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# Opportunity for very rapid phenotypic susceptibility testing of clinical specimens using the novel ASTar platform

Eva Hell, Jan Grawé, Tony Lindberg, Charlotta Göransson, Jenny Göransson, Ida Niklasson, Mats Gullberg. Q-linea, Uppsala, Sweden.

Q-linea
+46 18 444 36 10
contact@qlinea.com

## Introduction

Urinary tract infection (UTI) is one case where using rapid antimicrobial susceptibility testing (AST) could improve clinical practice by providing timely and accurate antimicrobial treatment, thus improving patient care and reducing the global threat of antimicrobial resistance. The majority of UTIs are caused by Gram-negative pathogens, commonly by *Escherichia coli* (86%), *Klebsiella pneumoniae* (4%), *Proteus mirabilis* (4%) and *Pseudomonas aeruginosa* (1%) (Fig 1).

In this study, we tested *E. coli* and *K. pneumoniae* isolates and showed that a prototype urine application for the ASTar™ AST system (Q-linea) can deliver a phenotypic AST for *E. coli* with a susceptible (S) or resistant (R) classification in only 30 to 60 minutes in a clinical matrix.

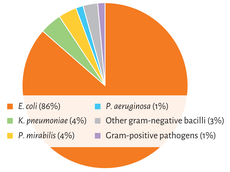


**Fig 1.** Prevalence of different bacterial species in UTI (1).

## Materials and methods

### *Consumables and automated imaging*

A prototype ASTar consumable adapted for rapid image-based detection of small changes in pathogen biomass was manually inoculated and placed in a prototype ASTar incubation and read-out unit. Time-lapse images were taken every 5 minutes for 90 minutes.

### *Samples and antimicrobial treatment*

Mid-stream urine from healthy individuals was collected and filtered to remove contaminants. Experimental conditions are shown in Figure 2. Processed bacterial solutions in cation-adjusted Mueller-Hinton broth (CAMHB) were mixed with antimicrobials prior to loading on the test-consumable. Ciprofloxacin (CIP), gentamicin (GEN), amoxicillin-clavulanate (AMC) and trimethoprim-sulfamethoxazol (TRS), all clinically-relevant antimicrobials in UTI with different modes of action, were selected for our tests. The concentrations of each were set according to the EUCAST breakpoint recommendations.

### *Analysis*

Biomass was extracted from time lapse images. Normalized biomass change was constructed from biomass data for visualization purposes. Untreated positive controls were used to determine normal growth variability. Mean values and standard deviation were calculated using five data sets.

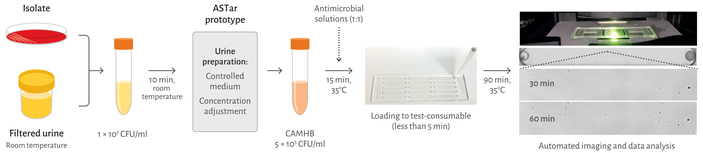


**Fig 2.** Experimental set-up.

## Results

Preliminary tests using the *E. coli* MG1655 isolate in CAMHB exposed to CIP and GEN showed that a response time to GEN of less than 30 min and to CIP of around 60 min can be detected with our method (Fig 3). Phenotypic AST results from urine were also attained for selected antimicrobials when *E. coli* isolates were exposed to CIP, GEN, AMC and TRS (Fig 3). By comparing the normalized biomass change of the two treated *E. coli* isolates to their untreated controls, we could correctly classify the tested isolate as susceptible or resistant (Fig 3). Short response times (30–40 min) were observed with exposed to AMC and GEN while longer times (50–60 min) were required to detect growth inhibition in response to CIP and TRS.

Experiments with a susceptible *K. pneumonia* isolate showed variable delayed response to CIP, GEN and AMC compared to susceptible *E. coli* isolate, while to the TRS, no consistent response for *K. pneumoniae* isolate was seen after 90 min (Fig 3). Subsequent tests revealed that this delay is likely due to a slower initial growth of *K. pneumoniae* compared to *E. coli*. Similar but more accentuated results were seen for *P. aeruginosa* under the experimental conditions used (data not shown). Adjusting assay conditions might improve the performance for these species.

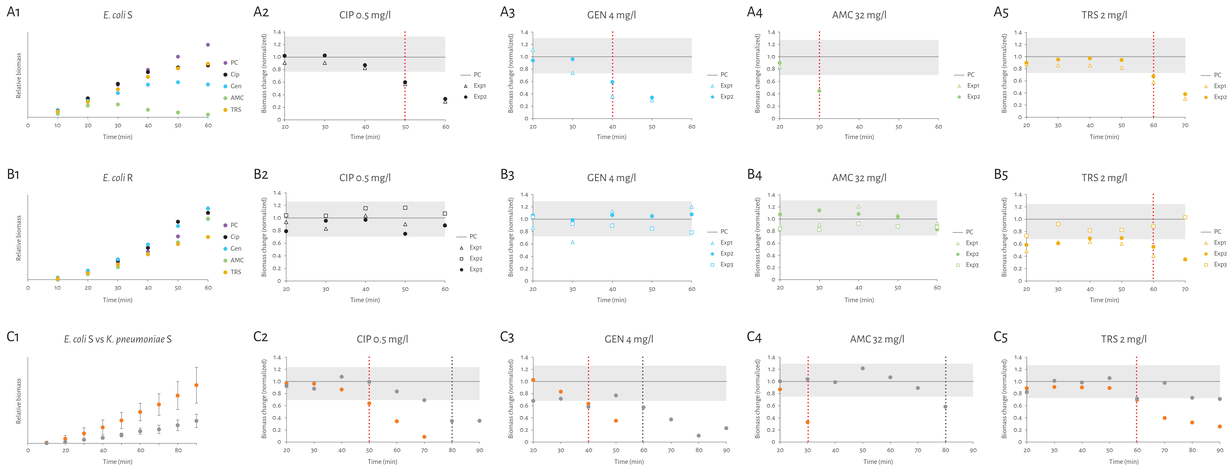


**Fig 3.** Relative biomass curves and normalized biomass change. A1–A5: Susceptible *E. coli* (MG1655) was exposed to CIP and GEN, AMC and TRS in urine; B1–B5: Resistant *E. coli* isolate was exposed to CIP, GEN, AMC and TRS in urine; C1–C5: Comparison of biomass and normalized growth rates of susceptible *E. coli* and susceptible *K. pneumoniae* isolates exposed to CIP, GEN, AMC and TRS in urine. Data is presented as median on A1, B1 and C1 while mean values are shown on C2–C5. Shadowed boxes represent 2 × SD (± 25%) intervals to set the transition from Susceptible to Resistant. Dashed lines indicate the earliest response to antimicrobials: red for *E. coli* and black for *K. pneumoniae*. Grey line: untreated control, orange dots: *E. coli*, grey dots: *K. pneumoniae*.

**Table 1.** Antimicrobial susceptibility of the isolates at the assay concentration used. Susceptibility testing was done using broth micro-dilution.

| Antimicrobials and assay concentration | *E. coli* S (MG1655) | *E. coli* R | *K. pneumoniae* S |
|---|---|---|---|
| Ciprofloxacin (CIP) 0.5 mg/l | S | R | S |
| Gentamicin (GEN) 4 mg/ml | S | R | S |
| Amoxicillin-clavulanate (AMC) 32 mg/l | S | R | S |
| Trimethoprim-Sulfamethoxazole (TRS) 2 mg/ml | S | S | S |

## Conclusions

- We can detect and quantify changes in pathogen growth for both resistant and sensitive strains within 30 minutes, delivering rapid phenotypic AST approaching point-of-care requirements for the most prevalent UTI pathogen *E. coli* (Fig 3.) with selected antimicrobials commonly used for UTI.
- For some species and antimicrobials, e.g *K. pneumoniae* with TRS, the timing of the biological response is such that clinically-relevant results were not obtained within the time span used.
- For other clinically-relevant Gram-negative bacteria (e.g *Pseudomonas* sp.) an even longer assay time might be needed, possibly up to 3 hours.

### *References*

1. Johnson *et al.* (1995) Direct Antimicrobial Susceptibility Testing for Acute Urinary Tract Infections in Women. *Journal of Clinical Microbiology*.

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