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ORIGINAL ARTICLE

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Direct detection of ESKAPEc pathogens from whole blood using the T2Bacteria Panel allows early antimicrobial stewardship intervention in patients with sepsis

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Abstract

In the microbiological diagnosis of bloodstream infections (BSI), blood culture (BC) is considered the gold standard test despite its limitations such as low sensitivity and slow turnaround time. A new FDA-cleared and CE-marked platform utilizing magnetic resonance to detect amplified DNA of the six most common and/or problematic BSI pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*; referred to as ESKAPEc) is available and may shorten the time to diagnosis and potentially improve antimicrobial utilization. Whole blood samples from hospitalized patients with clinical signs of sepsis were analyzed using the T2Bacteria Panel (T2Biosystems) and compared to simultaneously collected BC. Discrepant results were evaluated based on clinical infection criteria, combining supporting culture results and the opinion of treating physicians. A total of 55 samples from 53 patients were evaluated. The sensitivity and specificity of the T2Bacteria panel was 94% (16 out of 17 detections of T2Bacteria-targeted organisms) and 100%, respectively, with 36.4% (8 of 22) causes of BSI detected only by this method. The T2Bacteria Panel detected pathogens on average 55 hours faster than standard BC. In our study, 9 of 15 patients with positive T2Bacteria Panel results received early-targeted antibiotic therapy and/or modification of antimicrobial treatment based on T2Bacteria Panel findings. Given the high reliability, faster time to detection, and easy workflow, the technique qualifies as a point-of-care testing approach.

KEYWORDS
antimicrobial stewardship, bacteremia, blood culture, rapid diagnostics, sepsis, T2MR

1 | INTRODUCTION

Bloodstream infections (BSIs) are characterized by high morbidity and mortality, consequences that have been linked to delays in early and accurate administration of antimicrobials (Kumar et al., 2006).

Timely detection of BSI pathogens is an unmet need in today's medical microbiology practice, remaining largely dependent on blood culture (BC) (Idelevich et al., 2019). BC performance is being continuously improved with new protocols (Lamy et al., 2016) and new approaches for pathogen identification and antimicrobial susceptibility determination (Bookstaver et al., 2017; Buehler et al., 2016). However, BC still has inherent limitations including the requirements for large amounts of blood and long turnaround time. Low sample volumes and exposure to antimicrobials prior to BC sample collection considerably increase the risk of false-negative BC results. Despite these limitations, BC remains the gold standard and the first-line tool for detecting BSIs.

To improve BSI management, several innovative diagnostic techniques have been developed to optimize direct pathogen detection from whole blood samples (Peker et al., 2018; Poole et al., 2018). Some of them were introduced with limited success (Tkadlec et al., 2020) or with inconclusive validation study results (Tkadlec et al., 2019; Warhurst et al., 2015). In this study, we report real-world experience with the T2Bacteria Panel (T2Biosystems, USA) which utilizes T2 magnetic resonance (T2MR) to detect six bacterial BSI pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*, referred to as ESKAPEc pathogens) directly from whole blood (De Angelis et al., 2018). This culture-independent method can greatly shorten the time to positivity and like other existing rapid diagnostic methods, when integrated with an antibiotic stewardship program, may reduce the time to appropriate therapy and possibly improve infection-related outcomes such as duration of hospitalization or mortality.

2 | MATERIALS AND METHODS

2.1 | Study population

This prospective study was conducted between November 2018 and April 2019 at Motol University Hospital, a tertiary care teaching hospital with over 2200 beds (18% of them being intensive care) and roughly 80,000 hospitalizations per year. Patients were eligible for study enrolment if they (a) were being managed by Anesthesiology and Intensive Care Medicine Department or by intensive care units of Internal Medicine (including Hematology) or Surgery Departments, and (b) presented clinical signs of BSI, evaluated at the discretion of the managing physician. Initially, only patients whose samples were obtained during the standard operating hours of the microbiology laboratory (weekdays from 6 am to 4 pm) were included to ensure immediate processing of blood samples. To expand patient recruitment, eligibility hours in April 2019 were expanded from 6 am to midnight (except weekends).

2.2 | Laboratory methods

All blood samples were subjected to both culture and T2Bacteria Panel testing. BCs were processed using BACTEC FX™ Automated Blood Culture System (Becton Dickinson). Positive flagged BCs were primed for Gram stain. Subsequent pathogen identification from positive BC bottles was accomplished with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics) with the first attempt for identification made after 2 hours of pre-incubation on solid media (Nunvar & Drevinek, 2015).

Direct detection of six bacterial pathogens on the T2Dx instrument (T2Biosystems) requires 4 mL whole blood specimens. During processing on the T2Dx, bacteria are concentrated directly in whole blood, then lysed to release the DNA. After amplification, the target amplicon is hybridized with superparamagnetic particles and then detected by T2MR (Mylonakis et al., 2015). The results are available in as few as 3.5 hours. According to the manufacturer's instructions for use, the T2Bacteria Panel is indicated as an aid in the diagnosis of bacteremia and results should be used in conjunction with other clinical and laboratory data.

All results were called to the managing clinician by the medical microbiologist. Results were reported as target detected or target not detected for each of the six bacterial targets and accompanied with optional antimicrobial stewardship recommendations for patients with positive results.

2.3 | Study endpoints and statistical analysis

The primary outcomes of this study were the sensitivity and specificity of the T2Bacteria Panel, which were calculated using positive BC and/or T2Bacteria Panel results for a T2Bacteria-targeted organism as the reference. Species detected by BC but not included in the T2Bacteria Panel were excluded from sensitivity and specificity calculation. Similar to Nguyen *et al*. and De Angelis *et al*., we developed infection criteria to resolve any discrepancies between T2Bacteria Panel and BC results (De Angelis et al., 2018; Nguyen et al., 2019). "Proven BSI" was defined as a positive BC (excluding potential BC contaminants) using a concurrently drawn specimen. T2Bacteria Panel results were determined to be "Probable BSIs" when the T2Bacteria-detected organism was isolated within 21 days from another BC specimen collected at a different time or from a clinical sample taken from another site (such as abdominal fluid, urine, or bronchoalveolar lavage), indicating a plausible cause of infection. "Possible BSI" was defined by a negative BC, but a positive T2Bacteria Panel result (in the absence of supporting culture data if the T2Bacteria Panel detected organism was a plausible cause of disease). Finally, the category "Unlikely BSI" was introduced for positive BC that did not fulfill the criteria for

any of the above definitions and thus represented contaminated BC. Proven, probable, and possible BSIs were included in the final sensitivity and specificity calculation.

We also quantified the time to species identification (ID) between the BC and T2Bacteria Panel and noted any antibiotic stewardship interventions made as a result of the T2Bacteria Panel result. The time to species identification was defined as the number of hours between the time when a BC or T2Bacteria Panel sample was received in the lab and the time when the ID results were reported. Continuous variables were presented as means and standard deviations and were compared using a two sample t-test, as appropriate. Categorical variables were presented as counts and percentages and were compared using an adjusted Wald confidence interval. Differences were considered to be statistically significant for $p < 0.05$. Statistical analyses were performed by using Minitab®19 Statistical Software (Minitab, LLC.).

3 | RESULTS

During the study period, 55 samples were obtained and tested from 53 unique patient cases (Figure 1). Testing of blood samples by using the T2Bacteria Panel resulted in the recovery of 16 positive detections from 15 samples yielding a 27% rate of positivity (15 of 55). All 16 detections were considered proven, probable, or possible BSI based on the pre-specified definitions for BSI (see Methods). In total, 23 culture findings were recovered from 20 BC samples. As nine of them were deemed potential normal skin flora contaminants and not treated, only 14 findings from 14 BC samples were considered to be clinically relevant. However, five of them were not identifiable using the T2Bacteria Panel (Figures 1 and 2) and thus excluded from sensitivity and specificity calculation. Since probable and possible BSIs were assumed to be true positives that were missed by concurrent BC, sensitivity and positive predictive value were calculated to be 94% (95% CI, 71.1%–99.9%) and 100%, respectively (95% CI, 82.9%–100%). Only one false-negative T2Bacteria result (*E. coli* positive by BC) occurred in one sample that was T2Bacteria Panel-positive for another pathogen (*S. aureus*). There were 40 concordant negative detections (in regard to T2Bacteria Panel targeted organisms), yielding a specificity of 100% (95% CI, 92.4%–100%) and a negative predictive value of 98% (95% CI, 86.3%–99.9%).

The combined performance of BC and the T2Bacteria Panel for diagnosis of BSI is shown in Figure 3. Among all identified causes of proven, probable, and possible BSIs, 77% (17 of 22) of positive detections were included on the T2Bacteria Panel. The most commonly identified species were *E. coli* and *P. aeruginosa*.

The implementation of the T2Bacteria Panel decreased the time to species identification on average by 55 hours. The mean time from arrival to the laboratory to species identification was 6.1 hours (SD ± 5.4) for T2Bacteria Panel and 62 hours (SD ± 54) for the conventional BC-based identification ($p = 0.001$). The

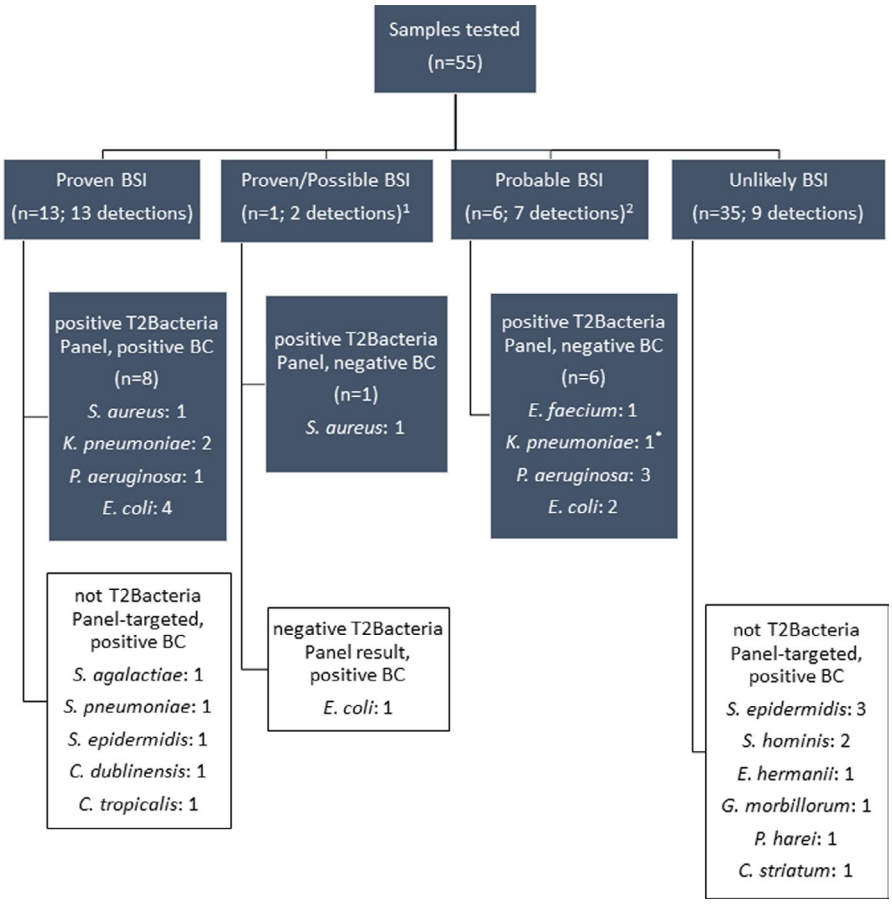

FIGURE 1 Performance of blood culture (BC) and the T2Bacteria Panel for diagnosis of BSI. For definitions of Proven BSI, Probable BSI, Possible BSI, and Unlikely BSI, please see the Methods. Samples with only BC positivity (and T2Bacteria Panel negativity) are in blank boxes. [1]In 1 sample, one T2Bacteria-targeted organism was identified by T2Bacteria Panel that was not identified in the paired blood culture. In this same sample, one T2Bacteria-targeted organism was identified in the paired blood culture, but not by the T2Bacteria Panel. [2]In 1 sample, two T2Bacteria-targeted organisms were identified (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). [*]Cross-reactivity between T2Bacteria *K. pneumoniae* channel and *Klebsiella variicola* was confirmed per the T2Bacteria Instructions For Use

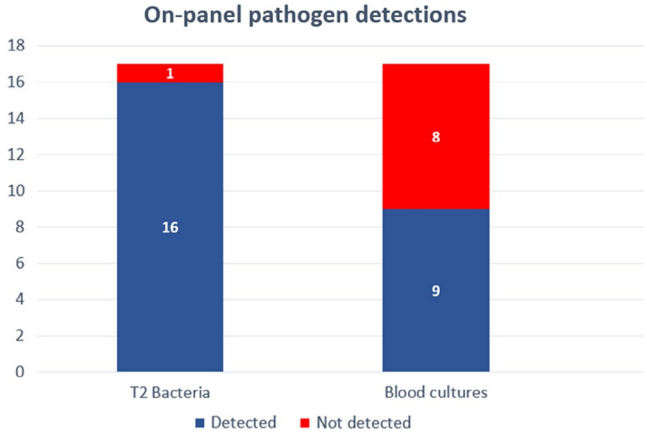

FIGURE 2 Comparison of on-panel pathogen detection (T2Bacteria vs. Blood cultures). 16 out of 17 proven, probable, and possible BSIs were detected by T2Bacteria Panel, whereas BC detected only 9 out of 17

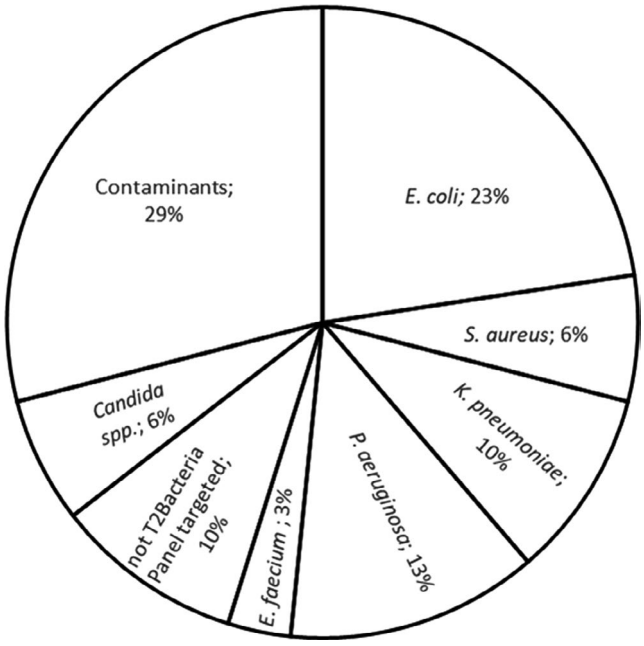

FIGURE 3 The distribution of all 31 positive detections comprising 15 different microbial species, detected by either method in 25 samples. A total of 55% of findings were detectable with the T2Bacteria Panel. If contaminants are excluded, the T2Bacteria Panel covers 77% of clinically relevant findings

mean time from arrival to negative result was 7.4 hours (SD ± 7) for T2Bacteria Panel and 119 hours (SD ± 56) for the conventional BC ($p < 0.001$).

Rapid identification with the T2Bacteria Panel permitted early antimicrobial stewardship interventions such as early initiation of targeted antibiotic therapy secondary to faster time to detection and/or new finding of BSI pathogen. Interventions were made in 9 of 15 patients with T2Bacteria Panel-positive samples as seen in Table 1 (more detailed information to support the change of antibiotic therapy is provided in Appendix 1). T2Bacteria Panel negative results were not evaluated for this analysis.

4 | DISCUSSION

The timely administration of effective antimicrobial therapy is crucial for the survival of patients with sepsis (Kumar et al., 2006). Rapid diagnostic assays have been associated with improvements in time to appropriate antibiotic therapy by enhancing the early identification of causative organisms for BSI (Bookstaver et al., 2017; Buehler et al., 2016). Such data support the coupling of rapid diagnostic technology with antimicrobial stewardship programs to optimize empiric antibiotic therapy and reduce time to targeted therapy.

In this study, we report the interventional experience of the T2Bacteria Panel in the Czech Republic which identified common ESKAPEc pathogens directly from whole blood. Despite the limited number of bacterial species included on the T2Bacteria Panel, we found that 77% of all identified causative pathogens of proven, probable, and possible BSI (i.e., excluding common BC contaminants) were detected by the T2Bacteria Panel. The high sensitivity of the T2Bacteria Panel was demonstrated by the detection of eight pathogens in seven samples that were missed by BC, representing 36.4% (8 of 22) of the total number of identified BSIs. All of these confirmed true positives were from patients who were previously exposed to antibiotic therapy which is consistent with previous findings in patients with sepsis demonstrating that BC sensitivity is reduced by approximately 50% after the initiation of antimicrobial therapy (Cheng et al., 2019). It also suggests the T2Bacteria Panel performance may have limited interference from empirical antimicrobial treatment, which continues to be an issue with culture-based diagnostics (Farrell et al., 2015).

The T2Bacterial Panel provided species identification in 6.1 hours on average, which was 55 hours faster compared to BC. These results are consistent with a multi-hospital survey demonstrating a median BC time to identification of 43.4 hours and replicate previous reports of the T2Bacteria Panel advantage for faster diagnosis compared to standard BC diagnostic methods (De Angelis et al., 2018; Nguyen et al., 2019; Tabak et al., 2018; Voigt et al., 2020). This allowed for early antibiotic stewardship interventions in 60% of our patients with T2Bacteria Panel-positive samples.

This evaluation is limited by the small number of bacteremic patients who were enrolled in our single-center cohort study. Not every blood culture was eligible for a parallel investigation with the T2Bacteria Panel (as stated in Methods). To target patients with the highest risk of BSI and to improve the rate of positive detections, we predefined testing criteria for blood samples from specific intensive care units to be tested by both culture and T2Bacteria Panel methods. As a result, the percentage of positive blood cultures (36.3%) was higher than expected from the usual clinical setting where a total of 10,843 blood culture bottles were ordered during the study

TABLE 1 Antimicrobial stewardship impact of positive T2Bacteria Panel results. Data from 15 patients with T2Bacteria Panel-positive results

Patient sample ID	T2Bacteria Panel result	BC result	ATB change	ATB before T2bacteria Panel result	ATB after T2bacteria Panel result
T2_30	*S. aureus*	*S. aureus*	Yes	Amikacin + cefepim	Linezolid + ceftriaxone
T2_20	*K. pneumoniae*	*K. pneumoniae*	Yes	Imipenem	Imipenem + amikacin
T2_25	*E. coli*	*E. coli*	Yes	piperacillin/ tazobactam + metronidazole	Imipenem + metronidazole
T2_61	*E. coli*	*E. coli*	Yes	Cefotaxime + metronidazole	Cefotaxime + metronidazole + gentamicin
T2_1	*P. aeruginosa*	negative	Yes	Meropenem + vancomycin	Meropenem + amikacin
T2_4	*K. pneumoniae*, *P. aeruginosa*	negative	Yes	Cotrimoxazole + tigecycline	Cotrimoxazole + tigecycline + amikacin
T2_7	*S. aureus*	*E. coli*	Yes	Amoxicillin/clavulanic acid	Linezolid + meropenem
T2_27	*P. aeruginosa*	*S. epidermidis*	Yes	Amikacin	Amikacin + imipenem
T2_46	*E. faecium*	negative	Yes	Piperacillin/tazobactam	Piperacillin/tazobactam + vancomycin
T2_47	*E. coli*	*E. coli*	No	Ceftriaxone	Ceftriaxone
T2_48	*K. pneumoniae*	*K. pneumoniae*	No	Meropenem	Meropenem
T2_51	*E. coli*	*E. coli*	No	Cefotaxime + gentamicin	Cefotaxime + gentamicin
T2_22	*E. coli*	negative	No	Meropenem	Meropenem
T2_36	*E. coli*	negative	No	Meropenem + ciprofloxacin	Meropenem + ciprofloxacin
T2_40	*P. aeruginosa*	*P. aeruginosa*	No	Meropenem	Meropenem

Note: Please note that T2Bacteria results were used in combination with other microbiological and diagnostic findings, local epidemiology, and clinical history of the patient to determine antibiotic treatment decisions (not reported in the Table).

Abbreviation: ATB, antibiotic.

period, from which 1,602 (14.8%) were positive. We did not assess the potential role of the T2Bacteria-negative result on early therapeutic decisions, namely the early de-escalation of unnecessary, broad-spectrum antibiotics. Our study did not assess the effect of the assay on patient outcomes, but it could be expected that faster time to effective antibiotic prescription would translate into reductions in length of stay and mortality based on previous reports that observed reductions in length of stay and mortality based on findings with alternative post-culture molecular diagnostic methods (Beganovic et al., 2017; Kumar et al., 2006; Perez et al., 2013, 2014; Timbrook et al., 2017). Lastly, the pathogen coverage by the T2Bacteria Panel is not inclusive of all causative organisms for BSI, does not provide antimicrobial susceptibility information, and is not intended to replace routine culture and susceptibility methods.

5 | CONCLUSIONS

In this study, T2Bacteria Panel proved high sensitivity (by identifying BSI in eight cases where BC produced false-negative results) and a significant reduction of time to species identification (55 hours on average). A larger study should be conducted to determine the exact clinical impact of earlier T2Bacteria Panel results on length of hospital stay and mortality benefit in patients with BSI. However, based on our experience with rapid BSI diagnostics, T2Bacteria Panel represents the most promising currently available diagnostic tool. Implementation of the T2Bacteria Panel at our institution led to faster time to reliable detection of selected BSI pathogens and decreased time to administration of species-directed antibiotic therapy.

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CONFLICTS OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

Pavel Drevinek: Conceptualization (lead); Data curation (supporting); Funding acquisition (lead); Methodology (equal); Validation (supporting); Writing-original draft (lead). **Jakub Hurych:** Data curation (equal); Formal analysis (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Software (lead); Writing-review & editing (equal). **Milena Antuskova:** Investigation (lead). **Jan Tkadlec:** Methodology (supporting); Writing-review & editing (equal). **Jan Berousek:** Conceptualization (supporting); Resources (equal). **Zuzana Prikrylova:** Resources (equal). **Jiri Bures:** Resources (equal). **Jaromir Vajter:** Resources (equal). **Martin Soucek:** Conceptualization (supporting); Resources (equal). **Jan Masopust:** Resources (equal). **Vendula Martinkova:** Conceptualization (supporting); Resources (equal). **Jaroslava**

Adamkova: Resources (equal). **Veronika Hysperska:** Resources (equal). **Eliska Bebrova:** Resources (equal).

ETHICS STATEMENT

This study was approved by the Ethics Committee of the University Hospital Motol and the 2nd Faculty of Medicine, Charles University (ref. No EK-337/21).

DATA AVAILABILITY STATEMENT

All raw data are available in the Zenodo repository at https://doi.org/10.5281/zenodo.4746206

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APPENDIX 1

Patient sample ID	T2Bacteria Panel result	T2 time from sample arrival to result (h:mm)	BC result	BC time to result (hh:mm)	ATB change	ATB before T2Bacteria Panel result (for how many days)	ATB after T2Bacteria Panel result	Reason for change
T2_30	*S. aureus*	3:40	*S. aureus*	17:00	Yes	Amikacin (1 day), cefepim (1 day)	Linezolid + ceftriaxone	Patient on empiric therapy with amikacin and cefepime. Switch to linezolid and ceftriaxone based on the T2Bacteria Panel result (*S. aureus* was a new finding). Suspicion of catheter-related bloodstream infection.
T2_20	*K. pneumoniae*	4:04	*K. pneumoniae*	22:00	Yes	Imipenem (1 day)	Imipenem + amikacin	Worsening of symptoms (fever, shivers) with leukocyturia on current antibiotics (imipenem). Amikacin was added based on the T2Bacteria Panel result (*K. pneumoniae* was a new finding, preceding the culture positivity from urine and blood culture).
T2_25	*E. coli*	3:51	*E. coli*	17:00	Yes	Piperacillin/tazobactam (1 day) + metronidazole (1 day)	Imipenem + metronidazole	Acute admission due to suspected cholangitis, pancreatitis in the septic state. Empiric therapy was changed due to worsening of clinical state and based on the T2Bacterial Panel result (*E. coli* was a new finding).
T2_61	*E. coli*	4:04	*E. coli*	9:00	Yes	Cefotaxime (1 day) + metronidazole (1 day)	Cefotaxime + metronidazole + gentamicin	Patient with suspected urosepsis, worsening of symptoms on current empiric antibiotics. Gentamicin was added based on the T2Bacteria Panel result (*E. coli* was a new finding preceding the culture positivity from urine and blood culture).
T2_1	*P. aeruginosa*	7:17	negative	NA	Yes	Meropenem (4 days) + vancomycin (13 days)	Meropenem + amikacin	Worsening of symptoms on current antibiotics. Amikacin added as usual combination therapy of *P. aeruginosa* bloodstream infection, based on T2Bacteria Panel result (*P. aeruginosa* detected in another blood culture, performed 11 days earlier).
T2_4	*K. pneumoniae*, *P. aeruginosa*	6:38	*negative*	NA	Yes	cotrimoxazole (9 days) + tigecycline (8 days)	Cotrimoxazole + tigecycline + amikacin	Amikacin was added based on the T2Bacteria Panel result (*Klebsiella* detected in another blood culture 14 days earlier; *P. aeruginosa* was a new finding).

Patient sample ID	T2Bacteria Panel result	T2 time from sample arrival to result (h:mm)	BC result	BC time to result (hh:mm)	ATB change	ATB before T2Bacteria Panel result (for how many days)	ATB after T2Bacteria Panel result	Reason for change
T2_7	*S. aureus*	4:34	*E. coli*	11:00	Yes	Amoxicillin/clavulanic acid (1 day)	linezolid +meropenem	Linezolid was added based on the T2Bacteria Panel result (*S. aureus* was a new finding).
T2_27	*P. aeruginosa*	4:31	*S. epidermidis*	28:00	Yes	Amikacin (7 days)	Amikacin + Imipenem	Patient 3 months after lung transplantation, chronically colonized with *P. aeruginosa*. Worsening of symptoms. Imipenem was added as a combination therapy of *P. aeruginosa* bloodstream infection. Furthermore, intermediate susceptibility of *P. aeruginosa* to amikacin was detected in earlier collected BAL and sputum.
T2_46	*E. faecium*	5:14	Negative	NA	Yes	Piperacillin/ tazobactam(3 days)	Piperacillin/tazobactam + vancomycin	Worsening of symptoms on piperacillin/ tazobactam, therefore vancomycin was added based on T2Bacteria Panel result (*E. faecium* was a new finding).
T2_47	*E. coli*	4:24	*E. coli*	10:00	No	Ceftriaxone	ceftriaxone	NA
T2_48	*K. pneumoniae*	3:50	*K. pneumoniae*	28:00	No	Meropenem	meropenem	NA
T2_51	*E. coli*	4:01	*E. coli*	38:00	No	Cefotaxime + gentamicin	cefotaxime +gentamicin	NA
T2_22	*E. coli*	26:14	Negative	NA	No	Meropenem	Meropenem	NA
T2_36	*E. coli*	3:38	Negative	NA	No	Meropenem + ciprofloxacin	Meropenem + ciprofloxacin	NA
T2_40	*P. aeruginosa*	5:20	*P. aeruginosa*	90:00	No	Meropenem	Meropenem	NA