**Rapid Identification and Antimicrobial
Susceptibility testing of Positive Blood
Cultures using MALDI-TOF MS and a
modification of the standardized disk
diffusion test - a pilot study.**

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Abstract

Aims

In an era when clinical microbiology laboratories are under increasing financial pressure, there is a need for inexpensive, yet effective, rapid microbiology tests. The aim of this study was to evaluate a novel modification of standard methodology for the identification and antimicrobial susceptibility testing (AST) of pathogens in positive blood cultures, reducing the turnaround time of laboratory results by 24 hours

Methods

277 positive blood cultures had a gram stain performed, were subcultured and incubated at 37°C in a CO₂ atmosphere for 4 to 6 hours. Identification of the visible growth was performed using matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS). Taking a modified approach to the CLSI standardised AST methodology, an inoculum density of 0.5McFarland was prepared from the early growth for disk diffusion testing. The standard AST method was also performed on the 18 to 24 hour culture.

Results

96% (n=73/76) of gram negative organisms were correctly identified by MALDI-TOF MS. Comparative analysis of the rapid and standard AST results showed an overall interpretive category error rate of

7.7% (6.7% minor errors, 0.6% major errors and 0.4% very major errors)

100% of *S.aureus* (n=41) and enterococcus isolates (n=9) were correctly identified after 4 to 6 hours incubation. The overall AST categorical agreement was also 100% for these isolates.

Conclusions

An incubation of 4-6 hours directly from positive blood cultures allowed both for a rapid species identification and an antimicrobial susceptibility result 24 hours earlier than is possible using standard methodology.

Funding: No external funding was received for this study

Ethical approval : As the intervention of this study was purely laboratory based without the involvement of patients, formal ethical approval was not necessary. Samples were collected as part of standard care and patients were not subjected to extra procedures or questions.

Competing Interest

None declared

Introduction

Sepsis, severe sepsis and septic shock affect millions of people worldwide each year. Estimates of incidence vary internationally but consensus points to an incidence of approximately 300 per 100000 population per annum. (2 replace/move blake et al with hall et al 2011) Blood culture is considered the 'gold standard' investigation for the detection of micro-organisms in blood, leading to the diagnosis of bacteraemia and sepsis.¹ The period of time within which the infection and the patient can both be successfully treated is known as the 'therapeutic window'. There comes a point outside this window period at which, even though the infection may be eradicated, the patient will not survive due to irreversible organ damage.³ Kumar et al, report a 7.6% mean decrease in survival for every hour that effective treatment is delayed following documented hypotension in septic shock.^{3,4,5} Empiric broad spectrum antimicrobial therapy is initiated immediately when there is a clinical suspicion of sepsis..³ However whilst effective, broad spectrum antimicrobial therapy is also associated with adverse effects, for example, *Clostridium difficile* associated disease. It can also potentially contribute to the selection of multidrug resistant organisms.^{3,6} Antibiotic resistance amongst pathogens (particularly gram negative) is the most common cause of empirical treatment failure in bloodstream infection and sepsis.² Early diagnosis and initiation of appropriate antibiotic treatment together with earlier de-escalation of broad spectrum antibiotics is critical to improve patient outcome^{2,3,7,}

Bearing this in mind, there is an onus on clinical microbiology laboratories to provide a more rapid robust identification and susceptibility testing system for the diagnosis of sepsis.³ Within the past decade, clinical microbiology laboratories have experienced

revolutionary changes in the way in which microorganisms are identified, moving away from slow, traditional microbial identification algorithms toward rapid molecular methods and mass spectrometry . With the introduction of new technologies and the modification of existing ones it is possible for an organism to be identified from blood cultures within a short time frame^{1,2,6,8,9} Significantly reducing the time to microbial identification and antimicrobial susceptibility results of blood culture isolates provides valuable clinical diagnostic information upon which appropriate antimicrobial therapy can be based, helping to reduce morbidity and mortality and improve patient care^{10,11,12,13,14,15}

The aim of this study was to evaluate a novel modification of standard methodology for the identification and antimicrobial susceptibility testing (AST) of pathogens in positive blood cultures, reducing the turnaround time of laboratory results by approximately 24 hours

u Matrix Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) coupled with a modification of the standardized disk diffusion test were employed in this study. We compared the results of the identification and antimicrobial susceptibility patterns performed from an early stage of bacterial growth, after 4 to 6 hours incubation, to the standard results from overnight culture after 18 to 24 hours incubation.

Materials and Methods

All consecutive blood culture bottles which were flagged as positive by the BacTalert 3D continuous blood culture monitoring system (*bioMerieux, Bruz, France*), over a 3 month period (November 2013 - February 2014) were analyzed in this study.

Rapid identification (rID)

Each positive bottle was subcultured according to our routine laboratory procedure. Agar plates were incubated at 37°C in an atmosphere containing 5% CO₂. One blood agar plate was also incubated at 37°C in an anaerobic atmosphere. Plates were reviewed after 4 to 6 hours incubation and examined for growth. Identification of any light visible growth (Figure 1) was performed via direct transfer using standard settings on the MALDI-TOF MS Microflex instrument (*Bruker, Mainheim Germany*)

Figure 1 Culture of *Escherichia coli* after (i) 4 - 6 hours incubation and (ii) 18 – 24 hours incubation

i)

(ii)

Using MALDI biotyper 2.0 (*Bruker*) , database version V3.1 as a reference, results were accepted if the identification was consistent among the database matches 1 to 10, and the score values of database match 1 and match 2 was >1.8 . Scores of <1.8 were considered to be unreliable and the identification was repeated. When a result of 'no reliable identification' or 'no peaks found' was obtained mass spectrometry was repeated after overnight incubation. For species where there were less than 10 spectra in the database, testing was done in duplicate. Results were also confirmed using Api Identification system (BioMerieux). The gram positive isolates which identified as *S.aureus* were subsequently tested for the presence of penicillin binding protein (PBP) using a PBP2a culture colony test (*Alere Scarborough Inc., Maine, US*) and Xpert MRSA/SA assay (*Cepheid, California, US*), in parallel, in accordance with the manufacturer's instructions.

Rapid Antimicrobial Susceptibility testing (rAST)

Antimicrobial susceptibility testing (AST) was performed by standardized disk diffusion methodology using the Clinical and Laboratory Standards Institute (CLSI) guidelines.^{10,11} The method was modified for the purpose of rAST by preparing an inoculum, equivalent to 0.5 McFarland (1×10^8 cfu/ml), using a Densi-CHEK densitometer (*bioMerieux*), from the light growth visible after 4 to 6 hours incubation (Fig.1 (i)) This was the only deviation made from the standard CLSI AST guidelines during the course of this study. Agar plates were reincubated and reviewed for purity after overnight incubation. Disk diffusion testing was repeated on the monomicrobial cultures according to the standard CLSI methodology.^{25,26}

S.aureus and enterococcus isolates were tested against some or all of the following antibiotics according to local protocol; cefoxitin (FOX), erythromycin (ERY), fusidic acid (FD), gentamicin (GM), linezolid (LZD), mupirocin MUP), rifampicin (RF), teicoplanin (TEC), tetracycline (TE), vancomycin (VA) on Mueller Hinton (MH) agar (*Oxoid*). Gram negative isolates were tested against some or all of the following antibiotics; amikacin (AK), ampicillin (AMP), amoxycillin/clavulanate (AMC), cephalothin (CF), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GM), meropenem (MEM), piperacillin-tazobactam (PTZ) on MH agar. Streptococci were tested against penicillin (P), erythromycin (ERY), teicoplanin (TEC) and vancomycin (VA) antibiotic disks on MH agar which was supplemented with 5% sheep blood (*Oxoid, Basingstoke, UK*). Antibiotic disks (*Becton Dickinson*,) and etest (*bioMerieux*) were used throughout this study.

Zone diameters were read after 18 to 24 hours incubation at 37°C, using the Mastascanelite automated zone reader (*Mast Group Ltd., Bootle, Merseyside, UK.*). Results were interpreted as susceptible (S), intermediate (I) or resistant (R) as defined in the CLSI guidelines.^{25,26} The results of the rapid method were compared with the results of the standard method and the categorical agreement was evaluated. Discrepancies were classified as very major, major and minor (table 1).²⁷

TABLE 1 Discrepancy classification (CLSI)²⁵

Error	Standard method	Modified method
Very major	R	S
Major	S	R
Minor	S	I
	R	I
	I	S/R

Error rates were calculated and discrepancy resolution testing of the major errors was performed according to standard protocol ISO20776-2:2007 'Evaluation of performance of antimicrobial susceptibility test devices'^{25,27}

Discrepancy resolution testing

ISO 20776-2 'Evaluation of performance of antimicrobial susceptibility test devices' states that if there is no obvious indication of a technical error, the discrepancy may be resolved by a one-time triplicate testing of the method using separate bacterial inoculum suspensions.²⁷ For each organism/antibiotic combination, 3 inoculums (0.5 McFarland) were prepared. Serial ten-fold dilutions in normal saline were performed to obtain a concentration of 1×10^6

cfu/ml.²⁸ 1ml of each suspension was inoculated into the relevant BacTalert blood culture bottle. 5mls of lysed horse blood (*Oxoid*) was then added to each bottle.⁶ Bottles were incubated on the BacTalert 3D system until they flagged positive. The rapid and standard AST methods were then repeated, as described above and confirmed by MIC testing using *Etest*. If at least two of the three results gave the same categorical agreement, the error could be deemed resolved.²⁷

Quality control

We validated the rID (including PBP2a and GenXpert assays) and rAST methods using quality control strains recommended in the CLSI guidelines.²⁶ *S.aureus* ATCC 25923, *MRSA* ATCC 43300, *S.epidermidis* ATCC 14990 and *E.faecalis* ATCC 29212 for validation of gram positive tests. *E.coli* ATCC 25922, and *P.aeruginosa* ATCC 27853 for validation of gram negative tests²⁶.

Clinical review of results

We performed a retrospective review of the clinical microbiologists clinical notes to determine if the information generated by the rapid methods, distinct from the information provided by gram stain alone would have an impact on the advice given to clinicians regarding the management of bacteraemia.

The criteria for evaluation in this analysis were;

- a) no mix on gram stain or culture
- b) first episode of bacteraemia
- c) the patient was alive at the time of culture positivity.

RESULTS

96% (n = 73/76) of the gram negative isolates were correctly identified from the light growth on culture after 4 to 6 hours incubation using MALDI-TOF MS (table 2). There were 679 antibiotic tests performed in total on the gram negative organisms in this study. The comparative analysis of the results showed 3 (0.4%) very major errors, 4 (0.6%) major errors and 46 (6.7%) minor errors. The 46 minor errors were distributed amongst all antimicrobial agents. (Table 2). The 3 very major errors we observed were detected in one strain of *Acinetobacter sp.* and in two strains of *E. coli*. The *Acinetobacter sp.* tested susceptible to meropenem by the rAST method and resistant by the standard AST method. One strain of *E.coli* tested susceptible to cephalothin by the rAST method and resistant by the standard AST method. The second strain of *E.coli* tested susceptible to co-amoxiclav by the rAST method and resistant by the standard method. 4 major errors were detected in one strain of *E.coli* and three strains of *K.pneumoniae*. The discordant agent in the *E.coli* strain was Piperacillin/tazobactam which tested resistant by the rAST method and susceptible by the standard method. The discordant agents observed for *K.pneumoniae* were piperacillin/tazobactam in one strain and cephalothin in two strains which tested resistant by the rAST method and susceptible by the standard method.

2 very major errors and 3 major errors were resolved by the discrepancy resolution testing protocol outlined in ISO20776-2 (table 3). 1 very major error and 1 major error resolved as minor errors.

Table 2 Rapid identification and antimicrobial susceptibility testing results of gram negative organisms

Gram negative strain	ID @ 4-6hrs (no. identified / no. tested)	No. of organism/ antibiotic combinations	No. of each type of error in antibiotic susceptibility testing (% of total number of tests)	Very major	Major	Minor
<i>Acinetobacter sp.</i>	1/1	5	1 ^a	0	0	
<i>Bacteroides sp.</i>	2/2	0				
<i>E.coli</i>	54/55 ^h	550	2 ^b	1 ^c	39 ^d	
<i>K.pneumoniae</i>	5/5	50	0	3 ^e	5 ^f	
<i>Moraxella sp.</i>	2/3 ⁱ	6	0	0	0	
<i>Pantoea agglomerans</i>	1/1	10	0	0	2 ^g	
<i>Pasteurella multocida</i>	1/1	3	0	0	0	
<i>Proteus mirabilis</i>	3/3	30	0	0	0	
<i>P.aeruginosa</i>	1/2 ^j	5	0	0	0	
<i>Salmonella sp.</i>	1/1	10	0	0	0	
<i>S.maltophilia</i>	2/2	10	0	0	0	
Total	73/76 (96%)	679	3 (0.4%)	4 (0.6%)	46 (6.7%)	

^a The discordant agent was MEM. Reported as S by rAST and R by the standard method. Discrepancy resolution testing resolved this error as S by both methods.

^b The discordant agents were CF in 1 strain and AMC in 1 strain. Reported as S by rAST and R by the standard method. Both errors resolved as R by both methods.

^c The discordant agent was PTZ. Reported as R by rAST and S by the standard method. Discrepancy resolution testing resolved this error as S by both methods.

^d The discordant agents were AM in 1 strain, AMC in 11 strains, CF in 12 strains, CIP in 1

strain, PTZ in 9 strains, CTX in 4 strains, CAZ in 1 strain and AK in 1 strain.

^e The discordant agents were PTZ in 1 strain and CF in 2 strains. Discrepancy resolution testing resolved the PTZ error and one of the CF errors. The 2nd CF error resolved as a minor error (R-I) when tested in triplicate

^f The discordant agents were AK in 1 strain, CF in 2 strains, CTX in 1 strain and PTZ in 1 strain.

^g The discordant agents were CTX in 1 strain and PTZ in 1 strain.

^h One strain of *E.Coli* did not generate a reliable identification score (<1.8) from 4-6hr culture. This strain was identified from culture after 24hrs incubation.

ⁱ One strain of *M.catarrhalis* did not identify as it was a mixed culture with *S.haemolyticus*

^j One strain of *P.aeruginosa* did not identify as it was a mixed culture with *S.capitis*.

90% (n = 175/194) of the gram positive isolates were correctly identified from culture after 4 or 6 hrs incubation using MALDI-TOF MS (table 3).

There were 491 antibiotic susceptibility tests performed in total on the gram positive organisms. The comparative analysis of the rapid and standard AST results showed no very major, major or minor errors (table 3). On review, we found 100% concordance for *S.aureus* (n=41), *Enterococcus* sp. (n=9) and the β -haemolytic streptococci (n=6).

40 of the 41 strains which identified as *S.aureus* tested negative for PBP2a. One strain was PBP2a positive. The combined testing of PBP2a, Xpert MRSA and Cefoxitin disk were used to exclude MRSA. 115 of 133 strains of Coagulase negative staphylococci (CoNS) were identified by MALDI-TOF MS after 4 to 6 hours incubation. The remaining 18 strains were identified after 18 to 24hrs incubation.

Table 3 Identification and antimicrobial susceptibility testing results of gram positive organisms

Gram	positive	ID @ 4-	No.	of	No. of each type of error in	
strain		6hrs	organism/		antibiotic	susceptibility
		(no.	antibiotic		testing(% of total	number of
		identified /	combinations		tests)	
		no. tested	tested			
					Very	Major
					major	Minor
<i>S.aureus.</i>		41/41 ^a	410		0	0
<i>E.faecalis</i>		6/6	30		0	0
<i>E.faecium</i>		3/3	15		0	0
<i>S.agalactiae</i>		1/1	4		0	0
<i>S. dysgalacticae</i>		4/4	16		0	0
<i>S.pyogenes</i>		1/1	4		0	0
<i>S.milleri</i>		1/1	4		0	0
<i>S.gallolyticus</i>		2/2	8		0	0
<i>S.parasanguinis</i>		0/1 ^b	0		0	0
<i>S. salivarius</i>		1/1 ^c	0		0	0
<i>Coagulase</i>	<i>neg</i>	115/133 ^d	n/a		0	0
<i>staph</i>						
Total		175/194	491		0	0
		(90%)				

^a 40/41 strains of *S.aureus* were PBP2a negative. One strain was PBP2a positive and was confirmed with Xpert MRSA/SA BC assay. This isolate was reported as MRSA 48hrs earlier than conventional methods

^b One strain of *S.parasanguinis* did not identify after 4-6hrs incubation. This strain was identified from culture after 24hrs incubation.

^c This *S.salivarius* isolate was considered to be a contaminant and did not require antimicrobial susceptibility testing.

^d 18/133 strains of Coagulase negative staphylococcus were not identified by MALDI-TOF-MS after 4-6hrs incubation All strains were identified from culture after 24hrs incubation

Table 4 Discrepancy Resolution Testing

Organism	Antibiotic	Rapid Disk Diffusion @4 to 6 hours			Standardized disk diffusion ' 16 -24 hours			Conclusion
		1	2	3	1	2	3	
<i>K.pneumo</i>	Tazo-Pip 110ug	I(20mm)	S(22mm)	S(22mm)	S(22mm)	S(22mm)	S(22mm)	Resolved
MID 2.362								
<i>K.pneumo</i>	Cephalothin 30ug	R(14mm)	R(14mm)	I(15mm)	I(15mm)	I(16mm)	I(16mm)	Minor(R-I)
MID 2.363								
<i>E.Coli</i>	Cephalothin 30ug	R(8mm)	R(10mm)	R(11mm)	R(11mm)	R(11mm)	R(10mm)	Resolved
MID2.536								
<i>K.pneumo</i>	Cephalothin 30ug	I(15mm)	R(14mm)	I(15mm)	I(17mm)	I(16mm)	I(16mm)	Resolved
MID 2.402								
<i>A.lwoffii</i>	Meropenem 10ug	S(20mm)	S(20mm)	S(23mm)	S(22mm)	S(23mm)	S(20mm)	Resolved
MID2.303								
<i>E.Coli</i>	Cephalothin 30ug	I(15mm)	R(12mm)	R(13mm)	I(17mm)	I(16mm)	I(15mm)	Minor(R-I)
MID 2.607								
<i>E.Coli</i>	Augmentin 30ug	S(21mm)	S(20mm)	S(19mm)	S(18mm)	S(18mm)	S(18mm)	Resolved
MID 2.363								

2 very major errors and 3 major errors were resolved by the discrepancy resolution testing protocol outlined in ISO20776-2 (table 4).1 very major error and 1 major error resolved as minor errors.

Clinical review of results

Table 5 describes the clinical impact analysis of results generated by the rID and rAST methodology for gram negative organisms. 43 episodes of gram negative bacteraemia were eligible for clinical impact analysis. Significant clinical impact was seen in 28% of these cases (n=12/43), outlined as follows: 3/12 were contaminants which did not require any further follow up. 3/12 identified the need for further investigations to determine the source of the infection. 2/12 required escalation of antimicrobial treatment based on the rapid identification result, indicating the likely failure of empiric therapy. 4/12 required escalation of treatment based on rAST result, indicating inadequate empiric therapy.

Table 5: Clinical impact analysis of rapid identification and rapid antimicrobial susceptibility testing of gram negative organisms

Gram negative strain	Initial Antibiotic prescribed	New Antibiotic recommended	Comment on clinical impact
<i>E. coli</i>	AMC	CIP	ESBL producer suspected with rAST results
<i>E. coli</i>	PTZ + GM	MEM	Inappropriate escalation of therapy due to a major error in rAST: see Table 1 subscript c
<i>E. coli</i>	AMC	MEM	PTZ resistance by rAST – initial change from AMC to PTZ with rID
<i>E. coli</i>	PTZ	CIP	PTZ resistance by rAST
<i>K.pneumoniae</i>	PTZ + GM	MEM	Escalation with rID (Multidrug resistant phenotype of

			<i>K.pneumoniae</i> circulating in community)
<i>S.maltophilia</i>	MEM	Co-trimoxazole	Critically unwell ICU patient, rID crucial to timely treatment
<i>M. osloensis</i>	None	None	Contaminant suspected, recall of patient from home avoided
<i>B. fragilis</i>	PTZ + GM	Metronidazole	Focused search for likely abdominal source of sepsis with rID
<i>A. lwoffii</i>	AMC	AMC	Contaminant suspected, recall of patient from home avoided
<i>Pasteurella multocida</i>	PTZ + GM	P	Focused search for osteomyelitis at site of infected cat bite with rID
<i>Salmonella sp.</i>	None	Ceftriaxone	Haemodialysis patient recalled from home, targeted treatment and isolation possible
<i>Moraxella sp.</i>	PTZ	PTZ	Initial gram stain reviewed as gram positive cocci reported , amended to gram variable cocco-bacilli on review, additional gram positive cover avoided

Figure 2 describes a clinical impact analysis of the results generated by the rID of *S.aureus* followed by rapid PBP2a culture colony test result. 12/41 patients with *S.aureus* were eligible for inclusion in this analysis. In each case the PBP2a culture colony test and Xpert SA/MRSA BC assay indicated meticillin susceptible *S.aureus* (MSSA). 50% of patients (n=6/12) had same day de-escalation from

broad spectrum antimicrobials to targeted therapy with flucloxacillin. 34%(n=4/12) were either commenced on targeted flucloxacillin (n=2) or continued on flucloxacillin (n=2). 16% of patients (n=2),admitted to the intensive care unit, were continued on broad spectrum antimicrobials

Figure 2 Clinical impact of the rapid identification of *S.aureus*, followed by PBP2a identification and a genXpert PCR assay

Time to reporting of final results

Figure 3 Demonstration of a reduction in turnaround time of up to 24hrs using the rapid disc diffusion method.

Discussion

In this study we describe a method to rapidly identify significant isolates from positive blood cultures using MALDI-TOF MS. A similar method of identifying organisms from positive blood cultures using MALDI-TOF MS subsequent to short term incubation on solid medium has also been described by Idelevich al. and we report similar findings for rapid identification.⁹ MALDI-TOF MS has been used in routine clinical laboratories since 2009 and has demonstrated excellent performance and cost effective benefits when compared to conventional methods for the rapid identification of bacteria and yeast from solid media.^{2,14,16,17,18.} For rapid antimicrobial susceptibility testing (rAST) there are several automated systems available which provide results within 8 to 12 hours but are very costly for a routine clinical laboratory with a high specimen throughput.^{19,20,21,22} A cheaper alternative to commercial and high technology platforms is required. The disk diffusion test which dates back to 1965 has been well standardized and is a simple, practical robust system. The advantages of the disk method is test simplicity that does not require any special equipment^{7,23,24} The is flexibility in selection of disks for testing and the provision of categorical results are easily interpreted and understood by clinicians. We performed rapid disk diffusion testing by preparing the 0.5mcF inoculums from the bacterial growth after 4 to 6 hours incubation using the Clinical and laboratory Standards Institute (CLSI) methodology and compared results to the standardized CLSI method^{25,26} As no calculations were performed 24 hours is an estimated time.

To date, several studies have utilised MALDI-TOF MS for identification of microorganisms directly from positive blood cultures and demonstrated identification rates of 80% to 90%, with 59% to 95% concordance with identification results directly from culture.^{2,9,29} The variability in MALDI-TOF MS performance described in the literature has been reported to be due to the various methods used for isolation of organisms from blood culture. The methods described include differential centrifugation, washing, gel based separation and protein extraction steps to isolate bacteria and remove substances that can interfere with MALDI-TOF MS analysis.^{2,17,29,30,31} These procedures, whilst more rapid than our method are costly and labour intensive and therefore more difficult use in a routine clinical microbiology laboratory. The method described in this paper is neither labour intensive nor costly, results in a relatively rapid turnaround time that is conducive to a routine microbiology laboratory workflow.

The protocol in this study involves bacterial cultures in the log phase of growth (4 to 6 hours incubation) rather than in the stationary phase (18 to 24 hours incubation). Our data supports the work of other research groups and concurs that the physiological phase of bacterial cell growth does not significantly influence the results of identification or standardized disk diffusion antimicrobial susceptibility tests.^{7,23,24} We found general categorical agreement for zone diameters obtained between the rapid and standardized AST methods in 100% of gram positive organisms and 92.9 % of gram negative organisms. Upon investigation of the discrepancies we found that these could be attributed to differences in inoculum density and technical interpretation of the zone diameters rather than

differences in the physiological phase of the bacterial cells, as all errors were resolved by discrepancy resolution testing.

The clinical and financial benefits of rapid identification and faster antimicrobial sensitivity results have been described by other authors, therefore we did not directly examine the impact of the new protocol on patient mortality, length of stay or hospital costs.^{2,17,27,28,37}

In the clinical impact analysis, we reviewed the medical microbiologists notes for each episode of bacteraemia to determine how the information obtained from the rapid laboratory tests would have influenced antimicrobial prescribing advice. We found that rapid identification allows for early discrimination of pathogens from contaminants. In our institution the use of glycopeptides was recommended to clinicians for patients with evidence of sepsis together with a positive blood culture showing gram positive cocci. As the rate of gram positive contaminants versus significant pathogens exceeds a 2:1 ratio, this resulted in the overprescribing of glycopeptides. With the new protocol, 87% (n=115/133) of CoNS would be correctly identified on the same day as the gram stain result. This would result in earlier identification of those isolates that were blood culture contaminants, prompting a timely review of the patients antimicrobial therapy. In 83% (n=10/12) of the patients with *S.aureus* isolated from blood cultures, we would be able to advise a change in therapy to targeted anti-staphylococcal treatment on the same day as blood culture positivity. This would result in the use of less costly antibiotics, i.e. flucloxacillin, and a reduced need for vancomycin therapeutic drug monitoring. It has been reported that patients treated with targeted therapy for MSSA bacteraemia using beta-lactam agents have higher cure rates than those treated with vancomycin.^{32,33}

The impact of rapid identification of gram negative bloodstream infection is similar to previously reported rates.^{8,22,34} The potential benefit to antimicrobial stewardship was evident, albeit less pronounced. In 28% (n=12/43) of patients with gram negative bloodstream infection, the rID would have a significant impact on the choice of antimicrobial therapy, with the most frequent impact being the broadening of the antibiotic spectrum by availability of AST results within 24hrs. In 21%(n=9/43) of patients the new method would result in advice to clinicians to escalate therapy or perform specific clinical investigations to identify the source of infection.

This study describes a modification of an established method of processing positive blood cultures. It requires very little additional processing time when compared to other strategies which have been evaluated to optimise rapid MALDI-TOF identification of isolates from patients with bloodstream infections.^{2,29,30,31,34} No additional reagents or training of laboratory personnel is required once a laboratory has access to a MALDI-TOF mass spectrometer. While identification results with this methodology may lag several hours behind the gram stain result they are still available on the same day. Laboratory workflow limitations may necessitate batching of these tests, particularly outside of routine laboratory hours, as we have found in our own laboratory. However it still results in far superior turnaround times when compared to conventional methods.¹⁴ While other protocols described may provide earlier identification results, methods are often more labour intensive.^{2,29,30,32,36} Our method is capable of achieving a turnaround time equivalent to a sepsityper or centrifugation/washing technique, batched 6 hourly.

While some errors did occur with this method, the error rates were within acceptable limits as outlined in ISO20776-2.²⁷ We have

introduced a 24/7 workflow scheme into our laboratory to incorporate the rMID and rAST methods described here, for positive blood cultures. This allows final blood culture results to be available within an average of 24 hours from positive blood culture, compared to approximately 48hrs with our previous methods. It also supports our hospital antimicrobial stewardship programme by facilitating earlier review and streamlining of antimicrobial therapy using the 'Start smart then focus' care bundle.³⁵ Other potential benefits of rapid species ID that have been reported include the optimisation of clinical outcomes, reducing costs of antimicrobial therapy and limiting the rapidly growing selection of antimicrobial resistant pathogens.³⁶

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Transparency Declaration

The authors declare no conflicts of interest

Author Contributions

C.F, E.P. and P.S. designed the study. C.F and E.P. performed the laboratory work. C.F., E.P. and P.S. compiled and analysed the data. C.F.and P.S. wrote the manuscript. M.D, E.P., P.M., B.C., M.H. and B.L, performed critical reading of the manuscript prior to submission.

Take home messages

Rapid laboratory results provide valuable clinical diagnostic information upon which appropriate antimicrobial therapy can be based, helping to reduce morbidity and mortality, improve patient care and reduce healthcare costs.

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