ORIGINAL ARTICLE

Early Identification of Bacterial Isolates from Positive Blood Culture Bottles using MALDI-TOF MS and Early Antimicrobial Susceptibility Testing on Pediatric Oncology Patients

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ABSTRACT

Background: Final identification and susceptibility results of positive blood cultures would take 24 to 48 h by conventional methods. Every hour of early treatment, could be life-saving for septic immunocompromised patients. We conducted a pilot study to determine the earliest timing for performing identification and antimicrobial susceptibility testing of microorganisms isolated, after positive blood culture bottles with the most accurate results. Materials and Methods: Positive blood culture samples were inoculated onto two blood agar plates at 37◦c. The growth on one plate was used every one hour repeatedly identification by MALDI-TOF MS (bioMérieux Vitek MS IVD system) and antimicrobial susceptibility testing Vitek 2 system. A total of 600 ID and MIC testing were done (13 Gram positive and 12 Gram negative). Results: Identification of all isolates showed 100% agreement between testing initial 3-hour growth and testing over-night growth. MIC showed 100% agreement when testing at mean hours of 5.6 (SD+/- 2.5) and 9 (SD +/- 2.5) for Gram negative and positive organisms respectively. The total time needed for accurate MIC results from start of positive BC sub-culturing was 16.1 h & 22 h for Gram negative and positive organisms respectively. Conclusion: Identification of causative pathogens using MALDI-TOF MS could be achieved accurately after 3h of sub-culturing. MIC testing can be performed after 5.6 h, 9 h incubation. Applying this methodology provided final accurate rapid results and early treatment of septic patients. (*Int J Biomed Sci* 2018; 14 (1): 26-31)

Keywords: MALDI TOF MS; Blood culture; bacterial identification; antimicrobial susceptibility

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INTRODUCTION

Bloodstream infections are associated with high mortality, longer hospital stays, and increased costs of care (1). Timely diagnosis of the etiology of infection is imperative, with increasing mortality of approximately 8% through every hour with unsuitable antimicrobial therapy administered to patients with sepsis (2). The current standard of practice for diagnosing bloodstream infections continues to be blood culture. Once a culture is positive for growth,

final organism identification and antimicrobial susceptibility testing require additional growth *in vitro*. Final results of positive blood cultures are generally not available to the treating physician for approximately 24 to 72 h after culture positivity is first noted (3). Early organism identification and detection of antimicrobial resistance using broth directly from blood cultures can therefore facilitate earlier optimization of treatment.

Recently, MALDI-TOF MS use for bacterial identification was introduced, which revolutionized the diagnostic microbiology service (4). MALDI-TOF MS theoretically has the potential to identify any organism from a positive blood culture. Unfortunately, this method showed variable results in this regard (5, 6).

Many studies compared different methods that can be used for rapid identification (ID) of microorganisms from blood culture broth and from colonies grown after the earliest incubation of culture plates. Few studies focused on early antimicrobial susceptibility testing (AST) that can be as accurate as AST performed from overnight incubation grown colonies. We aimed to conduct a pilot study to determine the earliest timing for performing identification and antimicrobial susceptibility testing of microorganisms isolated, after positive blood culture bottles sub-culturing, with the most accurate results.

MATERIALS AND METHODS

A total of 28 positive blood cultures (BC) (prospectively collected from 28 pediatric oncology patients admitted to CCHE 57357 Hospital clinically diagnosed with sepsis) were included. Automated blood culture systems used were; BD BACTEC™ 9240 Blood Culture System (Becton Dickinson), and the BacT/ALERT® 3D System (bioMeriéux). Paediatric BC bottles (BACTECTM PedsPlusTM/F, BD Diagnostics, Shanon County, Clare, Ireland) and BacT/ALERT®PF Plus, INC. Durham), inoculated with patients' blood were incubated in the corresponding BC system.

After reporting positive signal, Positive blood culture broths were inoculated into two blood agar plates and one MacConkey agar plate and incubated at 35°C and the plates were incubated at 35°C in air using an incubator StabiliTherm (ThermoScientific, Langenselbold, Germany).

MALDI-TOF MS was performed at time-points 3, 6, 8, 10 and 12 h until apathogen was successfully identified. The spectra were acquired using the MALDI-TOF Vitek MS (bioMérieux) and analysed on Vitek MS IVD system (bioMérieux; Marcy l'Etoile, France). Samples were performed in duplicate, with tests performed simultaneously on the same target slide. Part of single colony was transferred to an individual spot on the48-well Vitek MS-DS disposable target slide. Each spot was covered with 1 µl ready-to-use Vitek MS alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix (bioMérieux, France). The target plate was then read and analyzed by the Vitek MS IVD system. The protein profile of each specimen with an *m/z* of 3,000 to 15,000 was produced and profiles were further matched with the Vitek MS reference CE-IVD certified database (>20,000 spectra). Matching results with confidence percentages 90 to 98% confidence were considered for genus level, while results of>98% confidence, were considered for the species level, while <90% confidence were unacceptable identification. For specimens that showed different identification under the same genus, we identified to the genus level only, with more than one genus or family identifications, they were considered unacceptable.

Vitek 2 (bioMérieux SA, Marcy l'Étoile, France) testing was performed from the plates at 3, 6, 8, 10, 12 hours respectively. Steps of Vitek 2 inoculum-preparation was as recommended by the manufacturer for each organism. Vitek 2 AST cards GP67, GN73 were chosen according to Gram stain from BC broth or/and early MALDI-TOF MS identification. The detection of common resistance mechanisms was evaluated: *Staphylococci* methicillin resistance using cefoxitin screening, inducible clindamycin resistance in *Staphylococci* and *Streptococci* and extended-spectrum β-lactamases (ESBL) production in *Enterobacteriaceae*. Interpretation of results was done according to CLSI guidelines (7).

Results of identification and antimicrobial susceptibility testing at each time from the same day culture was compared to those from overnight growth in terms of; Very major error (VME) (false susceptible result), major error (ME) (false-resistant result), and minor error (mE) (false intermediate result) rates, and categorical agreement (results within the same category) and essential agreement (MIC difference ≤ 1 double dilution step) were determined for Gram positive cocci (GPC) and Gram negative rods (GNR) at different timings (8, 9).

Statistical methods

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 23. Data was summarized using frequency (count) and relative frequency (percentage), mean and standard deviation. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the

expected frequency is less than 5. P-values less than 0.05 were considered as statistically significant.

RESULTS

On a total of 28 BC bottles that signals positive on the automated blood culture, 3 samples were excluded after performing Gram stain and subculture as they were false positive alarms (technical errors) or mixed cultures. A total of 600 ID and MIC testing were done.

The mean time necessary for cultivating plated positive BC broth on agar until successful MALDI-TOF MS identification from shortly incubated solid medium cultures to species level for Gram positive and Gram negative organisms was 3 h, in all isolates (100%) after being cultivated for 3 h as control for 24 h.

A total of 25 isolates were tested; 3 *Staphylococcus aureus*, 2 *Staphylococcus hemolyticus*, 5 *Staphylococcus epidermidis*, 1 *Staphylococcus hominis*, 2 *Streptococcus agalactiae*, 7 *Escherichia coli*, 1 *Escherichia vulneris*, 1 *Klebsiella pneumoniae*, 1 *Pseudomonas stutzeri*, 2 *Acinetobacter baumannii*.

For GPC, inoculation of Vitek 2 cards was possible at 3 h in all but 2 (11/13) 92% of the isolates, with all specimens being inoculated within 6 h. At 3 h, inoculation was possible with all GNR isolates.

Regarding the MIC, 100% agreement was obtained when testing at mean hours of 5.6 (SD+/- 2.5) and 9 (SD +/- 2.5) for Gram negative and positive organisms respectively. The total time needed for accurate ID & MIC results from start of positive BC sub-culturing was 19.1 h & 26.5 h (*p* value= 0.006, 0.002) for Gram negative and positive organisms respectively. Details are shown in Table 1.

Comparison of AST results for categorical and essential agreement at different time intervals; 3, 6, 8, 10, 12 h, with overnight incubation, is shown in Table 2.

For the total errors rate within Gram positive organisms tested [13] against a panel of 19 antimicrobial agents (247 combination of organism/antimicrobial); VME 0.4%, ME 1.2%, mE 3.23%, while for Gram negative organisms tested [12] against a panel of 21 antimicrobial agents (252 combination of organism/antimicrobial); only mE 1.58%, with no major or very major errors. Details are shown in Table 3.

Gentamicin showed discrepant results in 2 cases (*S. hemolyticus* & *S. epidermidis*) in isolates tested before 12 hours incubation, 1 major error ME (sensitive was interpreted as resistant) and 1 minor error (intermediate was interpreted as resistant).

At 12 hours incubation (16/25) 64% of cases, showed 100% categorical and essential agreement in MIC values for the tested isolates. While at 10 hours incubation only (8/25) 32% and at 8 hours (7/25) 28% of cases showed this 100% agreement.

For resistance markers' screening using Vitek 2 AST at different timings; Methicillin resistant *S. aureus* (MRSA) screening was successful in *S. aureus* isolates tested as early as 3 h, clindamycin induced resistance was detected at 6 h and ESβL screening was also successful at 6 h.

DISCUSSION

Many studies attempted to evaluate the earliest possible method for releasing a valid result for blood culture samples (10-12).

In the present study, 25 isolates from positive blood

Table 1. Vitek 2 Antimicrobial Susceptibility Testing Time to results

Time period	Time (mean +/- SD) (h)					
	Gram-positive cocci (*n*=13)			Gram-negative rods (*n*=12)		
	Early culture	Control culture	*P* value	Early culture	Control culture	*P* value
Cultivation time until Vitek 2 inoculation	4.5 +/- 2.1	24	0.002[a]	3	24	0.006[a]
Cultivation time until Vitek 2 Accurate results	9 +/-2.58	24	0.000[a]	5.6 +/- 2.51	24	0.001[a]
Duration of Vitek 2 AST	13 +/- 3.31	12.5 +/- 2.73	0.361	10.5 +/- 2.44	10 +/- 1.58	0.378
Total time from positive BC subculture to accurate AST result	22	36.5	0.002[a]	16.1	34	0.000[a]

[a]*P* value <0.05 is significant.

Table 2. Microorganisms available for AST results comparison and their categorical and essential agreement between different time intervals and overnight incubation

	Categorical agreement (%)				
	3 h	**6 h**	**8 h**	**10 h**	**12 h**
Gram-positive cocci (13)	97.28 SD+/-1.59	97.64 SD+/-2	98.4 SD+/-2.37	98.4 SD+/-2.37	100
Staphylococcus aureus (3)	98.2	100	100	100	100
Staphylococcus epidermidis (5)	98.9	98.9	100	100	100
Staphylococcus hemolyticus (2)	97.3	97.3	97.3	97.3	100
Staphylococcus hominis (1)	94.7	94.7	94.7	94.7	100
Streptococcus agalactiae (2)	97.3	97.3	100	100	100
Gram-negative rods (12)	98.76 SD+/- 2.08	99.04 SD+/-2.14	100	100	100
Escherichia coli (7)	98.6	100	100	100	100
Escherichia vulneris (1)	100	100	100	100	100
Klebsiella pneumoniae (1)	100	100	100	100	100
Pseudomonas stutzeri (1)	95.2	95.2	100	100	100
Acinetobacter baumannii (2)	100	100	100	100	100

	Essential agreement (%)				
	3 h	**6 h**	**8 h**	**10 h**	**12 h**
Gram-positive cocci (13)	98.04 SD+/-2.2	98.94 SD+/-2.37	98.94 SD+/-2.37	100	100
Staphylococcus aureus (3)	98.2	100	100	100	100
Staphylococcus epidermidis (5)	100	100	100	100	100
Staphylococcus hemolyticus (2)	100	100	100	100	100
Staphylococcus hominis (1)	94.7	94.7	94.7	100	100
Streptococcus agalactiae (2)	97.3	100	100	100	100
Gram-negative rods (12)	100	100	100	100	100
Escherichia coli (7)	100	100	100	100	100
Escherichia vulneris (1)	100	100	100	100	100
Klebsiella pneumoniae (1)	100	100	100	100	100
Pseudomonas stutzeri (1)	100	100	100	100	100
Acinetobacter baumannii (2)	100	100	100	100	100

culture samples were tested by MALDI TOF MS at 3 h growth and showed correct identification (100% agreement) with 24 h growth.

This early method for identification from cultured plates can be used instead of using BC positive broth as results from most reports are variable. Gram negative organisms are better identified from BC positive broth than Gram positives or yeasts. Also, different preparation methods that were used; including adding detergent with different concentrations (e.g. saponin, sodium dodecyl sulfate, Tween 80) or use of the Sepsityper kit (Bruker Daltonik GmbH, Bremen, Germany) (11, 12). Those methods are labor intensive, costy and liable to contamination from the many steps of preparation.

Other studies using MALDI TOF from early culture growth showed similar results, as one study that evaluated the possibility of identifying young colonies (3 and 6 hours incubation) from solid agar after subculture from positive blood culture bottles. MALDI-TOF MS was used and the matched identification to the species level was 80.6%

Table 3. Performance of antimicrobial susceptibility testing of Gram-positive cocci and Gram negative rods that show errors

Antimicrobial agent	No. (%) of errors at 3 h			No. (%) of errors at 6 h			No. (%) of errors at 8 h			No. (%) of errors at 10 h		
	VME	ME	mE	VME	ME	mE	VME	ME	mE	VME	ME	mE
Oxacillin	1 (7.7%)[1]											
Gentamicin		1 (7.7%)[2]	1 (7.7%)[3]		1 (7.7%)[2]	1 (7.7%)[3]		1 (7.7%)[2]	1 (7.7%)[3]			2 (15.3%)[2, 3]
Doxycycline			1 (7.7%)[4]									
Ciprofloxacin			1 (7.7%)5[*]			1 (7.7%)[5]						
Piperacillin-tazobactam			1 (8.3%)[6]									
Amikacin			2 (16.6%)[6, 7]			1 (8.3%)[7]						

[1]one case of *S. aureus*; [2]one case of *S. hominis*; [3]one case of *S. hemolyticus*; [4]one case of *S. epidermidis*; [5]one case of *S. agalactiae*; [6] one case of *E.coli;* [7]one case of *Ps. Stutzeri.*

(141/175) for the 3-h cultures and 90.9% (159/175) for the 6-h cultures, compared with overnight cultures. The panel of organisms analyzed was commonly isolated Gram positive cocci and Gram negative bacilli while, *Candida spp.* and fastidious bacteria were not evaluated (10).

Time to accurate AST results was 6 & 8 h for Gram negative & Gram positive respectively, except for Aminoglycosides that show descript results, as Gentamicin showed mE in 2 cases 15.3% (*S. hemolyticus* & *S. epidermidis*) till 10 h incubation and Amikacin showed mE in 1 case 8.3% at 8 h incubation. Fortunately, Gentamicin is not the drug of choice for Gram positive cocci except for *Enterococcus spp.* as a combination therapy with β-lactams or glycopeptides. Our isolates are from cases of blood stream infections, where the use of aminoglycosides is limited to selected cases.

This can be explained by the nature and mode of action of aminoglycosides. Aminoglycosides display concentration-dependent bactericidal activity against the majority of gram negative rods either aerobic or facultative anaerobic bacilli" but not against gram negative anaerobes and most gram positive bacteria. They are most effective against susceptible bacterial populations that are rapidly multiplying (13). The inhibition of protein synthesis mediated by aminoglycosides is energy dependent, and occurs through irreversible binding, to the cytosolic, membrane-associated bacterial ribosome. The degree of protein synthesis affection is variable between different aminoglycoside agents, as can their affinity and degree of binding, aminoglycoside presence in the cytosol generally disturbs peptide elongation at the 30S ribosomal subunit, leading to improper mRNA translation (misread) and therefore biosynthesis of proteins that are truncated or have different arrangement of amino acids or even premature termination (14).

Another study conducted, used the earliest culture growth on solid media for ID and AST using Vitek 2 cards, showed that VME, ME, mE, categorical agreement (CA) and essential agreement (EA) were 1.6%, 0.3%, 0.1%, 99.2%, and 99.1% for GPC and 0%, 0.5%, 0.8%, 99.2%, and 99.3% for GNR, respectively. In that study they used the same definitions and methods of calculating errors and agreement (15).

In that study conducted on 104 isolates (68 GPC and 36 GNR), they used the earliest culture growth for AST in comparison with 24 h culture and detected the mean time to earliest available AST results that was 3.8 h for GPC and 2.4 h for GNR (15) but in our study time intervals for testing were set prior to AST inoculation, so all GNR and GPC were inoculated in cards at 3 h except 2 isolates GPC that were inoculated at 6 h.

Also different resistance patterns were correctly detected from early culture tested as MRSA, induced Clindamycin resistance and ESBL in our study and other studies (15).

Early identification of bacterial species after 3 hr of subculture adds to the simple information of Gram positive or negative reported after BC bottle flag positive. Different species require variable treatment regimen as *S. aureus* will differ from *Enterococcus spp.* or Coagulase negative *Staphylococci*. Also, *E.coli* and *K. pneumoniae* will require a different empiric treatment than *A. baumannii* or *Pseudomonas spp.* Another add value for identification of the causative organism, it may identify other pathologies that were not suspected clinically (16). Moreover, early administration of correct antibiotics to septic

patients improves outcome and delay in antibiotics when patients are in septic shock recognition leads to increased mortality (17).

This work represents an advance, as identification of causative pathogens for BSIs could be achieved accurately after 3h sub-culturing using MALDI-TOF MS. MIC testing can be performed at 5.6 h, 9 h incubation after subculture of Gram negative and positive organisms respectively. Applying this methodology provided final accurate early available results and early treatment of septic patients. A large scale study with the same idea and methodology is recommended.

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

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