Phenotypic resistance evolution in *E. coli* after prolonged exposure in urine

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Aim

We aimed to study how a urine culture medium influenced evolution of phenotypic antibiotic resistance compared to Mueller-Hinton broth (MHB). We studied 5 commonly used antibiotics in treating UTIs caused by *E. coli.*

Introduction

- Antimicrobial resistance (AMR) poses a challenge for urinary tract infections (UTIs), including complicated UTIs.[1,2]
- UTIs are characterized by bacterial proliferation within the urinary tract, including urine.
- The nutritional composition and pH of urine affects microbial physiology, growth rates and susceptibility.
- These alterations could influence evolution of AMR. [3,4,5]
- It remains unclear how urine influences resistance evolution in UTIs under prolonged antibiotic exposure.

Approach

- A clinical *E. coli* UTI isolate was serially passaged for 21 days under exposure to ciprofloxacin (CIP), nitrofurantoin (NIT), amoxicillin (AMX), gentamicin (GM) and cefotaxime (CTX).
- Cultures were passaged in fresh MHB or pooled human urine every 48 hours.
- Antibiotic concentrations were increased step-wise when growth was observed.
- The minimum inhibitory concentration (MIC) in MHB was compared at the end of the experiment for both culturing conditions to the MIC of the parental strain.

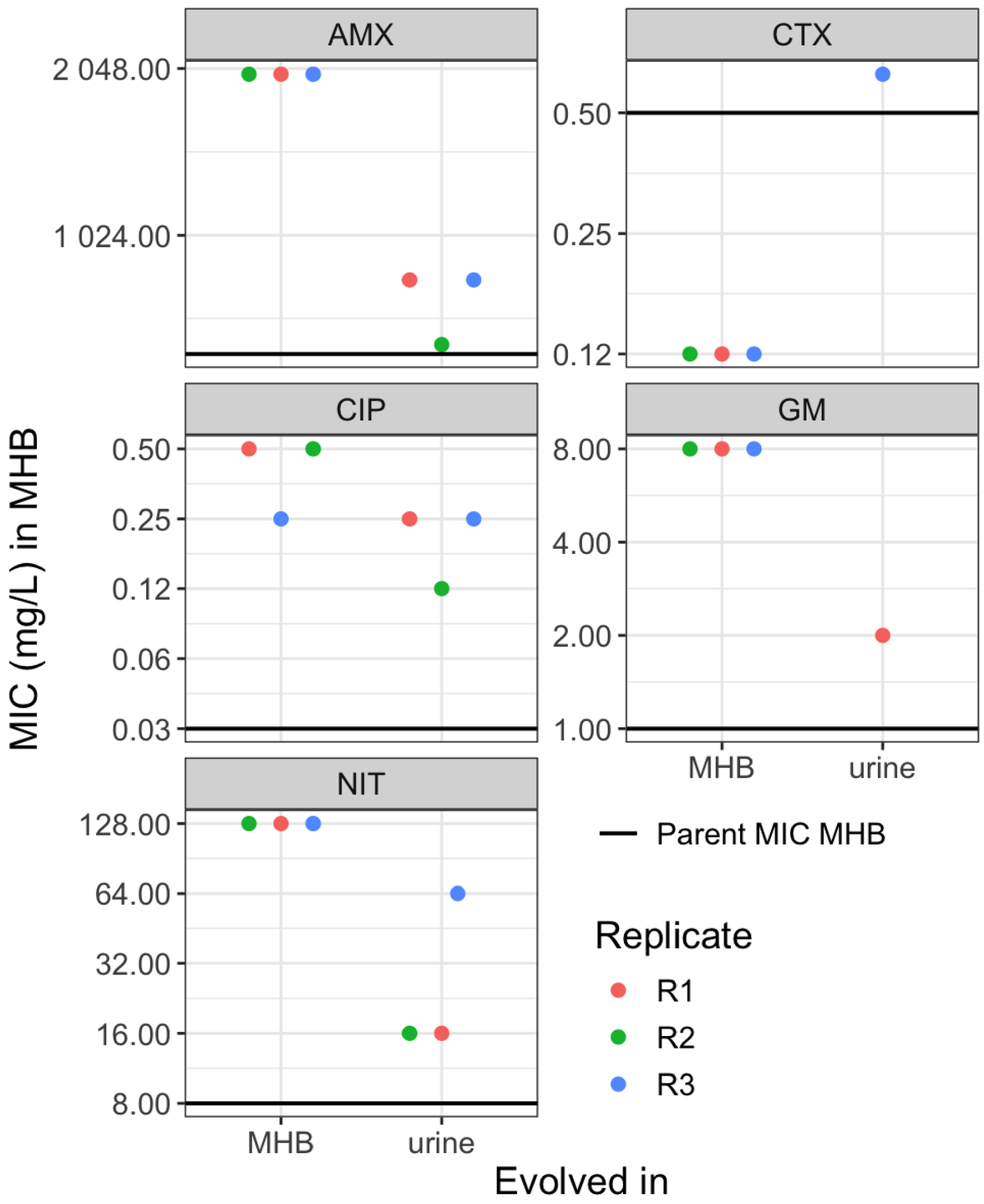

Figure 1. MIC determination in MHB of three E. coli replicates for amoxicillin (AMX), cefotaxime (CTX), ciprofloxacine (CIP), gentamicin (GM) and nitrofurantoin (NIT) after adaptive evolution. Parent MIC MHB = MIC in MHB before adaptive evolution.

Results

Phenotypic resistance evolution proceeds slower in urine for AMX, GM, CIP and NIT

- Four out of five antibiotics reached higher MICs in MHB compared to urine: amoxicillin (2.6-fold), gentamicin (4-fold), ciprofloxacin (2-fold), and nitrofurantoin (4-fold).
- Cefotaxime showed a 5-fold MIC decrease in MHB compared to urine (Figure 1).

Parental MIC patterns do not predict evolved MIC patterns

- For gentamicin and ciprofloxacin, parental MICs were lower in MHB compared to urine. After passaging this effect reversed.
- Amoxicillin and nitrofurantoin showed higher MICs in urine compared to MHB. After passaging this pattern remained.
- Cefotaxime maintained a similar difference between MICs before and after passaging.

Conclusions and perspectives

- Urine growth environment might constrain resistance development. This might be due to differences in nutrient composition and pH, compared to MHB.
- Further studies across multiple strains are warranted to confirm these findings.

References

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