Rapid Identification and Multiple Susceptibility Testing of Pathogens from Positive-Culture Sterile Body Fluids by a Combined MALDI-TOF Mass Spectrometry and Vitek Susceptibility System

Yueru Tian¹ †, Bing Zheng² †, Bei Wang¹, Yong Lin¹* and Min Li²*

¹ Department of Laboratory Medicine, Shanghai Medical College, Huashan Hospital, Fudan University, Shanghai, China,
² Department of Laboratory Medicine, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

Infections of the bloodstream, central nervous system, peritoneum, joints, and other sterile areas are associated with high morbidity and sequelae risk. Timely initiation of effective antimicrobial therapy is crucial to improving patient prognosis. However, standard final identification and antimicrobial susceptibility tests (ASTs) are reported 16–48 h after a positive alert. For a rapid, effective and low-cost diagnosis, we combined matrix-assisted laser desorption/ionization time of flight mass spectrometry with a Vitek AST system, and performed rapid microbial identification (RMI) and rapid multiple AST (RMAST) on non-duplicated positive body fluid cultures collected from a hospital in Shanghai, China. Sterile body fluid positive culture and blood positive culture caused by Gram negative (GN) or polymicrobial were applied to the MALDI–TOF measurement directly. When positive blood culture caused by Gram positive (GP) bacteria or yeasts, they were resuspended in 1 ml brain heart infusion for 2 or 4 h enrichment, respectively. Regardless of enrichment, the RMI (completed in 40 min per sample) accurately identified GN and GP bacteria (98.9 and 87.2%, respectively), fungi (75.7%), and anaerobes (94.7%). Dominant species in multiple cultures and bacteria that failed to grow on the routing plates were correctly identified in 81.2 and 100% of cases, respectively. The category agreements of RMAST results, determined in the presence of various antibiotics, were similarly to previous studies. The RMI and RMAST results not only reduce the turnaround time of the patient report by 18–36 h, but also indicate whether a patient’s antibiotic treatment should be accelerated, ceased or de-escalated, and adjusted the essential drugs modification for an optimized therapy.

Keywords: MALDI-TOF mass spectrometry, Vitek AST system, sterile body fluids positive culture, rapid diagnosis, clinical impact

Abbreviations: A list of abbreviations is shown in Table S2 in Supplementary Material.
INTRODUCTION

Infections of the bloodstream, central nervous system, peritoneum, joints, and other sterile areas are associated with high morbidity and risk of sequelae (Thigpen et al., 2011; Goto and Al-Hasan, 2013; Chon et al., 2014; Ascione et al., 2015; Bagheri-Nesami et al., 2015). Among these, bloodstream infection (BSI) is most serious, because it can rapidly deteriorate into sepsis, severe sepsis, or septic shock. BSIs have become a major cause of death in European intensive care units, incurring a mortality rate of 30–50% (Vincent et al., 2006). To improve this prognosis, timely initiation of effective antimicrobial therapy is essential (Kumar et al., 2006; Vincent et al., 2006; Delling et al., 2013; Chon et al., 2014; Ascione et al., 2015; Bagheri-Nesami et al., 2015). Currently, culture remains the gold standard of infection diagnosis. However, standard final identification (ID) and ASTs are reported 16–48 h after a positive alert. During this delay, the clinician must administer an empirical antimicrobial therapy, typically a broad-spectrum antibiotic or an antibiotic cocktail to cover all likely pathogens. However, inappropriate antimicrobial therapy will worsen the outcome (Ibrahim et al., 2000; Tumbarello et al., 2010; Cain et al., 2015). Moreover, the long-term use of dispensable broad-spectrum antibiotics promotes antibiotic resistance and spread, increases cost and lengthens hospital stays (Blot et al., 2002; Tumbarello et al., 2010).

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI–TOF MS) promises a revolutionary breakthrough in clinical microbiology. The technology identifies bacteria within 6 min (Seng et al., 2009), with a species-level accuracy of 84.1–93.6% (Bizzini and Greub, 2010) and high sensitivity (∼10^5 CFU). Currently, MALDI-TOF MS has shown the ability to distinguish vanB-positive Enterococcus faecium from isolates that do not possess this resistance gene (Griffin et al., 2012) and demonstrate the carbapenemase activity by detection of meropenem and the relevant degradation products (Hrabak et al., 2011). Another study focused on a proteome analysis of ampicillin-resistant Fusobacterium nucleatum was reported (Al-Haroni et al., 2008). Moreover, MALDI-TOF MS fingerprinting has been used to distinguish the different expression levels of cell wall components between resistant isolates and sensitive isolates (Xu et al., 2006). Recently, RMI by MALDI-TOF MS has been adopted in various protocols, including the commercial Sepsityper kit (Bruker Daltonics, Bremen, Germany; Martiny et al., 2012; Hazelton et al., 2014; Idelevich et al., 2014; Martinez et al., 2014; Schieffer et al., 2014; Morgenthaler and Kostrzewa, 2015), serum separator tubes (Stevenson et al., 2010; Schubert et al., 2011) and in-house methods (Martiny et al., 2012). These techniques return the RMI from positive bottles within 30 min to a few hours. They also reliably identify species-level GN and GP bacteria (with accuracies of 90 and 76%, respectively) and yeasts (66%) (Morgenthaler and Kostrzewa, 2015). However, the Sepsityper kit is expensive, whereas the in-house method involves multi-step washing/centrifugation and yields relatively low RMI accuracy for GP bacteria and yeasts.

Rapid ASTs have been attempted in several studies. For instance, methicillin-resistant Staphylococci and vancomycin-resistant enterococci have been detected in real-time PCR–melt curve analysis (Chan et al., 2015). MALDI–TOF MS can detect subtle differences in isologenic Staphylococcus aureus, which determine the organism’s resistance to methicillin or teicoplanin (Majcherczyk et al., 2006), ampicillin susceptibility (Grundt et al., 2012), and carbapenemase (Hrabáková et al., 2012). All of the above methods are efficient; however, molecular methods cannot identify the expression of resistance genes, and other methods are limited to one or a few specific antibiotic determination profiles.

To overcome these difficulties, we determined the RMI and RMAST by a combined MALDI–TOF MS/Vitek AST system. Bacterial cells were directly extracted from positive sterile body fluid cultures in serum separator tubes, and proliferated in brain heart infusion broth (BHI) for 2–4 h.

This study aims to evaluate the reliability and accuracy of this protocol in fast pathogen diagnosis without additional costs and efforts.

MATERIALS AND METHODS

Location

This study was conducted in Huashan Hospital (affiliated with Fudan University), located in the center of Shanghai, China. Huashan Hospital is one of the largest (1300 beds) comprehensive teaching hospitals in China, handling ∼8000 admissions per day.

Clinical Samples

Sterile body fluids, including blood, cerebrospinal fluid (CSF), pleural fluid, ascitic fluid, pericardial effusion, joint cavity fluid, and vitreous fluid, were injected into blood culture bottles to improve the positive rate of clinical samples. From September 2014 to August 2015, we enrolled the first positive culture from each patient. Thus, we collected 485 non-duplicated positive cultures.

Blood Culture (BC) Bottles and BC System

The BC bottles were BacT/Alert aerobic/SA and anaerobic/SN (bioMérieux, Marcy l’Etoile, France), and BACTEC Plus Aerobic/F, BACTEC Plus Anaerobic/F and BACTEC Mycosis-IC/F (Becton Dickinson, USA; Figure S1). All bottles were incubated in automated BC systems (BacT/Alert 3D, bioMérieux and BACTEC FX, Becton Dickinson; Figure S1) until they tested positive. Positive cultures were analyzed immediately after a positive alert during laboratory hours (8 a.m. to 5 p.m.). Cultures that became positive later than this period were stored in the BC systems and analyzed the next morning.

Positive Cultures Processing for RMI Analysis and RMAST

Based on the Gram staining results, 5 ml (sterile body fluid positive culture and blood positive culture caused by GN or polymicrobial) or 10 ml (blood positive culture caused by other bacteria) were drawn into one or two serum separator tubes
Tian et al. Rapid Diagnosis of Sterile Body-Fluids (BD Vacutainer SSTII Advance, USA). Bacteria were pelleted by centrifugation at 4000 g for 10 min. The bacteria became aggregated at the surface of the polymeric gel, while the red-blood-cell component was sedimented beneath the gel layer. The supernatant was discarded and the bacterial pellet was gently resuspended in 1 ml sterile distilled water, without disrupting the gel layer. The suspension was transferred to a sterile Eppendorf tube, mixed thoroughly, and centrifuged at 16,000 g for 1 min. The supernatant was discarded and the washing/centrifugation steps were repeated once. The pellets were resuspended in 100 µl sterile distilled water. A portion of the resuspension was drawn and subjected to an ethanol/formic acid extraction procedure. The rest of the resuspension was prepared for 0.5 McFarland (McF) and subjected to RMAST as described for standard AST.

Prior to the ethanol/formic acid extraction, pellets of blood cultures caused by other bacteria (but not those caused by GN or polymicrobial) were resuspended in 1 ml BHI for enrichment. GP bacteria and yeasts were inoculated at 37°C with shaking at 200 rpm for 2 and 4 h, respectively. The enrichments were pelleted at 16,000 g for 1 min. After discarding the supernatant, the pellets were washed with 1 ml sterile distilled water, and re-centrifuged at 16,000 g for 1 min. Again, the supernatant was discarded.

In the ethanol/formic acid extraction procedure, a portion of the bacterial pellet was resuspended in 300 µl water by vortexing. The suspension was thoroughly mixed with 900 µl absolute ethanol and then centrifuged at 16,000 g for 1 min. The supernatant was discarded and the residual ethanol was removed after a repeat centrifugation. The cell pellet was air dried and dissolved in 30 µl of 70% formic acid by thorough vortexing. After adding 30 µl acetonitrile, the dissolved pellet was centrifuged at 16,000 g for 2 min and 1 µl supernatant was spotted onto a steel target plate for MALDI–TOF MS (Bruker Daltonics, Bremen, Germany) analysis (Figure 1).

**Standard Identification and Antimicrobial Susceptibility Testing**

The positive broths were sub-cultured onto routing plates of 5% sheep blood agar, chocolate, or anaerobic blood agar. Bacteria that failed to grow on the routing plates were inoculated on self-made plates composed of 20 ml sterile blood culture broth (bioMérieux, Marcy l’Etoile, France; Becton Dickinson, USA) and 3.9% agar powder (Oxoid, Thermo Fisher Scientific, England). Plates were grown in the incubator (Thermo Scientific Forma, USA) at 35°C in 5% CO₂ or an anaerobic atmosphere until visible colonies appeared. A pure bacterial colony was smeared onto a steel target plate for identification by MALDI–TOF MS. For yeasts, 1 µl of formic acid was added to the plate and air dried for 5 min.

In the antimicrobial susceptibility testing, Vitek cards AST-GN13, AST-Gp67, and AST-Gp68 were used for GN bacteria, staphylococci/enterococci and *Streptococcus pneumoniae*, respectively. Other streptococci and yeasts were tested with ATB-STREP5 and ATB-FUNGS3 (bioMérieux, Marcy l’Etoile, France), respectively. The GN bacteria, staphylococci, enterococci, streptococci, or yeast pellets were dissolved in 0.45% saline solution to prepare 0.5–0.63 McF or 2 McF suspension, respectively. The Vitek card AST-GN13 or AST-Gp67/AST-Gp68 were filled with suspension composed of 3 ml 0.45% saline solution and 145 or 280 µl 0.5–0.63 McF suspension, respectively. The ATB-STREP5 strip was filled with suspension composed of ATB S medium and 200 µl 0.5 McF suspension. The ATB-FUNGS3 strip was filled with suspension composed of ATB F2 medium and 20 µl 2McF suspension.

**FIGURE 1 | Workflow of positive culture processing for RMI/RMAST analysis.**

Gram staining: 5 ml sterile body fluid positive culture or blood positive culture caused by GN or polymicrobial was drawn into the serum separator tube.

Centrifuged at 4000g for 10min.

Pellet was washed in 1ml sterile water. Centrifuged at 16000g for 1 min and discarded the supernatant.

Repeated the washing/centrifugation step.

Blood positive culture caused by GP bacteria was inoculated in 1ml BHI for 2 h enrichment.

Blood positive culture caused by yeast was inoculated in 1ml BHI for 4 h enrichment.

The enrichments were centrifuged at 16000g for 1 min and discarded the supernatant.

Repeated the washing/centrifugation step.

RMI/RMAST analysis

Frontiers in Microbiology | www.frontiersin.org 3 April 2016 | Volume 7 | Article 523
Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry

The spot was overlaid with $1 \mu l$ MALDI matrix (a saturated solution of $\alpha$-cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid). After drying at room temperature for 5 min, sample was subjected to analysis of the bacteria protein using MALDI-TOF MS system. The spectrum was obtained in linear positive-ion mode range from 2000 to 20 000 Da. Each spot was measured manually on five different positions by using 1000 laser shots at 25 Hz in groups of 40 shots. The MALDI Bruker Biotyper 3.0 software and library (Bruker Daltonics) were used for spectra analysis. According to the spectra matching and score criterion, MALDI Bruker Biotyper 3.0 software obtained appropriate scores and identification results. The workflow was shown in Figure 2A.

Interpretation of RMI and RMAST

RMI results were scored by the manufacturer’s standard criterion (cut-off values of 1.7 and 2.0 for acceptable identification to the genus and species levels, respectively) and by the modified criterion (cut-off values of 1.5 and 1.8 for at least three identical results in the list at the genus and species levels, respectively). These criteria have been described in previous studies (Schmidt et al., 2012; Machen et al., 2014; Martinez et al., 2014; Morgenthaler and Kostrzewa, 2015). The RMI results were compared with the final reports. Non-identifiable samples included correctly identified samples with scores below the genus level cut-off value and samples that presented no peak or a very weak signal. Discordant identification included samples that the RMI results were inconsistent with the final reports which proved correct. Discrepancies were resolved by 16S rRNA (for bacteria; primer F, 5′-AGAGTTTGATGATGGCTCAG-3′; primer R, 5′-ACCAGGACTGCTTGGCAC-3′; expected PCR product size: ~800 bp) or 18S rRNA (for fungi; primer F, 5′-GATACGTCGAGTAGCTT-3′; primer R, 5′-ATTCTCATTGGAAGACG-3′; expected PCR product size: ~800 bp) amplification. DNA was extracted from colonies sub-cultured for 24 h using Genomic DNA isolation kit (Sangon Biotech, Shanghai, China). Thermal cycler conditions were 94°C for 5 min, followed by 35 cycles of 94°C (30 s), 55°C (30 s), and 72°C (30 s), with a final extension at 72°C (7 min).

RMAST results were compared with those obtained from the standard method. The minimum inhibitory concentrations (MIC) obtained by both methods were translated into clinical

![Figure 2A](image.png)

**FIGURE 2 | (A)** The RMI workflow of MALDI-TOF MS system. **(B)** RMI spectra of representative bacterial species.
categories (susceptible, intermediate, resistant), following the CLSI recommendations. The comparison between the direct and standard inoculation methods was categorized as agreement, very major error (VME, false susceptibility), major error (ME, false resistance), or minor error (mE, susceptible/resistant versus intermediate susceptibility). Discrepancies in MICs were resolved by broth dilution methods according to the CLSI (2015) guidelines.

**Evaluation of Clinical Relevance**

To prevent the development of resistance, to reduce toxicity, and to reduce costs, the antimicrobial regimen should be ceased when detected typical contaminants (Monoculture grew Coagulase-negative *staphylococcus* among multiple cultures, *Micrococcus* spp, *Corynebacterium* spp, *Bacillus* spp, *Propionibacterium acnes*) and assessed for potential de-escalation (De-escalation refers to narrow the spectrum of antimicrobial coverage and choose the most appropriate single-agent therapy). Antibiotic therapy should be installed when detected pathogenic species that cannot be distinguished by Gram staining and modified when detected intrinsic resistant bacteria (Bacteria were intrinsic antibiotic-resistance. *Stenotrophomonas maltophilia*, *Enterococcus casseliflavus*, and *Candida glabrata* are intrinsic resistant to carbapenem, vancomycin, and fluconazole, respectively) or acquired resistant bacteria (Antibiotic-resistance was mediated by plasmid, resistant enzymes, or other resistance mechanisms). RMI/RMAST results were reported to the clinician. To assess the impact of our approach on the management of sterile body infections, we determined whether the results can instructively recommend an optimal therapeutic scheme. Unreliable results were excluded. Indications leading to a treatment change are listed in Table 1.

**Statistical Analysis**

Statistical time comparisons were conducted by a Wilcoxon signed-rank test (GraphPad Prism 5.0, CA, USA). \( P < 0.05 \) was considered statistically significant. All statistical tests were two-tailed.

**RESULTS**

**Rapid Microorganism Identification**

Regardless of enrichment, the RMI test of each sample was completed in 40 min (15 min for the pellet collection/washing/centrifugation steps, 15 min for the extraction procedure, 5 min for the sample spotting/drying steps, 5 min for MALDI–TOF MS measurement). The RMI was evaluated in 485 non-duplicated positive cultures

| TABLE 1 | Indications for optimized antibiotic therapy and recommendations based on RMI /RMAST results. |
|-----------------|---------------------------------------------------------------|
| **Cessation of antibiotic therapy** | Detection of typical contaminants: |
| RMI result (0.58–4.58 h) | * Monoculture grew Coagulase-negative *staphylococcus* among multiple cultures |
| | * Micrococcus spp. |
| | * Corynebacterium spp. |
| | * Bacillus spp. |
| | * Propionibacterium acnes |
| **Installation of antibiotic therapy** | Detection of pathogenic species that cannot be distinguished by Gram staining: |
| RMAST result (8.4–34 h) | * Staphylococcus aureus |
| | * Staphylococcus lugdunensis |
| | * Listeria monocytogenes |
| **Modification of antibiotic therapy** | Detection of intrinsic resistant bacteria: |
| | * Stenotrophomonas maltophilia with carbapenem resistance |
| | * Enterococcus casseliflavus with vancomycin resistance |
| | * Candida glabrata with fluconazole resistance |
| **De-escalation antibiotic therapy** | De-escalation of broad spectrum antibiotics or last-resort antibiotics (carbapenems, vancomycin, linezolid) or combination therapy in case of results indicates low-risk intrinsic resistant bacteria (e.g., *Escherichia coli*, *Staphylococci*) associated with negative acquired antibiotic-resistant history. |
| **Modification of antibiotic therapy** | Detection of acquired resistant bacteria (e.g., carbapenem resistant GN bacteria, vancomycin, or linezolid non-susceptible *Enterococci* and *Staphylococci*). |
The compositions of the positive cultures are listed in Table S1.

The RMI spectra of representative bacterial species were shown in Figure 2B. Scored by the standard criterion, the correct identification rates of RMI were 96.3% for GN bacteria (182/189), 82.8% for GP bacteria (168/203), 11.8% for fastidious bacteria (2/17), 62.2% for fungi (23/37), and 89.5% for anaerobic bacteria (17/19). In cultures of polymicrobial, the dominant bacterium was correctly identified in 81.2% (13/16) of cases and bacteria that failed to grow on the routing plates were identified with a success rate of 50.0% (2/4). Scored by the modified criterion, the correct identification rates of RMI were 98.9% (187/189) for GN bacteria, 87.2% (177/203) for GP bacteria, 11.8% (2/17) for fastidious bacteria, 75.7% (28/37) for fungi, and 94.7% (18/19) for anaerobic bacteria (18/19). In cultures containing polymicrobial and bacteria that failed to grow on the routing plates, the correct identification rates were 81.2% (13/16; dominant species) and 100.0% (4/4), respectively. The modified criterion provided more accurate RMI scores than the standard criterion, with no relevant misidentification at the genus level (Tables 2, 3). The RMI accuracy was lower in blood samples than in other sterile body fluids (Table S1). The mean RMI scores and the RMI results of polymicrobial are listed in Tables 2, 3, respectively.

Rapid Antimicrobial Susceptibility Test

Among the 320 non-duplicated positive cultures, 140 GN bacteria, 105 Staphylococcus spp., 28 Enterococcus spp., 14 Streptococcus spp., 33 fungi, and 3349 bacterial/antimicrobial combinations, were analyzed in the RMAST investigation (Table 4).

The RMAST was actually performed on 142 GN bacteria (109 Enterobacteriaceae, 14 Acinetobacter spp., and 19 non-fermentative bacteria), but two cultures (1 Acinetobacter junii, 1 Brevundimonas diminuta) grew poorly so were excluded from the analysis. In total, 1828 bacterial/antimicrobial combinations were analyzed with a category agreement of 96.77%, a mE rate of 2.52%, an ME rate of 0.22%, and a VME rate of 0.49%. Errors were found in Escherichia coli (1.09% mE, 0.05% ME, 0.27% VME), Klebsiella pneumoniae (0.66% mE, 0.05% ME, 0.71% VME), Enterobacter cloacae (0.22% mE), Enterobacter aerogenes (0.05% mE), Morganella morganii (0.16% mE), Raoultella ornithinolytica (0.11% mE), Providencia rettgeri (0.05% mE), Pseudomonas aeruginosa (0.22% mE, 0.05% ME, 0.11% VME), and other non-fermenters (0.22% mE, 0.11% ME, 0.05% VME).

Although, RMAST results were available for all 28 Enterococcus spp., they were available for only 105 of the 114 Staphylococcus spp.; 10 cases failed because of poor growth (4 Staphylococcus epidermidis, 2 S. hominis, 1 S. capitis, 1 S. auricularis, 1 S. simulans, and 1 S. caprae). In total, 1246 bacterial/antimicrobial combinations were analyzed with a category agreement of 93.50%, an mE rate of 3.69%, an ME rate of 0.72%, and a VME rate of 2.09%. Errors were found in S. aureus (0.32% mE, 0.16% VME), Coagulase-negative staphylococcus (1.28% mE, 0.56% ME, 1.76% VME), and Enterococci spp. (0.32% mE, 0.16% ME, 0.16% VME).

RMAST was originally performed on 21 Streptococcus spp., including 5 S. pneumoniae, 3 S. agalactiae, 1 S. pyogenes, and 12 S. viridans, but 7 of the S. viridans failed because of poor growth, yielding 14 RMAST results. In total, 140 bacterial/antimicrobial combinations were analyzed with a category agreement of 98.57%, an ME rate of 0.71%, and a VME rate of 0.71%.

RMAST results were available for 33 of the 37 fungal isolates (7 Candida albicans, 6 C. tropicalis, 3 C. parapsilosis, 3 C. glabrata, 1 C. lusitaniae, 1 Trichosporon asahii, 15 Cryptococcus neoformans, and 1 C. albidos). Four cases (1 C. albicans, 1 C. parapsilosis, 1 C. glabrata, and 1 Cryptococcus albidos) grew poorly and were thus excluded. In total, 90 bacterial/antimicrobial combinations were analyzed with a category agreement of 95.56%, an mE rate of 3.33% and a VME rate of 1.11% in the yeast group. Forty-five bacterial/antimicrobial combinations were also analyzed with a category agreement of 100% in the C. neoformans group.

Forty-three multi-drug resistant bacteria were detected in this study, including 1 vancomycin-intermediate Staphylococcus epidermidis, 1 vancomycin-resistant Enterococcus faecium, 1 linezolid-resistant S. aureus, 8 linezolid-resistant S. capitis, 16 carbapenem non-susceptible Enterobacteriaceae, 8 carbapenem non-susceptible Acinetobacter spp., and 6 carbapenem non-susceptible non-fermenting spp. The RMAST category agreement of multi-drug resistant bacteria was 97.68%, with 1 mE in carbapenem non-susceptible Enterobacteriaceae.

Time to Identification and Antimicrobial Susceptibility Testing

The average times of the RMI (vs. final identification report) were 0.58 h for GN, polymicrobial and sterile body fluid bacteria (vs. 18.1 h), 2.58 h for GP bacteria (vs. 18.1 h), 3.53 h for fungi (0.58 h in sterile body fluid and 4.58 h in blood, vs. 32.2 h). 1.36 h for anaerobic bacteria (0.58 h for GN and 2.58 h for GP bacteria, vs. 27.8 h), and 2.5 h for bacteria that failed to grow on the routing plates (vs. 64 h). The results are presented in Figure 3.

The average times of RMAST (vs. the conventional method) were 8.4 ± 2.6 h for GN bacteria (vs. 26.4 ± 2.6 h; p < 0.0001), 13.1 ± 3.0 h for GP bacteria (vs. 31.1 ± 3.0 h; p < 0.0001), 34 ± 12 h for fungi (vs. 58 ± 12 h; p < 0.0001; Figure 3).

Analysis of Clinical Relevance

Among 485 RMI cases (Figure 4), 14.85% of the results indicated that clinicians should adjust their treatment. Specifically, antibiotic therapy should be ceased in 3.71% of patients, initiated or modifying (13.44%) the antibiotic therapy.

This study incorporated a combined MALDI–TOF MS/Vitek AST system into the clinical microbiology laboratory workflow.
| Species                        | Standard cut-off values | Modified cut-off values | Not identified | Discordant ID | Mean scores |
|-------------------------------|-------------------------|-------------------------|----------------|---------------|-------------|
|                               | n (%)                   | species                | genus          | n (%)         | species     | genus         | (min-max) |
|                               |                        |                        |                |               |             |               |           |
|                               | Correct ID             | Not identified         | Discordan ID   | Correct ID    | Not identified | Discordant ID |
|                               |                        |                        |                |               |               |               |           |
| AEROBIC GN BACTERIA           |                         |                        |                |               |             |               |           |
| Enterobacteriaceae            |                         |                        |                |               |             |               |           |
| Escherichia coli              | 63 (100)                | 55 (87.3)              | 8 (12.7)       | 0             | 0            | 63 (100)      | 57 (90.5)  | 6 (9.5)    | 0 | 2.097 (1.712–2.417) |
| Klebsiella pneumoniae         | 38 (100)                | 33 (86.8)              | 5 (13.2)       | 0             | 0            | 38 (100)      | 37 (97.4)  | 1 (2.6)    | 0 | 2.076 (1.799–2.380) |
| Klebsiella oxytoca            | 1 (100)                 | 1 (100)                | 0              | 0             | 1 (100)      | 1 (100)       | 0            | 0           | 0 | 2.261 |
| Enterobacter cloacae          | 7 (100)                 | 4 (57.1)               | 3 (42.9)       | 0             | 0            | 7 (100)       | 5 (71.4)   | 2 (28.6)   | 0 | 1.952 (1.762–2.067) |
| Enterobacter aerogenes        | 1 (100)                 | 1 (100)                | 0              | 0             | 1 (100)      | 1 (100)       | 0            | 0           | 0 | 2.183 |
| Salmonella                    | 4 (100)                 | 2 (50.0)               | 2 (50.0)       | 0             | 0            | 4 (100)       | 2 (50.0)   | 2 (50.0)   | 0 | 1.928 (1.733–2.154) |
| Morganella morganii           | 3 (100)                 | 3 (100)                | 0              | 0             | 3 (100)      | 3 (100)       | 0            | 0           | 0 | 2.217 (2.059–2.415) |
| Proteus mirabilis             | 3 (100)                 | 2 (66.7)               | 1 (33.3)       | 0             | 0            | 3 (100)       | 3 (100)    | 0          | 0 | 2.079 (1.828–2.233) |
| Proteus vulgaris              | 2 (100)                 | 1 (50.0)               | 1 (50.0)       | 0             | 0            | 2 (100)       | 1 (50.0)   | 1 (50.0)   | 0 | 1.901 (1.787–2.015) |
| Salmonella                    | 2 (100)                 | 0                      | 2 (100)        | 0             | 0            | 2 (100)       | 1 (50.0)   | 1 (50.0)   | 0 | 1.752 (1.703–1.800) |
| Pantoea                       | 2 (100)                 | 1 (50.0)               | 1 (50.0)       | 0             | 0            | 2 (100)       | 2 (100)    | 0          | 0 | 2.079 (1.828–2.233) |
| Raoultella ornithinolytica    | 2 (100)                 | 1 (50.0)               | 1 (50.0)       | 0             | 0            | 2 (100)       | 1 (50.0)   | 1 (50.0)   | 0 | 2.083 (1.794–2.372) |
| Providencia rettgeri          | 2 (100)                 | 0                      | 2 (100)        | 0             | 0            | 2 (100)       | 0          | 2 (100)    | 0 | 1.720 (1.712–1.727) |
| Providencia stuartii          | 1 (100)                 | 0                      | 1 (100)        | 0             | 1 (100)      | 1 (100)       | 0            | 0           | 0 | 1.890 |
| Citrobacter freundii          | 2 (100)                 | 2 (100)                | 0              | 0             | 2 (100)      | 2 (100)       | 0            | 0           | 0 | 2.044 (2.001–2.087) |
| Citrobacter braakii           | 1 (100)                 | 1 (100)                | 0              | 0             | 1 (100)      | 1 (100)       | 0            | 0           | 0 | 1.924 |
| Subtotal                      | 134 (100)               | 106 (79.1)             | 28 (20.9)      | 0             | 0            | 134 (100)     | 118 (88.1) | 16 (11.9)  | 0 | 0 |
| ACINETOBACTER SPP             |                         |                        |                |               |             |               |           |           |
| Acinetobacter baumannii       | 21 (100)                | 17 (81.0)              | 4 (19.0)       | 0             | 0            | 21 (100)      | 18 (85.7)  | 3 (14.3)   | 0 | 2.054 (1.738–2.237) |
| Acinetobacter junii           | 3 (100)                 | 2 (66.7)               | 1 (33.3)       | 0             | 0            | 3 (100)       | 2 (66.7)   | 1 (33.3)   | 0 | 1.947 (1.720–2.067) |
| Acinetobacter haemolyticus    | 3 (100)                 | 0                      | 3 (100)        | 0             | 0            | 3 (100)       | 2 (66.7)   | 1 (33.3)   | 0 | 1.755 (1.702–1.817) |
| Acinetobacter pittii          | 1 (100)                 | 0                      | 1 (100)        | 0             | 0            | 1 (100)       | 1 (100)    | 0          | 0 | 1.826 |
| Subtotal                      | 28 (100)                | 19 (67.9)              | 9 (32.1)       | 0             | 0            | 28 (100)      | 23 (82.1)  | 5 (17.9)   | 0 | 0 |
| NON-FERMENTERS                |                         |                        |                |               |             |               |           |           |
| Pseudomonas aeruginosa        | 12 (100)                | 8 (66.7)               | 4 (33.3)       | 0             | 0            | 12 (100)      | 10 (83.3)  | 2 (16.7)   | 0 | 1.999 (1.701–2.214) |
| Stenotrophomonas              | 5 (20.0)                | 0                      | 1 (20.0)       | 4 (80.0)      | 0             | 5 (20.0)      | 3 (60.0)   | 1 (20.0)   | 1 (20.0) | N/A |
| Pseudomonas fulva             | 1 (100)                 | 0                      | 1 (100)        | 0             | 0             | 1 (100)       | 1 (100)    | 0          | 0 | 1.994 |
| Myroides odoratimus           | 1 (100)                 | 1 (100)                | 0              | 0             | 1 (100)      | 1 (100)       | 0            | 0           | 0 | 2.266 |
| Pseudomonas oryzihabitans     | 1 (100)                 | 0                      | 1 (100)        | 0             | 0             | 1 (100)       | 1 (100)    | 0          | 0 | 1.878 |

(Continued)
| Species | Standard cut-off values | Modified cut-off values | Not identified | Discordant ID | Mean scores | N/A |
|---------|--------------------------|-------------------------|----------------|---------------|-------------|-----|
|         | No ID | Correct ID | Not identified | No ID | Correct ID | (min-max) |
|---------|-------|------------|----------------|-------|------------|-----------|
| ID     | species | genus | Score ≥ 2.0 | Score ≥ 1.7 | Score ≥ 1.8 | Score ≥ 1.5 | |

**Brevendimonas diminuta**  
1 (0) 0 0 1 (100) 0 0 1 (100) 0 N/A

**Aeromonas hydrophila**  
2 (100) 1 (50.0) 1 (50.0) 0 0 2 (100) 2 (100) 0 0 1.986 (1.927–2.045)

**Aeromonas caviae**  
1 (0) 0 0 1 (100) 0 1 (100) 0 1 (100) 0 0 1.682

**Aeromonas veronii**  
1 (100) 0 1 (100) 0 0 1 (100) 0 1 (100) 0 0 1.765

**Methylobacterium**  
1 (0) 0 0 1 (100) 0 1 (100) 0 1 (100) 0 0 1.623

**Vibrio cholerae**  
1 (100) 0 1 (100) 0 0 1 (100) 1 (100) 0 0 0 1.876

**Subtotal**  
27 (74.0) 10 (37.0) 10 (37.0) 7 (26.0) 0 27 (92.6) 19 (70.4) 6 (22.2) 2 (7.4) 0

**Total**  
189 (96.3) 135 (71.4) 47 (24.9) 7 (3.7) 0 189 (98.9) 160 (84.7) 27 (16.9) 2 (1.1) 0

**AEROBIC GP BACTERIA**

**Staphylococci**

**Staphylococcus aureus**  
41 (100) 19 (46.3) 22 (53.7) 0 0 41 (100) 31 (75.6) 10 (24.4) 0 0 1.989 (1.700–2.410)

**Staphylococcus epidermidis**  
29 (82.8) 6 (20.7) 18 (62.1) 3 (10.3) 2 (6.9) 29 (86.2) 14 (48.3) 11 (37.9) 2 (6.9) 2 (6.9) 1.850 (1.658–2.098)

**Staphylococcus capitis**  
28 (85.7) 6 (21.4) 18 (64.3) 2 (6.9) 2 (6.9) 28 (89.3) 12 (42.6) 13 (46.4) 1 (3.8) 2 (6.9) 1.865 (1.616–2.159)

**Staphylococcus hominis**  
23 (82.6) 4 (17.4) 15 (65.2) 2 (8.7) 2 (8.7) 23 (82.6) 9 (33.3) 10 (43.5) 2 (8.7) 2 (8.7) 1.857 (1.703–2.195)

**Staphylococcus haemolyticus**  
9 (88.9) 0 8 (88.9) 1 (11.1) 0 9 (88.9) 0 8 (88.9) 1 (11.1) 0 1.748 (1.701–1.862)

**Staphylococcus cohnii**  
3 (66.7) 0 2 (66.7) 1 (33.3) 0 3 (66.7) 0 2 (66.7) 1 (33.3) 0 1.769 (1.741–1.797)

**Staphylococcus warneri**  
1 (100) 0 1 (100) 0 0 1 (100) 1 (100) 0 0 0 1.934

**Staphylococcus xylosus**  
1 (100) 1 (100) 0 0 0 1 (100) 1 (100) 0 0 0 2.068

**Staphylococcus saprophyticus**  
1 (100) 0 1 (100) 0 0 1 (100) 1 (100) 0 0 0 1.920

**Staphylococcus aureus**  
1 (100) 0 1 (100) 0 0 1 (100) 1 (100) 0 0 0 1.912

**Staphylococcus simulans**  
1 (100) 0 1 (100) 0 0 1 (100) 1 (100) 0 0 0 1.819

**Staphylococcus caprae**  
1 (0) 0 1 (100) 0 0 1 (100) 0 1 (100) 0 0 1.857 (1.703–2.195)

**Subtotal**  
139 (88.5) 36 (25.9) 87 (62.6) 10 (7.2) 6 (4.3) 139 (89.9) 71 (51.1) 54 (38.8) 8 (5.8) 6 (4.3) N/A

**ENTEROCOCCI**

**Enterococcus faecium**  
13 (84.6) 2 (15.4) 9 (69.2) 1 (7.7) 1 (7.7) 13 (82.3) 8 (61.5) 4 (30.8) 0 1 (7.7) 1.856 (1.606–2.113)

**Enterococcus faecalis**  
10 (100) 4 (40.0) 6 (60.0) 0 0 10 (100) 8 (80.0) 2 (20.0) 0 0 1.926 (1.742–2.186)

**Enterococcus casseliflavus**  
4 (100) 3 (75.0) 1 (25.0) 0 0 4 (100) 3 (75.0) 1 (25.0) 0 0 1.989 (1.710–2.097)

**Enterococcus avium**  
3 (86.7) 0 2 (66.7) 1 (33.3) 0 3 (86.7) 0 2 (66.7) 1 (33.3) 0 1.778 (1.760–1.795)

**Subtotal**  
30 (90.0) 9 (30.0) 18 (60.0) 2 (6.7) 1 (3.3) 30 (90.3) 19 (63.3) 9 (30.0) 1 (3.3) 1 (3.3) (Continued)
| Species                              | No ID | Correct ID | Not identified | Modified cut-off values | Not identified | Discordant ID | Mean scores |
|--------------------------------------|-------|------------|----------------|--------------------------|----------------|---------------|-------------|
|                                      | n (%) | species    | genus          |                           | species        | genus         | (min–max)    |
|                                      |       | Score ≥ 2.0| Score ≥ 1.7    |                           | Score ≥ 1.8    | Score ≥ 1.5  |             |
| **STREPTOCOCCUS**                    |       |            |                |                           |                |               |             |
| Streptococcus pneumoniae             | 5 (40.0) | 0 | 2 (40.0) | 2 (40.0) | 1 (20.0) | 5 (60.0) | 2 (40.0) | 1 (20.0) | 1 (20.0) | N/A |
| Streptococcus agalactiae             | 3 (100) | 0 | 3 (100) | 0 | 0 | 3 (100) | 3 (100) | 0 | 0 | 0 | 1.898 (1.870-1.934) |
| Streptococcus pyogenes               | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (0) | 0 | 0 | 1 (100) | 0 | N/A |
| Streptococcus salivarius             | 5 (60.0) | 1 (20.0) | 2 (40.0) | 2 (40.0) | 0 | 5 (80.0) | 1 (20) | 3 (60.0) | 1 (20) | 0 | 1.862 (1.726-2.088) |
| Streptococcus sanguis               | 2 (0) | 0 | 2 (100) | 0 | 0 | 2 (100) | 0 | 2 (100) | 0 | 0 | 1.729 (1.702-1.755) |
| Streptococcus mitis               | 2 (100) | 0 | 2 (100) | 0 | 0 | 2 (100) | 0 | 2 (100) | 0 | 0 | N/A |
| Streptococcus gordonii             | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 1 (100) | 0 | 1 (100) | 0 | N/A |
| Streptococcus oralis               | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| Streptococcus massiliensis         | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| **OTHER GP BACTERIA**               |       |            |                |                           |                |               |             |
| Listeria monocytogenes            | 4 (100) | 4 (100) | 0 | 0 | 0 | 4 (100) | 4 (100) | 0 | 0 | 0 | 2.125 (2.078-2.168) |
| Micrococcus spp                  | 2 (50.0) | 0 | 1 (50.0) | 0 | 0 | 1 (50.0) | 2 (50.0) | 0 | 1 (50.0) | 0 | 1.773 |
| Corynebacterium spp              | 6 (16.7) | 0 | 1 (16.7) | 5 (83.3) | 0 | 6 (50.0) | 0 | 3 (50.0) | 3 (50.0) | 0 | N/A |
| Bacillus amyloydidefaciens        | 1 (100) | 1 (100) | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 0 | 2.083 |
| **FASTIDIOUS BACTERIA**            |       |            |                |                           |                |               |             |
| Haemophilus influenzae             | 2 (50.0) | 0 | 1 (50.0) | 1 (50.0) | 0 | 2 (50.0) | 1 (50.0) | 0 | 1 (50.0) | 0 | 1.888 |
| Erysipelothrix rhusiopathiae       | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | 1.795 |
| Actinobacillus                    | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | N/A |
| Actinomyces pyogenes               | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | N/A |
| Actinomyces neuii                  | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (0) | 0 | 0 | 1 (100) | 0 | N/A |
| Actinomyces meyeri                 | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (0) | 0 | 0 | 1 (100) | 0 | N/A |
| Nocardia spp                      | 4 (0) | 0 | 4 (100) | 0 | 0 | 4 (100) | 0 | 0 | 4 (100) | 0 | N/A |
| Streptomyces                       | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| Granulicatella adiacens           | 2 (0) | 0 | 2 (100) | 0 | 0 | 2 (100) | 0 | 2 (100) | 0 | 0 | N/A |
| Moraxella spp                     | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| Cardiobacterium hominis           | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| Lactobacillus                     | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| Microbacterium liquefaciens       | 1 (0) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | N/A |
| **Total**                          | 17 (11.8) | 0 (0.0) | 2 (11.8) | 15 (88.2) | 0 | 17 (11.8) | 1 (5.9) | 1 (5.9) | 15 (88.2) | 0 | (Continued) |
| Species                        | Standard cut-off values | Modified cut-off values | Not identified | Discardant ID | Mean scores (min-max) |
|-------------------------------|-------------------------|-------------------------|----------------|---------------|---------------------|
|                               | No ID | Correct ID | Not identified | Discardant ID |                     |
|                               | n (%) | species | genus | Score ≥ 2.0 | Score ≥ 1.7 | n (%) | species | genus | Score ≥ 1.8 | Score ≥ 1.5 |                     |
| **FUNGI**                     |       |         |       |            |            |       |         |       |            |            |                     |
| Candida albicans              | 7 (42.9) | 2 (28.6) | 1 (14.3) | 3 (42.8) | 1 (14.3) | 7 (71.4) | 3 (42.9) | 2 (28.6) | 1 (14.3) | N/A |
| Candida tropicalis            | 6 (50.0) | 0 | 3 (50.0) | 2 (33.3) | 1 (16.7) | 6 (83.3) | 3 (60.0) | 2 (33.3) | 0 | 1 (16.7) | 1.808 (1.696-1.884) |
| Candida glabrata              | 3 (33.3) | 0 | 1 (33.3) | 1 (33.3) | 1 (33.3) | 3 (33.3) | 0 | 1 (33.3) | 1 (33.3) | N/A |
| Candida parapsilosis          | 3 (33.3) | 0 | 1 (33.3) | 2 (66.7) | 0 | 3 (66.7) | 1 (33.3) | 1 (33.3) | 0 | N/A |
| Candida lusitaniae            | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | N/A |
| Cryptococcus neoformans       | 15 (86.7) | 9 (60.0) | 4 (26.7) | 1 (6.7) | 1 (6.7) | 15 (86.7) | 12 (80.0) | 1 (6.7) | 1 (6.7) | N/A |
| Cryptococcus albidos          | 1 (0) | 0 | 0 | 1 (100) | 0 | 1 (0) | 0 | 0 | 1 (100) | 0 |
| Trichosporon asahii           | 1 (100) | 1 (100) | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 2.029 |
| **ANAEROBIC BACTERIA**        |       |         |       |            |            |       |         |       |            |            |                     |
| Bacteroides fragilis          | 9 (100) | 5 (55.6) | 4 (44.4) | 0 | 0 | 9 (100) | 9 (100) | 0 | 0 | 0 | 2.016 (1.820-2.283) |
| Propionibacterium acnes       | 3 (100) | 2 (66.7) | 1 (33.3) | 0 | 0 | 3 (100) | 2 (66.7) | 1 (33.3) | 0 | N/A |
| Fusobacterium necrophorum     | 2 (50.0) | 0 | 1 (50.0) | 1 (50.0) | 0 | 2 (50.0) | 0 | 1 (50.0) | 1 (50.0) | 1.798 |
| Bacteroides uniformis         | 1 (100) | 1 (100) | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | N/A |
| Veillonella                   | 1 (100) | 1 (100) | 0 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 2.038 |
| Staphylococcus saccharolyticus| 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 1.828 |
| Prevotella bivia              | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 1.894 |
| Campylobacter fetus           | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 1 (100) | 0 | 0 | N/A |
| Polymicrobial                 | 19 (89.5) | 9 (47.4) | 8 (42.1) | 2 (10.5) | 0 | 19 (94.7) | 15 (78.9) | 3 (15.8) | 1 (5.3) | 0 | 1.924 (1.703-2.241) |
| **BACTERIA GROW IN THE SPECIAL MEDIUM** |       |         |       |            |            |       |         |       |            |            |                     |
| Methylobacterium              | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 1 (100) | 0 | 0 | 1.588 |
| Streptococcus salivarius      | 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 1.573 |
| thermophilus                  |       |         |       |            |            |       |         |       |            |            |                     |
| Staphylococcus saccharolyticus| 1 (100) | 0 | 1 (100) | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 | 1.828 |
| Campylobacter fetus           | 1 (100) | 0 | 0 | 1 (100) | 0 | 1 (100) | 0 | 1 (100) | 0 | N/A |
| Total                         | 4 (50.0) | 0 (0.0) | 2 (50.0) | 2 (50.0) | 0 | 4 (100) | 2 (50.0) | 2 (50.0) | 0 (0.0) | 0 |                     |

FID, final identification; FAST, final AST; N/A, not available.
The aim was to provide clinicians with fast and accurate identification results and multiple precise MIC values for optimizing the therapeutic regimen.

Numerous strategies can identify RMI pathogens from positive blood samples by MALDI-TOF MS (Hazelton et al., 2014; Idelevich et al., 2014; Martinez et al., 2014; Schieffer et al., 2014; Chan et al., 2015; Morgenthaler and Kostrzewa, 2015). The RMI accuracy of GN and GP bacteria or yeasts identified by these strategies has been widely reported, but the investigated species were mostly single. Comprehensive analyses are relatively rare. In this study, we collected 485 non-duplicated positive cultures from blood, csf, ascitic fluid, vitreous fluid, and eye tissues samples, and initiated by GN and GP bacteria, streptococci, fungi, fastidious bacteria, anaerobic bacteria, polymicrobial, and bacteria that failed to grow on the routing plates, and systematically evaluated the accuracy and feasibility of RMI. Data showed the RMI accuracies of CSF, ascitic fluid, vitreous fluid, and eye tissues (almost 100%) were obviously higher than blood sample (57.1–100%), especially for CSF, ascitic fluid, vitreous fluid, and eye tissues samples which might attribute to the blood protein interference. Moreover, the correct RMI rate of GN and GP bacteria reached 98.9 and 87.2%, respectively, similar to the results of previous studies (Hazelton et al., 2014; Schieffer et al., 2014), whereas that of fungi (75.7%) was slightly higher than in previous studies (Idelévich et al., 2014; Machen et al., 2014). Unlike the former methods, the Vitek AST system could provide sufficient precise MIC values for the targeted species. The obtained data were relatively scarce and unable to provide accurate MIC values for clinicians (Idelevich et al., 2014; Machen et al., 2014). Unlike theformer methods, the Vitek AST system was able to prevent hospital infections.

A variety of studies have focused on the resistance determinants and activities of resistant enzymes (Majcherczyk et al., 2006; Grundt et al., 2012; Hrabáková et al., 2012; Chan et al., 2015), but the obtained data were relatively scarce and unable to provide accurate MIC values for clinicians (Idelevich et al., 2014; Machen et al., 2014). Unlike the former methods, the Vitek AST system is highly sensitive and well-standardized. In this study, the category agreements of GN bacteria, Staphylococcus and Enterococcus spp., and fungi reached 96.77, 93.5, 98.57, and 95.56%, respectively, similarly to previous studies (Romero-Gómez et al., 2012; Wimmer et al., 2012). The percentage of major and very major errors was low among the 3349 bacteria/antimicrobial combinations. In a RMAST of GN bacteria, most of the VMEs (0.49%, n = 9) occurred in amikacin, gentamicin, cefazolin, cefotetan, cefepime, and piperacillin-tazobactam, as observed for E. coli and P. aeruginosa in a previous study (Wimmer et al., 2012). Moreover, the present study obtained VMEs from K. pneumoniae and other non-fermenters. In the RMAST of Staphylococcus spp., most of the VMEs (n = 26) occurred in the presence of gentamicin, levofloxacin, clindamycin, and sulfamethoxazole/trimethoprim. It is worth mentioning that the VITEK ATB AST system could read the data automatically or semi-automatically. The automatic interpretation of low-growth streptococcal and fungal species could be complemented by manual assistance. Thus, the RMAST category agreement of fungi is obviously higher in our study than in previous study (Idelevich et al., 2014). Importantly, the RMAST category agreement of C. neoformans reached 100%.

Accurate RMI/RMAST results are useful for optimizing a therapeutic regimen. First, the RMI and RMAST results are

| Species | Correct ID | Scores |
|---------|------------|--------|
| Stenotrophomonas maltophilia/Staphylococcus haemolyticus | Stenotrophomonas maltophilia | 1.732 |
| Klebsiella pneumoniae/Enterococcus faecalis | Klebsiella pneumoniae/Enterococcus faecalis | 2.316/2.482 |
| Enterococcus cloacae/Streptococcus oralis | Not identified (very weak signal) | N/A |
| Candida tropicalis/Acinetobacter baumannii | Acinetobacter baumannii | 1.703 |
| Streptococcus oralis/Staphylococcus epidermidis | Not identified (Misidentified for Staphylococcus warneri) | 1.752 |
| Acinetobacter baumannii/Klebsiella pneumoniae | Klebsiella pneumoniae | 2.176 |
| Staphylococcus capitis/Klebsiella pneumoniae | Staphylococcus capitis | 1.739 |
| Pseudomonas aeruginosa/Klebsiella pneumoniae | Klebsiella pneumoniae | 2.219 |
| Pseudomonas aeruginosa/Klebsiella pneumoniae | Klebsiella pneumoniae | 2.22 |
| Staphylococcus epidermidis/Streptococcus salivarius | Staphylococcus epidermidis | 1.902 |
| Enterococcus faecalis/Staphylococcus haemolyticus | Not identified (very weak signal) | N/A |
| Staphylococcus haemolyticus/Streptococcus salivarius | Staphylococcus haemolyticus | 1.749 |
| Acinetobacter baumannii/Klebsiella pneumoniae | Klebsiella pneumoniae | 2.241 |
| Staphylococcus aureus/Pantoea spp | Staphylococcus aureus | 1.715 |
| Acinetobacter baumannii/Klebsiella pneumoniae | Klebsiella pneumoniae | 2.241 |
### TABLE 4 | Bacterial/antimicrobial combinations and errors in bacterial isolates from positive-culture sterile body fluids.

| Antimicrobial agent                  | No | Category agreement | mE | ME | VME |
|-------------------------------------|----|--------------------|----|----|-----|
| **GN BACTERIA (n = 140)**          |    |                    |    |    |     |
| Amikacin                            | 122| 120                | 98.36% | 1 | 0.82% | 1 | 0.82% |
| Gentamicin                          | 135| 131                | 97.04% | 3 | 2.22% | 1 | 0.74% |
| Ciprofloxacin                       | 135| 131                | 97.04% | 3 | 2.22% | 1 | 0.74% |
| Ampicillin                          | 117| 117                | 100.0% |    |     |     |
| Cefazolin                           | 135| 127                | 94.07% | 7 | 5.19% | 1 | 0.74% |
| Cefotetan                           | 135| 129                | 95.56% | 4 | 2.96% | 2 | 1.48% |
| Cefazidime                          | 135| 127                | 94.07% | 6 | 4.44% | 2 | 1.48% |
| Cefotaxime                          | 135| 134                | 99.26% | 1 | 0.74% |    |
| Cefepime                            | 135| 123                | 91.11% | 9 | 6.67% | 3 | 2.22% |
| Imipenem                            | 135| 133                | 98.52% | 2 | 1.48% |    |
| Piperacillin-tazobactam             | 112| 112                | 100.0% |    |     |     |
| Sulfamethoxazole/trimethoprim       | 140| 137                | 97.86% | 2 | 1.43% | 1 | 0.71% |
| Tobramycin                          | 135| 132                | 97.78% | 3 | 2.22% |    |
| **Total**                           | 1828| 1769              | 96.77% | 46 | 2.52% | 4 | 0.22% | 9 | 0.49% |

**STAPHYLOCOCCUS SPP (n = 105), ENTEROCOCCUS SPP (n = 28)**

| Antimicrobial agent                  | No | Category agreement | mE | ME | VME |
|-------------------------------------|----|--------------------|----|----|-----|
| Penicillin                          | 105| 104                | 99.05% | 1 | 0.95% |    |
| Oxacillin                           | 105| 104                | 99.05% | 1 | 0.95% |    |
| Gentamicin                          | 105| 83                 | 79.05% | 10 | 9.52% | 5 | 4.76% | 7 | 6.67% |
| Levofloxacin                        | 133| 124                | 93.23% | 6 | 4.51% | 3 | 2.26% |
| Erythromycin                        | 133| 130                | 97.74% | 2 | 1.50% | 1 | 0.75% |
| Clindamycin                         | 133| 127                | 95.49% | 4 | 3.01% | 2 | 1.50% |
| Linezolid                           | 133| 133                | 100.0% |    |     |     |
| Vancomycin                          | 133| 133                | 100.0% |    |     |     |
| Rifampicin                          | 105| 105                | 100.0% |    |     |     |
| Sulfamethoxazole/trimethoprim       | 105| 93                 | 88.57% | 1 | 0.95% | 2 | 1.90% | 9 | 8.57% |
| Ampicillin                          | 28 | 4                  | 14.29% | 23 | 82.14% | 1 | 3.57% |
| Gentamicin-High level               | 28 | 25                 | 89.29% | 2 | 7.14% | 1 | 3.57% |
| **Total**                           | 1246| 1165              | 93.50% | 46 | 3.69% | 9 | 0.72% | 26 | 2.09% |

**STREPTOCOCCUS SPP (n = 14)**

| Antimicrobial agent                  | No | Category agreement | mE | ME | VME |
|-------------------------------------|----|--------------------|----|----|-----|
| Penicillin                          | 14 | 13                 | 92.86% | 1 | 7.14% |
| Cefotaxime                          | 14 | 14                 | 100.0% |    |     |
| Ceftriaxone                         | 5  | 5                  | 100.0% |    |     |
| Imipenem                            | 5  | 5                  | 100.0% |    |     |
| Meropenem                           | 5  | 5                  | 100.0% |    |     |
| Levofloxacin                        | 14 | 14                 | 100.0% |    |     |
| Erythromycin                        | 14 | 14                 | 100.0% |    |     |
| Clindamycin                         | 14 | 14                 | 100.0% |    |     |
| Linezolid                           | 5  | 5                  | 100.0% |    |     |
| Vancomycin                          | 14 | 14                 | 100.0% |    |     |
| Quinupristin-Dalfos                 | 9  | 8                  | 88.89% | 1 | 11.11% |
| Tetracycline                        | 9  | 9                  | 100.0% |    |     |
| Chloramphenicol                     | 9  | 9                  | 100.0% |    |     |
| Sulfamethoxazole/trimethoprim       | 9  | 9                  | 100.0% |    |     |
| **Total**                           | 140| 138               | 98.57% | 1 | 0.71% | 1 | 0.71% |

(Continued)
TABLE 4 | Continued

| Antimicrobial agent | No | Category agreement | mE | ME | VME |
|---------------------|----|---------------------|----|----|-----|
| **YEAST (n = 18)**  |    |                     |    |    |     |
| 5-Fluorocytosine    | 18 | 17                  | 94.44% | 1  | 5.56% |
| Fluconazole         | 18 | 17                  | 94.44% | 1  | 5.56% |
| Voriconazole        | 18 | 18                  | 100.0% |    |     |
| Amphotericin B      | 18 | 18                  | 100.0% |    |     |
| Itraconazole        | 18 | 16                  | 88.89% | 2  | 11.11% |
| **Total**           | 90 | 86                  | 95.56% | 3  | 3.33% |
| **CRYPTOCOCCUS NEOFORMANS (n = 15)** |    |                     |    |    |     |
| 5-Fluorocytosine    | 15 | 15                  | 100.0% |    |     |
| Fluconazole         | 15 | 15                  | 100.0% |    |     |
| Amphotericin B      | 15 | 15                  | 100.0% |    |     |
| **Total**           | 45 | 45                  | 100.0% |    |     |

The total numbers of isolates differed for each specific antimicrobial agent tested according to the CLSI (2015) guidelines.

![FIGURE 3 | Comparison between the average times of RMI/RMAST and FID/FAST. The average times of RMAST vs. the conventional method were of great statistical significant (p < 0.0001). **p < 0.0001. Bacteria failed to grow: bacteria failed to grow on the routing plates.](image)

13.44% of the cases showed resistance to last-resort antibiotics (such as vancomycin, linezolid, and carbapenem), further alerting clinicians to adjust the treatment or initiate essential combination therapy. In contrast, 51.56% of the bacteria were susceptible to third-generation cephalosporin and carbapenem (GN bacteria) or vancomycin and linezolid (GP bacteria), suggesting that antibiotic therapy might be de-escalated in patients infected by these organisms.

In this study, we theoretically examined the utility of RMI/RMAST in optimizing therapeutic regimens. We did not conduct a practical investigation. Several studies (Martiny et al., 2013; Perez et al., 2013) prospectively demonstrated that rapid pathogen identification and antimicrobial stewardship reduces the hospital length of stay and total costs. However, these studies were limited to BSI caused by GN bacteria. Therefore, we tentatively propose a protocol that establishes the control and intervention groups and detects bacterial, yeast and fungal infections in diverse sterile body fluids. The impact of RMI/RMAST in decreasing the hospital length of stay, cost, and antibiotic selective pressure deserves further investigation.

CONCLUSIONS

The combined MALDI–TOF MS and Vitek AST system could obtain a rapid, accurate, reliable identification, and ASTs reports. The RMI and RMAST results not only reduce the turnaround time of the patient, but also guide clinicians whether a patient’s antibiotic treatment should be accelerated, ceased or de-escalated, and adjusted the essential drugs modification for an optimized therapy.

ETHICS STATEMENT

This study was approved by the ethics committee of Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai,
People's Republic of China (protocol HS-H-2014-0213). All subjects provided written informed consent before their inclusion in the study.

**AUTHORS CONTRIBUTIONS**

YT and BZ performed all experiments. BW assisted in antimicrobial susceptibility testing. ML, YL, YT, and BZ conceived the study and analyzed the results. ML and YL supervised the study and wrote the manuscript. All authors read and approved the final manuscript.

**FUNDING**

This study was supported by the National Natural Science Foundation of China (grants 81322025, 81171623 and 81371875), Outstanding Young Talent Plan of Shanghai (XYQ2011039), and Shanghai Shuguang Talent Project (12SG03).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2016.00523
Seng, P., Drancourt, M., Gouriet, F., La Scola, B., Fournier, P. E., Rolain, J. M., et al. (2009). Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin. Infect. Dis.* 49, 543–551. doi: 10.1086/600885

Stevenson, L. G., Drake, S. K., and Murray, P. R. (2010). Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J. Clin. Microbiol.* 48, 444–447. doi: 10.1128/JCM.01541-09

Thigpen, M. C., Whitney, C. G., Messonnier, N. E., Zell, E. R., Lynfield, R., Hadler, J. L., et al. (2011). Bacterial meningitis in the United States, 1998-2007. *N. Engl. J. Med.* 364, 2016–2025. doi: 10.1056/NEJMoa1005384

Tumbarello, M., Spanu, T. D., Bidino, R., Marchetti, M., Ruggeri, M., Trecarichi, E. M., et al. (2010). Costs of bloodstream infections caused by *Escherichia coli* and influence of extended-spectrum-β-lactamase production and inadequate initial antibiotic therapy. *Antimicrob. Agents Chemother.* 54, 4085–4091. doi: 10.1128/AAC.00143-10

Vincent, J. L., Sakr, Y., Sprung, C. L., Ranieri, V. M., Reinhart, K., Gerlach, H., et al. (2006). Sepsis in European intensive care units: results of the SOAP study. *Crit. Care Med.* 34, 344–353. doi: 10.1097/01.CCM.0000194725.48928.3A

Wimmer, J. L., Long, S. W., Cernoch, P., Land, G. A., Davis, J. R., Musser, J. M., et al. (2012). Strategy for rapid identification and antibiotic susceptibility testing of Gram-negative bacteria directly recovered from positive blood cultures using the Bruker MALDI Biotyper and the BD Phoenix system. *J. Clin. Microbiol.* 50, 2452–2454. doi: 10.1128/JCM.00409-12

Xu, C., Lin, X., Ren, H., Zhang, Y., Wang, S., and Peng, X. (2006). Analysis of outer membrane proteome of *Escherichia coli* related to resistance to ampicillin and tetracycline. *Proteomics* 6,462–473. doi: 10.1002/pmic.200500219

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.