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Evaluation of Immulex *S. pneumoniae* Omni test for the direct detection of *S. pneumoniae* from positive blood cultures

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ABSTRACT

Rapid and early identification of *Streptococcus pneumoniae* from positive blood cultures is crucial for the management of patients with bloodstream infections (BSI). Many identification systems in microbiology laboratories have difficulty differentiating *S. pneumoniae* from other closely related species in the *Streptococcus mitis* group. To overcome this limitation, we developed a rapid workflow in our laboratory combining direct MALDI-TOF MS identification with the Immulex *S. pneumoniae* Omni test (SSI Diagnostica, Denmark) for rapid detection of *S. pneumoniae* directly from positive blood cultures. The workflow was evaluated using 51 *Streptococcus* isolates. Compared to conventional biochemical testing, our new workflow demonstrates 100 % specificity and sensitivity for the detection and differentiation of *S. pneumoniae* from other closely related species. Our new workflow is accurate, cost-effective, and can easily be implemented in microbiology laboratories that already perform direct MALDI-TOF identification from positive blood cultures to improve the management of patients with invasive pneumococcal disease.

Importance: Invasive pneumococcal disease remains a major public health problem worldwide. Reducing the time to identify *Streptococcus pneumoniae* in positive blood cultures allows patients to be treated sooner with more targeted and effective antibiotics. We evaluated a two-step protocol where positive blood cultures are first tested directly by MALDI-TOF MS and any samples containing *Streptococcus* species are tested by Immulex *S. pneumoniae* Omni test to both detect and differentiate *S. pneumoniae* from other closely related *Streptococcus* species. Our study results showed 100 % sensitivity and specificity, and a much faster turn-around time than conventional methods.

1. Introduction

Streptococcus pneumoniae is a major cause of severe infections such as community-acquired pneumonia (CAP), bacteremia, and meningitis worldwide in young children and elderly [1,2]. The incidence of invasive pneumococcal infections is tracked by the Centers

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for Disease Control and Prevention (CDC) in the United States. Overall, there are over 2 million pneumococcal infections per year in the United States, which are responsible for more than 6000 deaths, and more than $4 billion in healthcare costs [3]. Pneumococcal conjugate vaccines (PCVs) introduced in the early 2000s were very effective, helping in preventing and reducing infections (more than 90 % in children), and slowing down the development of pneumococcal resistance [3].

S. pneumoniae is one of the 13 know species of *Streptococcus mitis* group which includes *S. mitis, S. oralis, S. pneumoniae, S. australis, S. gordonii, S. cristatus, S. pseudopneumoniae, S. infantis, S. oligofermentans, S. parasanguinis, S. sanguinis*, *S. peroris,* and *S. tigurinus* [4]. This group of organisms are commensals of the mouth and upper respiratory tract. The finding of *S. pneumoniae* in the bloodstream is always clinically significant, but the finding of non-pneumococcal *S. mitis* group species in the blood may often indicate specimen contamination and requires further clinical correlation [5]. Accurate and rapid identification of *S. pneumoniae* is essential and differentiating *S. pneumoniae* from other members in the *S. mitis* group is important to ensure that the infected patients are on the appropriate treatment regimen.

S. pneumoniae is often identified in laboratories using conventional methods such as optochin disc and/or bile solubility test [6,7]. Clinical & Laboratory Standards Institute (CLSI) recommends using bile solubility testing for rapid and definitive identification of *S. pneumoniae* [6] although 1 % of *S. pneumoniae* can be bile negative [8]. Optochin testing is another method for differentiating *S. pneumoniae* from closely related *S. mitis* group species but recently, resistance has been reported in some isolates [9] and it fails to differentiate *S. pneumoniae* from *S. pseudopneumoniae* [10]. Several promising molecular methods have been developed for the identification and differentiation of *S. pneumoniae* from other *S. mitis* but these methods remain inconvenient for clinical laboratories to use for routine diagnostic testing as they are labor intensive and have longer turn-around times [5]. The introduction of MALDI-TOF MS technology (MatrixAssisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) has revolutionized the identification of microorganisms in microbiology laboratories due to its speed, cost-effectiveness, and accuracy. However, the ability to differentiate *S. pneumoniae* from other closely related species within the *S. mitis* group is a known limitation of the current MALDI-TOF MS systems [5,7,11].

MALDI-TOF MS has recently been used to directly test positive blood culture bottles prior to isolation of the bacteria on agar plates, either using commercially-available kits such as the Sepsityper kit (Bruker Daltonik, Bremen, Germany) or using laboratory-developed protocols [12]. However, the same limitation of differentiating *S. pneumoniae* and other closely related species within the *S. mitis* group still exists. To overcome this limitation, we developed a rapid workflow in our laboratory combining direct MALDI-TOF MS identification from positive blood cultures with the Immulex *S. pneumoniae* Omni test (SSI Diagnostica, Denmark) for rapid detection of *S. pneumoniae*. This study aims to evaluate the performance of this new workflow and compare it to the conventional methods of the identification of *S. pneumoniae* from blood cultures.

2. Results

Our approach for direct identification of bacteria from positive blood cultures using MALDI-TOF was validated in-house, using 505 monomicrobial positive blood cultures. A total of 473 (93.7 %) and 316 (62.6 %) monomicrobial bottles were correctly identified to the genus and species levels, respectively [13]. A total of 51 samples identified by direct MALDI-TOF to contain Streptococcus species. These blood culture samples were tested directly with the Immulex *S. pneumoniae* Omni test (SSI Diagnostica, Denmark) for the rapid detection of *S. pneumoniae,* and the results were compared against conventional testing with optochin and bile solubility (Becton Dickinson, Maryland, United States) from colonies after 18–24 h of incubation. The isolates assessed were *S. pneumoniae* (n = 27), *S. mitis* (n = 13), *S. oralis* (n = 9), *S. salivarius* (n = 1) and, *S. parasanguinis* (n = 1).

As illustrated in Table 1 and Supplemental Table 1, using direct identification by MALDI-TOF only, 43 *S. pneumoniae* and other closely related *S. mitis* group species were correctly identified, 1 *S. mitis* was wrongly identified as *S. pneumoniae*, and 7 *S. pneumoniae* were unidentifiable or wrongly identified as *S. mitis*. Overall, direct identification by MALDI-TOF alone showed 74.1 % sensitivity (95 % confidence interval [CI], 53.7 %–88.9 %) and 95.8 % specificity (95 % CI, 78.9 %–99.9 %) when compared to the results from conventional biochemical testing.

The new protocol combining identification by MALDI-TOF and the Immulex *S. pneumoniae* Omni test (SSI Diagnostica, Denmark) correctly identified all 27 *S. pneumoniae* organisms, with an overall sensitivity of 100 % (95 % confidence interval [CI], 87.2 %–100 %) and specificity of 100 % (95 % CI, 85.7 %–100 %) compared to conventional biochemical testing.

To further confirm the correct identification of the isolates, *S. pneumoniae* PCR targeting the *lytA* gene was performed. Of the 51

Table 1
Performance characteristics of the Immulex *S. pneumoniae* Omni test on positive blood cultures compared to conventional tests (optochin and bile solubility).

Test (No.)	Results (No.)				Sensitivity	Specificity	95 % CI of the sensitivity		95 % CI of the specificity	
	TP	FP	FN	TN			Low	High	Low	High
Direct MALDI TOF (51)	20	1	7	23	74.1 %	95.8 %	53.7 %	88.9 %	78.9 %	99.9 %
Direct MALDI TOF and The Immulex *S. pneumoniae* Omni Immulex (51)	27	0	0	24	100 %	100 %	87.2 %	100 %	85.7 %	100 %

No. = number of samples; TP = True Positive; FP=False Positive; FN=False Negative; CI=Confidence Interval.

isolates, 37 were available for *S. pneumoniae* PCR testing. All 17 of 17 isolates which were positive by the Immulex *S. pneumoniae* Omni test were also *S. pneumoniae* PCR positive, and all 20 of 20 Immulex-negative isolates were also negative by *S. pneumoniae* PCR.

3. Discussion

Our new rapid diagnostic testing approach was demonstrated to be fast, highly specific and sensitive, and user-friendly for the differentiation of *S. pneumoniae* from other closely related organisms in the *S. mitis* group. This new workflow can be easily adapted in microbiology laboratories that already perform direct MALDI-TOF identification from positive blood cultures using commercial or lab-developed assays. The Immulex *S. pneumoniae* Omni test is very easy to perform directly from positive blood cultures and requires less than 5 min of technologist time. Once a positive blood culture bottle is flagged by the automated blood culture system, this two-step protocol can be completed in less than 60 min. This new workflow provides reliable identification for *S. pneumoniae* 24–48 h faster than traditional methods using optochin tests or bile tests which are performed from colonies. In terms of time to detection of *S. pneumoniae*, this new workflow is comparable to commercial PCR assays available in the market, but the advantage over PCR commercial assays is that this workflow is much cheaper; the cost of multiplex PCR can be up to six times the cost of the Immulex *S. pneumoniae* Omni kit used in our workflow. Additionally, our workflow is more targeted and specific toward the detection of *S. pneumoniae* in comparison with PCR commercial assays which are usually multiplex assays and contains extra organisms as targets (gram-positive and gram-negative) which may be wasteful to use for this purpose.

Combining direct MALDI-TOF identification from positive blood cultures with MALDI-TOF peak analysis methods [7,14] or highly sensitive molecular methods such as multiplex PCR or sequencing methods [15,16] have been described in the literature. Although these methods are useful, they have not been validated from direct blood culture, have a higher cost, and more importantly, require longer turnaround time which is not ideal for physicians to make a clinical decision. Further studies are needed to explore the usefulness of these methods. Our new rapid workflow will assist antimicrobial stewardship efforts by reducing the time to optimal antimicrobial therapy for cases of pneumococcal bacteremia. Providing the clinician with the identity of the organism (pneumococcal versus non-pneumococcal) within 60 min of a blood culture bottle turning positive, using our workflow, will prompt the clinicians to take early and appropriate decisions about the use of antibiotics. Timely differentiation of *S. pneumoniae* from other closely related species is also important for the control and treatment of the disease in general. Many *Streptococcus* organisms are commensal microflora and might be considered contaminants in blood cultures. In contrast, *S. pneumoniae* is clinically significant and associated with high morbidity and mortality, and is never considered a contaminant in positive blood cultures, and should always be treated appropriately with an antibiotic [17,18].

Our study had several limitations. First of all, we used a small number of positive blood cultures (n = 51) which may result in selection bias. A study with more isolates may reveal some discrepant results. Second, only 37 of the 51 isolates were available for PCR confirmation. Third, the strains used in this study were not serotyped so cannot rule out the potential for clonal bias.

Mass spectrometry systems from different manufacturers (Bruker versus Vitek) and/or different spectra databases may also yield differences in performance [7,16]. Newer mass spectrometry instruments such as Vitek MS Prime or MALDI Biotyper sirius with updated databases and algorithms could provide better performance in differentiating *Streptococcus* species. In our study, we did not examine the clinical impact of our findings on antibiotic usage, but this is a very important aspect that should be examined in future studies.

The combination of MALDI-TOF MS with the Immulex *S. pneumoniae* Omni test for the direct assessment of positive blood culture bottles is not only rapid and accurate, but also inexpensive and user-friendly. This rapid workflow, combined with timely antibiotic stewardship interventions, has the potential to improve the management of septicemia due to *S. pneumoniae* or other closely related *Streptococcus* organisms.

4. Materials and methods

Blood culture system: Blood samples were collected in BD BACTEC Peds Plus/F blood culture bottles (Becton Dickinson, Franklin Lakes, NJ) and incubated in the BD BACTEC FX blood culture system (Becton Dickinson). The routine microbiological workflows for the assessment of positive blood culture bottles are aligned with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Gram stain and subculture: Gram stains were prepared for all positive blood culture bottles. An aliquot from each bottle was streaked onto blood, MacConkey, and chocolate agar plates, as well as being directly tested byMALDI-TOF MS. The Immulex test was performed when the MALDI-TOF identification of the organism was *S. pneumoniae*, *S. mitis*, *S. oralis*, or other streptococci within the *S. mitis* group.

Blood culture pre-processing for direct MALDI-TOF: A 1.5 ml aliquot was withdrawn from each positive blood culture followed by the addition of 0.3 ml of 5 % SDS to lyse the blood cells. The tube was then vortexed, incubated for 3–5 min at room temperature, and centrifuged for 2 min at 13,000×*g*. After removing the supernatant, the pellet was resuspended in 1 ml of water. This was repeated three more times to wash the bacterial pellet using 1 ml of water, 1 ml of 70 % ethanol, and 0.3 ml of water, successively. From the final wash, 1 μL of the bacterial pellet was used to smear the MALDI-TOF target plate and allowed to dry at room temperature. Next, 1 μL of 70 % formic acid was added to the target plate and allowed to dry at room temperature. At the end, 1 μL of HCCA matrix (α-Cyano-4-hydroxycinnamic acid) was added to the target plate and allowed to dry at room temperature before performing MALDI-TOF (Bruker Daltonik, Bremen, Germany) testing on the instrument according to the manufacturer's instructions. A MALDI-TOF score of =>2.0 was considered as an acceptable score for identification [19]. A score <2.0 was not considered acceptable for the purpose of this study for species differentiation of *Streptococcus* as it only accurate to the genus level [19]. However, four samples with a score <2.0 were

subjected to the Immulex *S. pneumoniae* Omni test as they were deemed to have a high clinical suspicion for *S. pneumoniae* by the microbiologist.

4.1. Immulex S. pneumoniae Omni test

Sample preparation is not required for the positive blood culture. The Immulex *S. pneumoniae* Omni test was performed according to the manufacturer's instructions in the package insert (SSI Diagnostica, Denmark). One drop of the latex reagent is placed on the reaction card and one drop of the positive blood culture is added next to the drop of the latex reagent. After both drops are mixed using a mixing stick, the reaction card is moved gently back and forth vertically for 10 s and observed for any agglutination.

4.2. Optochin test

One 5 μg optochin disc (6 mm discs; BD BBL Taxo P Discs, Becton Dickinson) was positioned on blood agar plates streaked with the organisms. The plates were incubated aerobically for 24 h. An inhibition zone of 14 mm or more was considered sensitive and consistent with *S. pneumoniae*.

4.3. Bile solubility test

The tube method was used according to the manufacturer's instructions (Desoxycholate Reagent Droppers, Becton Dickinson). A heavy suspension equivalent to 1.0–2.0 McFarland standard was prepared in 1 ml of 0.85 % sterile saline. The suspension was then divided into two test tubes (0.5 ml per tube). One tube was labeled as a test tube and the other as a control tube. 10 drops (0.5 ml) of sterile 0.85 % saline were added to the tube labeled control and 10 drops of desoxycholate reagent were added to the tube labeled test. Both tubes were incubated at room temperature for 15 min. If the test tube appeared clear or less turbid than the control tube then the result was considered positive and consistent with *S. pneumoniae*.

4.4. S. pneumoniae PCR

Single colonies of the bacterial isolates were resuspended in sterile saline and DNA was extracted using the KingFisher Flex (Thermo Fisher Scientific, Waltham, MA, USA). Samples were subjected to PCR against the *lytA* gene of *S. pneumoniae* using the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific) [20].

4.5. Result comparison against reference methods

All conventional microbiological methods were in compliance with CLSI guidelines [6]. The Immulex *S. pneumoniae* Omni test results were compared against biochemical test results (optochin disc and bile solubility tests) performed the next day on the pure isolated colonies. For PCR confirmation, available isolates were retrieved from storage at −80 °C and re-cultured on blood agar plates prior to DNA extraction and PCR.

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Author contribution statement

MOHAMMED SULEIMAN, Andrés Pérez-López, Patrick Tang, Jill Roberts: Conceived and designed the experiments.

MOHAMMED SULEIMAN, Nazik Elamin, Rhanty Nabor: Performed the experiments.

MOHAMMED SULEIMAN, Patrick Tang, Rhanty Nabor, Nazik Elamin, Mohammad Rubayet Hasan: Analyzed and interpreted the data.

MOHAMMED SULEIMAN, Patrick Tang: Contributed reagents, materials, analysis tools or data.

MOHAMMED SULEIMAN, Nazik Elamin, Rhanty Nabor, Andrés Pérez-López, Patrick Tang, Jill Roberts, Mohammad Rubayet Hasan; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22106.

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