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Rapid antimicrobial susceptibility testing in patients with bacteraemia due to Enterobacterales: an implementation study

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Summary

AIMS OF THE STUDY: The goal of this descriptive study was to assess the performance as well as the extent of the clinical impact of rapid automated antimicrobial susceptibility testing in patients with bacteraemia due to Enterobacterales. We also aimed to analyse how rapid automated antimicrobial susceptibility testing influences clinical decision-making.

METHODS: This single-centre study conducted at the University Hospital of Zurich included data from all consecutive patients with Enterobacterales bacteraemia from November 2019 to October 2020. There was no control group. The primary outcome was the effect of rapid automated antimicrobial susceptibility testing on antibiotic therapy (no adjustment, escalation to a broader-spectrum antibiotic or de-escalation to a narrower-spectrum antibiotic). Rapid automated antimicrobial susceptibility testing results were further compared to susceptibility tests using European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard methods and erroneous results were noted. Additionally, we investigated turnaround times for rapid automated antimicrobial susceptibility testing and routine diagnostic testing.

RESULTS: We analysed 106 patients with 116 episodes of bacteraemia due to Enterobacterales, with *Escherichia coli* and *Klebsiella pneumoniae* being the most frequent isolates. Almost 8% of pathogens were multidrug resistant. Rapid automated antimicrobial susceptibility testing showed category agreement in 98.4% of all interpretable cases. A significant reduction of more than 20 h in turnaround times could be achieved with rapid automated antimicrobial susceptibility testing compared to the routine diagnostic workflow. In the majority of cases, rapid automated antimicrobial susceptibility testing had no effect, given that the empirical therapy was already correct or circumstances did not allow for de-escalation. In 38.8% of cases, antimicrobial therapy was adjusted, whereas eight cases were de-escalated based on rapid automated antimicrobial susceptibility testing alone.

CONCLUSIONS: Rapid automated antimicrobial susceptibility testing may be a valuable and safe way to accelerate diagnosis. In particular, time to suitable therapy can be shortened in cases of incorrect therapy. However, physicians are reluctant to de-escalate antibiotic therapy based on rapid automated antimicrobial susceptibility testing alone, limiting its impact in everyday clinics. To further explore the potential of rapid automated antimicrobial susceptibility testing, a stringent/compulsory antibiotic stewardship programme would be a valuable next step.

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Introduction

Emerging antimicrobial resistance has been progressively complicating antibiotic treatment, forcing treating physicians to use broad-spectrum antibiotics as an empirical therapy [1, 2]. The consequent use of second- or third-line antibiotics promotes undesired side effects, among others drug toxicity, selection of antimicrobial resistance and dysbiosis thus facilitating subsequent infections [3–5]. Moreover, increasing bacterial resistance can delay the institution of effective treatment and therefore endanger the patient, especially in case of blood infections [6, 7].

A major factor prolonging the time to optimal treatment has traditionally been microbiological diagnosis. Time from pathogen growth detection to identification is often 24 h to 48 h, mainly because subculturing and overnight incubation are necessary. In recent years, several new microbiological diagnostic methods have been introduced, reducing the time between sample collection and completion of microbiological diagnosis [8, 9]. These methods concern rapid pathogen identification such as Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) and rapid automated antimicrobial susceptibility testing. Recently, a method to rapidly detect phenotypic resistance using the WASPLab^{TM} system has been developed. The WASPLab^{TM} system is a fully automated platform for inoculation, incubation, digitisation and storage, thus allowing full automation of microbiological examinations [8, 10, 11]. A few studies have assessed the clinical impact of rapid pathogen identification and susceptibility

testing [12–15], with a focus on the amount of time that can be saved until antibiotic therapy can be correctly escalated or de-escalated. As real-life practices often differ from the controlled setting of a study, we aimed to assess the performance as well as the extent of the clinical impact of rapid automated antimicrobial susceptibility testing at University Hospital Zurich regarding treatment adjustment, reduction of broad-spectrum antibiotics and its role in the clinical decision-making process.

Methods

This single-centre study was conducted from November 2019 to October 2020 at University Hospital Zurich, Switzerland, a tertiary care hospital with approximately 900 beds, 44 clinics and institutes and 42,000 inpatients per year. The Institute of Medical Microbiology of the University of Zurich processes the microbiological samples of all University Hospital Zurich inpatients.

Infectious diseases consultation service

At University Hospital Zurich, blood culture results are reviewed on a daily basis by members of the Infectious Diseases consultation service jointly with the microbiologists. Moreover, highly complex situations often requiring second- or third-line antibiotics are jointly discussed. A formal infectious diseases consultation service has been established; however, an infectious diseases consultation is not mandatory and if there is an infectious diseases consultation, the primary service is not required to follow the recommendation of the infectious diseases expert. Since November 2019, a team of infectious diseases specialists and microbiologists prospectively monitors the results and the effect of rapid automated antimicrobial susceptibility testing among inpatients with Enterobacterales bacteraemia.

Workflow

Positive blood cultures with a clear Gram-stain result and available before 9:00 am were discussed in the rapid automated antimicrobial susceptibility testing team and suitable isolates were included. Criteria for exclusion were cultures from repeat isolates in cases of persistent bacteraemia, growth of non-Enterobacterales, lack of growth after 6 h, mixed-Gram stains, organ donors, cultures where the routine result could be received the same day, cultures which became positive on the weekend and cultures from deceased patients that had not been cancelled by the treating physician. Blood cultures that became positive after 9:00 am were not included. Identification and rapid automated antimicrobial susceptibility testing results were communicated in the afternoon and, in parallel, the treating physician and the infectious diseases consultation service were informed. The infectious diseases physician could then choose to make recommendations to the primary service regarding therapy adjustment. The members of the rapid automated antimicrobial susceptibility testing team themselves did not interfere in the clinical workflow due to the observational nature of the study.

During the study period, a night shift was introduced at the Institute of Medical Microbiology. Samples were transported and loaded into the Virtuo System. Further analysis was performed in the morning. The effect of this on transport time and turnaround times was also examined.

Pathogen identification and antimicrobial susceptibility testing

Incubation, streaking and imaging were done using the fully automated WASPLab^{TM} system for all blood cultures containing Gram-negative rods. Inhibition zones were read after 6h. MALDI-TOF was used for pathogen identification. When Enterobacterales were identified, rapid automated antimicrobial susceptibility testing was evaluated as previously published [8, 10, 11]. Briefly, after Gram staining, bacterial counts were obtained using flow cytometry (UF-4000, Sysmex Europe GmbH, Germany). A standardised inoculum of 10^6 colony-forming units/mL was prepared by dilution in 0.9% NaCl solution. 60 microL of the inoculum was then plated on MH agar (Oxoid Limited, Basingstoke, UK) by the COPAN WASP system (Copan Italia S.p.A., Brescia, Italy). Antibiotic discs (Oxoid) were added manually. The plates were incubated and read after 6 hby the COPAN WASPLab^{TM} system.

Standard antimicrobial susceptibility testing according to EUCAST/CLSI (Clinical & Laboratory Standards Institute) guidelines [16, 17] was performed in parallel.

Study participants and data collected

All inpatients >16 years with rapid automated antimicrobial susceptibility testing and detection of Enterobacterales in blood cultures were potentially eligible for the study (1 Nov 2019 to 31 October 2020). A specific patient could be included more than once if their blood infections were distinct clinical events (hence not repeat isolates), for example two hospital stays due to bacteraemias with different pathogens and different foci. We performed a chart review of these patients and collected coded information on demographic, clinical and laboratory data, antimicrobial treatment, and follow-up in a spreadsheet. All clinical and microbiological data were checked by an independent infectious diseases physician and a clinical microbiologist.

Patients were considered to be immunosuppressed if they had been receiving corticosteroids at a dosage of at least 20 mg/d for at least 2 weeks or had received any other immunosuppressive agent within the last 4 weeks (in case of systemic rheumatic disease, solid organ transplantation or stem cell transplantation), cytotoxic chemotherapy within the last 4 weeks or had other conditions that lead to an immunosuppressed state such as HIV infection with a CD4 cell count below 200 cells/mm^3. Further, we collected information on the source of infection in case of a clear focus (e.g. urogenital tract infection and intra-abdominal infection). We recorded all antimicrobial agents administered on the day of rapid automated antimicrobial susceptibility testing except for prophylactic antimicrobial agents.

Concerning rapid automated antimicrobial susceptibility testing, we noted the results of the Gram stain at the time of the morning call, species identification at the time of the afternoon call and the relevant antibiotic resistance. The latter was defined as the resistance to the broadest antibiotic class or substance. For example, a pathogen resistant to 3rd- and 4th-generation cephalosporins but susceptible to carbapenems was rated as resistant to 4th-generation

cephalosporins. Further, only visible phenotypic resistance was taken into consideration. For example, pathogens known to possess chromosomal AmpC enzymes were only recorded as resistant to 4th-generation cephalosporins or carbapenems if the phenomenon was already visible on the agar plates. We further assessed every microbiological case for differences in category agreement between conventional and rapid antimicrobial resistance testing. Additionally, the rates of *minor errors*, *major errors* and *very major errors* were determined as previously described [8, 10]:

- *major error*: a pathogen is classified as resistant to a given substance using rapid automated antimicrobial susceptibility testing but as sensitive according to EUCAST (therefore falsely resistant),
- *very major error*: a pathogen is classified as sensitive to a substance using rapid automated antimicrobial susceptibility testing and as resistant according to EUCAST,
- *minor error*: erroneous classifications involving category (i) (susceptible, increased exposure).

For each case, the effect of rapid automated antimicrobial susceptibility testing was categorised into one of three categories: escalation (adjustment to a broader-spectrum antibiotic), de-escalation (adjustment to a narrower-spectrum antibiotic) or no effect. For the "no effect" category, we further collected the reasons why the regimen was not changed.

To gain additional insights into whether rapid automated antimicrobial susceptibility testing might help optimisation of anti-infective therapy, we noted the time of the morning and afternoon calls and, when the therapy was adjusted, the time of the new prescription.

Methods and statistical analyses

Our aim was to describe the real-life performance and feasibility of rapid automated antimicrobial susceptibility testing during routine clinical work and its role in the decision-making process, especially regarding treatment adjustment and reduction of broad-spectrum antibiotics usage. Secondary outcomes were turnaround times and the time elapsed between the rapid automated antimicrobial susceptibility testing result and antimicrobial treatment adjustment.

Descriptive statistical analyses were performed with IBM SPSS Statistics 26 and R (version 4.0.3). The Wilcoxon rank-sum test or where applicable the paired Wilcoxon signed-rank test were used as implemented by R; 95% confidence intervals (95% CI) were calculated as described in Baur and the Hodges-Lehmann estimator was used [18]. To counteract the multiple comparisons problem, multiple testing correction was performed by the Holm method [19]. A significance level of 0.05 was used.

Ethical consent

The study was presented to the local ethics committee (BASEC-Nr. Req 2020-00615). They regarded it as a quality improvement programme and therefore ethical approval was waived by KEK Zürich; hence patient informed consent was not required.

Information from microbiological and clinical files was analysed anonymously and did not require informed consent from patients.

Results

From 1 November 2019 to 31 October 2020, 202 patients with 226 microbiological isolates were eligible for analysis. Of these, 93 patients with 110 bacterial isolates were excluded due to positivity at the weekend (63 isolates, 27.9%), detection of non-Enterobacterales pathogens (9 isolates, 4.0%), repeat isolates (6 isolates, 2.7%), mixed-Gram stain (5 isolates, 2.2%), death of the patient or switch to palliation (5 isolates, 2.2%), unknown reasons (15 isolates, 6.6%) or other reasons (no growth, out of hospital, organ donor; 7 isolates in total, 3.1%).

Baseline data

The study included 106 patients with 116 positive Enterobacterales blood cultures. Their median age was 67 years (interquartile range 56–76 years) and 68 (64.2%) were male. Table 1 shows the baseline clinical data including the suspected source of infection, which was mostly urogenital or intra-abdominal. In three patients, blood cultures were drawn to rule out bacterial infection but the main suspected cause of fever and elevated inflammatory markers was either a non-bacterial infection (for example, patients who were hospitalised with COVID-19) or not infection-related at all. Twenty-eight patients (24.1%) were treated in the intensive care unit at the time of the Enterobacterales bacteraemia.

Antimicrobial therapy

The most frequent empirical antimicrobial agents were piperacillin/tazobactam (42, 36.2%), ceftriaxone (38, 32.8%) and meropenem (17, 14.7%), whereas in the remaining cases other antimicrobial agents such as amoxicillin/clavulanic acid, ceftazidime/avibactam, colistin, gentamicin, metronidazole and/or ciprofloxacin were used. In total, 26 (22.4%) patients received a 4th-generation cephalosporin, a carbapenem, ceftazidime/avibactam or colistin. Sixteen patients received additional antimicrobial agents with Gram-positive activity (e.g. vancomycin, daptomycin, linezolid) for proven or suspected concomitant infections. Four patients received no empirical antimicrobial treatment at all.

Pathogens

Table 2 shows the frequency of each pathogen as well as the relevant resistances. Nine (7.8%) Enterobacterales were multidrug-resistant (5 *E. coli*, 3 *K. pneumoniae* and 1 *Enterobacter cloacae*). Rapid automated antimicrobial susceptibility testing was able to correctly detect five of these multidrug-resistant strains. The difference was due to the area of technical uncertainty and was resolved by an 18 h reading. Of note, rapid automated antimicrobial susceptibility testing was capable of identifying all ESBL (extended-spectrum beta-lactamase) strains phenotypically without additional testing.

Agreement with conventional microbiology

In 10 cases (8.8%), the inhibition zone on the rapid automated antimicrobial susceptibility testing discs was within the area of technical uncertainty and therefore uninterpretable. Therefore, rapid automated antimicrobial susceptibility testing achieved category agreement in 103 of 105 (98.1%) interpretable cases. In the remaining 2 (1.7%) cases, the pathogen was considered susceptible using rapid automated antimicrobial susceptibility testing but was within the "susceptible at increased exposure range" in conventional microbiology and was therefore rated a minor error.

In total, 1478 antibiotic tests were performed. Of these, 1330 (90.0%) were in category agreement. A further 126 (8.5%) were in the area of technical uncertainty and thus not interpretable at the 6 htimepoint. Discounting these, category agreement was achieved in 1330/1352 (98.4%) tests with 3 very major errors, 15 major errors and 4 minor errors remaining. The very major errors occurred for piperacillin/tazobactam (n = 1), imipenem (n = 1) and amikacin (n = 1). In the piperacillin/tazobactam case, growth of *Citrobacter koseri* and delayed growth of *Citrobacter freundii* resulted in the error. For the imipenem case, the antibiotic disc was placed incorrectly on the agar during routine testing. For the amikacin case, the inhibition zone was within normal variation around the breakpoint. None of the errors led to an error in the clinical decision.

Table 1:
Baseline data of patients with Enterobacterales bacteraemia.

Patient characteristics		**n = 106**
Age (years)	Median (interquartile range)	67 (56–76)
Sex	Female, n (%)	38 (35.8)
	Male, n (%)	68 (64.2)
Comorbidities	Chronic kidney disease, n (%)	10 (8.6)
	Chronic pulmonary disease, n (%)	4 (3.4)
	Chronic liver disease, n (%)	6 (5.2)
	Systemic rheumatic disease, n (%)	3 (3.4)
	Solid organ or stem cell transplantation, n (%)	20 (17.2)
	Neoplasia (solid or haematological), n (%)	30 (25.9)
	Immunosuppressive medication, n (%)	20 (17.2)
	Neutropenia*, n (%)	9 (7.8)
	Treatment in intensive care unit, n (%)	28 (24.1)
Source of infection		n = 116
Urogenital tract, n (%)		48 (41.4)
Intra-abdominal tract, n (%)		26 (22.4)
Respiratory tract, n (%)		6 (5.2)
Skin and soft tissue, n (%)		5 (4.3)
Intravascular, n (%)		1 (0.9)
Multiple possible infectious foci or unknown, n (%)		27 (23.2)
No bacterial infection suspected, n (%)		3 (2.5)

* = absolute neutrophil count <500 cells/mm^3. Source of infection was listed separately for each blood infection as 10 patients were included twice because of different episodes of bacteraemia. Percentages of suspected source of infection might not sum to 100% due to rounding.

Table 2:
Frequency of each pathogen as a percentage of all Enterobacterales as well as antibiotic resistance in each pathogen.

Pathogen (number of isolates, % of detected Enterobacterales)	**Relevant resistance**	**n**	**% of pathogens**
Escherichia coli (69, 59.5)	ESBL phenotype	12	17.4
	Piperacillin/tazobactam	3	4.3
	4th-generation cephalosporins	10	14.5
Klebsiella pneumoniae (20, 17.2)	ESBL phenotype	7	33.3
	Piperacillin/tazobactam	4	5.7
	4th-generation cephalosporins	8	38.1
	All carbapenems	2	9.5
	Ertapenem only	2	9.5
Klebsiella aerogenes (2, 1.7)	Piperacillin/tazobactam	1	50
Other *Klebsiella* spp. (4, 3.4)	3rd-generation cephalosporins	1	33.3
Enterobacter cloacae (9, 7.8)	Piperacillin/tazobactam	4	33.3
	4th-generation cephalosporins	2	22.2
Citrobacter freundii (1, 0.9)	ESBL phenotype	1	100
	4th-generation cephalosporins	1	100
Other *Citrobacter* spp. (4, 3.4)	No relevant resistance		
Serratia marcescens (4, 3.4)	No relevant resistance		
Proteus mirabilis (2, 1.7)	No relevant resistance		
Morganella morganii (1, 0.89)	No relevant resistance		

ESBL = Extended-spectrum beta-lactamase; Spp = species

Effect of rapid automated antimicrobial susceptibility testing

The effects of rapid automated antimicrobial susceptibility testing are shown in table 3. In the majority of analysed Enterobacterales bacteraemias (71, 61.2%), rapid automated antimicrobial susceptibility testing did not have an effect. In most cases, the empirical therapy was correct (54.9%) or circumstances did not allow de-escalation of therapy (e.g. concomitant intra-abdominal infections with one pathogen in blood culture but multiple pathogens in tissue samples). The two cases where pathogen identification would have warranted an escalation were due to the detection of *E. cloacae* AmpC. In the nine cases where reasons were unknown, a de-escalation would have been justifiable in seven cases (improving clinical condition). The other two cases were patients with *E. cloacae* who were treated with piperacillin/tazobactam. Therefore, an escalation of therapy would have been advisable.

In 45 cases, rapid automated antimicrobial susceptibility testing had an effect; antibiotic therapy was adjusted in 26 cases (57.8%) because the empirical therapy proved to be ineffective.

Time elapsed

Blood culture results using rapid automated antimicrobial susceptibility testing were available after a mean of 26.7 h (interquartile range 22.5–28.6 h), whereas routine diagnostics required 47.2 h (42.5–48.8 h). Thus, rapid automated antimicrobial susceptibility testing reduced the time to result by 20.5 h, a statistically significant difference (median 20 h; 95% confidence interval [CI] 19.6–20.3 h, p <0.001). Additionally, we examined whether an around-the-clock transfer service could reduce turnaround times. We saw a non-significant reduction in transport times after the introduction of a night shift collecting samples directly at the wards (mean 2.7 h, median 0.83 h, 95% CI 0.03–1.8 h , p = 0.081). Similarly, we saw no difference in turnaround times (mean 2.44 h, median 2.5 h, 95% CI 0.13–5 h, p = 0.081). This finding is most probably due to low numbers (78 pre- vs 35 post-introduction) and needs further evaluation.

The median interval from the morning call to the afternoon call was 6.9 h (range 3.1–9 h) with 10 cases taking less than 6 hand 5 cases taking more than 8 h. The time elapsed from the second call to antibiotic therapy adjustment in cases where escalation was necessary was 0.8–5.4 h (median 0.8 h). De-escalation took 0.3–121 h (median 28.7 h). In only 8 of 19 (0.42) cases, therapy was de-escalated within 24 hours. Strictly speaking, only these eight therapy adjustments can be considered a true effect of rapid automated antimicrobial susceptibility testing compared to antimicrobial resistance testing in general. Of note, in seven of these eight cases, an infectious diseases specialist had been involved.

Discussion

In this study we set out to analyse the clinical impact of rapid automated antimicrobial susceptibility testing in clinics with regard to decision-making, adjustment of antibiotic therapy —especially reduction of broad-spectrum antibiotics— and to evaluate its performance in everyday practice.

In general, our data reflect a very ill patient population, of whom a quarter was immunosuppressed and in need of intensive care. The main pathogens isolated were *E. coli* and *K. pneumoniae*, with nine pathogens being multidrug resistant. While most patients received either piperacillin/tazobactam or ceftriaxone, almost a quarter were given agents with a broader spectrum of activity such as carbapenems. Rapid automated antimicrobial susceptibility testing performed very well with: only two cases of major errors relevant to treatment; no minor errors or very major errors relevant to treatment; and interpretable results after 6 hours of incubation in most cases. In 61.2% of bacteraemia cases, rapid automated antimicrobial susceptibility testing did not have an effect on empirical anti-infective therapy. In 22.4% of cases, patients were switched to a broader-spectrum antibiotic based on rapid automated antimicrobial susceptibility testing results. In 16.4% of cases, therapy could be de-escalated although the de-escalation occurred within a day in less than half of them.

The Enterobacterales isolated over the study period tended to be more resistant than the regional average: in 2019, the *Antibiotikaresistenz Überwachung Schweiz* (ANRESIS, Swiss Centre for Antibiotic Resistance) [20] recorded resistance to 3rd-generation cephalosporins in 10.8% of all *E. coli* and 9.8% of all *K. pneumoniae* as well as resistance to 4th-generation cephalosporins in 6.3% of all *E. coli* and

Table 3:
Effect of rapid automated antimicrobial susceptibility testing as a percentage of all Enterobacterales bacteraemias. Reasons are provided for “no effect” cases.

Effect of rapid automated antimicrobial susceptibility testing (n, %)	Reason	n (%)
No effect (71, 61.2)	Empirical therapy correct	39 (54.9)
	Circumstances do not allow de-escalation	14 (19.7)
	Start of palliative therapy or death	5 (7.0)
	Rapid automated antimicrobial susceptibility testing did not work	1 (1.4)
	Rapid automated antimicrobial susceptibility testing result within area of technical uncertainty	1 (1.4)
	Identification of pathogen warranted escalation*	2 (2.8)
	Unknown	9 (11.0)
Escalation (26, 22.4)		26 (100)
De-escalation (19, 16.4)		19 (100)

* This refers to the detection of *Enterobacter cloacae* in Matrix-Assisted Laser Desorption/Ionization-Time Of Flight spectrometry. Patients were switched to a substance active against AmpC beta-lactamase before susceptibility results were known.

7.7% of all *K. pneumoniae* in patients hospitalised in central and eastern Switzerland [21]. Notwithstanding the low numbers in our study that limit comparability, we saw a higher proportion of resistant bacteria. This higher prevalence of resistance may be due to the fact that the patient population is more complex in our tertiary care institution than in surrounding, smaller hospitals.

The low error rate is consistent with our previous publication [11] demonstrating that rapid automated antimicrobial susceptibility testing is a safe way to accelerate diagnostics. Rapid automated antimicrobial susceptibility testing accelerated therapy escalation in 22% of all cases, which is a relevant proportion of our patient population, especially considering that in the majority of cases empirical therapy was already correct. Although this project was not designed to draw statistical conclusions, we are convinced that rapid automated antimicrobial susceptibility testing will play a key role in the management of Gram-negative bacteraemia.

However, when therapy could be de-escalated, rapid automated antimicrobial susceptibility testing did not seem to accelerate antibiotic adjustment. Only in 8 of 19 cases was therapy de-escalated within 24 h and in 8 cases therapy was not de-escalated at all without any apparent reason. In fact, many studies have demonstrated that the best clinical outcomes are achieved when rapid diagnostics (source of infection, pathogen and/or antimicrobial susceptibility testing) are linked to stringent stewardship programmes [13, 22–26]. Therefore, a logical next step would be to design a compulsory antibiotic stewardship programme that seamlessly links diagnostics, infectious diseases consultants (and possibly other specialists such as pharmacologists) and treating clinicians while integrating rapid diagnostics into clinical decision-making. This was previously reported by Banjeree et al. [27] for a rapid PCR approach. We believe our approach to be more versatile as it relies on phenotypic resistance testing. Moreover, the most important drawback of phenotypic resistance, overnight incubation, was successfully eliminated.

Strengths and limitations

Our data are derived from real-world practice and therefore our findings about the advantages and limitations of this diagnostic tool are applicable to everyday practice. Rapid automated antimicrobial susceptibility testing has been shown to be performant in a hospital setting. Our study also demonstrates that the challenges of rapid automated antimicrobial susceptibility testing or microbiologic diagnostics in general do not currently reside in speed or technical issues but in clinical and microbiological workflows and therefore reveal possible next steps for strengthening its impact (i.e. implementation of antimicrobial stewardship).

The most important limitation of this study is the heterogeneous patient population with some patients being in a stable condition and having a rather simple problem (such as urinary tract infection and bacteraemia with pan-susceptible *E. coli*) and others having a severe systemic disease (such as burns) and multi-organ failure. This renders comparisons, especially concerning the impact of rapid automated antimicrobial susceptibility testing, difficult. Furthermore, to assess the impact of rapid automated antimicrobial susceptibility testing and to investigate hard clinical endpoints, a larger sample size plus a control group of patients would have been necessary. However, given that many unsuitable therapies could have been quickly adjusted, a control group was for us ethically unjustifiable, as we would have withheld potentially lifesaving information from this control group. A further limitation is the fact that not all positive blood cultures could be included (namely, those that became positive during the weekend or after 9:00 am), which unfortunately further reduced the sample size. The introduction of a night shift at the laboratory site was associated with additional costs. However, night shifts and weekends were not sufficiently staffed to fully exploit the potential of the method. In a future analysis, cost-effectiveness of rapid automated antimicrobial susceptibility testing will have to be addressed.

We also emphasise that there are technical differences between our protocol and the one published by EUCAST in 2019 [28] and 2020 [29] concerning rapid resistance testing directly from positive blood cultures. Briefly, we first measure bacterial density with flow cytometry in positive blood cultures, standardise the inoculum and streak the plates with an automated platform (WASPLab^{TM}) as published previously [11]. This allows for standardised results with the additional possibility of reading the plates after 6 h, 8 h and 18 h thus removing the necessity of parallel testing which is needed when performing the EUCAST method. EUCAST rapid automated antimicrobial susceptibility testing has become a well-established additional method of resistance testing with the main drawbacks of parallel testing and limited species and antibiotic breakpoint inclusion [28, 29]. Namely, EUCAST provides breakpoints for *Escherichia coli* and *Klebsiella pneumoniae* only, while we also applied breakpoints for other Enterobacterales. Further, these EUCAST breakpoints were published in November 2020 and therefore at the end of our study period. Since we were interested in the clinical implications of rapid automated antimicrobial susceptibility testing, we have decided not to retrospectively assess rapid automated antimicrobial susceptibility testing results according to the new EUCAST method. However, we still believe that we have developed a highly standardised and reliable method of rapid automated antimicrobial susceptibility testing covering all Enterobacterales with at least comparable results. We acknowledge that further investigation is still required, including multiple testing sites and direct comparison to EUCAST rapid automated antimicrobial susceptibility testing.

Conclusions

Rapid automated antimicrobial susceptibility testing may be an important asset in clinical decision-making especially in our patient population that has often been exposed to multiple antibiotics and a hospital environment and that is prone to infection by resistant pathogens. However, without a strong antibiotic stewardship programme, the full potential will not be realised.

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Potential competing interests
All authors have completed and submitted the International Committee of Medical Journal Editors form for disclosure of potential conflicts of interest. No potential conflict of interest was disclosed.

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