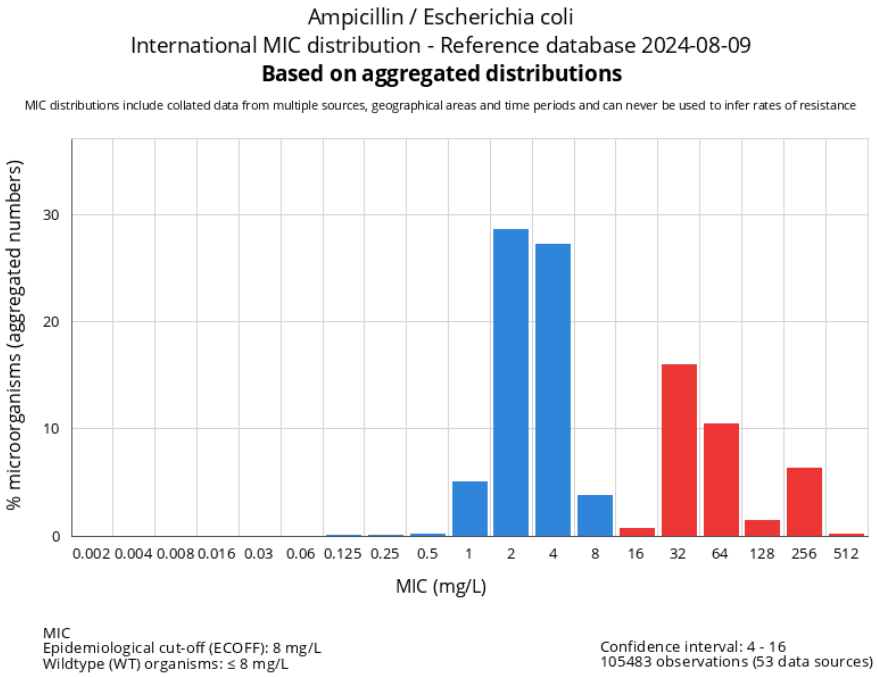
Culture Dilutions and Final Plating:

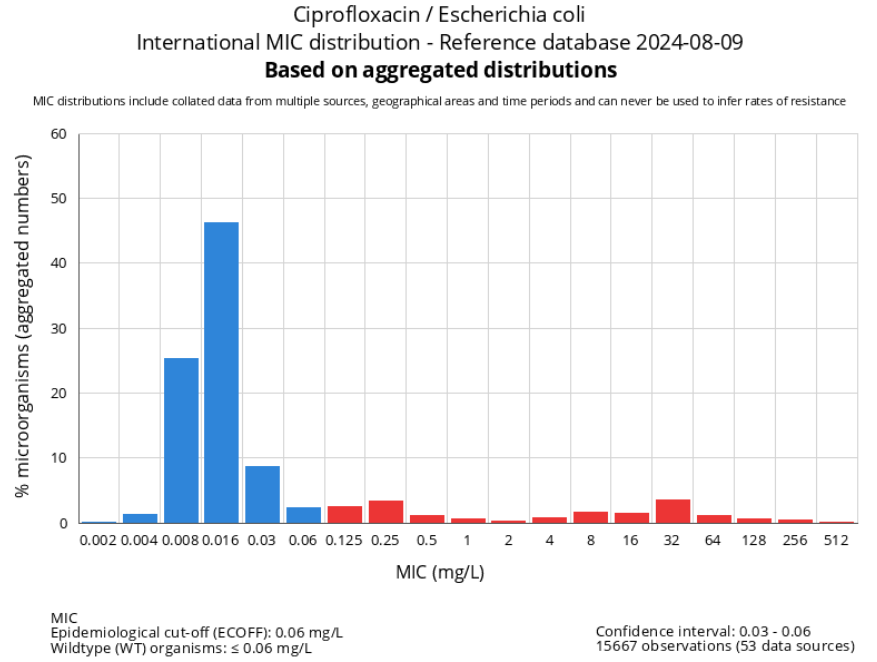
6. Add 100 µL of your 18-hour overnight cultures to a different clean clear 96 well plate. Include a LB blank. Measure the OD600.
    a. The spectrophotometer measures both alive and dead cells in a broth culture. Because of this a standard line needed to be created to calculate the CFU/mL at a given absorbance value.
    b. Create 6-8 dilutions of your culture and measure their absorbances. Plot these values against CFU (cells/mL) x 10^6. With the slope of the line, you can determine the CFU/mL at the experimental OD.
    c. For a full explanation on determining CFU/mL, see this link here → 1.15: Determination of Bacterial Numbers - Biology LibreTexts
7. Dilute your overnight cultures to 10^6 CFU/mL in LB (2x the desired bacterial concentration).
8. Add 50 µL of your diluted cultures to all wells on the plate except for those in column 12.
9. Incubate plates at 37°C without shaking for 20 hours.

Determine Experimental MIC:

Timing: 20 min

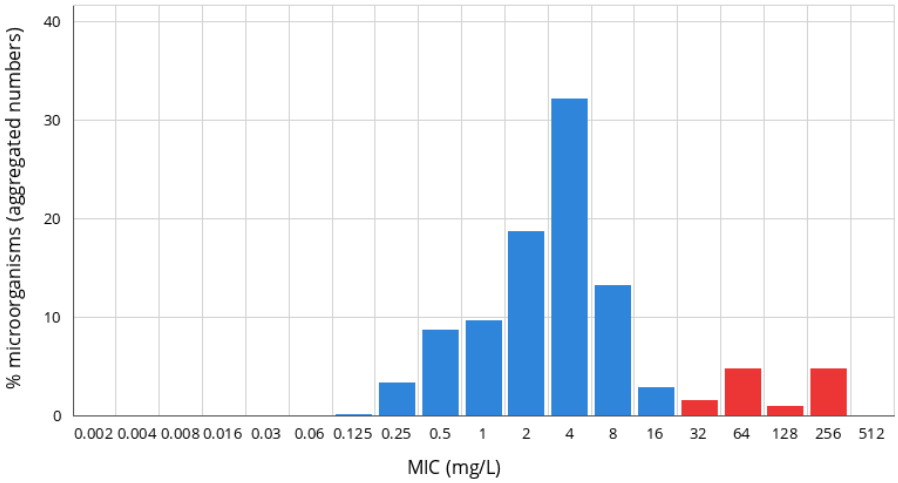
10. Score the plate based on
    a. Presence/absence of turbidity in test wells
    b. Confirm growth in bacteria only positive control wells
    c. Confirm no growth in media only negative control wells
11. Interpret the MIC values with respect to the clinical breakpoints determined through EUCAST.





Kanamycin / Escherichia coli
International MIC distribution - Reference database 2024-08-31
Based on aggregated distributions

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): (16) mg/L
Wildtype (WT) organisms: ≤ 16 mg/L

Confidence interval: 4 - 32
3860 observations (4 data sources)