

Perspectives on Improved Therapy of Septicemia by Rapid Bacterial Identification Using MALDI-TOF MS and Rapid Susceptibility Testing

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Commentary

The most serious clinical manifestations of bloodstream infections (BSI), sepsis and septic shock, are urgent conditions requiring prompt initiation of adequate antimicrobial therapy [1]. In septic shock, every hour of delayed upstart of effective antimicrobial therapy was associated with a decrease in survival rate, thus falling from 82.7% initially to 42% after six hours of hypotension duration [2]. Rapid identification of microorganisms directly from blood cultures after flagging positive is the first step in shortening the diagnostic process. A number of molecular methods for direct identification from blood cultures have been described, including multiplex real-time PCR [3]. After matrix-assisted laser desorption ionization- time of flight (MALDI-TOF) mass spectrometry (MS) was introduced in microbiological laboratories in the last decade [4], development of rapid in-house [5,6] and commercial methods (Sepsityper Kit, Bruker Daltonics, Germany) for direct identification of organisms from positive blood cultures have been reported.

We have previously described the development of two in-house methods for rapid identification of microorganisms directly from positive blood cultures by MALDI-TOF MS [7]. A modification of the method described by Martini et al. [8] by adding a formic acid extraction step directly on the MALDI-TOF plate provided the higher level of identification obtained by the first in-house method. By adjustment of cut-off score values to >1.7 for species and >1.5 for genus level identification, 89.3% of organisms were identified, including 94% of Gram negative and 85.4% of Gram positive bacteria. Overall concordance to definitive identification was 99.3% to genus and 96.6% to species level. Genus discrepancy was observed only in one case of *Citrobacter freundii* misidentified as *Lactobacillus helveticus*, with the score 1.513 close to the lower limit. Five minor discrepancies were observed in differentiating among *Streptococcus mitis* group isolates and insufficient identification of *Salmonella* to species level, which are known limitation of MALDI-TOF MS identifications.

Due to its excellent applicability and reliability, this rapid in-house method providing identification results in less than 30 minutes, is now implemented in our routine laboratory work. Since September 2015, the laboratory opening hours are extended to 10 pm and positive blood cultures are analyzed directly by MALDI-TOF MS three times a day. After updating of Bruker Daltonics database in October of 2015, all analysis are being performed on the new software Compass 4.1 comprising 5989 different microorganisms strains in its database (5291 bacterial strains and 691 eukaryotic-mainly fungal-strains). A higher level of confident identification to species level with score values >2.0 was noticed since updating, including less frequent organisms as *Pasteurella multocida*, *Bacillus cereus* group, *Burkholderia cepacia complex*. Almost all isolates of *Enterobacteriaceae*, *Pseudomonas spp*, staphylococci, β -hemolytic streptococci, enterococci and anaerobes are now routinely reliably identified directly with confident genus and species score values, >1.7 and >2.0, respectively. Identification problems are still noticed with *Propionibacterium spp.* and *Corynebacterium spp.*,

supposed to be caused by insufficient concentration of organisms in blood culture flasks. In our routine laboratory work, this observation have led to exclusion from direct identification of those almost always single positive blood culture flasks flagging positive after 5 days of incubation as probable contaminants. Direct identification of *Pneumococci* is still based on Gram microscopy and agglutination test due to previously described problems [7,9].

The most important purpose of rapid direct identification in our laboratory was gaining information leading to adequate antimicrobial therapy in septic patients, based on knowledge of intrinsic antimicrobial resistance and national resistance patterns of the bacterial species identified [10]. In our laboratory, the results of antimicrobial susceptibility testing (AST) are still obtained the next day after the blood cultures are flagged positive. The method presently used is direct disk diffusion test from blood culture flasks, where resistance results are read after 18 hours of incubation. Briefly, different volumes of blood from blood culture flasks are added to 3 ml of 0.9% sterile saline (volume dependent on results of Gram stain and direct MALDI-TOF MS species identification), floated on adequate resistance agar media by swabbing followed by placing of antibiotic disks on the surface. The interpretation of results is performed by using NordicAST breakpoints (version 6.0; 2016) based on European Clinical Antimicrobial Susceptibility Testing Breakpoints (EUCAST), but with the comments adjusted to local Nordic antimicrobial patterns. This method has been employed for several years in our laboratory, validated internally and assessed as reliable in comparison with AST performed from bacterial growth after subcultivation on agar media. Direct disk diffusion method was recently compared with the VITEK-2 based definitive AST and acceptable accuracy of the disk diffusion test system was reported [11].

Due to increasing number of resistant bacterial isolates, especially those with plasmid transferable resistance genes, more rapid AST results are required. We have considered different options for this approach, and one that could be readily employed in our routine laboratory work is early reading of direct disk diffusion test results, approximately after 6 hours of incubation. This approach is widely used in some laboratories for getting rapid preliminary AST results, but publications based on validation of this method are scarce.

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Received April 27, 2016; Accepted May 05, 2016; Published May 15, 2016

Citation: Jakovljevic A, Bergh K (2016) Perspectives on Improved Therapy of Septicemia by Rapid Bacterial Identification Using MALDI-TOF MS and Rapid Susceptibility Testing . J Antimicro 2: 115. doi:10.4172/2472-1212.1000115

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Recently, oral and poster presentations [12] from Kahlmeter et al. at ESCMID 2016 reported the results of disk diffusion AST directly from blood cultures read at different time points: after 6, 8 and 12 hours of incubation. The conclusion was that regular breakpoints cannot be applied if early AST reading is performed as the corridor between wild type and non-wild type population widens with longer incubation time, and some resistance mechanisms can be expressed only after the recommended length of incubation. These researchers are currently working on establishment of breakpoints for early disk diffusion AST reliable reading. Automated AST options (Phoenix system, Vitek 2) were reported as successful for direct AST of Gram negative bacterial isolates from blood cultures [13,14]. The results of our internal validation of AST using automated Phoenix system performed on Gram negative bacterial urine isolates during autumn 2015 were more similar to those reported by Romero-Gómez et al. [15], with sporadic very major and major errors for cefuroxime, piperacillin-tazobactam and trimethoprim-sulfamethoxazole.

Considering our requirements for rapid detection of resistant isolates, the major resistance problems are associated to *Escherichia coli* and *Klebsiella pneumoniae* isolates bearing plasmid located genes for extended-spectrum β -lactamase (ESBL) enzyme production, represented mainly by CTX-M enzymes [16]. ESBL producing isolates are resistant to most of β -lactam antibiotics, except carbapenems and rapid adjustment of empirical antimicrobial therapy is necessary. The prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolated from blood cultures in Norway is still low, reported to be 5.8% and 3.9%, respectively, in 2014 [10]. Due to more travel activity and migration this figure is expected to increase. The few isolates of carbapenemase producing bacteria diagnosed in Norway were imported cases [17,18]. Molecular diagnostics of ESBL CTX-M, TEM, SHV and CARBA KPC, OXA-48, VIM, NDM genes for detection of ESBL and carbapenemase producing bacteria by real-time PCR is feasible by commercial tests and in-house methods [19,20], but is considered to be an expensive approach requiring both time, facilities and in many instances trained personnel. Additionally, PCR will only detect the known genes encoding most frequent enzymes, while resistance caused by other mechanisms (e.g. porin modification, efflux pump) will not be detected.

Development and evaluation of inexpensive, reliable and rapid methods for direct detection of ESBL and carbapenemase producing bacteria from positive blood cultures by using MALDI-TOF MS [21-26] warrants consideration. This approach is based on detection of cephalosporine or carbapenem degradation into hydrolyzed products. Establishment of software database for detection of specific peaks is necessary for this method, which then may provide results within 1 to 4 hours. The results obtained in these studies varied in relation to class of ESBL or carbapenemases detected and whether resistance was chromosomally or plasmid encoded, but were generally assessed promising. In the study performed by Mirande et al., the attempt to understand the kinetics of hydrolysis of antibiotics was done by monitoring the detection of hydrolysis of ertapenem and faropenem by using the VITEK MS RUO system during 24 hours. The authors presented an interesting discussion about strengths and weaknesses of previously described MALDI-TOF MS protocols with respect to applicability and reliability for work in routine microbiological laboratories [27].

Nordmann et al. [28] recently described a test for rapid diagnostic of ESBL-producing *Enterobacteriaceae* based on biochemical detection of hydrolysis of the β -lactam ring of cefotaxime. This test, named

Nordmann-Dortet-Poirel (NDP) test was recently evaluated by Dortet et al. [29] for rapid detection of ESBL-producing *Enterobacteriaceae* directly from blood cultures. The results of NDP testing compared with molecular diagnostic techniques have shown complete concordance and ESBL NDP test had excellent sensitivity and specificity of 100%. This inexpensive test is suitable for bench work and can be directly performed on positive blood cultures with results provided within 30 minutes. These encouraging results should be confirmed in other laboratories. The rapid test (CARBA-NP Assay) based on the similar biochemical principle was developed by the same group of authors for rapid detection of carbapenemase producing *Enterobacteriaceae* and evaluated also directly from blood cultures with sensitivity and specificity of 97.9% and 100% respectively [30]. However, these excellent results were not completely reproduced in the study performed by Chong et al. [31] where MALDI-TOF MS detection of carbapenemase activity in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter spp.* was compared against CARBA-NP-Assay. MALDI-TOF MS based diagnostic have shown better performance by detecting carbapenemase activity from 99% of tested isolates, while CARBA-NP assay detected activity from 85% of isolates. From the aspect of clinical microbiologists, both MALDI-TOF MS based diagnostics and CARBA-NP-Assay methods are considered attractive due to its rapidness, cost effectiveness and reliability.

The increase of antimicrobial resistance worldwide is worrying, and to improve patient care clinical microbiologists should emphasise the impact of rapid methods for identification and AST of bacteria causing BSI. The field of rapid methods for AST is gaining wide interest, and an array of future technologies, including microarray, nanotechnology and whole genome sequencing, are expected to contribute significantly [32]. In this report we have focused on the combined use of direct MALDI-TOF MS identification from positive blood cultures and rapid cost effective AST methods that can be readily implemented in routine laboratory work providing reliable results.

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