



Enfermedades Infecciosas y Microbiología Clínica

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Brief report

Enhancing pneumococcal bacteraemia diagnosis: A comparative assessment of culture-independent assays (MALDI-TOF-MS Sepsityper[®] module and a lateral flow immunochromatography test)



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ARTICLE INFO

Article history:

Received 22 September 2023

Accepted 12 February 2024

Available online 4 May 2024

Keywords:

Streptococcus pneumoniae

Bacteremia

Sepsityper module

Standard F[®]

ICT

ABSTRACT

Introduction: Pneumococcal bacteraemia is a major contributor to global morbidity and mortality. Traditional culture-based methods lack sensitivity and are time-consuming. This study aimed to assess the effectiveness of two culture-independent assays, the MALDI-TOF-MS Sepsityper[®] module and the lateral flow immunochromatography test (LFICT) with the Standard F[®] *Streptococcus pneumoniae*, directly from positive blood culture (BC) bottles.

Methods: A prospective study was conducted from December 2021 to July 2022. For all BC positives for *S. pneumoniae* a double centrifugation protocol was implemented. The resulting pellet was subsequently processed using both techniques.

Results: The LFICT showed exceptional performance with 100% sensitivity and specificity, outperforming the MALDI-TOF-MS Sepsityper[®] module, which achieved 85.2% sensitivity and 100% specificity. Nevertheless, the combination of these assays offers a robust and comprehensive approach to diagnosis.

Conclusions: The simultaneous use of both techniques offers a promising alternative that can be integrated into routine practices directly from BC samples.

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Mejora en el diagnóstico de la bacteriemia neumocócica: evaluación comparativa de ensayos cultivo/independientes (MALDI-TOF-MS módulo Sepsityper[®] y un test de inmunocromatografía lateral flow)

RESUMEN

Palabras clave:

Streptococcus pneumoniae

Bacteriemia

Módulo Sepsityper[®]

Standard F[®]

Inmunocromatografía

Introducción: La bacteriemia neumocócica es un gran contribuidor en la morbilidad global. Métodos tradicionales basados en el cultivo carecen de sensibilidad y demandan mucho tiempo. Este estudio tiene como objetivo comparar la efectividad de dos ensayos cultivo/independientes, el MALDI-TOF-MS módulo Sepsityper[®] y el test de inmunocromatografía lateral flow (LFICT) con Standard F[®] *S. pneumoniae*, directamente desde frascos de hemocultivos (HC) positivos.

Métodos: Se realizó un estudio prospectivo desde diciembre del 2021 a julio del 2022. Para todos los HC positivos de *S. pneumoniae* se implementó un protocolo de doble centrifugación. El pellet resultante fue posteriormente procesado utilizando ambas técnicas.

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Resultados: La LFICT mostró una actuación excepcional con un 100% de sensibilidad y especificidad, superando al MALDI-TOF-MS módulo Sepsityper®, el cual consiguió un 85,2% de sensibilidad y un 100% de especificidad. No obstante, la combinación de estos ensayos ofrece una aproximación diagnóstica robusta y amplia.

Conclusiones: El uso simultáneo de ambas técnicas ofrece una alternativa prometedora que puede ser integrada en la práctica rutinaria directamente desde muestras de HC.

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Introduction

Streptococcus pneumoniae is part of the commensal flora of the upper respiratory tract. It is classified into various serotypes based on the type of capsular polysaccharide produced. It is an important pathogen responsible of major public health problems.¹ Invasive pneumococcal disease (IPD) is defined as an infection that is confirmed by the identification of *S. pneumoniae* from normally sterile sites. This primarily includes pneumococcal meningitis, bacteraemia resulting from pneumococcal pneumonia and pneumococcal bacteraemia without a specific focus. Pneumonia remains the most common infectious source, despite the introduction of pneumococcal conjugate vaccination programs.² In adults, a descriptive analysis revealed that pneumonia accounted for most of the cases (53%), making it the predominant source of infection in individuals over 18 years of age. Additionally, bacteraemia without a specific focus were observed in 40% of the cases.³ Hospital mortality rates in IPD have been estimated at 15%, affecting individuals across all age groups. Vulnerable populations, such as children under 2 years old and individuals aged 65 and older, are particularly at risk.⁴ Between 2020 and 2022, our center reported a total of 83 positive blood cultures (BC) for *S. pneumoniae* among hospitalized patients, resulting in a positivity rate of 1.4%.

The identification of *S. pneumoniae* from culture relies on methods such as optochin susceptibility, latex agglutination, lateral flow immunochromatography test (LFICT) and/or matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). However, cultured-based methods often suffer from limited sensitivity due to the fastidious nature of this organism.⁵ This limitation is attributed to the production of an autolysin, a cell wall enzyme responsible for self-lysis during the stationary grow phase. Consequently, false-negative cultures can occur, compromising the accuracy of results.⁶ Additionally, culture is slow, requiring 24–48 h for definitive identification. Besides, the antimicrobial susceptibility test (AST) adds an additional 24 h to the process.

Direct application of MALDI-TOF-MS from positive BC can significantly reduce the turnaround time for identification and may enable the earlier implementation of appropriate antimicrobial treatment.⁷ However, the identification of Gram-positive bacteria can present greater difficulties, primarily due to the resilient characteristics of their cell wall and the slow growth of certain species, which can result in a notably smaller pellet after the extraction procedure. Furthermore, distinguishing *S. pneumoniae* and viridans group streptococci poses a significant challenge due to their close phenotypic and genotypic similarities.^{6,8} In order to improve identification accuracy, a commercial tool known as Sepsityper® module (Bruker-Daltonik, Germany) was developed.⁷

An additional alternative diagnostic is Standard F® *S. pneumoniae* antigen (SD Biosensor, South Korea). It is a LFICT based on immunofluorescence technology which detects capsular polysaccharide antigen shared by all *S. pneumoniae* serotypes in just ten minutes. While this test has been recommended for use in urine and cerebrospinal fluid, its applicability to BC samples warrants further validation.

The aim of this study was to assess the diagnostic accuracy of culture-independent assays, specifically MALDI-TOF-MS Sepsityper® module and LFICT using the Standard F® *S. pneumoniae* directly from positive BC bottles.

Material and methods

A prospective study was conducted from December 2021 to July 2022. BC bottles were collected from patients at our hospital and incubated in the automated Bactec® FX system (Becton-Dickinson®, USA). All samples with Gram-positive cocci in pairs or chains compatible with streptococcal morphology and time to positivity below 12 h were included in the study. Subsequently, these samples were sub-cultured on blood agar plates (Thermo-Scientific®, UK) with optochin disks (Oxoid®, UK) and incubated at 37 °C with 5% CO₂ for 48 h. Identification was performed by MALDI-TOF-MS (Bruker Daltonics®, Germany). Alpha-haemolytic colonies displaying inhibition zones around the optochin disk of ≥14 mm were identified as *S. pneumoniae*.

Simultaneously, in case of positivity, the previously described double centrifugation protocol for MALDI-TOF-MS identification was performed.⁸ The resulting pellet was processed with the Sepsityper® module. Concurrently, LFICT was conducted using the Standard F® LFICT. The same pellet obtained in the previous step was applied with 100 µL of sterile water to the sample well of the test device, following then the manufacturer's instructions. The analyser automatically displays the test result after ten minutes. A positive result presented a cut-off index (COI) above 1.0 that indicates the presence of *S. pneumoniae* antigen (see Fig. 1).

Statistical analysis

The frequency data was described by type of isolates identified. Categorical data are presented as numbers and percentages. Sensitivity, specificity, and kappa correlation were calculated using the optochin disk method as the reference technique. All the calculations were performed using R Studio package version 4.0.5.

Results

Among the 2554 positives BC, 378 (14.8%) were Gram-positive cocci in pairs/chain and 71/378 (18.8%) met the inclusion criteria. *S. pneumoniae* was identified in 27/71 (38%), while 44/71 (62%) samples were positive for the following non-pneumococcal microorganisms: 24 (33.8%) *Enterococcus* spp., 14 (19.7%) Viridans group streptococci, 3 (4.2%) *Streptococcus gallolyticus*, 1 (1.4%) *Streptococcus agalactiae*, 1 (1.4%) *Streptococcus dysgalactiae* and 1 (1.4%) *Streptococcus pyogenes*.

The Standard F® LFICT correctly identified all 27 *S. pneumoniae* positive samples, achieving a sensitivity and specificity of 100% each, indicating almost perfect agreement (Kappa correlation: 1.00). For the remaining 44 isolates, no false positive was detected. In the case of MALDI-TOF-MS Sepsityper® module, it detected 23 out of the 27 positive *S. pneumoniae* samples (4 remaining samples

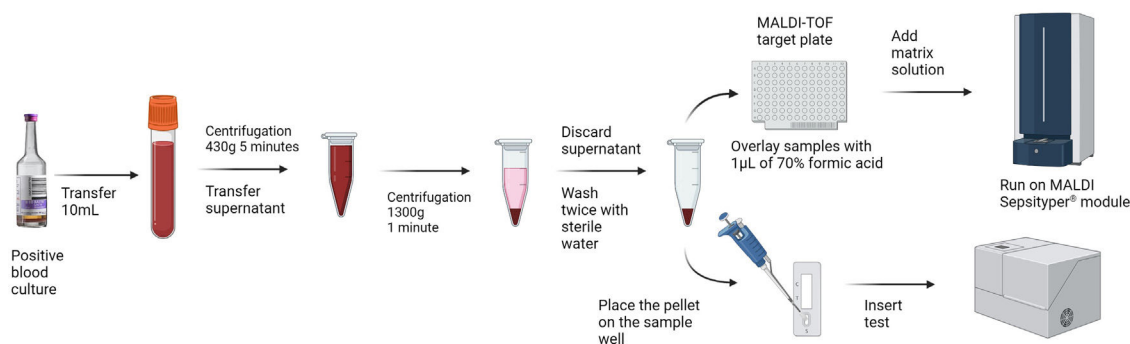


Fig. 1. Identification workflow using MALDI-TOF-MS Sepsityper® module and Standard F® LFICT.

were identified as viridans group streptococci), resulting in a sensitivity of 85.2% (95% CI: 65.4–95.1%) and a specificity of 100% (95% CI: 90.0–100%). The Kappa correlation for this method also demonstrated almost perfect agreement (0.95). When both techniques were combined, the sensitivity and specificity reached 100%, again indicating almost perfect agreement (Kappa correlation: 1.00).

Discussion

Diagnosing *S. pneumoniae* bacteraemia remains a significant challenge.⁹ *S. pneumoniae* is a fastidious growing microorganism, making microbiological diagnostic difficult. Despite the advancements brought by MALDI-TOF-MS, which has greatly improved microbiological diagnostics in a relatively short time, it has not been possible to reach a reliable identification.¹⁰ To the best of our knowledge, this is the first study of the Standard F® LFICT and MALDI-TOF-MS Sepsityper® module using direct BC samples.

Compared to prior studies using a different LFICT, our analysis had similar sensitivity but higher specificity.^{5,6,11} These variations may be attributed to potential cross-reactivity between viridans group streptococci and the pneumococcal C polysaccharide antigen. Moreover, the utilization of immunofluorescence technology along with automated sample reading likely enhances test specificity while reducing the likelihood of false positive results.

Ponderand et al.¹² evaluated a rapid MALDI-TOF Sepsityper® protocol directly from positive BC. The sensitivity of their protocol for *S. pneumoniae* (1/6, 16.7%) was substantially lower compared to our performance (23/27, 85.2%). We employed an alternative protocol for sample processing, yielding a sensitivity of 85.2% alongside a flawless specificity of 100%.

When comparing both methods, the Standard F® LFICT exhibited superior sensitivity and specificity among MALDI-TOF-MS Sepsityper® module. Nonetheless, our results suggest that integrating both methods into a workflow routine can improve the rapid identification of this microorganism. The Standard F® LFICT is priced at 9.68€ per determination, making it a cost-effective choice for integration into routine clinical laboratory procedures.

The main limitation of this study is the relatively small number of samples analyzed. Furthermore, further studies are needed to gain a comprehensive understanding and assess the clinical implications of these rapid identifications systems within the microbiology workflow.

In conclusion, this study underscores the efficacy of the Standard F® LFICT and MALDI-TOF-MS Sepsityper® module in the direct detection of *S. pneumoniae* from positive BC bottles, exhibiting both high sensitivity and specificity. The synergy of these two techniques highlights their efficiency in diagnostics, marked by reliability and rapidity when applied to direct positive BC samples.

References

- Engholm DH, Kilian M, Goodsell DS, Andersen ES, Kjergaard RS. A visual review of the human pathogen *Streptococcus pneumoniae*. FEMS Microbiol Rev. 2017;41:854–79. <http://dx.doi.org/10.1093/femsre/fux037> [cited 14.7.23].
- Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. Lancet Glob Health. 2018;6:e744. Available from: /pmc/articles/PMC6005122/ [cited 17.5.23].
- Drijckoning JJ, Rohde GGU. Pneumococcal infection in adults: burden of disease. Clin Microbiol Infect. 2014;20 Suppl. 5S5:45–51. Available from: <https://pubmed.ncbi.nlm.nih.gov/24313448/> [cited 10.1.23].
- Factsheet about pneumococcal disease [Internet]. Available from: <https://www.ecdc.europa.eu/en/pneumococcal-disease/facts> [cited 3.5.23].
- Baggett HC, Rhodes J, Dejsirilert S, Salika P, Wansom T, Jorakate P, et al. Pneumococcal antigen testing of blood culture broth to enhance the detection of *Streptococcus pneumoniae* bacteremia. Eur J Clin Microbiol Infect Dis. 2012 May 7;31:753–6. <http://dx.doi.org/10.1007/s10096-011-1370-3> [cited 11.1.23].
- Altun O, Athlin S, Almuhayawi M, Strålin K, Özenci V. Rapid identification of *Streptococcus pneumoniae* in blood cultures by using the Immulex, Slidex and Wellcogen latex agglutination tests and the BinaxNOW antigen test. Eur J Clin Microbiol Infect Dis. 2016;35:579–85. <http://dx.doi.org/10.1007/s10096-015-2573-9> [cited 11.1.23].
- Cordova M, Zignoli A, Ambretti S. Rapid sepsityper in clinical routine: 2 years' successful experience. J Med Microbiol. 2020;69:1398–404. <http://dx.doi.org/10.1099/jmm.0.001268> [cited 16.7.23].
- Zboromyrska Y, Rubio E, Alejo I, Vergara A, Mons A, Campo I, et al. Development of a new protocol for rapid bacterial identification and susceptibility testing directly from urine samples. Clin Microbiol Infect. 2016;22, 561.e1–e6.
- Song JY, Eun BW, Nahm MH. Diagnosis of pneumococcal pneumonia: current pitfalls and the way forward. Infect Chemother. 2013;45:351. Available from: /pmc/articles/PMC3902818/ [cited 10.6.23].
- Singhal N, Kumar M, Kanaujia PK, Virdi JS. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front Microbiol. 2015. Available from: /pmc/articles/PMC4525378/ [cited 10.6.23].
- Tootla HD, Bamford C, Centner CM, Moodley C. The BinaxNOW pneumococcal antigen test: an adjunct for diagnosis of pneumococcal bacteraemia. S Afr J Infect Dis. 2021;36. Available from: /pmc/articles/PMC8378051/ [cited 12.1.23].
- Ponderand L, Pavese P, Maubon D, Giraudon E, Girard T, Landelle C, et al. Evaluation of rapid Sepsityper® protocol and specific MBT-Sepsityper module (Bruker Daltonics) for the rapid diagnosis of bacteraemia and fungemia by MALDI-TOF-MS. Ann Clin Microbiol Antimicrob. 2020;19:60. Available from: /pmc/articles/PMC7727196/ [cited 12.1.23].