Reading technique – MIC determination

1. Check purity control

2. Check growth in the MIC-panel

   - Growth in control wells okay ? If weak → too low MICs → do not read.
   - More than one skip → do not read.
   - Volume okay ? Dark circles indicate < 50 µl →too high MICs → do not read.
   - Rows of different kind of pellets indicate contamination.
     (except for beta-lactams; cause pellet to "splash out" close to the end point)
   - Colistin-resistance for E.coli or Salmonella → do not read.

3. Read the panel according to the guidelines for reading (Figure 1)

4. Growth might appear different close to the end point of growth:

   - Pellet might look like a star
   - Pellet might be absent, but there is diffuse growth in the broth that appears cloudy.
   - Pellet might "splash out" and seems bigger (typical for beta-lactams)
   - If pellet is less than a dot, it should be ignored, but only for E.coli/Salm (typical for chloramphenicol and florfenicol).

5. Remember special reading for trimethoprim, sulphonamides and for

   the combination of the two: Ignore growth when growth is less than

   20 % compared to the control wells.

Acceptable deviation for MIC-determination is +/- one dilution step.

Evaluating MIC-results

Tools

- Is the observed resistance profile as expected for the bacteria?
- Are the observed resistance markers consistent with the knowledge about resistance genes and cross-resistance?

Suggested action when observing atypical or rare resistance profiles

- Check the reading
- Check purity control for contaminations and correct morphology
- Take out material from well with high MIC and from control wells, check morphology next day before re-testing.
- Sub cultivate before re-testing (from top of 3-4 colonies)
- Verify ID before re-testing (microscope / API)

Action differs and depends on the specific problem – “case-by-case” handling.

Contaminations

Tools for identifying contaminations

- Purity control
- Growth pattern in the MIC-panel
- The observed resistance profile

Avoid contamination

- Check the purity before the MIC-testing
- Make sure you have single colonies to pick from (3-4 colonies)
- When you transfer material from McFarland standard to broth: Tip the tube and use long tips for the pipette.
- Don’t touch the dosing head at the dosing tip
- Open MIC-panel in the bench and inoculate in the bench if possible
- Test the broth and saline for contaminations before use.

Quality Control with the ATCC reference strains

Daily performance is needed if the lab do not have any routine or experience in MIC-testing. After a longer period (CLSI states 30 days) of QC testing without any deviations, weekly QC testing would be okay.

The number of QC reference strains to use varies, but if routinely testing Salmonella and Campylobacter, the minimum QC testing should include the ATCC *E. coli* strain and the ATCC *C. jejuni* strain.

QC acceptable ranges are not defined for all antimicrobials. In house reference values can be used instead.

The QC reference strain Ps. aeruginosa is recommended because it reveals if the cationic concentration in the broth (or agar) is not appropriate (the MIC-values for tetracycline and aminoglycosides would differ).

The QC reference strain E. faecalis is recommended because it reveals if the antagonist content of the broth (or agar) is not appropriate for testing trimethoprim and sulphonamides.

Trouble-shooting

- Switch of strains ?
- Contamination ?
- Growth weak or inoculation wrong ?
- Incubation temperature and -time ?
- Change of broth or other media/reagents ?
- Use of expired broth, MIC-panels or other reagents ?
- Etc. etc.

Action on QC deviations

- Re-test (after subcultivation)
- One dilutionstep outside the acceptable QC range is okay, but….
- If the same deviation appears over time or for other QC strains too or for other types of panels, you have to take action

Relevant information on antibiotics, genes and cross-resistance

Tetracyclines

100 % cross-resistance for all types including the semisynthetic mino- and doxycycline.

Amphenicols

Chloramphenicol

Florfenicol (*100 % cross-resistance to chloramphenicol, S. Tm DT104 always express the gene)*

- Rule for E.coli and Salmonella: If FFN-R, also CHL-R
- Salmonella Typhimurium DT104 resistance profile: Amp Chl Ffn Spe Str Smx Tet

Aminoglycosides

Streptomycin *(first aminoglycoside)*

Spectinomycin

Gentamicin

Apramycin *(100 % cross-resistance to gentamicin due to the aac(3)-IV gene, that is the only b reported gene conferring resistance to apramycin)*

Neomycin

Kanamycin

Except for apramycin, there is no specific pattern of cross-resistances due to the many different resistance genes

- Rule for E.coli and Salmonella: If APRA-R, also GEN-R

Polymyxins

Colistin *(= polymyxin E, resistance not observed for E.coli / Salm, great marker for contamination!)*

100% cross-resistance for polymyxins.

- Rule for E.coli and Salmonella: If COL-R, either contamination or wrong identification.

Folic acid pathway inhibitors

Trimethoprim

Sulphonamides *(100 % cross-resistance for all sulphonamides)*

Huge synergi-effect when used in combination (SXT). Only when resistance to both Tmp and Su is observed, when tested separately, the isolate will also be resistant to the combination of the drug.

- Rule: TMP-R + SU-S → SXT-S
- Rule: TMP-S + SU-R → SXT-S
- Rule: TMP-R + SU-R → SXT-R
- Remember special reading for these antimicrobials !!!

Quinolones/fluoroquinolones

Nalidixic acid (quinolone)

Ciprofloxacin (fluoroquinolone)

Until recently only mutational resistance was known: One point-mutation in the DNA gyrase gene → NAL-resistance and reduced susceptibility to CIP (typically MICs of 0.12, 0.25 and 0.5). And with one more point-mutation in the gyrase gene → resistance to both NAL and CIP (MICs >2).

Because of observations of therapy failure with this reduced susceptibility to CIP, we need to change the clinical breakpoint from >2 to >0.06.

Now also transferrable genes (qnr and aac) have been reported. They confer resistance to CIP with MIC of 0.12 and 0.25 – and isolates with these genes are fully sensitive to NAL !

- Rule for E.coli and Salmonella: Traditionally rules can not be used any longer, but the MICs give information on the type of resistances.
- Rule for Campylobacter: CIP-R should be NAL-R, and NAL-R should be CIP-R.

Beta-lactam antibiotic

Ampicillin *(beta-lactamase sensitive, gramneg., 100 % cross-resistance to amoxicillin)*

Amoxicillin+clavulanic acid *(clavulanic acid is a beta-lactamase-inhibitor)*

Cefotaxime *(3. generation cephalosporin, beta-lactamase stable – ESBL screening)*

Ceftiofur *(3. generation cephalosporin, beta-lactamase stable – ESBL screening)*

- Rule for E.coli and Salmonella: AUG-R or AUG-I should be AMP-R
- Rule for E.coli and Salmonella: FOT-R should be AMP-R
- Rule for E.coli and Salmonella: XNL-R should be AMP-R and FOT-R