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Original article

Impact of MALDI-TOF-MS-based identification directly from positive blood cultures on patient management: a controlled clinical trial

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ABSTRACT

Objectives: Rapid identification of pathogens directly from positive blood cultures (BC) in combination with an antimicrobial stewardship programme (ASP) is associated with improved antibiotic treatment and outcomes, but the effect of each individual intervention is less clear. The current study investigated the impact of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) versus conventional identification on antibiotic management in a setting with a well-established ASP and low resistance rates.

Methods: In this single-centre open label, controlled clinical trial 425 patients with positive BCs were allocated by weekday during a 1-year period to either MALDI-TOF directly from positive BCs or conventional processing. ASP was identical throughout the study period. The primary outcome was duration of intravenous antimicrobial therapy and was analysed in an intention-to-treat approach.

Results: In all, 368 patients were analysed (MALDI-TOF $n = 168$; conventional $n = 200$) with similar baseline characteristics. Mean duration of intravenous antimicrobial therapy (12.9 versus 13.2 days, p 0.9) and length of stay (16.1 versus 17.9 days, p 0.3) were comparable. In the clinically significant bloodstream infection subgroup ($n = 242$) mean time from Gram-stain to active treatment was significantly shorter (3.7 versus 6.7 h, p 0.003). Admission to the intensive care unit after bloodstream infection onset was less frequent in the MALDI-TOF group (23.1 versus 37.2%, p 0.02).

Conclusions: Rapid identification of contaminated BCs ($n = 126$) resulted in a shorter duration of intravenous antimicrobial therapy (mean 4.8 versus 7.5 days, p 0.04). Rapid identification using MALDI-TOF directly from positive BCs did not impact on duration of intravenous antimicrobial therapy, but provided fast and reliable microbiological results and may improve treatment quality in the setting of an established ASP. **M. Osthoff, CMI 2017;23:78**

Introduction

Bloodstream infections are a major cause of sepsis, affecting approximately 1.2 million people in Europe each year [1]. Any delay in appropriate antibiotic treatment is a major risk factor for sepsis-related mortality [2]. Therefore, initial empiric therapy covers a broad range of bacteria. In sepsis, blood cultures (BCs) are an important part of the diagnostic process [3], although <10% of obtained BCs become positive and a considerable number are contaminated with skin flora [4]. Identification and susceptibility testing of positive BCs using conventional methods may require up to 72 h after sample collection [5]. In addition, initial Gram staining does not allow a clear-cut decision between contamination and a clinically significant bloodstream infection (BSI). Consequently, broad-spectrum empiric treatment is often continued until the final identification and/or antibiotic susceptibilities are available, although clinical outcomes are at least equal with targeted

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antibiotic therapy compared with continuation of broad-spectrum therapy [6]. Moreover, targeted therapy is associated with significant reductions in antibiotic-related costs and episodes of nosocomial multi-drug-resistant infection [7].

Recently, rapid identification of BSI pathogens has emerged as a valuable tool to reduce unnecessarily broad and prolonged antibiotic treatment in the context of emerging antibiotic resistance and *Clostridium difficile* infections, but its impact on morbidity and mortality is still controversial [5,8–10]. Previous studies exploring the impact of rapid identification of pathogens directly from positive BCs have demonstrated improvements in antibiotic treatment and patient outcomes only in combination with an antimicrobial stewardship programme (ASP) and/or have used a before–after study design [8,9,11–14]. In addition, data on its impact in low-resistance settings are lacking.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) has revolutionized the routine diagnostic approach in microbiology laboratories and allows rapid identification of bacterial species [15], which is predominantly based on the profiling of bacterial ribosomal proteins. MALDI-TOF is typically used for direct identification of single colonies from solid cultures. Recent studies have reported successful and rapid identification of bacterial species directly from positive blood cultures [16–19].

Given the lack of prospective, controlled studies investigating the effect of rapid identification by MALDI-TOF and ASP separately and the expansions of ASPs around the world, the present pragmatic, controlled single-centre clinical study aimed to compare the impact of MALDI-TOF-based identification versus conventional identification on antibiotic management in a setting with a well-established ASP and low resistance rates. We hypothesized that rapid MALDI-TOF-based identification on top of a well-established ASP would significantly improve antibiotic management compared with an ASP provided with conventional identification, even in a hospital setting with a low prevalence of resistant organisms.

Materials and methods

Patients and study design

The University Hospital of Basel is a tertiary hospital with 670 beds, two intensive care units (ICUs) and around 33 000 inpatients per year. The prevalence of multidrug-resistant (MDR) organisms is low (<2% of *Staphylococcus aureus* BSI isolates are methicillin-resistant (MRSA) [20], and BSIs due to vancomycin-resistant enterococci, MDR *Enterobacteriaceae* or MDR *Pseudomonas aeruginosa* are exceptionally rare ($\leq$5 episodes/year). We performed a prospective, non-blinded, controlled clinical trial from April 2014 to April 2015. A total of 425 patients with positive BCs (BactAlert FA/FN plus, bioMérieux, Marcy l'Etoile, France) were assigned by weekday into two arms: (i) rapid identification by MALDI-TOF (Microflex; Bruker, Bremen, Germany) directly from positive BCs using the Sepsityper Kit (Bruker) versus (ii) conventional processing. An ASP was already established in our centre before the study. The study was approved by the Ethics Committee of Northwest and Central Switzerland (EKNZ 2014–176) with a waiver of informed consent.

Inclusion criteria

All positive BCs from patients $\geq$ 18 years old which became positive between 18.00 and 10.00 on the subsequent day (i.e. after hours and overnight) Sunday to Friday were included. This timeframe was chosen to reduce bias related to different laboratory resources allocated to the two identification processes and related to the daily routine of the ASP. Initially, we established MALDI-TOF from BCs into the routine diagnostics only on Tuesday and Thursday (April to August 2014) and then switched to Monday, Wednesday and Friday (September 2014 to April 2015). Hence, patients with positive BCs on Tuesday and Thursday were allocated to the MALDI-TOF group from April to August 2014 and to the conventional identification group from September 2014 to April 2015, whereas patients with positive BC on Monday, Wednesday and Friday were allocated to the conventional identification group during the first period and to the MALDI-TOF group during the second period. MALDI-TOF from included BCs was performed once per day at 10.00, i.e. included BCs becoming positive between 18.00 and 10.00 were batched and analysed at 10.00. Conventional identification including Gram staining is routinely performed every day during working hours (Monday–Friday 08.00 to 18.00), i.e. those BCs turning positive after 18.00 are processed at 08.00 on the subsequent day. This allowed comparison of the diagnostic approaches without introducing any additional significant delay for the two approaches compared with the standard of care in our hospital. Positive BC results are reported to the treating team immediately after results from Gram staining are available. In addition, the ASP team reviews all positive BCs once daily and provides additional immediate feedback to the treating team (see below). Patients could be included multiple times if BCs became positive more than 5 days apart and were related to a different infective episode. However, only the first episode was analysed for clinical end points.

Although the mode of identification (rapid versus conventional) was not communicated to the treating unit, full blinding of the treating team (and the ASP) to the intervention was not feasible because physicians (in particular ASP physicians) were able to guess the intervention as a consequence of a much faster species identification after BC turned positive. During the study period, physicians were not able to ask for changes of the algorithm.

Exclusion criteria

Individuals who were transferred to another hospital or who died before positive BCs could be reviewed by the ASP; individuals whose antibiotic treatment was withdrawn; and outpatients whose clinical details could not be verified were excluded from the study. Attending physicians responsible for the patients did not prevent inclusion in the trial in a single case.

Study end points

The primary study end point was duration of intravenous antimicrobial therapy in patients with clinically significant bacteraemia. The power calculation suggested that in each diagnostic group 100 patients with clinically significant bacteraemia have to be recruited to show a significant 2-day reduction ($p < 0.05$) in antibiotic treatment duration with a power of 80% (standard deviation (SD) of 5). Based on the local epidemiology and the rate of contaminated BCs, we selected a 1-year period to accomplish the required sample size. Secondary study end points included time to Gram stain, identification and resistance testing, active, definitive and optimal antibiotic treatment within 48 h after collection of BCs, quality of antimicrobial stewardship team intervention, in-hospital length of stay, and 30-day all-cause mortality. Exploratory analyses based on identified organisms were planned ahead of the study. Post-hoc analyses included analysis of the time from reporting of Gram stain to active treatment and analyses of the primary and end points in the whole cohort (including contaminated blood cultures). All analyses were performed on an intention-to-treat approach. For the analysis of clinical end points, only the first BSI episode was considered. Primary and secondary end points were calculated from the time of BC collection if not stated otherwise. Data on co-

morbidities and outcome were extracted retrospectively from chart review, whereas ASP recommendations were recorded prospectively. Patients were followed for 30 days or until death.

Antimicrobial stewardship programme

Advice on ASP, including same-day audit and feedback for all positive BCs, is provided by the Infectious Diseases Service and was unchanged during the study period. All positive BCs are reviewed once per day, and formal advice is provided to the treating unit every day until final identification and susceptibility results are available. During the study period, results of Gram staining (of all included BCs) and MALDI-TOF identification (only intervention group) were available at the first audit (at approximately 11.00), and advice was communicated to the treating physician immediately by telephone followed by a formal written consultation note including full assessment of the patient if deemed necessary (e.g. in the case of *S. aureus* BSI) or requested by the treating unit. In summary, results from Gram staining were reported to the treating unit at 08.30 for the included BCs becoming positive overnight and as soon as possible for BCs becoming positive until 10.00 in both groups, and results from Gram staining and MALDI-TOF identification were reported to the ASP at approximately 11.00.

Definitions

Active therapy: active antimicrobial therapy according to *in-vitro* susceptibility testing.

Streamlining: reduction of the antibiotic spectrum including narrowing the coverage and/or discontinuation of unnecessary antibiotics (see Supplementary material, Table S1 for further explanations).

Optimal therapy [12]: optimal antibiotic treatment including escalation or de-escalation, switch to oral treatment, need to cover polymicrobial/concomitant infections, discontinuation of antibiotics in case of unnecessary coverage and taking into account allergies.

Definitive intravenous therapy: active antibiotic therapy for at least 3 days after BCs became positive (as opposed to empirical treatment).

Quality of antibiotic therapy was determined retrospectively by two Infectious Diseases specialists blinded to the method of identification.

Blood cultures were determined as contaminated according to the judgement of the ASP after consideration of clinical and microbiological information including number of positive blood cultures and identified pathogen (e.g. only one BC out of two or more positive for organisms commonly associated with contamination such as coagulase-negative staphylococci (CNS) or *Propionibacterium acnes*), time to positivity, results from repeat BCs, presence of prosthetic material and focus of infection.

In contrast 'clinically significant bacteraemia' was defined as BSI and excluded cases with only contaminated blood cultures.

MALDI-TOF-based identification

After initial Gram staining, the SepsiTyper kit was used for identification directly from BCs by MALDI-TOF as previously described [16]. We used a MALDI-TOF Microflex system from Bruker Daltonics (Faellanden, Switzerland). The database was the MALDI Biotyper 3.1 software at default settings for identification. For the purpose of this study, we have lowered the MALDI-TOF score to >1.7 as being valid for identification on the species level [21]. As a diagnostic backup, all samples in the MALDI-TOF diagnostic group were further identified by conventional methods.

Conventional identification

We used our routine protocol to identify positive BCs by conventional methods as described previously [22], which included MALDI-TOF-based identification of colonies grown on subcultures. In case of a mismatch between MALDI-TOF and conventional identification, we had planned to use 16S ribosomal RNA gene sequencing. However, this was not required in a single case.

Resistance testing was performed with the VITEK2 system (bioMérieux), and in some selected cases using an Etest (bioMérieux). EUCAST guidelines (v6, January 2016) were used for interpretation in most instances.

Statistical analysis

Categorical variables were analysed using a chi-square test or Fisher's exact test where appropriate. Continuous non-normally distributed data were analysed using a Mann–Whitney *U* test. In the case of a normally distributed data set, a Student *t*-test was used. Bivariate comparison of Kaplan–Meier curves (log-rank test) was used to compare the impact of the different diagnostic approaches over time. All p values are two-sided with a 95% level of significance. We managed and analysed all data with the use of SPSS Statistics software (version 22.0; IBM, Chicago, IL, USA) and GraphPad Prism (version 4.0; GraphPad, La Jolla, CA, USA).

Results

Patient characteristics

From April 2014 to April 2015 a total of 43 330 aerobic and anaerobic BC flasks were submitted to the microbiology laboratory, of which 3221 (7.4%) became positive, representing 1027 patients with distinct infective episodes. After exclusion of patients whose BC flagged positive outside the specified time period, 425 (41.4%) patients were allocated to the diagnostic groups. After exclusion of 57 (13.4%) patients, 368 patients with positive BCs were analysed, including 242 episodes of clinically significant BSI and 126 episodes of contaminated BCs (Fig. 1). Follow-up was complete in all included patients. Overall, mean age was 65.6 (SD 17.1) and 220/368 (59.8%) patients were male. Clinical characteristics in patients with BSI were similarly distributed between the diagnostic groups (Table 1). MALDI-TOF-based identification was successful in 150/168 (89%) patients with BSI, and in agreement with conventional identification in all cases.

Impact of rapid MALDI-TOF-based identification in the entire cohort

Mean time to Gram staining and susceptibility reporting were similar in the MALDI-TOF and the conventional identification groups. Mean time to bacterial species identification was significantly shorter in the MALDI-TOF group (33.2 (SD 22.3) versus 59.1 (SD 34.0) hours, p <0.0001) (Table 1 and Fig. 2a).

Overall, *Escherichia coli* (72/368, 19.6%), *Klebsiella pneumoniae* (26/368, 7.1%) and *S. aureus* (25/368, 6.8%; no case of MRSA) were identified most commonly in both groups (Table 2). β-Lactam/β-lactamase inhibitor combinations and third-/fourth-generation cephalosporins were the most frequently prescribed empirical regimens in both groups (p 0.7).

Duration of intravenous antimicrobial therapy, total antibiotic treatment duration, 30-day all-cause mortality, and length of hospital stay were comparable (Table 3). However, we observed a lower rate of ICU admission (27.3% versus 37.3%, p 0.05) in the MALDI-TOF group.

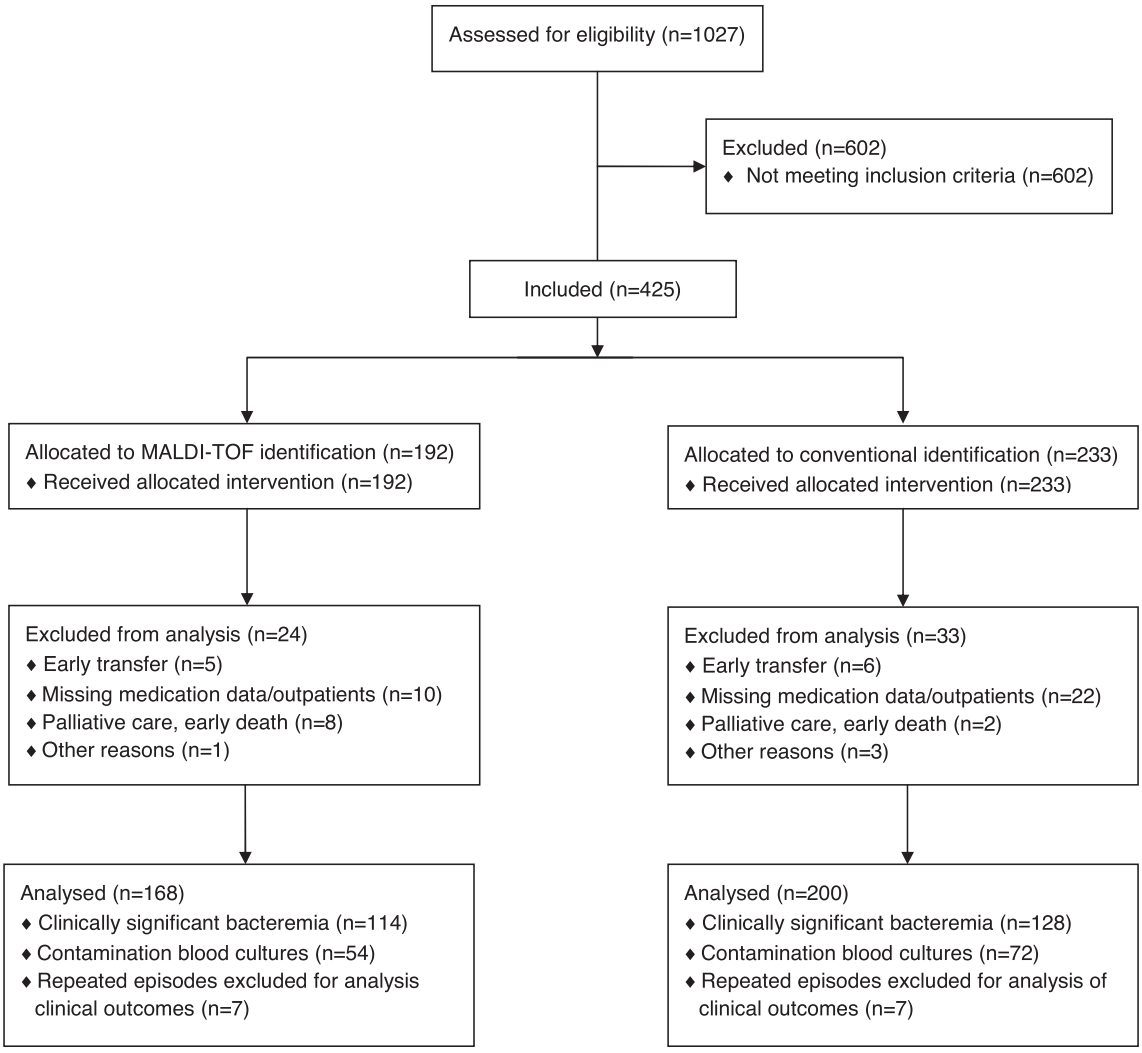

Fig. 1. Enrolment of participants. Early death and transfer were defined as event occurring before blood culture results could be reviewed by the antimicrobial stewardship team. Abbreviation: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Rapid MALDI-TOF-based identification in patients with clinically significant bloodstream infection

MALDI-TOF-based identification was successful in 106/114 (93.0%) patients. Overall, times from BC collection to active, optimal, definitive treatment and streamlining were similar (Fig. 2b). However, time from reporting of Gram stain to active treatment was significantly shorter (mean 3.7 (SD 8.4) versus 6.7 (SD 19.3) hours, p 0.003), whereas active treatment was administered at a non-significantly higher rate within 48 h after BC collection (95.6% versus 89.8%, p 0.09) in the MALDI-TOF group. In addition, definitive treatment was more frequently established within 48 h (76.3% versus 61.7%, p 0.02).

Duration of intravenous antimicrobial therapy, total antibiotic treatment duration and length of hospital stay were comparable (Table 1). Frequency of ICU admission (23.1% versus 37.2%, p 0.02) and 30-day all-cause mortality (9.6 versus 16.4%, p 0.06; hazard ratio 0.5, 95% CI 0.3–1.1, p 0.08) were numerically lower in the MALDI-TOF group.

As results from resistance testing are a major determinant for antibiotic selection and streamlining in the two most commonly encountered pathogens *E. coli* and *K. pneumoniae*, we separately analysed a subgroup of clinically significant patients with BSI (100/242, 41.3%) including Ampicillin C-producing and non-fermenting organisms, *S. aureus, Enterococcus* and *Streptococcus* spp. In this subgroup, rapid identification with MALDI-TOF not only resulted in improved active treatment (100% versus 87.9%, p 0.02) but also in a non-significantly higher number of patients on optimal treatment within 48 h (57% versus 38%, p 0.06), whereas it had no impact on *E. coli* or *Klebsiella* spp. bacteraemias (98/242) (Fig. 3). Quantity and type of ASP recommendations at the time of the first audit were similar in both groups (data not shown) as was their acceptance rate (96.7% and 95.0%).

Rapid MALDI-TOF-based identification in patients with contaminated blood cultures

Blood cultures from 126/425 (29.6%) patients were judged as contaminations (Table 1). CNS and other Gram-positive bacteria were isolated most frequently in both groups (78 versus 75% (CNS) and 20.4% versus 18.1% (Gram-positive bacteria), respectively). Again, median time to Gram staining and susceptibility reporting were comparable (Table 1). Rapid identification failed in 18.5%. Mean time to identification was significantly shorter in the MALDI-TOF group (46.3 (SD 30.9) versus 76.1 (SD 49.4) hours, p <0.001). At the time of BC collection, 32/126 (25.4%) patients were already on

Table 1
Clinical characteristics of the entire cohort and separately in patients with clinically significant bacteraemia and with contaminated blood cultures according to the mode of identification

Variable	Entire cohort ($n = 368$)			Clinically significant bacteraemia ($n = 242$)			Contaminated blood cultures ($n = 126$)		
	MALDI-TOF ($n = 168$)	Control ($n = 200$)	p value	MALDI-TOF ($n = 114$)	Control ($n = 128$)	p value	MALDI-TOF ($n = 54$)	Control ($n = 72$)	p value
Age in years, mean (SD)	64.6 (17.8)	65.8 (16.4)	0.5	64.1 (17.9)	66.4 (15.9)	0.3	65.5 (17.5)	64.8 (17.1)	0.8
Male sex, *n* (%)	92 (54.8)	128 (64.0)	0.1	57 (50.0)	79 (61.7)	0.07	35 (64.8)	49 (68.1)	0.7
Co-morbidities, *n* (%)									
Chronic lung disease	23 (13.7)	38 (19.0)	0.2	17 (14.9)	21 (16.4)	0.8	6 (11.1)	17 (23.6)	0.1
Cardiovascular disease	108 (64.3)	120 (60.0)	0.4	72 (63.2)	79 (61.7)	0.8	36 (66.7)	41 (56.9)	0.3
Diabetes mellitus	47 (28.0)	48 (24.0)	0.4	31 (27.2)	26 (20.3)	0.2	16 (29.6)	22 (30.6)	0.9
Chronic kidney disease	44 (26.2)	46 (23.0)	0.5	30 (26.3)	29 (22.7)	0.6	14 (25.9)	17 (23.6)	0.8
Stem cell and organ transplantation	22 (13.1)	15 (7.5)	0.1	15 (13.2)	13 (10.2)	0.5	7 (13.0)	2 (2.8)	**0.04**
Immunosuppression[a]	27 (16.1)	23 (11.5)	0.2	19 (16.7)	21 (16.4)	1[e]	8 (14.8)	2 (2.8)	**0.02**
Neutropenia[b]	16 (9.5)	15 (7.5)	0.5	10 (8.8)	13 (10.2)	0.7	6 (11.1)	2 (2.8)	0.07
Infection characteristics									
Hospital-acquired infection, *n* (%)	44 (26.2)	51 (25.5)	0.9	44 (38.6)	51 (39.8)	0.8	n/a	n/a	
Focus of BSI, *n* (%)			0.4			0.4	n/a	n/a	
Catheter and/or foreign material	18 (10.7)	19 (9.5)		18 (15.8)	19 (14.8)				
Intra-abdominal infection	20 (11.9)	30 (50.0)		20 (17.5)	30 (23.4)				
Respiratory tract infection	5 (3.0)	14 (7.0)		5 (4.4)	14 (10.9)				
Skin and soft tissue infection	15 (8.9)	14 (7.0)		15 (13.2)	14 (10.9)				
Urinary tract infection	34 (20.2)	30 (15.0)		34 (29.8)	30 (23.4)				
Other[c]	22 (13.1)	21 (10.5)		22 (19.3)	21 (16.4)				
Empiric antibiotic treatment, *n* (%)			0.7			0.9			0.2
Amoxicillin-clavulanic acid	27 (16.1)	31 (15.5)		17 (14.9)	13 (10.2)		10 (18.5)	18 (25.0)	
Ceftriaxone	39 (23.2)	49 (24.5)		31 (27.2)	37 (28.9)		8 (14.8)	12 (16.7)	
Piperacillin-tazobactam	40 (23.8)	54 (27.0)		30 (26.3)	40 (31.3)		10 (18.5)	14 (19.4)	
Cefepime	6 (3.5)	7 (3.5)		6 (5.3)	7 (5.5)		0 (0)	0 (0)	
Carbapenems	18 (10.7)	23 (11.5)		12 (10.5)	16 (12.5)		6 (11.1)	7 (9.7)	
Other[d]	38 (22.6)	36 (18.0)		18 (15.8)	15 (11.7)		20 (37.0)	21 (29.2)	

Abbreviations: BSI, bloodstream infection; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; n/a, not applicable; SD, standard deviation.

[a] Immunosuppression was defined as treatment with immunosuppressive medication including steroids at a dose equivalent to at least 0.5 mg/kg/day prednisolone, monoclonal antibodies, calcineurin inhibitors, mycophenolate mofetil and methotrexate, and solid or haematological tumours treated in the last 30 days.

[b] Neutropenia was defined as absolute neutrophil count <500 /μL.

[c] "Other" focus of BSI includes endocarditis and unknown/primary source.

[d] Includes no administration of empiric treatment.

[e] Fisher's exact test.

antibiotic treatment. After report of a positive BC, 12/126 (9.5%) patients were started on empiric antibiotic therapy, and a treatment change was initiated in 4% of patients. Mean time to determination of BCs as contaminated was comparable in both groups (49.5 (SD 32.2) versus 57.7 (SD 38.6) hours, p 0.2). A stewardship recommendation was given in 14.8% (MALDI-TOF) versus 18.1% (control group) (p 0.63) of patients on the day BCs became positive.

Duration of antimicrobial therapy in the group of patients with antibiotic treatment already established before BC collection was comparable (mean 12.8 (SD 10.1) versus 19.02 (SD 13.3) days, p 0.1). However, when antibiotic treatment was initiated only after the collection of BCs, duration of antibiotic treatment was significantly shorter in the MALDI-TOF group (mean 7.1 (SD 4.6) versus 10.1 (SD 6.6) days, p 0.04) as was duration of intravenous treatment (mean 4.8 (SD 3.4) versus 7.5 (SD 6.4) days, p 0.04). Vancomycin or Daptomycin was initiated infrequently after BCs became positive (ten patients) with comparable duration (data not shown).

Discussion

In the present controlled clinical trial, rapid identification of organisms by MALDI-TOF directly from positive BCs provided fast and reliable microbiological results, improved treatment quality in particular in BSIs not caused by *E. coli* and *Klebsiella* spp. and resulted in reduced antibiotic treatment duration in contaminated BCs. Rapid identification of causative pathogens in patients with BSIs has the potential to improve patients' outcome by optimizing antibiotic treatment. However, differences in mortality were only observed if rapid tests were combined with an ASP [23]. Previous studies were limited by design [9,11–14,18,24–27] and more importantly by only comparing rapid identification in combination with ASP versus conventional identification without ASP [8,11–14,24]. Our trial is notable because it assessed the effect of rapid identification by MALDI-TOF in a low-resistance setting on top of an established ASP in a prospective, controlled clinical study design.

Several reasons might account for the limited impact of rapid identification in our setting compared with previous studies as observed for duration of intravenous treatment, length of stay and time to optimal therapy. First, antibiotic resistance rates are low in our hospital, and hence fewer patients will receive unnecessary empiric combination therapies (covering MDR Gram-positive or Gram-negative pathogens) compared with institutions with a high prevalence of antibiotic resistance. As a result, opportunities to tailor treatment are less frequent.

Second, whereas in most institutions without an ASP clinicians are notified immediately following positive Gram stain results but not after availability of identification/susceptibility results, our ASP provides guidance on several occasions until final results are released. In our control group, the proportion of patients on active treatment rose from 62.5% before availability of Gram staining to 90.6% before conventional identification, and mean time to active treatment in our control group was already shorter (18.4 h versus 20.4 h) compared with the 'rapid' identification group in a recent study [12], which highlights the limited additional impact of rapid diagnostics on active treatment in a setting with an experienced

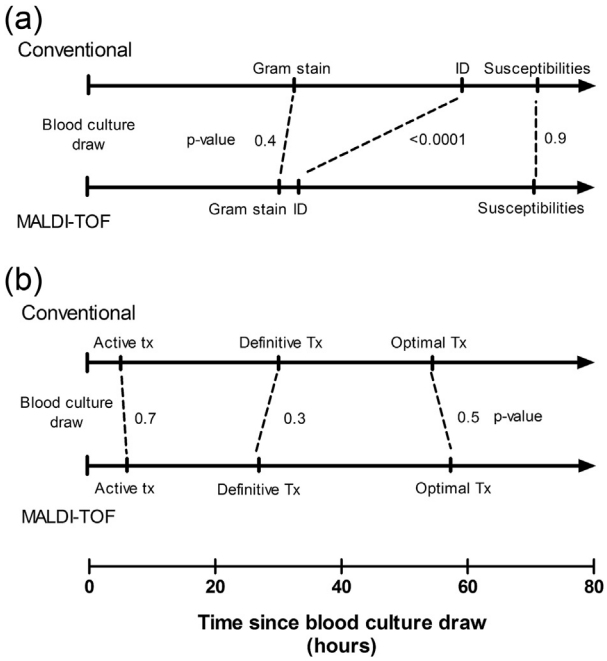

Fig. 2. Timeline (mean) of (a) microbiological procedures and (b) antibiotic management starting from blood culture collection according to study group (entire cohort). Abbreviation: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Table 2
Distribution of isolated organisms in the entire cohort according to the mode of identification

Variable	MALDI-TOF ($n = 168$)	Control ($n = 200$)	p value
Gram-positive organisms, *n* (%)			
Staphylococcus aureus[a]	14 (8.3)	11 (5.5)	0.3
Coagulase-negative *Staphylococcus*[b]	50 (29.8)	61 (30.5)	0.9
Streptococcus spp.	8 (4.8)	18 (9.0)	0.2
Enterococcus faecalis	3 (1.8)	3 (1.5)	0.7
Enterococcus faecium[a]	4 (2.4)	10 (5.0)	0.3
Other Gram-positive organisms	12 (7.1)	15 (7.5)	1.0[d]
Gram-negative organisms, *n* (%)			
Escherichia coli	38 (22.6)	34 (17.0)	0.2
Klebsiella spp.	12 (7.1)	14 (7.0)	1.0[d]
Serratia, *Citrobacter*, *Enterobacter* spp.	7 (4.2)	11 (5.5)	0.6
Pseudomonas aeruginosa	4 (2,4)	2 (1.0)	0.4
Other Gram-negative organisms	5 (3.0)	9 (4.5)	0.6
Yeasts, *n* (%)	3 (1.8)	1 (0.5)	0.3
Polymicrobial culture,[c] *n* (%)	8 (4.8)	11 (5.5)	0.8

Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
[a] Methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecium* were not detected.
[b] Eight (7.0%) and 7 (5.5%) episodes were confirmed as clinically significant bacteraemias in the MALDI-TOF and control groups, respectively.
[c] MALDI-TOF identified a single organism in 7/8 patients and was not successful in 1/8 patient.
[d] Fisher's exact test.

ASP. Conversely, rapid identification before BCs become positive (i.e. directly from whole blood) may have the potential to significantly shorten the time to active treatment in a relevant proportion of patients [28], albeit at higher costs.

Third, clinicians may be reluctant to change antibiotic treatment in the first 48 h before results from resistance testing are available because they may judge continuation of broad-spectrum antibiotic treatment for an additional 24 h as not harmful or even beneficial [9]. In particular, this may hold true for Gram-negative organisms with less predictable resistance patterns. Reluctance of clinicians, including infectious diseases physicians, to change antibiotic management as a result of a newly introduced test may explain our observation of similar duration of intravenous antibiotic therapy and similar utilization of broad-spectrum antibiotics.

Last, despite including many BCs that turned positive overnight but were only processed in the morning, mean time to identification in our control group was already shorter compared with the 'rapid' identification group in a recent study (49.6 versus 55.9 h) [12]. Our results are in agreement with Buss *et al.* [25], who did not observe a reduced time to active treatment or de-escalation in 49 patients compared with a historical control group in a low-resistance setting with an active ASP.

We were able to identify a subgroup of BSI organisms whose rapid identification by MALDI-TOF may improve antibiotic treatment quality in a setting with an active ASP and a low resistance rate. Within 48 h, active therapy was administered more frequently in patients with BSIs caused by Ampicillin C-producing, non-fermenting organisms, *S. aureus*, *Streptococcus* and *Enterococcus* spp. These organisms display a predictable resistance pattern in our hospital setting (in particular, multi-drug resistance is observed rarely), and hence clinicians may be more inclined to de-escalate (in the case of *S. aureus*, *Streptococcus* and *Enterococcus* spp., as incidence of MRSA or vancomycin-resistant *Enterococcus* BSIs is very low) or escalate antibiotic treatment (in the case of Ampicillin C-producing and non-fermenting organisms) even before results from resistance testing are available. In contrast, rates of ceftriaxone-resistant *E. coli* and *Klebsiella* spp. are 9% and 8%, respectively (unpublished data), and hence modification of therapy only as a result of identification is rare.

Differential impact of rapid MALDI-TOF-based diagnosis in BSIs according to the isolated pathogen has been reported infrequently [11,14,18]. For example, Huang *et al.* [12] noted that benefits of rapid identification and ASP were most pronounced in Gram-negative BSIs. Unfortunately, these studies do not identify the separate effects of ASP and rapid identification.

In our prospective setting, we observed a lower rate of ICU admission after BSI onset and a non-significant lower 30-day all-cause mortality in patients with clinically significant BSIs, which is in agreement with data from Huang *et al.* [12] and Perez *et al.* [14] after combined introduction of rapid identification and ASP intervention. Although time to active treatment has been directly associated with mortality in observational studies [2], many other rapid diagnostic studies failed to report a mortality benefit, which may be related to having been underpowered [8,11,13,25].

Regarding contaminated BCs, overall and intravenous antibiotic treatment was significantly shorter after rapid identification of potential contaminants. Less than 10% of patients were initiated on antibiotic treatment after BCs became positive, and most patients with contaminated BCs were continued on antibiotic treatment because they suffered from a serious infection. Although treatment duration should have been determined by the underlying infection independent of contaminated BCs, positive BCs may have influenced clinicians' decisions regarding treatment duration or switch to oral treatment even if a contamination is likely [29]. This is particular the case for CNS, which are the most common contaminants but also a very frequent cause of serious BSIs [30]. Rapid identification might lead to earlier follow-up investigations (such as repeat BCs) in order to determine the significance of BCs positive for CNS. Our results are in line with two small studies [11,31] that demonstrated decreased total antibiotic exposure after the introduction of rapid identification of CNS in combination with an ASP. Similarly, Nagel *et al.* [32] observed a reduced duration of inappropriate antibiotic administration of vancomycin or daptomycin after rapid identification of BCs contaminated by CNS. We did not

Table 3
Diagnostic characteristics and outcome of the entire cohort and separately in patients with clinically significant bacteraemia and with contaminated blood cultures according to the mode of identification

Variable	Entire cohort ($n = 368$)			Clinically significant bacteraemia ($n = 242$)			Contaminated blood cultures ($n = 126$)		
	MALDI-TOF ($n = 168$)	Control ($n = 200$)	p value	MALDI-TOF ($n = 114$)	Control ($n = 128$)	p value	MALDI-TOF ($n = 54$)	Control ($n = 72$)	p value
Diagnostic results									
Time to reporting of Gram stain, mean (SD) hours	30.1 (22.7)	32.5 (27.8)	0.4	22.8 (10.1)	22.9 (11.1)	0.9	45.7 (32.0)	49.4 (38.5)	0.6
Time to species identification, mean (SD) hours	33.2 (22.3)	59.1 (34.0)	<0.0001	28.2 (15.3)	49.7 (14.2)	<0.001	46.3 (30.9)	76.1 (49.4)	<0.001
Time to susceptibility reporting, mean (SD) hours	70.5 (32.5)	71.1 (28.9)	0.9	61.3 (23.3)	82.2 (23.7)	0.8	92.5 (40.4)	89.2 (30.0)	0.6
Outcome[a]									
30-day all-cause mortality, *n* (%)	17 (10.6)	24 (12.4)	0.6	9 (8.3)	20 (16.5)	0.06	8 (15.1)	4 (5.6)	0.1
In-hospital all-cause mortality, *n* (%)	15 (9.3)	18 (9.3)	1[d]	8 (7.4)	15 (12.4)	0.2	7 (13.2)	3 (4.2)	0.1
Admission to ICU after BSI onset, *n* (%)	44 (27.3)	72 (37.3)	0.05	25 (23.1)	45 (37.2)	0.02	19 (35.8)	27 (37.5)	0.9
Length of stay after BSI onset,[b] mean (SD) days	16.1 (15.5)	17.9 (17.0)	0.3	16.2 (15.6)	19.0 (18.6)	0.2	15.8 (15.5)	15.9 (13.9)	1[d]
Duration of IV antimicrobial therapy, mean (SD) days	12.9 (12.3)	13.2 (11.5)	0.9	13.1 (12.3)	13.7 (11.6)	0.7	4.8 (3.4)[c]	7.5 (6.4)[c]	0.04
Duration of total antimicrobial therapy, mean (SD) days	15.9 (15.3)	16.4 (14.8)	0.8	18.5 (16.8)	18.7 (16.4)	0.9	7.1 (4.6)[c]	10.1 (6.6)[c]	0.04

Abbreviations: BSI, bloodstream infection; ICU, intensive care unit; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SD, standard deviation.
[a] For outcome, only the first episode was analysed ($n = 161$ and $n = 193$ in the MALDI-TOF and control groups, respectively).
[b] Only survivors were included in the analysis of length of stay.
[c] Analyses only include patients in whom antibiotic treatment was initiated only after collection of the blood culture (28/53 and 47/72 in the MALDI-TOF and the conventional groups, respectively).
[d] Fisher's exact test.

observe any difference in the duration of vancomycin or daptomycin treatment in patients with contaminations. This is probably explained by the fact that vancomycin or daptomycin is not recommended as empirical therapy in our in-house guidelines, given that <2% of all BSIs are caused by MRSA [20].

Limitations of this study include the single-centre design and sample size that precluded us from detecting smaller differences in clinical outcomes. Second, results may not be generalizable, but depend on local patient populations, stewardship practices and resistance rates. Third, we only captured 41% of all patients with positive BCs, compromising generalizability. Apart from the financial and logistic aspects, our prospective controlled clinical trial design was chosen to reduce several kinds of bias, e.g. bias related to working hours of the microbiology laboratory or the ASP service, bias related to changes in the standard of care or bias related to shifts in resistance patterns or the seasonal variation of pathogens, and to identify the exact impact of rapid identification in our setting. However, due to the lack of randomization, residual confounding, e.g. in baseline characteristics or choice of antimicrobial treatment, cannot be excluded. Blinding of clinicians caring for the patients was not possible, but determination of quality of antibiotic treatment was conducted in a blinded fashion.

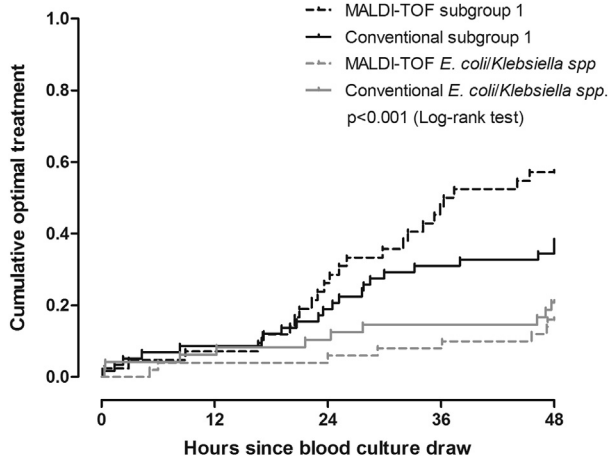

Fig. 3. Analysis of time to optimal treatment investigated at 48 h after blood culture collection according to study group and according to confirmed microorganism: *Escherichia coli/Klebsiella* spp. versus a combination of Ampicillin C-producing and non-fermenting organisms, *Staphylococcus aureus* and *Enterococcus/Streptococcus* spp. (subgroup 1). Kaplan–Meier estimate. Abbreviation: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Strengths of our study include the prospective and controlled design with blinded outcome adjudication. To the best of our knowledge, this is the first prospective, controlled clinical trial to compare diagnostic approaches on top of a well-established ASP. Previous studies have suggested that most improvements in antibiotic treatment are probably a consequence of an active ASP rather than rapid identification [12,23], and studies such as ours are the only way to address the influence of rapid identification separately.

In our opinion, rapid identification and antibiotic susceptibility testing methods will without doubt impact on the clinical management of patients in the near future. The benefits of rapid diagnosis observed in our study have to be weighed against costs. Previous studies have suggested cost savings by using MALDI-TOF-based identification of BSIs, which were mainly related to a reduced length of study [12,14]. Our study was not designed to analyse the impact of rapid identification on hospital costs, but a reduction in ICU admission rates as observed in the current study would certainly translate into important cost savings. In this regard, MALDI-TOF-based identification directly from BC is certainly more labour intensive (in particular the extraction step) but with lower reagent costs compared with fully automated multiplex PCR systems, which offer additional information regarding susceptibilities. Alternatively, MALDI-TOF-based identification after short incubation (2–5 h) of blood cultures may provide fast, reliable results at a low cost [33]. Results from our study may aid clinicians in settings with limited resources to judge the benefits of implementing MALDI-TOF-based rapid identification once (or twice) daily only, and even to judge the benefits of other rapid tests (e.g. MALDI-TOF from briefly incubated solid medium cultures), as results from our study may to some extent be generalizable to other modes of rapid identification. As a consequence of this study, we have implemented MALDI-TOF-based identification directly from positive BCs in our daily routine diagnostic management (including weekends).

In conclusion, rapid identification by MALDI-TOF directly from positive BCs did not impact on duration of intravenous antimicrobial treatment but provided fast and reliable microbiological results

and may be useful in the case of contaminated BCs and BSIs not caused by *E. coli* and *Klebsiella* spp. even in a setting with an established ASP.

Transparency declaration

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Appendix A. Supporting information

Additional Supporting Information may be found in the online version of this article at http://dx.doi.org/10.1016/j.cmi.2016.08.009.

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