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Rapid antimicrobial susceptibility testing from positive blood cultures based on Stimulated Raman Scattering Imaging analysis

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Abstract. The existing identification (ID) and antimicrobial susceptibility testing (AST) method requires at least two to three days to detect blood infection, and a fast and accurate detection method is very necessary for sepsis patients or Intensive Care Unit (ICU) patients. Here, we describe a direct isolated bacteria from a positive blood culture bottle (PBCB), and rapid AST method by femtosecond stimulated Raman scattering (SRS) imaging of deuterium oxide (D2O) metabolism, which can determine the antimicrobial susceptibility of bacteria from PBCB in 5-6 hours. The positive blood culture sample is passed through a filter membrane and mixed with cell lysis, after through the centrifugal which can directly isolated bacterium in order to identification by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), following by antibiotic susceptibility testing by SRS imaging within a day. Overall, this rapid and rapid process combination of MALDI-TOF MS and SRS imaging of deuterium oxide (D2O) metabolism can solve the direct identification and antibiotic susceptibility testing of pathogen in positive blood cultures.

1 Page layout

More than a million people are infected with bloodstream infections (BSIs) each year in Europe (1), BSIs have higher mortality rates , sequelae risk and more serious complications, becoming an increasing health care concern (2). Besides, Sepsis is a systemic response to infection, which not only kills a large number of people each year, but also increases the mortality rate for acute disease by 9% every hour. The administration of the correct antibiotic treatment including identification and determination of the antibiotic susceptibility profiles is delayed (3). To forestall the rise of antimicrobial obstruction, the solution of wide range antimicrobial medications is overseen. Subsequently, to improve the clinical forecast in patients with anti-infection safe bacterial infection, quick and precise assurance of the antimicrobial powerlessness of microbes is obligatory (3, 4). To address this general wellbeing emergency, rapid antimicrobial susceptibility testing is emerging to combat antimicrobial resistance, so as to reduce deaths from drug-resistant infections (5). Rapid, direct and accurate diagnosis of the bloodstream infections of correct susceptibility testing can improve the precise therapy in antimicrobial resistance (6-9).

The positive blood culture bottles (PBCBs) still includes an amount of blood cells (red cells, white cells and so on) and bacteria, it is often very difficult to separate the bacteria after identification (ID) and AST. PBCBs microbes are blended in with enormous number of blood cells and impurities which the bacterial concentrations from 107 to 109 CFU/ml, erratically (4). In actual clinical samples, rapid determination of the positive blood culture bottles bacteria, which the primary organism of ID and AST is considerable important for the patient. At present, in order to acquire the identification of bacteria, the bacterial of subcultures is very necessary (10). The commonly used clinical detection methods are including disk diffusion or broth dilution methods (11), another some commercial testing equipment even took a long time (12, 13). The above methods are difficult to test blood samples directly and carry out bacteria ID and AST has combined with rapid detection.

The matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is a powerful tool for identification bacteria of rapid and accurate technology (14). It has been proven to be a cost-effective and time-consuming method in the routine the clinical microbiology laboratory for identification (15). Some studies have been applied to direct separated bacteria from PBCBs samples of MALDI-TOF MS for ID, which had used centrifugation and filters (16, 17) methods of few hours whether took few days. However, these methods are either time-consuming or expensive, or they solve the problem of rapid identification, but they still require longer time to respond to antimicrobial susceptibility testing results.

In recent years, Raman spectroscopy has been proven to be used in this field to obtain AST results quickly, by

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deuterium oxide (D2O) metabolic in order to quantifying the C-D vibrational signal in the bacteria (18, 19). Due to the limitations, such as weaker signal and longer integration time. Stimulated Raman scattering (SRS) microscopy solves the above problems, SRS imaging have been applied to metabolic imaging in cells (16) and to detect accurately the MIC in single cells by using SRS imaging (20). A study has made it possible to quickly isolate bacteria for antibiotic susceptibility tests (21), but testing on known strains is still a long way for clinical application.

Here, the bacteria can be isolated by simple cell lysis and centrifuged for identification by MALDI-TOF MS and the AST value can be obtained rapidly due to SRS D2O metabolism imaging method, which can be combined with mass spectrometry to obtain ID and AST results quickly. The positive blood culture bottle sample is passed through filter membrane and mixed with cell lysis, after through the centrifugal can directly separate bacterium in order to identification by MALDI-TOF MS, following by antibiotic susceptibility testing by SRS imaging within a day.

2 Methods

E. coil (ATCC 25922) and S. aureus (ATCC 29213) was purchased from American Type Culture Collection (ATCC), which standard bacterial strain was used. Antibiotics used were also adopted to against amipicillin, ceftazidime, tobramycin, gentamicin and imipenem.

In this study, the positive blood culture bottles (BacT/ALERT FA Plus, Mérieux, France) were made as positive for microbial growth with automated incubation system (VITEK® MS, Mérieux, France).

Firstly, the positive blood culture bottle was used to extract 2 mL clinical sample, and the blood sample was filtered with 5μm filter (Miillipore Millex) to remove impurities. Then, the filtered sample was added to triple volume red blood cell lysis buffer (Solarbio, China) for 5-10 minutes. After, it was centrifuged for 3,500 × rpm for 5mins, and added to the re-suspend 0.45% saline solution to wash the cells and the rotation of the centrifugal chip, 3,500 × rpm for 5mins. For the experimental preparation of SRS, the automatic device was absorbed 2μl. Subsequent procedures were same as that of pure bacterial and standard strains for the preparation of SRS imaging. With the appearance of direct separated bacteria from PBCBs procedure, the bacteria from the centrifuge tube, added 1 μL HCCA was added on the steel target plate as described above. After a few minutes (5mins) drying, the sample was subjected to analyze the bacteria protein with MALDI-TOF MS system, the whole total time within 1.5-2 hour.(16).

When the identification results are known, others bacteria were prepared for AST by its SRS imaging after the identification. The solution and the 70 D2O LB broth (LB broth powder, 2%weight, Sigma Aldrich) was sterilized by 0.22 μm filter. The bacteria was first cultured in normal LB broth and different antibiotics for 1 hour. After, the bacteria was cultured in 70% D2O LB broth and Serial gradient concentration antibiotics for another 1 hour. The samples was centrifuged 2 minutes and washed twice by purified water, which deposited to an agarose gel pad (1~2% weight agarose powder to 5 ml purified water).

The SRS microscope apparatus is that Dual output femtosecond (fs) pulsed laser (InSight DeepSee, Spectra Physis) with a repetition rate 80 MHz was used for the SRS microscope. The SRS imaging at C-D vibration region (2126cm-1) required a 120 s laser in Figure 1) with tunable from 680-1100 nm wavelength, served as the pump beam.

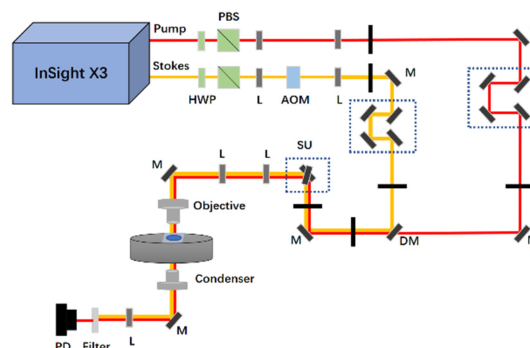

Fig 1. Schematic of a own lab-built SRS imaging setup. HWP, half wave plate; PBS, polarizing beam splitter; L, lens; M, mirror; AOM, acousto-optic modulator; DM, dichroic mirror; GM, galvo mirror; MS, motored stage; PD, photodiode.

The identification and AST were subjected to clinical laboratory conventional method MALDI-TOF MS identification and Vitek 2 AST which the sample from PBCBs. All results were compared with conventional clinical methods and interpreted according the Clinical and Laboratory Standards Institute (CLSI).

3 Results

3.1 The results of identification by MALDI-TOF MS

Positive culture bottles were followed by the laboratory MALDI-TOF MS identification methods. By simple separation, positive blood culture bottle includes amount of cells, rapid simple separation for identification by MALDI-TOF MS. A total 38 positive blood cultures were analyzed in this study (Table 1), including 18 gram-negative and 20 gram-positive direct isolates from PBCBs. The direct MALDI-TOF MS identification method results were compared with the conventional method, which were shown a high concordance between accordance. The rapid isolation of PBCB bacteria, can be obtained 92.2% isolates correctly identified from positive direct blood culture by MALDI-TOF MS.

TABLE I. THE NUMBER AND PERCENTAGE OF ISOLATES CORRECTLY IDENTIFIED WITH CENTRIFUGAL CHIP FROM POSITIVE DIRECT BLOOD CULTURE BY MALDI-TOF MS

Orgainism	Number of isolates	Number (%) of isolates correctly identified from positive direct blood culture by MALDI-TOF MS
E. coil	18	17 (94.4%)
S. aureus	20	18 (90%)
Total	38	35 (92.2%)

3.2 Direct AST isolates from blood bottles against different antibiotics

In order to verify the feasibility of this method for direct rapid AST, SRS imaging of D_2O metabolic incorporation for clinical bloodstream infections (BSI) samples after lysis and ID of samples. The minimal inhibitory concentration (MIC) value for *E. coil* ATCC 25922 in blood after 2-h culture was determined to be different concentrations of ceftazidime (Figure 2. A-B), which agreed with the MIC or MIC for *E. coil* ATCC 25922 in growth pure medium (Figure 2. C-D). These results demonstrated that SRS imaging of D_2O metabolic incorporation can rapidly determine MIC for simple separation after identification of bacteria in positive blood culture bottle. In order to verify the feasibility of this method, different antibiotics (against amipicillin, ceftazidime, tobramycin, gentamicin and imipenem) were tested and the results were consistent.

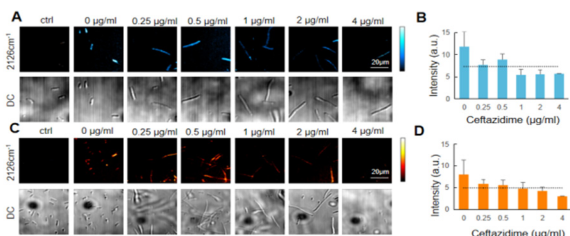

Fig 2. Direct and rapid AST isolates from blood bottles against ceftazidime .MIC deterimination of E. coil ATCC 25922, (A) C-D SRS imaging at ~2162 cm-1 and transmission images against ceftazidime. (B) Average C-D signal intensity of individual against ceftazidime in (A). The strans isolated from positive culture blood bottle (PBCB) E. coil ATCC 25922 against ceftazidime.(C) C-D SRS imaging at ~2162 cm-1 and transmission images of the strans isolated from PBCB against ceftazidime. (D) Average C-D signal intensity of individual of the strans isolated from PBCB against ceftazidime in (C).

TABLE II. COMPARISON OF SC-MIC AND SUSCEPTIBILITY CATEGORY FOR E. COIL ATCC 25922 DIRECT CULTURED AND E. COIL ATCC 25922 CULTURED IN BLOOD WITH SIMPLE ISOLATED FROM PBCB.

Antibiotics	Direct culture SRS MIC (μg/ml)	Direct culture SRS MIC (μg/ml)	MIC/SC-MIC Category
Amipicillin	2	4	S/S
Ceftazidime	1	1	S/S
Tobramycin	2	1	S/S
Gentamicin	1	1	S/S
Imipenem	0.5	0.5	S/S

4 Conclusion

Simple and direct separation bacteria from positive blood culture bottle for identification and AST, is critically required by BSI patients and ICU patients. However, this method takes time, wastes manpower and may cause unnecessary pollution, these problems will be solved if automated methods can be developed. A direct and rapid AST method by stimulated Raman scattering (SRS) imaging of D2O metabolism, which can detect the antimicrobial susceptibility of bacteria from a positive blood culture bottle (PBCB), shorten the time (almost 5-6 hours). With simple isolation to identify the bacteria and AST, this approach has great potential for clinical applications, and it can directly separate bacterium in order to identification by MALDI-TOF MS, following by antibiotic susceptibility testing by SRS imaging within a day. However, the current scheme still adopts manual method, which still cannot avoid the problems of samples contamination and labor cost. By rapid automating the separation of bacteria from samples and using SRS imaging, this method will be an effective method process to solve rapid and direct AST.

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References

1. M. Goto, M. N. Al-Hasan, Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clinical Microbiology & Infection the Official Publication of the European Society of Clinical Microbiology & Infectious Diseases **19**, (2013).
2. O. Opota, A. Croxatto, G. Prod'hom, G. Greub, Blood culture-based diagnosis of bacteraemia: state of the art. Clin Microbiol Infect **21**, 313-322 (2015).
3. J. Garnacho-Montero, T. Aldabo-Pallas, C. Garnacho-Montero…, Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Critical Care **10**, R111-R111 (2006).
4. J. Choi et al., Direct, rapid antimicrobial susceptibility test from positive blood cultures based

on microscopic imaging analysis. Sci Rep **7**, 1148 (2017).

5. A. R. Kumar, D.; Wood, K. E.; Light, B.; Parrillo, J. E.; Sharma, S.; Suppes, R.; Feinstein, D.; Zanotti, S.; Taiberg, L., Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Critical care medicine 1589-1596 (2006).
6. B. C. Pien et al., The clinical and prognostic importance of positive blood cultures in adults. Am J Med **123**, 819-828 (2010).
7. P. E. Eleftheria, A. Nikolaos, S. Fadi, P. D. Ziakas, M. Eleftherios, The Cost-Effectiveness of Rapid Diagnostic Testing for the Diagnosis of Bloodstream Infections with or without Antimicrobial Stewardship. Clinical Microbiology Reviews **31**, e00095-00017 (2018).
8. A. Rhodes et al., Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med **43**, 304-377 (2017).
9. A. W. Karchmer, Bloodstream infections: the problem and the challenge. Int J Antimicrob Agents 34 Suppl **4**, S2-4 (2009).
10. K. Syal et al., Current and emerging techniques for antibiotic susceptibility tests. Theranostics 7, 1795-1805 (2017).
11. I. Wiegand, K. Hilpert, R. E. Hancock, Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc **3**, 163-175 (2008).
12. N. Fanjat, A. Leclercq, H. Joosten, D. Robichon, Comparison of the phenotyping methods ID 32E and VITEK 2 compact GN with 16S rRNA gene sequencing for the identification of Enterobacter sakazakii. J Clin Microbiol **45**, 2048-2050 (2007).
13. G. Gherardi et al., Comparative evaluation of the Vitek-2 Compact and Phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Gram-positive isolates. Diagn Microbiol Infect Dis **72**, 20-31 (2012).
14. J. N. J. de Almeida et al., Rapid identification of moulds and arthroconidial yeasts from positive blood cultures by MALDI-TOF mass spectrometry. Med Mycol **54**, 885-889 (2016).
15. M. Kostrzewa, K. Sparbier, T. Maier, S. Schubert, MALDI-TOF MS: an upcoming tool for rapid detection of antibiotic resistance in microorganisms. Proteomics Clin Appl **7**, 767-778 (2013).
16. H. W. Pan et al., Simple Sample Preparation Method for Direct Microbial Identification and Susceptibility Testing From Positive Blood Cultures. Front Microbiol **9**, 481 (2018).
17. M. Christner et al., Rapid identification of bacteria from positive blood culture bottles by use of matrix-assisted laser desorption-ionization time of flight mass spectrometry fingerprinting. J Clin Microbiol 48, 1584-1591 (2010).
18. K. Yang et al., Rapid Antibiotic Susceptibility Testing of Pathogenic Bacteria Using Heavy-Water-Labeled Single-Cell Raman Spectroscopy in Clinical Samples. Anal Chem **91**, 6296-6303 (2019).
19. Y. Tao et al., Metabolic-Activity-Based Assessment of Antimicrobial Effects by D2O-Labeled Single-Cell Raman Microspectroscopy. Anal Chem **89**, 4108-4115 (2017).
20. W. Hong et al., Antibiotic Susceptibility Determination within One Cell Cycle at Single-Bacterium Level by Stimulated Raman Metabolic Imaging. Anal Chem **90**, 3737-3743 (2018).
21. M. Zhang et al., Rapid Determination of Antimicrobial Susceptibility by Stimulated Raman Scattering Imaging of D2O Metabolic Incorporation in a Single Bacterium. Adv Sci (Weinh) **7**, 2001452 (2020).