The molecular basis of antibiotic treatment failure in chronic urinary tract infections

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Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections and are likely to become recurrent. In young women, the risk of relapse within 6 months is 24%, which may lead to the development of a **chronic infection**.[1]

Unlike in the acute condition, chronic UTI patients often **fail to respond to antibiotic treatment**. Our clinical experience is that antibiotics predicted to be effective on culture may be unsuccessful in the clinical setting, while antibiotics predicted to be unsuitable can succeed. This suggests that phenotypic sensitivity and resistance profiles of strains that are actually causing the infection might differ from profiles obtained in diagnostic laboratory conditions.

Hypothesis

In this study we hypothesized that diagnostic growth media can influence the outcome of sensitivity testing, leading the same strain to show **different Minimum Inhibitory Concentrations** (MICs) for a given antibiotic depending on the nutrients available.

We decided to test this hypothesis using the following approaches:

- The use of a more **biologically relevant condition** (i.e. urine, human bladder organoid) could reveal sensitivity profiles that are more likely to match what happens in chronic UTI patients.
- The application of **Next Generation Sequencing** (NGS) techniques could reveal the genomic and transcriptional signatures of pathogens after antibiotic administration.

Results

- **Evolution of resistance to amoxicillin-clavulanic acid** (AMC) was performed using the clinical isolate *Escherichia coli* 10129 in either M9, ISO or LB medium. A significant reduction in cell density was observed during incubation in M9 containing sub-inhibitory concentrations of AMC (see Fig. 1) and it was not possible to recover any resistant isolate from agar plates.
- **MICs** of evolved strains in different media showed **very high variability in sensitivity profiles** (see Fig 2).
- Culturing of ancestral and evolved strains in LB and the human bladder organoid showed **discrepancies in relative fitness and growth** in the two systems (see Fig. 3).

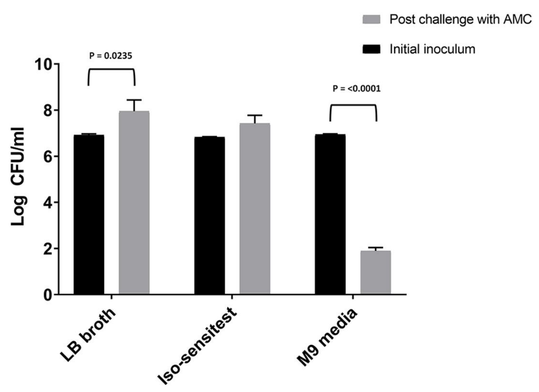


Fig. 1 Difference in cell density (Log CFU/ml) of *E. coli* 10129 after 24h exposure with sub-inhibitory concentrations of AMC. Compared with the initial inoculum, there was a significant increase of *E. coli* 10129 cell density of 1.03 log CFU/ml in LB in the presence of AMC (p-value = 0.0235), while there was an insignificant growth of 0.592 log CFU/ml in ISO containing AMC (p-value = 0.1339). Following exposure of *E. coli* 10129 to AMC in M9, we observed a significant reduction in cell density of 5.049 log CFU/ml (p-value < 0.0001) and we did not recover any resistant isolates from agar plates containing 8µg/ml or 16µg/ml AMC. Error bars represent the standard error of the mean.[2]

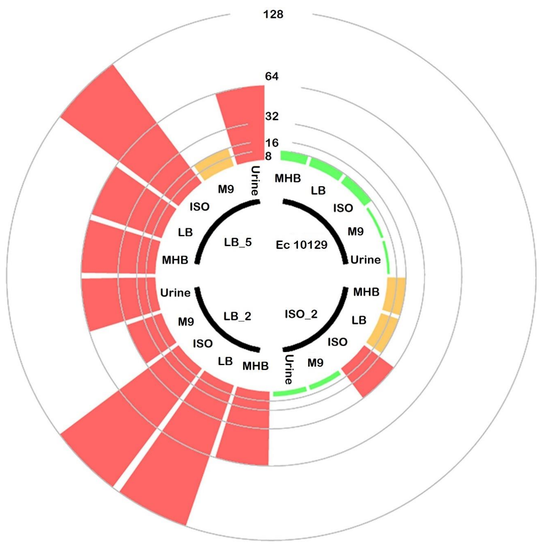


Fig. 2 Circular barplot chart showing the MIC values of the *E. coli* 10129 and three evolved resistant strains. Clinical breakpoint for sensitivity and resistance is 8 and 16µg/ml, respectively. The scale represents the MIC value. [2]

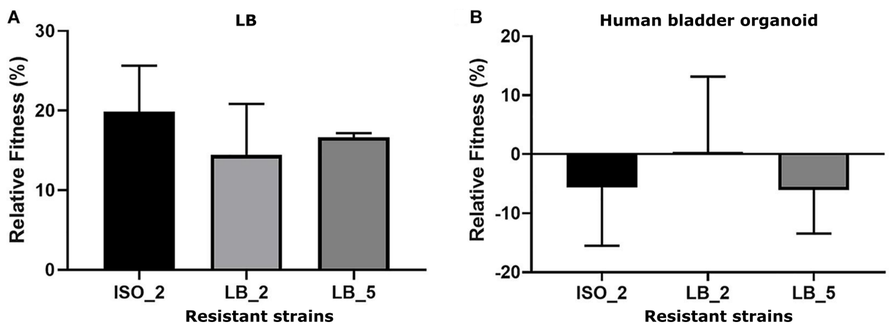
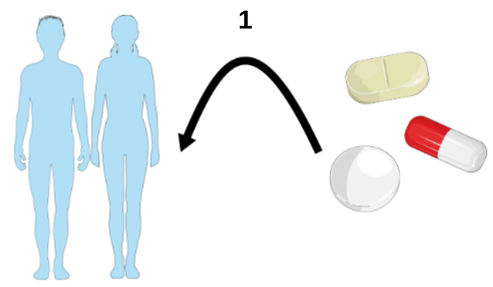
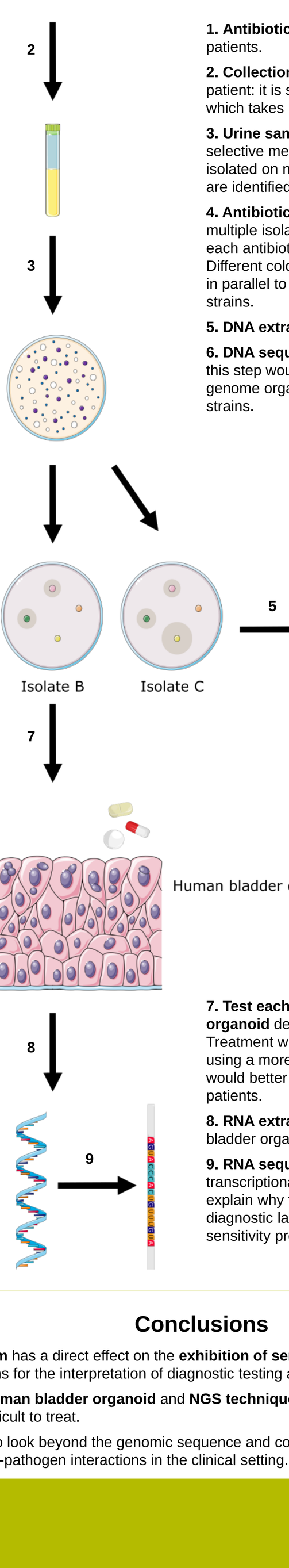


Fig.3 Relative fitness and growth of *E. coli* isolates. (A) Relative fitness of *E. coli* 10129-derived resistant isolates ISO_2, LB_2 and LB_5 compared to the ancestral isolate in LB. **(B)** Relative fitness of *E. coli* 10129-derived resistant isolates ISO_2, LB_2 and LB_5 compared with the ancestral isolate in urothelial organoids. Error bars represent standard error of the mean.[2]

Future perspectives





1. Antibiotics administration to chronic UTI patients.

2. Collection of the urine sample from the patient: it is stored at 4 degrees until processing, which takes place within 5h from collection.

3. Urine sample processing and plating on selective media: after 24h the colonies are isolated on non-selective media and the species are identified.

4. Antibiotic susceptibility testing (AST): multiple isolates are independently tested against each antibiotic administered to the patients. Different colonies of the same species are tested in parallel to reveal possible differences among strains.

5. DNA extraction

6. DNA sequencing and genome assembly: this step would highlight main differences in genome organisation compared with control strains.

7. Test each strain in a human bladder organoid developed previously in the lab.[3] Treatment with the antibiotics used in the clinic using a more biologically relevant environment would better mimic the conditions found in patients.

8. RNA extraction from bacteria grown in the bladder organoid.

9. RNA sequencing: identification of transcriptional signatures of key genes that might explain why the AST traditionally performed in diagnostic laboratories is not able to predict the sensitivity profiles in the clinical setting.

Conclusions

- **Type of medium** has a direct effect on the **exhibition of sensitivity profiles**: this finding may have implications for the interpretation of diagnostic testing and for treatment outcome.
- The use of a **human bladder organoid** and **NGS techniques** may shed light on why chronic UTIs can be difficult to treat.
- We may need to look beyond the genomic sequence and consider expression profiles when unravelling host-pathogen interactions in the clinical setting.

References

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[2] Hubbard A. et al., Front Microbiol. 2019
[3] Horsley H. et al., Sci Rep. 2018