Treatment of chronic relapsing urinary tract infection with antibiotics selected by AtbFinder

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INTRODUCTION

Conventional phenotypic or genotypic antimicrobial susceptibility testing (AST) frequently fails to identify optimal and effective antibiotics (1, 2). In patients with recurrent urinary tract infections (UTIs), antibiotics selected with these tests frequently fail to eradicate infections resulting in relapses (3). One of the reasons for such failure is the reliance on the antibiotic response of the lead UTI pathogen within a pure bacterial culture. For example, conventional AST neglects the occurrence of multispecies biofilms during UTI, where bacteria are up to 1,000 times more tolerant to antimicrobials than corresponding planktonic cells (4, 5, 6). Moreover, the lead pathogen in multispecies biofilms could be additionally protected by collective antibiotic resistance, when an antibiotic resistance factor released by even non-virulent bacteria, which are often fewer in number, may protect an entire community (7). Another issue concerning the selection of antibiotics effective only against the lead pathogen is related to the difficulty in definitively establishing the pathogenicity of certain bacteria. For example, rare pathogens such as *Bacillus spp., Kluyvera spp., and Herbaspirillum spp.* have only recently been classified as pathogenic (8–10). Finally, standard AST is unable to detect persisters or account for inter-microbial communication via quorum sensing, Teazeled (TezR) receptors, and the TR-receptor system that upregulate resistance genes (11, 12, 13).

The recently developed AtbFinder overcomes the above limitations (14). By recapturing polymicrobial biofilms from the biosamples it can identify effective and ineffective antibiotics by employing a "whole community response" to antibiotics instead of filtering a single lead bacterium. AtbFinder takes into consideration critical "real-life" factors required for the effective selection of antibiotics, such as biofilm growth, the presence of persisters, modulation of antibiotic resistance by quorum sensing and TezRs, and collective antibiotics resistance, not taken into consideration by routine AST.

AtbFinder is a 48-well plate filled with proprietary developed TGV agar that supports growth of a diverse bacterial population. In each well, the agar is supplemented with one or several antibiotics at a concentration that reflects their penetration into different tissues. Biosamples are plated directly on the agar and do not require isolation of a pure culture. Following incubation at 37 °C for 4 h, bacterial growth on the agar surface determines the effectiveness of antibiotic treatment. AtbFinder delivers a result within 4 h, which allows patients with serious bacterial infections to receive effective antibiotic therapy within a day. Different types of AtbFinder have been developed for the treatment of lung, urinary, skin, and soft tissue infections, differing in the content of antibiotics tested and their concentration added to the agar which reflect the particularities of their PK/PD for different tissues.
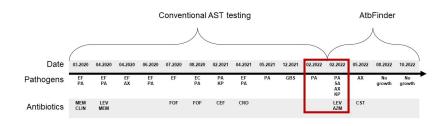
In pilot trials on patients with lung infections, antibiotics selected with AtbFinder developed for pulmonary diseases successfully eradicated multidrug-resistant gram-positive and gram-negative bacteria in patients with cystic fibrosis, who had been unsuccessfully treated with multiple antibiotic courses for years (15). Moreover, optimization with AtbFinder halved the total number of antibiotics administered to these patients. In the

1

present report, we describe a clinical case, whereby AtbFinder was used successfully to select antibiotics for a patient with recurrent UTI.

CASE PRESENTATION

A 46-year-old man underwent resection of the right kidney in February 2020 due to a cystic variant of renal cell carcinoma complicated with a urinary fistula. Three weeks post-surgery, the patient developed a UTI. A standard microbiological laboratory test identified *Enterococcus faecalis* and *Pseudomonas* spp. in urine and, based on disk-diffusion AST, meropenem and clindamycin were prescribed. The response was insufficient and the bacteria persisted for the next two weeks, which prompted treatment with meropenem and levofloxacin (Figure 1).

Figure 1. Timeline of recurrent UTIs.

Timeline of the disease. The column for each timepoint describes the pathogen identified with conventional microlab or AtbFinder methods, such as *E. faecalis* (EF), *P. aeruginosa* (PA), *A. xylosoxidans* (AX), and *K. pneumoniae* (KP), as well as the antibiotics used for the corresponding treatment, including levofloxacin (LEV), meropenem (MEM), fosfomycin (FOF), cefepime (CEF), ceftriaxone (CRO), azithromycin (AZT), and colistin (CST). The red square identifies the initiation point of AtbFinder-based therapy.

Nevertheless, the patient was still infected with *E. faecalis* and reinfected with *Achromobacter xylosoxidans*. In June 2020, *E. faecalis* and reinfection with *Pseudomonas aeruginosa* were confirmed, but due to the absence of clinical symptoms the patient was left untreated. Within a month, clinical symptoms of UTI appeared and *P. aeruginosa* was detected in urine; hence, the patient was administered fosfomycin, as suggested by conventional AST. However, the infection recurred and in August 2020 the patient was administered another course of fosfomycin due to persistence of *P. aeruginosa* along with reinfection with *Escherichia coli*. In February 2021, *P. aeruginosa* and reinfection with *Klebsiella pneumoniae* were identified, prompting treatment with a course of cefepime according to AST data. By April 2021, recurrence of *P. aeruginosa* and *E. faecalis* was noted and the patient was treated with ceftriaxone. During the next months, *P. aeruginosa* was detected in urine and, based on AST results, levofloxacin and cefepime were administered, but the infection recurred every time. Finally, in March 2022, the treating doctor prescribed a therapy based on antibiotic identification with AtbFinder.

Whereas routine AST identified only *P. aeruginosa*, AtbFinder detected *P. aeruginosa*, *Staphylococcus aureus*, *A. xylosoxidans*, and *K. pneumoniae* in the same urine sample. Accordingly, cefepime, moxifloxacin, cefepime + amikacin, meropenem + amikacin, and piperacillin/tazobactam + tobramycin were identified as effective by microbroth dilution, but ineffective by AtbFinder (Table 1).

Table 1. Comparison of antibiotic efficacy between conventional AST and AtbFinder.

Well number	Antibiotic	Antibiotic efficacy	Antibiotic efficacy
		Conventional AST	**AtbFinder**

Well number	Antibiotic	Antibiotic efficacy	Antibiotic efficacy
		PA	**PA; SA; AX; KP**
A1	Azithromycin	−	−
A2	Amikacin	−	−
A3	Amoxiclav	−	−
A4	Cefalexin	−	−
A5	Cefepime	+	−
A6	Cefotaxime	−	−
A7	Ciprofloxacin	−	−
A8	Clindamycin	−	−
B1	Colistin	−	−
B2	Co-trimoxazole	−	−
B3	Fosfomycin	−	−
B4	Furagin	−	−
B5	Furazidine	−	−
B6	Levofloxacin	−	−
B7	Meropenem	−	−
B8	Moxifloxacin	+	−
C1	Nitrofurantoin	−	−
C2	Piperacillin/tazobactam	−	−
C3	Teicoplanin	−	−
C4	Tigecycline	−	−
C5	Tobramycin	−	−
C6	Cefepime + amikacin	+	−
C7	Cefepime + ciprofloxacin	−	−
C8	Cefepime + meropenem	−	−
D1	Cefepime + piperacillin/tazobactam	−	−
D2	Cefepime + tobramycin	−	−
D3	Cefepime + levofloxacin	−	−
D4	Ceftriaxone + piperacillin/tazobactam	−	−
D5	Ceftriaxone + amikacin	−	+
D6	Ceftriaxone + levofloxacin	−	−
D7	Ceftriaxone + Meropenem	−	−
D8	Ceftriaxone + tobramycin	−	−
E1	Levofloxacin + amikacin	−	−
E2	Levofloxacin + azithromycin	−	+
E3	Levofloxacin + tobramycin	−	−
E4	Meropenem + amikacin	+	−
E5	Meropenem + azithromycin	−	−
E6	Meropenem + ciprofloxacin	−	−
E7	Meropenem + levofloxacin	−	−
E8	Meropenem + tobramycin	−	−
F1	Piperacillin/tazobactam + amikacin	−	−
F2	Piperacillin/tazobactam + azithromycin	−	−
F3	Piperacillin/tazobactam + ciprofloxacin	−	−
F4	Piperacillin/tazobactam + levofloxacin	−	+
F5	Piperacillin/tazobactam + meropenem	−	−
F6	Piperacillin/tazobactam + tobramicin	+	−
F7	Piperacillin/tazobactam + Fosfomycin	−	−
F8	Control	**N/A**	−

3

"+" antibiotic is effective (absence of bacterial growth)

"-" antibiotic is ineffective (presence of bacterial growth)

Instead, the latter identified ceftriaxone + amikacin, levofloxacin + azithromycin, and piperacillin/tazobactam + levofloxacin as effective (Figure 2).

Figure 2. Image of a 48-well AtbFinder plate, showing bacterial gowth after 12 h of cultivation at 37 °C.

The agar in each well is supplemented with one or several antibiotics at concentrations deemed effective in urine. Well F8 represents an antibiotic-free control. No bacterial growth is displayed by wells C4 (ceftriaxone + amikacin), D5 (levofloxacin + azithromycin), and E2 (piperacillin/tazobactam + levofloxacin).

The patient was eventually treated with a combination of levofloxacin and azithromycin, which resulted in the rapid disappearance of clinical symptoms. At the check-up visit in May 2022, *A. xylosoxidans* was detected; however, no clinical data supported ongoing UTI. AtbFinder identified colistin, cefepime + levofloxacin, and tigecycline as effective, and the patient was treated with colistin for 5 days. At the next follow-up visits in August and October 2022, no bacterial growth in urine was detected using either conventional AST or AtbFinder.

DISCUSSION

In this study, a variant of AtbFinder designed for the selection of antibiotics in patients with UTIs was successfully used to identify antibiotics effective against recurrent infection. Antibiotics selected by AtbFinder terminated UTIs that had been unsuccessfully treated with multiple courses of antibiotics indicated as effective by routine AST. Besides *P. aeruginosa* , which was identified in urine by a conventional microlab assay, AtbFinder identified also *S. aureus* ,*A. xylosoxidans* , and *K. pneumoniae.*

CONCLUSION , AtbFinder guided a successful course of antibiotic treatment. The first round of antibiotic therapy resulted in the eradication of all pathogens except *A. xylosoxidans* , which was eliminated in the second round, leading to negative urine culture results. Interestingly, the antibiotics suggested as effective by AtbFinder for the treatment of a mixture of *P. aeruginosaS. aureus* , *A. xylosoxidans* , and *K. pneumoniae*differed from those identified as effective against *A. xylosoxidans* alone. This discrepancy confirms how the antibiotic response of a single microorganism can differ entirely from that of the same pathogen within

a mixed microbial community. The presented case report highlights the advantage of the "whole microbial community response" to antibiotics utilized by AtbFinder for patients with UTIs.

Consent:

This study was approved by the Institutional Board Review (PA-3340/22, 2022) and followed the principles outlined in the Declaration of Helsinki. The patient provided written informed consent. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Declaration of interest:

Nothing to declare

Author contributions:

KK, VT, GT conceived and supervised the research. VM, KK, GT, VT and MT analyzed the data, and wrote the manuscript. KK and GT edited and helped to draft the final manuscript. All authors contributed to the article and approved the submitted version.

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