GenEpi presentation

Postdoc GENEPI

- Background:
  - AMR susceptibility testing done at very standardized condition (pH, temperature, media).

  Problem: Infection sites are not standardized – tests don't relate to reality
  - We don't understand why bacteria carry multiple/combinations of beta-lactamase

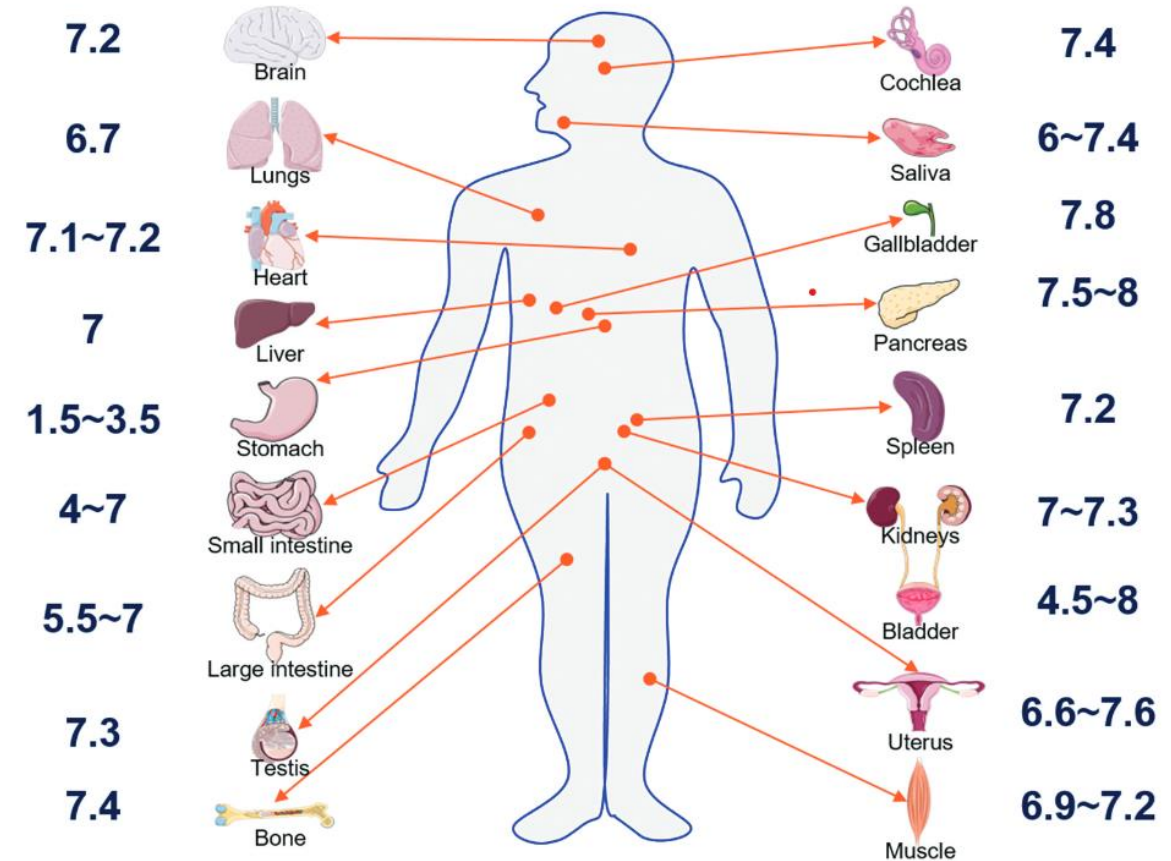 **Problem: redundant & fitness cost?**

Figure 2. Physiological pH values in the human body.

Gaohua L, Miao X, Dou L. 2021. Crosstalk of physiological pH and chemical pKa under the umbrella of physiologically based pharmacokinetic modeling of drug absorption, distribution, metabolism, excretion, and toxicity. Expert Opinion on Drug Metabolism & Toxicology.

Research questions

- Is the activity of beta-lactamases impacted by changes in pH or temperature like any other enzyme?

- Are these effects dependent on the specific beta-lactam antibiotic (enzyme substrate)?

- Will bacteria with specific combinations of beta-lactamases prevail under specific and especially changing environmental conditions? i.e. change in infection site/host
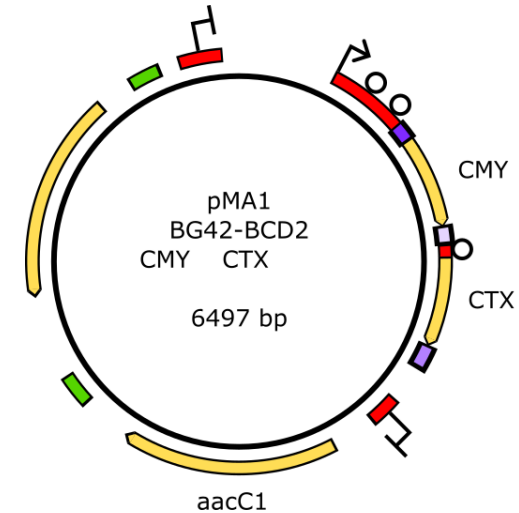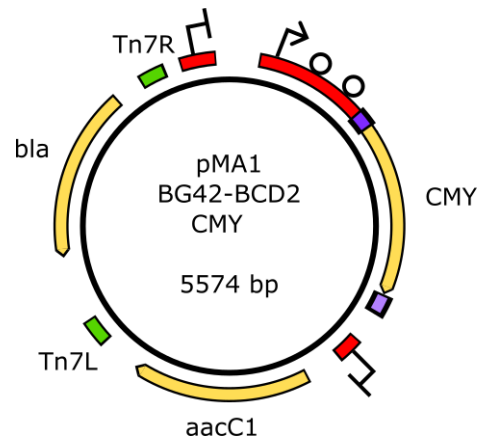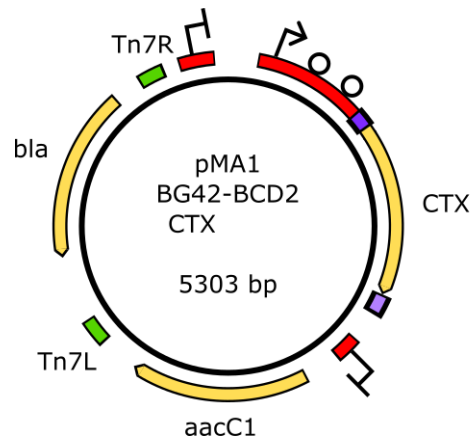
Preliminary data: cephalosporins
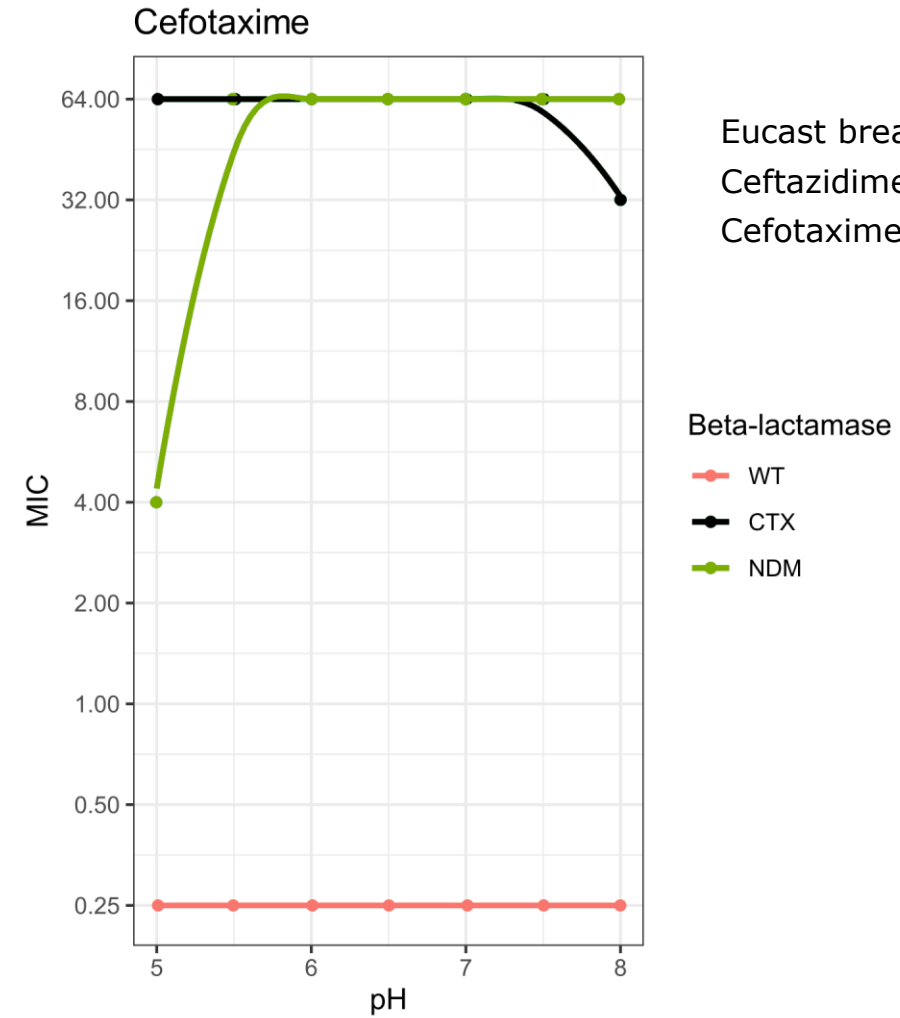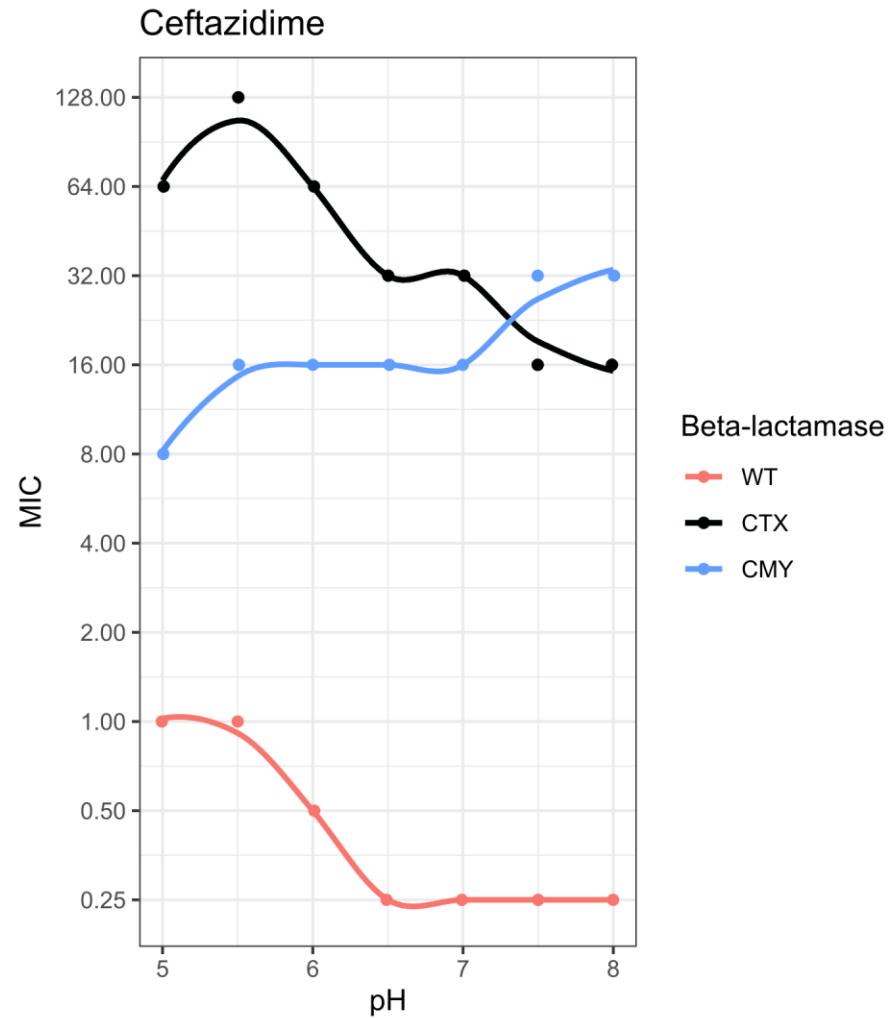
Eucast breakpoints:

Ceftazidime S <= 1, R > 4

Cefotaxime S <= 1, R > 2

Preliminary data: carbapenems

Eucast breakpoints:
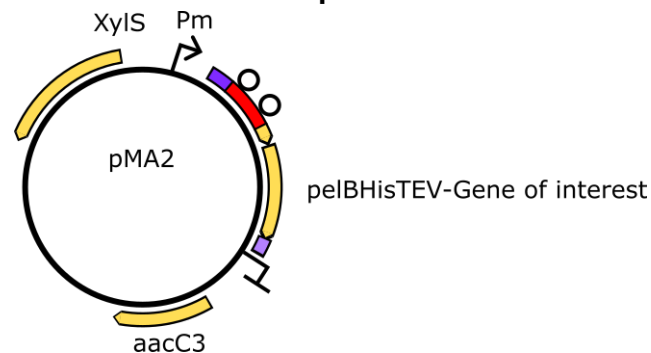Imipenem S <= 2, R > 4
Meropenem S <= 2, R > 8
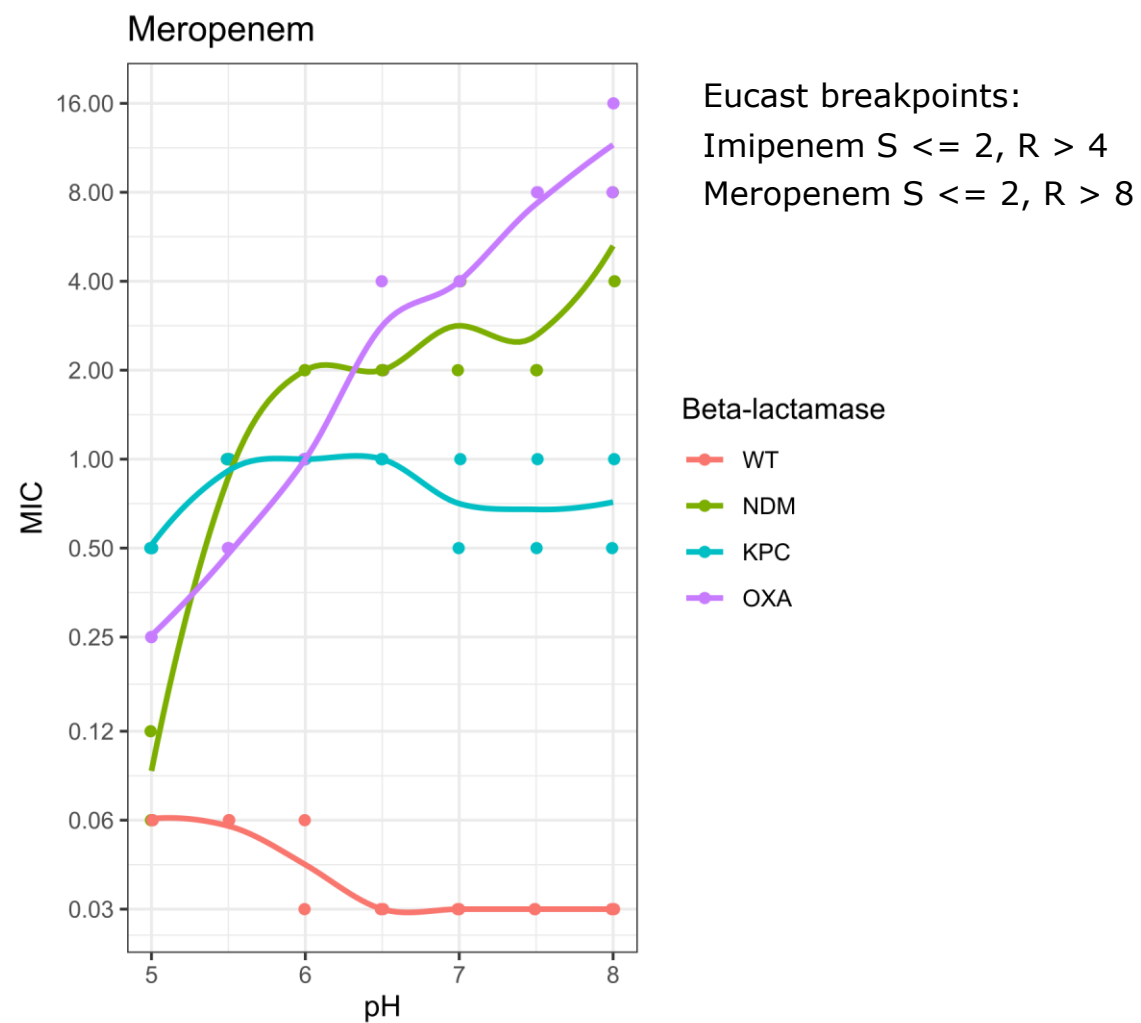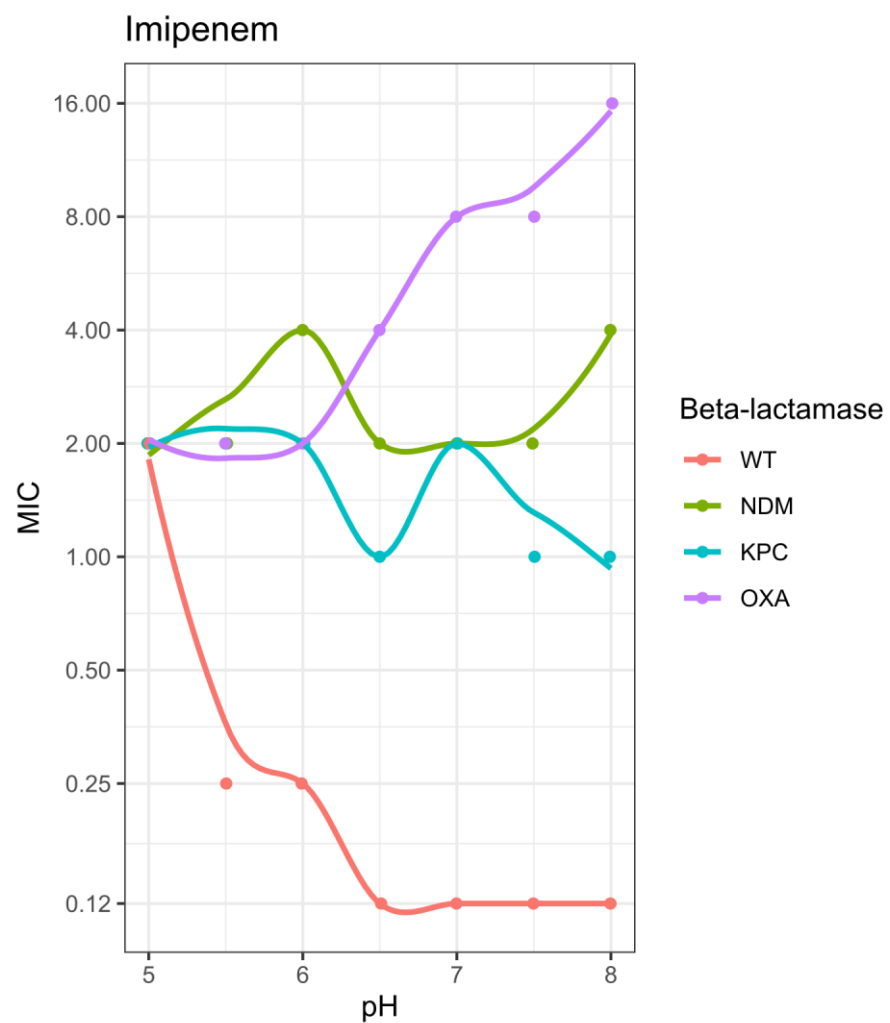
Plan

- Verify preliminary data
  - Genomic integration of beta-lactamases under controlled transcriptional and translational control for phenotypic testing


  - Inducible over-expression of beta-lactamases with pelB-His-TEV-tag for purification and enzyme kinetics
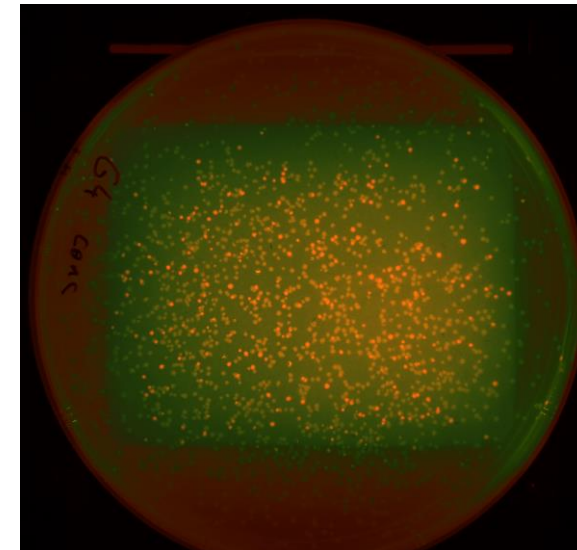
Progress & Todo

- Promoter variants with translational coupler:
  - 6 different promoters

- Single gene constructs and some synthetic operons
  - **CTX, CMY, NDM**, msfGFP, mKate
  - msfGFP-mKate & mKate-msfGFP
  - Todo: CTX-CMY & CMY-CTX, other *bla* genes
  - Todo2: integrate and test phenotypic effect of genes

- Inducible constructs w/ purification tags:
  - CTX, CMY, msfGFP, mKate

msfGFP (green fluorescent) construct (control)
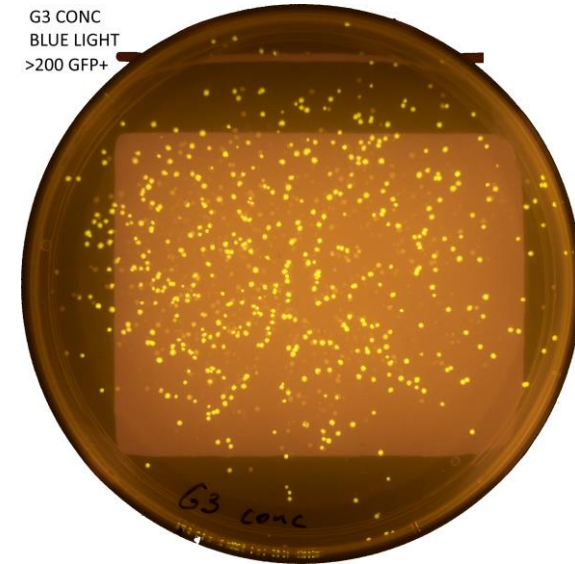
mKate (red fluorescent) construct (also a control)

Thanks for listening