Original Investigation / *Özgün Araştırma*

DOI: 10.5578/ced.20250403 • *J Pediatr Inf 2025;19(4):e233-e242*

Pilot Study for Cost-Effective and Rapid Serotyping of *Streptococcus pneumoniae* by FTIR-ATR

Streptococcus pneumoniae'nın FTIR-ATR ile Maliyet Etkin ve Hızlı Serotiplendirme Pilot Çalışması

Gizem Öztürk[1](İD), Emine Arzu Başkale[1](İD), Ayşe Nur Yurdcu[1](İD), Figen Teker[1](İD), Ayşegül Bülbül[1](İD), Muhammed Emin Demirkol[2](İD), Nuriye Ünal Şahin[2](İD), Selin Nar Otgun[2](İD), Ateş Kara[3](İD)

[1] *Türkiye Vaccine Institute, Aziz Sancar Research Center, Health Institutes of Türkiye, Ankara, Türkiye*
[2] *Microbiology Reference Laboratories Biological Products Directorate, General Directorate of Public Health, Ankara, Türkiye*
[3] *Division of Pediatric Infectious Diseases, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Türkiye*

Cite this article as: Öztürk G, Başkale EA, Yurdcu AN, Teker F, Bülbül A, Demirkol ME, et al. Pilot study for cost-effective and rapid serotyping of *Streptococcus pneumoniae* by FTIR-ATR. J Pediatr Inf 2025;19(4):e233-e242.

Abstract

Objective: *Streptococcus pneumoniae* is a pathogen that causes widespread and contagious diseases and poses a serious global public health threat, especially for infants, children and the elderly in the first two years of life. *S. pneumoniae* is a bacterium that can colonize the nasopharynx of adults, especially children, and can cause acute otitis media, respiratory tract infections and even life-threatening invasive infections (serious clinical conditions such as sepsis and meningitis). Serotyping of *S. pneumoniae* plays a critical role especially in infection management, epidemiological studies and vaccine development. There are more than one hundred known serotypes of *S. pneumoniae*. Each serotype has different pathogenicity, prevalence, infectivity and antimicrobial resistance patterns. Understanding the serotype distribution is important for monitoring the prevalence of serotypes covered by existing vaccines in the community and for planning future vaccines.

Material and Methods: Considering all these issues, this study aimed to develop a rapid and cost-effective analysis method for pneumococcal serotyping using Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR) spectroscopy. For this purpose, four different *S. pneumoniae* isolates (serotype 3, serotype 19A, serotype 23B and serotype 9L) were provided by the General Directorate of Public Health. Cultivated bacterial isolates were incubated at 37 °C in a 5% carbon dioxide atmosphere for 24 hours. Then, these culture samples were analyzed

Öz

Giriş: *Streptococcus pneumoniae*, yaygın ve bulaşıcı hastalıklara neden olan bir patojen olup, özellikle yaşamın ilk iki yılındaki bebek, çocuk ve yaşlılar için ciddi bir küresel halk sağlığı tehdidi oluşturmaktadır. *S. pneumoniae*, özellikle çocuklarda olmak üzere yetişkinlerin de nazofarenksinde kolonize olabilen bir bakteri olup; akut orta kulak iltihabı, solunum yolu enfeksiyonları ve hatta yaşamı tehdit edebilecek düzeyde invaziv enfeksiyonlara (sepsis ve menenjit gibi ciddi klinik tablolara) yol açabilir. *S. pneumoniae*'nin serotiplendirilmesi, özellikle enfeksiyon yönetimi, epidemiyolojik çalışmalar ve aşı geliştirmede kritik bir rol oynar. *S. pneumoniae*'nin bilinen yüzden fazla farklı serotipi vardır. Her serotipin ise farklı patojenitesi, yaygınlığı, bulaşıcılığı ve antimikrobiyal direnç kalıpları vardır. Mevcut aşıların kapsadığı serotiplerin toplumdaki yaygınlığını izlemek ve gelecekte geliştirilmesi gereken aşıları planlamak için serotip dağılımını anlamak önemlidir.

Gereç ve Yöntemler: Tüm bu konuları göz önünde bulundurarak, bu çalışmada; Fourier Dönüşümlü Kızılötesi-Zayıflatılmış Toplam Yansıma (FTIR-ATR) spektroskopisi cihazı kullanılarak, pnömokok serotiplendirme çalışması gerçekleştirebilmek için hızlı, uygun maliyetli bir analiz yöntemi geliştirilmesi amaçlamıştır. Bu doğrultuda, Halk Sağlığı Genel Müdürlüğünden dört farklı *S. pneumoniae* izolatı (serotip 3, serotip 19A, serotip 23B ve serotip 9L) temin edilmiştir. Ekimi yapılan bakteri izolatları %5 karbondioksit atmosferinde 37 °C'de 24 saat inkübe edilmiştir. Daha sonra, bu kültür örnekleri Shimadzu FTIR-ATR cihazı kullanılarak

Correspondence Address/Yazışma Adresi

Gizem Öztürk

Türkiye Vaccine Institute,
Aziz Sancar Research Center,
Health Institutes of Türkiye
Ankara, Türkiye

E-mail: ozturk9189@gmail.com

Received: 17.02.2024 **Accepted:** 25.07.2025
Available Online Date: 25.12.2025

using Shimadzu FTIR-ATR device and a method was developed to create a serotype library

Results: According to the results obtained from FTIR-ATR spectra of different *S. pneumoniae* isolates, peaks between 3100 and 950 nanometers belong to different regions that can allow serotyping of *S. pneumoniae* bacteria. Because the peaks in these regions belong to carbohydrates, lipids, nucleic acids and proteins that can present the unique fingerprint of each serotype.

Conclusion: However, since the changes between the peaks in all analyzed data were minimal, it was seen that the sensitivity of the methods in distinguishing serotypes was limited. On the other hand, the overlapping of the spectra in the analysis of the obtained data indicates the high reproducibility of the method.

Keywords: *Streptococcus pneumoniae*, bacterial infection, FTIR-ATR, serotyping, library preparation

analiz edilmiş ve serotip kütüphanesi oluşturmak için bir yöntem geliştirilmiştir.

Bulgular: Farklı *S. pneumoniae* izolatlarının FTIR-ATR spektrumlarından elde edilen sonuçlarına göre, 3100 ile 950 nanometre arasındaki pikler *S. pneumoniae* bakterisinin serotiplendirilmesine olanak sağlayabilecek farklı bölgelerine aittir. Çünkü bu bölgelerdeki pikler her serotipin kendine özgü parmak izini sunabilecek karbonhidrat, lipid, nükleik asit ve proteinlerine aittir.

Sonuç: Analiz edilen tüm verilerdeki pikler arasındaki değişimler minimal olduğundan, yöntemlerin serotipleri ayırt edebilme konusunda duyarlılığının sınırlı kaldığı görülmüştür. Öte yandan elde edilen verilerin analizlerinde spektrumların örtüşmesi, yöntemin yüksek tekrarlanabilirliğine işaret etmektedir.

Anahtar Kelimeler: *Streptococcus pneumoniae*, bakteriyel enfeksiyon, FTIR-ATR, serotipleme, kütüphane hazırlama

Introduction

Streptococcus pneumoniae is a gram-positive, extracellular pathogen. It is primarily spread through droplets (1). *S. pneumoniae* can cause non-invasive pneumococcal infections such as otitis media, sinusitis, and pneumonia without bacteremia, as well as invasive infections with a high risk of mortality. Sepsis, meningitis, and bacteremic pneumonia are among these serious conditions (2). Invasive pneumococcal disease primarily affects children under five years of age, individuals over 65 years of age, and those with comorbidities or compromised immune systems (3,4).

Serotype is the classification of a microorganism based on antigenic structures (usually polysaccharides or proteins) found on its surface. This classification, based on surface antigens that enable different strains within the same species to be recognized differently by the immune system, is particularly important for vaccine development, epidemiological surveillance, and diagnosis (5). The polysaccharide capsule surrounding the bacterial cell is the primary virulence factor of *S. pneumoniae* and determines its serotype. Each serotype has structurally distinct capsular polysaccharides, making them antigenically different. Over 100 distinct pneumococcal serotypes have been identified to date (6). Monitoring the distribution of these serotypes is crucial for understanding local epidemiology, determining vaccination strategies, and tracking serotype distribution. Although the Quellung reaction, one of the traditional serotyping methods, is widely used today, it is labor-intensive and time-consuming, requiring special reagents and expertise (7). Although the Quellung reaction (capsule swelling test) is classically used today, latex agglutination tests, polymerase chain reaction-based methods, DNA sequencing (whole genome sequencing, WGS), and Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)-based methods can also be used due to their different advantages. Each method has its advantages and limitations, and the choice depends on the context of application. New techniques, such as FTIR-ATR, in particular, have the potential to make serotyping more accessible and less costly in the future (8).

Although the introduction of conjugate pneumococcal vaccines into clinical use has been shown to be very effective in reducing the burden of pneumococcal disease, the high number of serotypes and changes in serotype distribution mean that new serotypes not covered by the vaccine are still detected at a higher rate as infectious agents, which remains a significant problem (9). Therefore, serotyping plays a vital role in epidemiological studies and the evaluation of vaccine effects. Table 1 shows the serotypes covered by vaccines that have entered clinical use.

FTIR-ATR analysis is based on measuring the absorption of radiation in the infrared (IR) region of the electromagnetic spectrum, which has a longer wavelength (approximately 2.5-25 μm) and lower frequency (approximately 4000-400 cm^{-1}) than visible light. In this technique, when the sample is exposed to IR radiation, the covalent bonds at the molecular level vibrate at characteristic frequencies and absorb the radiation (10). Each functional group creates a unique absorption band corresponding to a specific vibration mode. These vibration modes -such as stretching, bending, scissoring, and twisting- differ depending on the type of intramolecular bonds, bond strength, and atomic mass (11). The absorption spectrum obtained by FTIR spectroscopy provides a qualitative profile specific to the chemical structure of a compound, referred to as its "molecular fingerprint (12)."

FTIR analysis of bacterial isolates enables the acquisition of microorganism-specific spectral profiles by covering the specific vibrations of many biomolecules, including cell wall components (e.g., polysaccharides, proteins, lipids, and nucleic acids) (13). Thus, this technique offers rapid, label-free, and reagent-free characterization and classification at the microorganism level. FTIR-ATR spectroscopy is used to distinguish bacterial and yeast species at different taxonomic levels (14). This method is advantageous because it is simple, fast, and does not require special reagents for analysis (15).

Table 1. Serotypes covered by pneumococcal vaccines in clinical use

Vaccine	Serotypes Covered
PCV7	4, 6B, 9V, 14, 18C, 19F, 23F
PCV10	PCV7 + 1, 5, 7F
PCV13	PCV10 + 3, 6A, 19A
PCV15	PCV13 + 22F, 33F
PCV20	PCV15 + 8, 10A, 11A, 12F, 15B
PCV21	PCV20 + 9N, 17F, 20, 15A, 15C, 16F, 23A, 23B, 24F, 31, 35B

PCV7: 7-valent pneumococcal conjugate vaccine, PCV10: 10-valent pneumococcal conjugate vaccine, PCV13: 13-valent pneumococcal conjugate vaccine, PCV15: 15-valent pneumococcal conjugate vaccine, PCV20: 20-valent pneumococcal conjugate vaccine, PCV21: 21-valent pneumococcal conjugate vaccine.

In bacterial differentiation applications, identification studies have been conducted at the capsular type, serotype, or serogroup level for microorganisms such as *Cryptococcus neoformans* and enterococci (16).

Considering that identification at the serotype or serogroup level is possible, this study was planned to meet the need for serotyping through a reference library created using a low-cost, easy-to-implement method. The potential for widespread use of the serotyping method developed using the FTIR-ATR device, which is fast, low-cost, label-free, and has a wide range of applications, was investigated; the initial findings obtained have also made significant contributions to the literature. In this context, a comparative analysis of isolates containing different serotypes was performed, and the high reproducibility of the obtained spectra was also evaluated.

Materials and Methods

Isolates Obtained

The *S. pneumoniae* isolates used in this study were obtained from the Microbiology Reference Laboratories Department of the Turkish Public Health Agency. A total of eight isolates belonging to four different serotypes (3, 19A, 23B, and 9L) were selected. Serotype selection was based on the fact that these strains exhibit different infection characteristics from a microbiological, clinical, and epidemiological perspective. Although included in the current 13-valent pneumococcal conjugate vaccine, serotype 3 is known to be more resistant to antibody-mediated elimination than other serotypes, likely due to its thicker capsule (17). Serotype 19A, on the other hand, is particularly common in the pediatric age group and is associated with high rates of antibiotic resistance (18). Serotype 23B has been identified as one of the serotypes relatively frequently isolated from pneumococcal infections in Türkiye (19). Serotype 9L, which is not included in the vaccine, has been reported as one of the predominant secondary serotypes associated with multi-serotype pneumonia cases (20).

Bacterial Culture Preparation

The *S. pneumoniae* serotypes used in the study (3, 19A, 23B, 9L) were stored at -86 °C in skim milk powder. Prior to the study, the isolates were inoculated onto sheep blood agar (medium containing 5% defibrinated sheep blood) under sterile conditions and then incubated at 37 °C for 24 hours under atmospheric conditions containing 5% carbon dioxide (CO_2). Figure 1A shows the inoculation of *S. Pneumoniae* serotypes and Figure 1B shows their appearance after incubation.

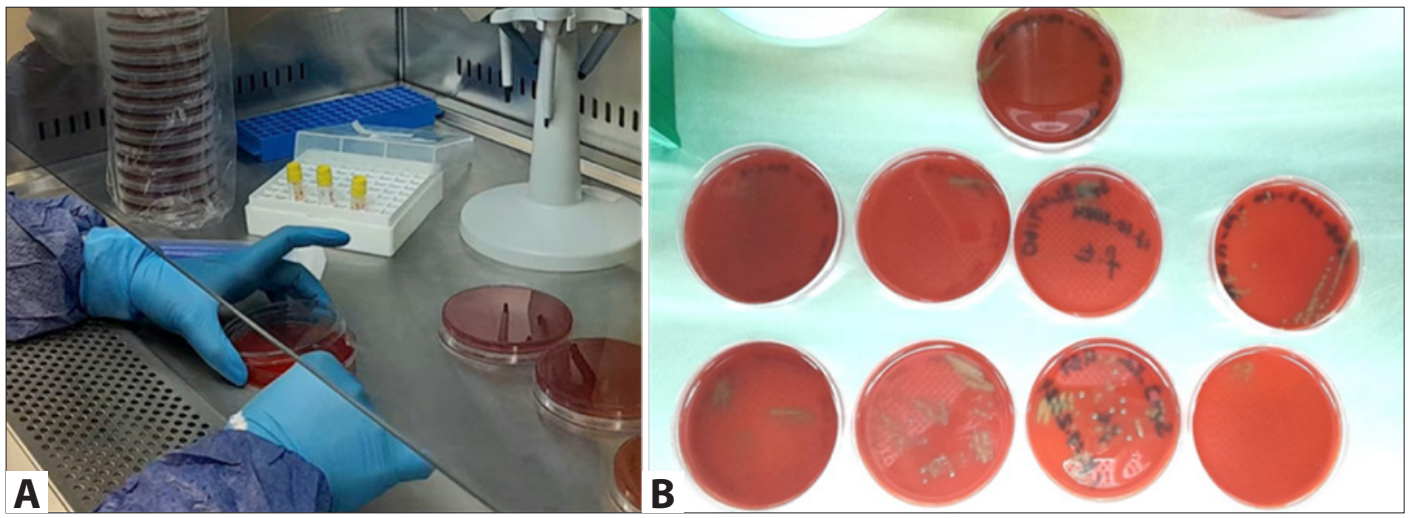

Figure 1. (A) *S. pneumoniae* serotypes culture and **(B)** appearance after incubation at 37 °C for 24 hours in an atmosphere containing 5% CO_2.

FTIR Spectrum Measurement and Analysis of the Serotype Library

FTIR-ATR provides information about the presence or absence of chemical structures and specific functional groups in the samples. FTIR-ATR spectroscopy has long been used as a technique for identifying microorganisms based on their unique spectral fingerprint profiles. FTIR spectra of microbial cells, including bacteria, produce characteristic peaks indicating the presence of carbohydrates, proteins, lipids, and their functional groups. These spectral patterns enable the identification of microorganisms at the species or serotype level by comparing them with reference spectra in databases. Especially for bacterial identification, the obtained FTIR spectra can be compared with reference spectra to distinguish samples based on their unique spectral characteristics (21). Within the scope of this study, a method was developed to create a spectral library for the isolates used, and the analyses were performed using a Shimadzu FTIR-ATR device.

FTIR-ATR analyses of *Streptococcus* species were performed using two different methods. In the first method, samples taken directly from the blood agar surface using a sterile cotton swab were applied to the ATR crystal, and spectrum measurements were performed. In the second method, samples taken from blood agar were transferred to a bacterial transport medium, and this suspension was dripped directly onto the ATR crystal for measurements. All spectra obtained by both methods were combined, analyzed, and evaluated for the creation of a serotype library.

Results

Culturing Different *S. pneumoniae* Isolates in an In Vitro Environment

Bacteria belonging to four different *S. pneumoniae* serotypes (3, 19A, 23B, and 9L), comprising a total of eight isolates, were obtained from samples stored at -86 °C. These isolates were inoculated onto sheep blood agar plates and incubated at 37 °C for 24 hours under atmospheric conditions containing 5% CO_2 to allow bacterial colonies to form. At the end of the incubation period, microbial growth was observed on all plates.

Analysis of FTIR-ATR Spectra

The lipid, protein, amino acid, and carbohydrate regions in the FTIR-ATR spectra of *S. pneumoniae* serotypes (3, 19A, 23B, and 9L) are shown in Figure 2, respectively. The absorption peaks observed in this spectrum in the range of 3100 to 2700 cm^{-1} (wavenumber unit is preferred instead of nanometer) correspond to the symmetric and asymmetric stretching vibration modes of the methyl ($-CH_3$) and methylene ($-CH_2$) groups present in the lipid components of the isolates. This finding demonstrates that the lipid profiles in bacterial cell membranes and internal structures can be identified spectroscopically.

The peaks observed in the 1800-1400 cm^{-1} (wavenumber) range of the spectrum represent the amide I and amide II bands belonging to the protein components of the isolates. These bands provide information about the secondary structures of proteins.

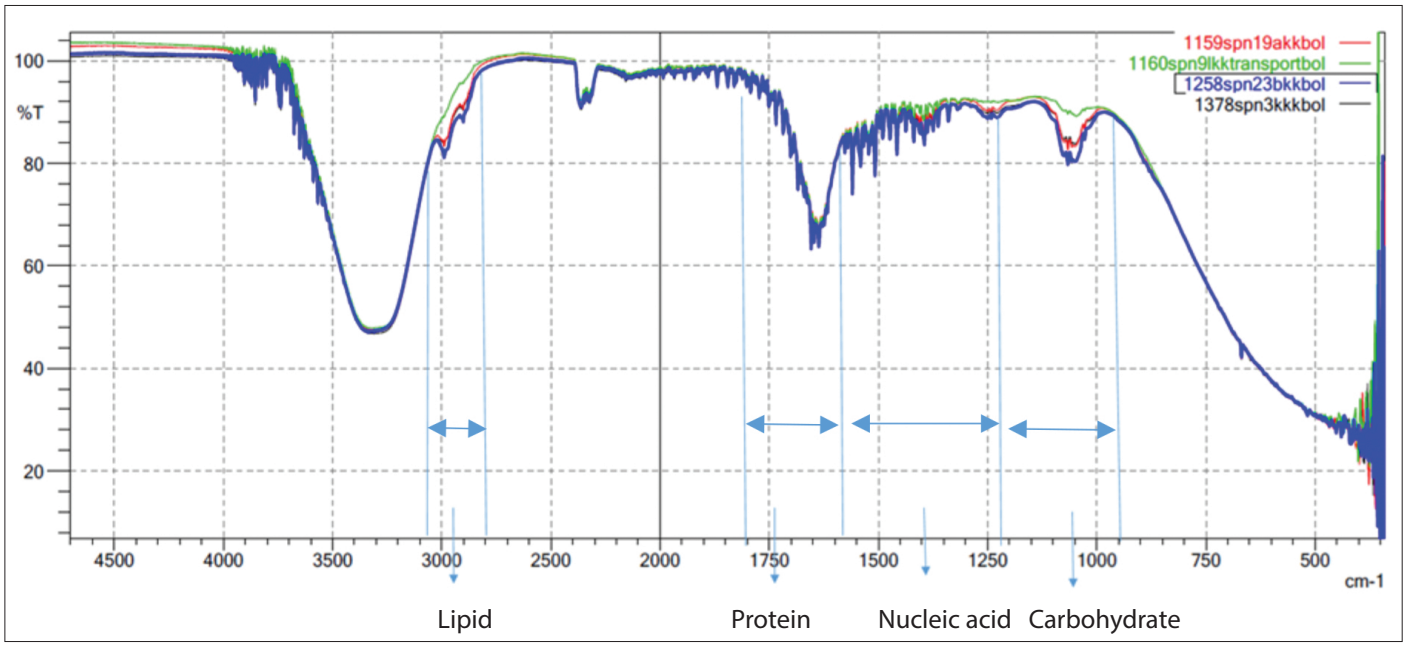

Figure 2. Lipid, protein, amino acid, and carbohydrate regions in the FTIR-ATR spectra of *S. pneumoniae* serotypes, respectively.

The 1400-1200 cm^{-1} range has a more complex structure. In this region, the bending vibrations of -CH_2 groups, the symmetric stretching vibrations of carboxylate (COO^-) groups, and the asymmetric stretching vibrations of phosphodiester (P=O) bonds specific to the nucleic acids of the isolates overlap.

Analyses using two different methods were performed with three technical replications for each isolate belonging to all serotypes, and the obtained FTIR spectra were comparatively evaluated in terms of analytical reproducibility, spectral regional variations that could enable serotyping, and functional group similarities in the capsular polysaccharide regions.

The chemical characterization of *S. pneumoniae* serotypes using FTIR-ATR technique was performed using two different sample preparation methods.

In the first method, colony samples directly isolated from blood agar medium were transferred to the ATR crystal surface for spectral analysis, and the resulting spectra are presented in Figure 3. This spectrum shows the FTIR-ATR spectra of isolates belonging to serotypes 3, 19A, 23B, and 9L, taken directly from blood agar and placed on the ATR crystal, and enlarged versions of the regions where differences between serotypes were observed. In the second method, colony samples taken from blood agar were suspended in a sterile bacterial transport medium and then transferred to the ATR crystal. The FTIR-ATR spectra obtained with this sampling procedure are shown in Figure 4. Additionally, Figure 5 presents detailed views of the spectral regions where variations between serotypes became apparent. These two different sampling approaches enabled the spectroscopic evaluation of potential changes in bacterial cell wall components and metabolic profiles.

Figure 5 presents close-up views of regions in the FTIR-ATR spectra of spn 3, spn 19A, spn 23B, and spn 9L isolates obtained from the bacterial transport medium that show differences that could enable serotyping. Additionally, FTIR-ATR spectrum images obtained from three consecutive sets of analyses performed on serotype 9L, serotype 23B, and serotype 19A in the bacterial transport medium are shown in Figures 6, 7, and 8, respectively. All these spectra were combined and examined to create a serotype library.

Discussion

The use of Fourier Transform Infrared Spectroscopy, particularly in ATR mode, has become a powerful tool in microbiological analysis. This technique provides discriminatory analysis at the species and strain level by

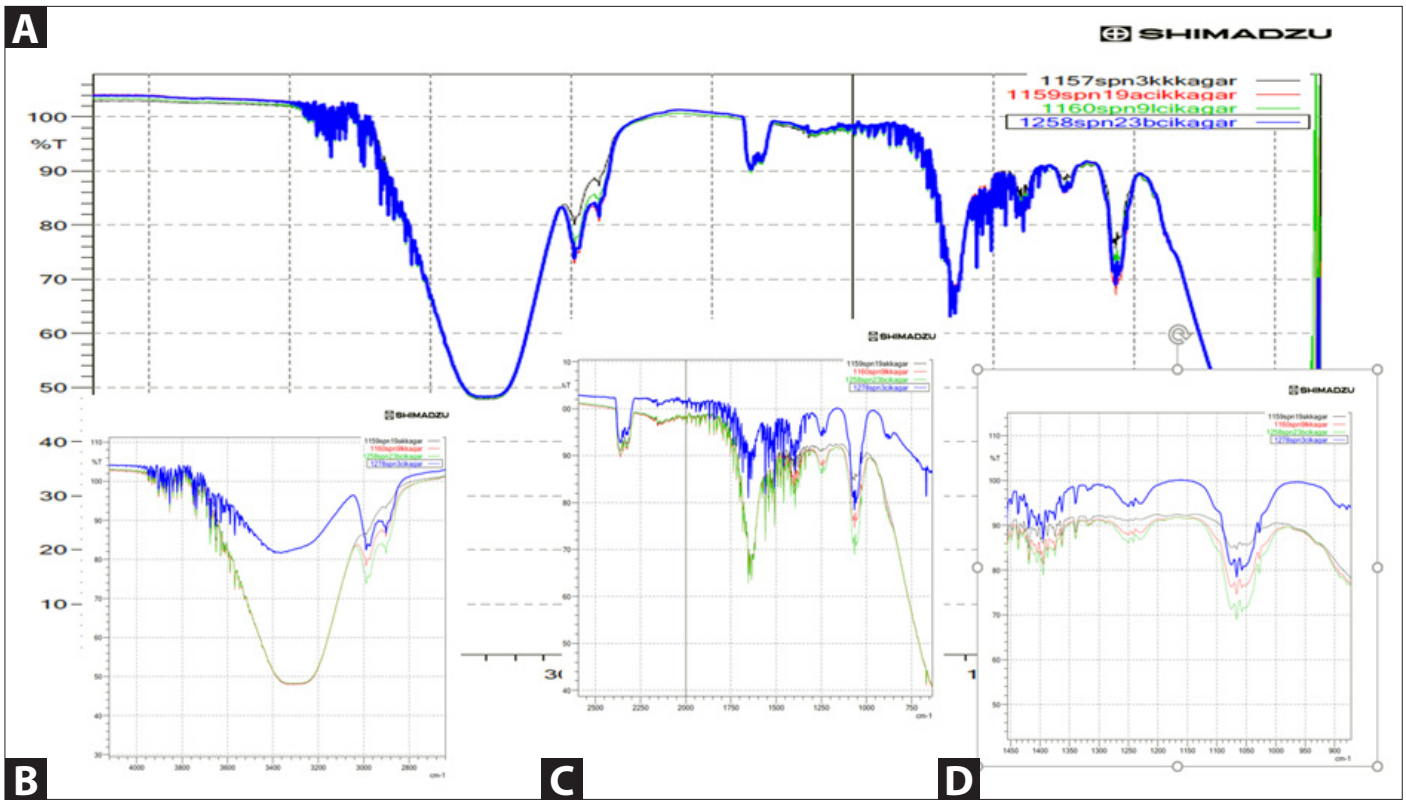

Figure 3. Fourier transform infrared spectroscopy – FTIR-ATR spectra of isolates directly obtained from blood agar: **(A)** Full spectrum of isolates spn 3, spn 19A, spn 23B, spn 9L directly obtained from blood agar. **(B, C, D)** Close-up views of the distinct regions of the FTIR-ATR spectra of spn 3, spn 19A, spn 23B, and spn 9L isolates obtained directly from blood agar.

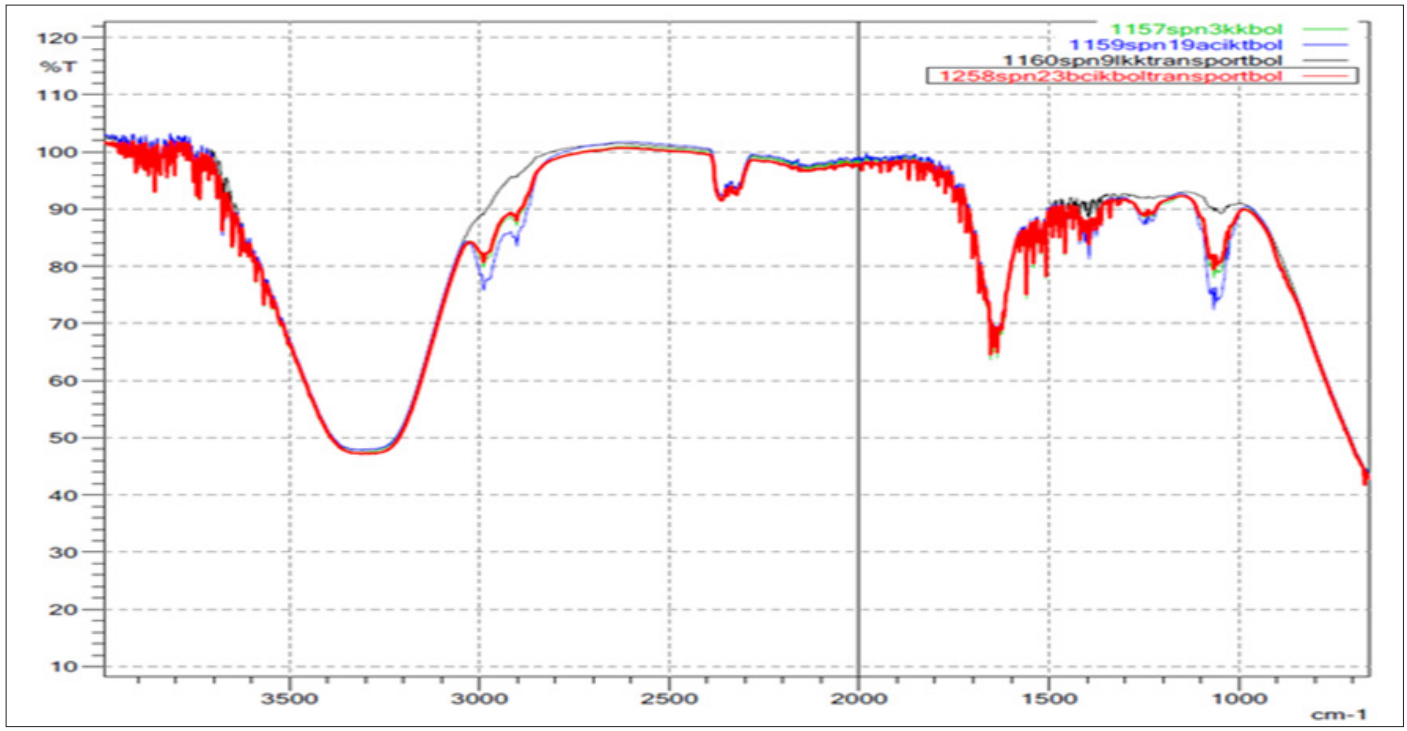

Figure 4. Fourier transform infrared spectroscopy – FTIR-ATR spectra obtained from serotypes 3, 19A, 23B, and 9L suspended in a bacterial transport medium.

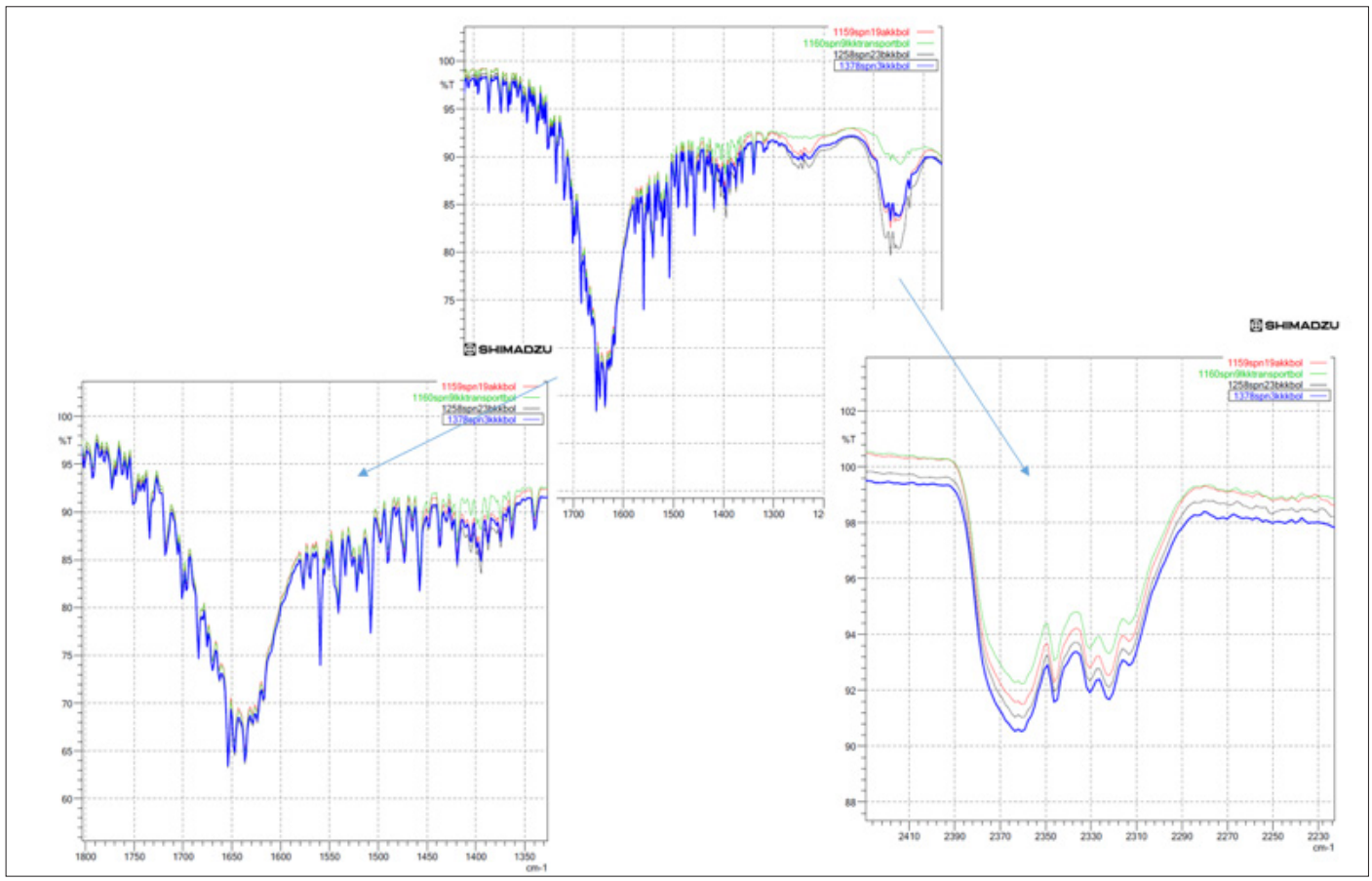

Figure 5. Close-up views of the FTIR-ATR spectra of the spn 3, spn 19A, spn 23B, and spn 9L isolates obtained in suspension in the bacterial transport medium.

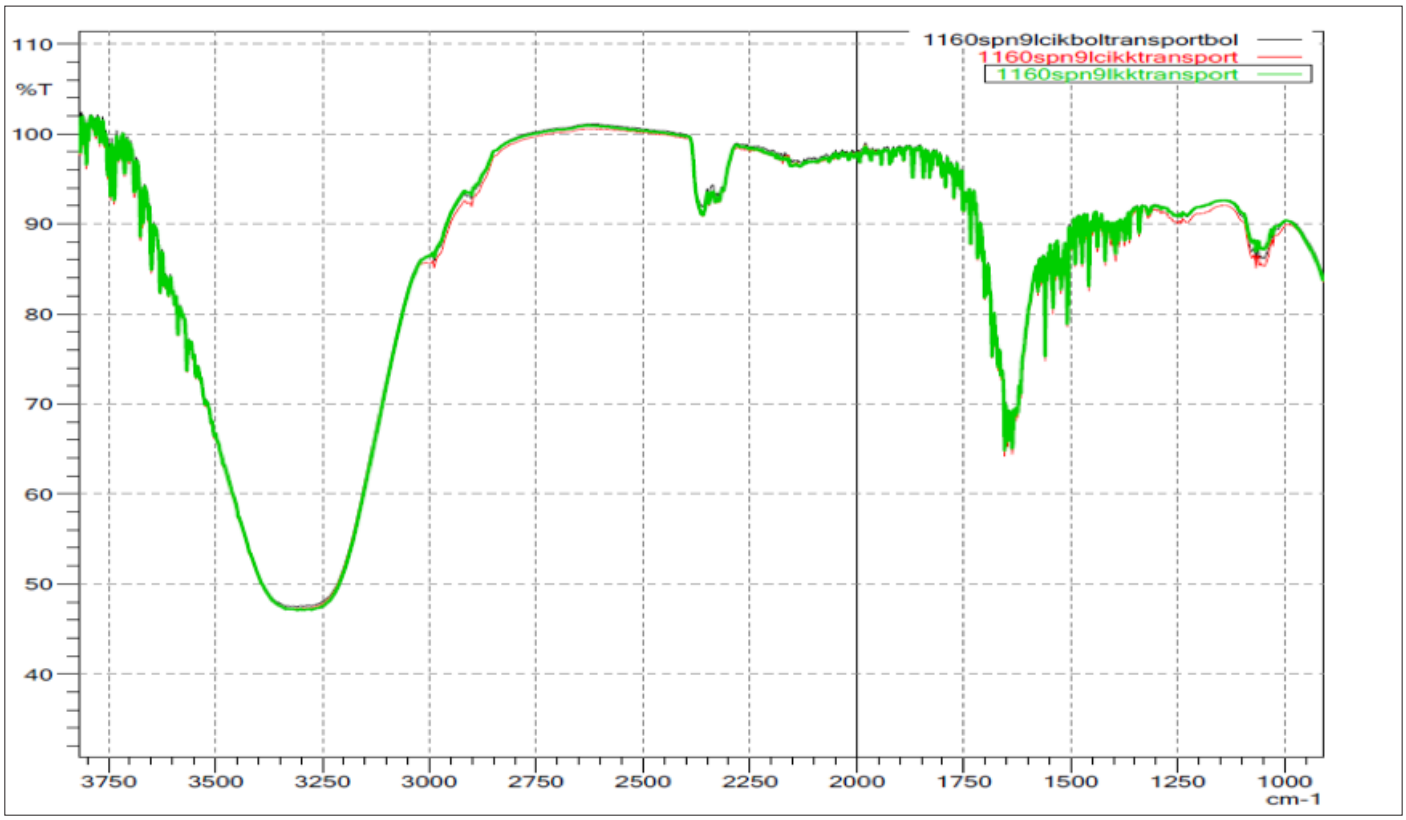

Figure 6. Fourier transform infrared spectroscopy – FTIR-ATR spectra obtained from serotype 9L suspended in a bacterial transport medium, corresponding to three consecutive sets of analyses.

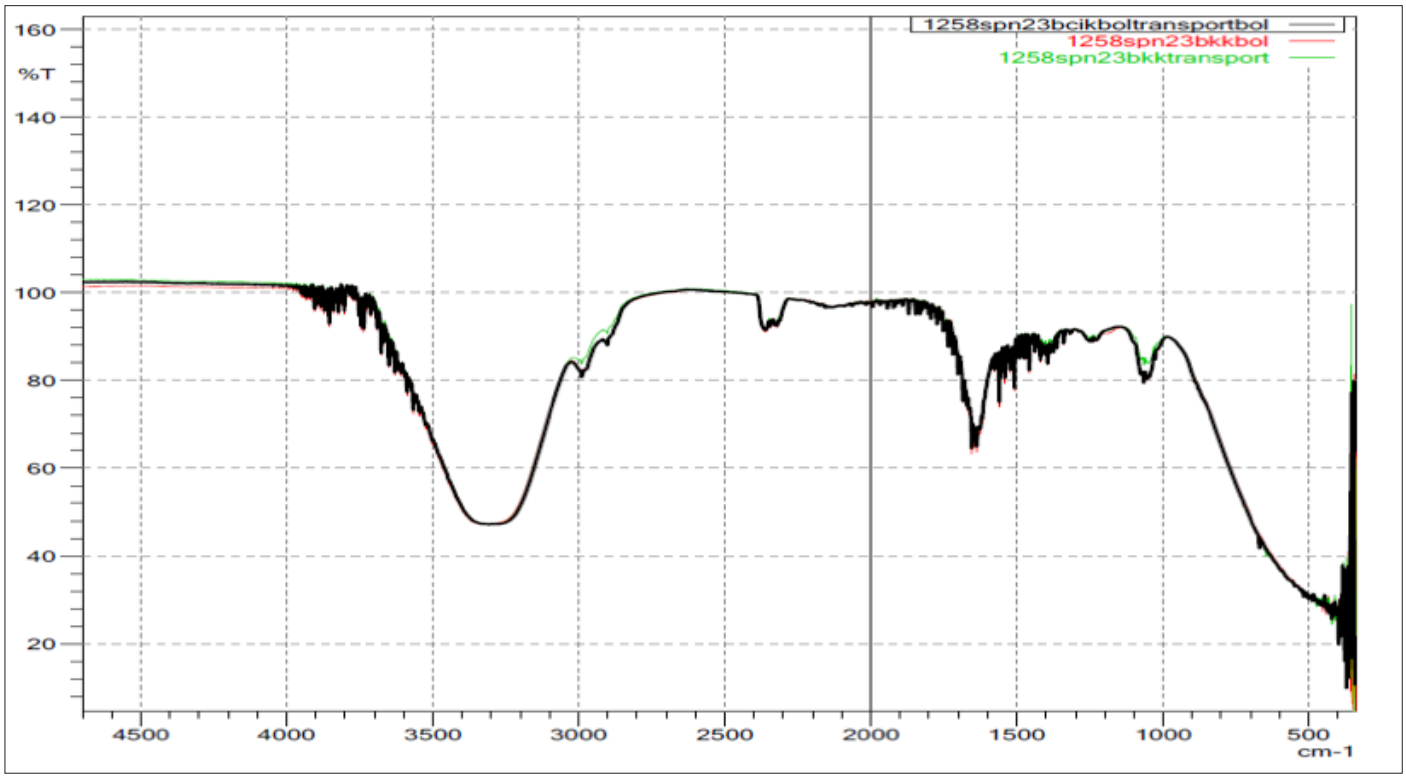

Figure 7. Fourier transform infrared spectroscopy – FTIR-ATR spectra obtained from serotype 23B suspended in a bacterial transport medium, corresponding to three consecutive sets of analyses.

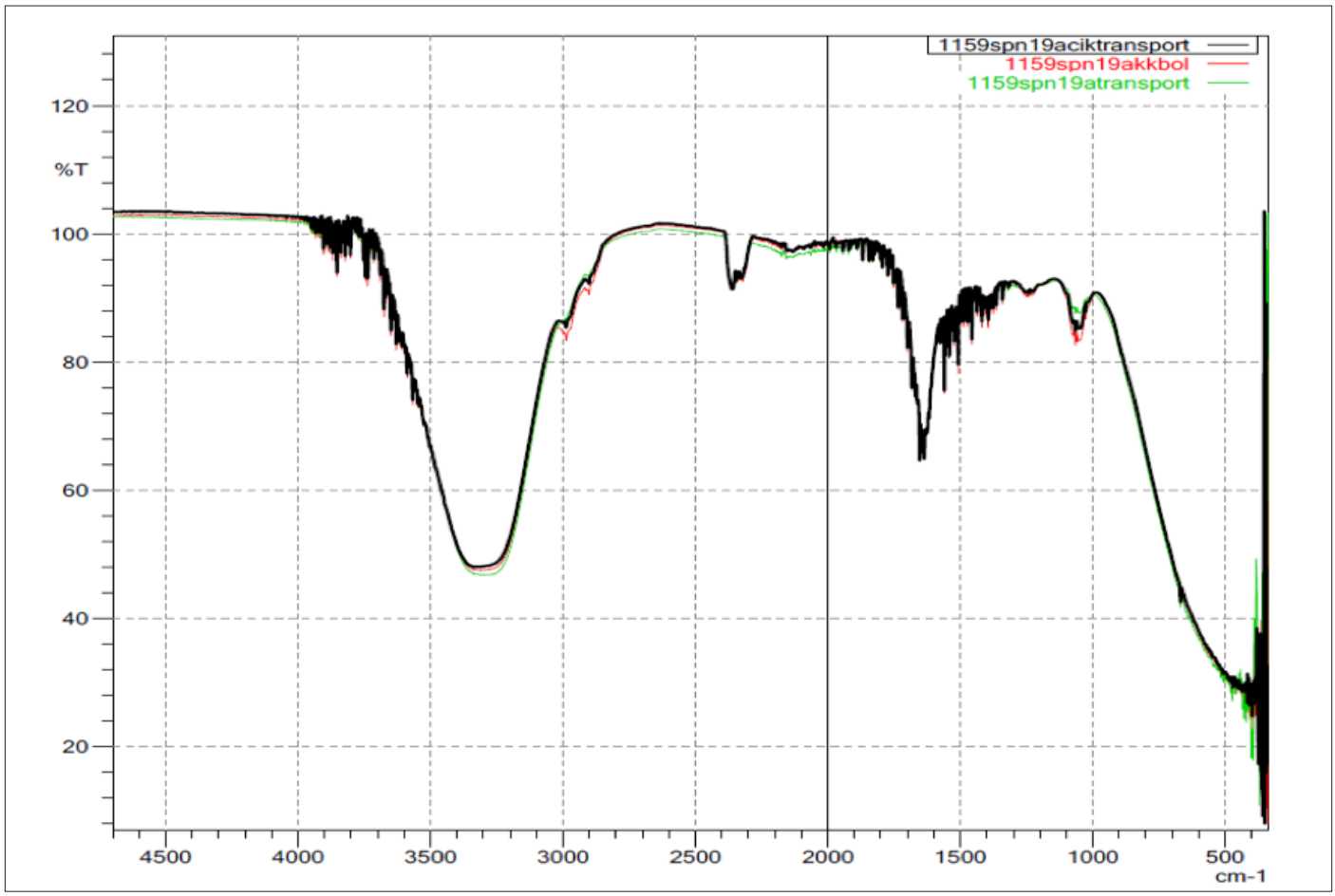

Figure 8. Fourier transform infrared spectroscopy – FTIR-ATR spectra obtained from serotype 19A suspended in a bacterial transport medium, corresponding to three consecutive sets of analyses.

detecting the unique biochemical "fingerprints" of bacterial cell arrangements and polysaccharide/capsule structures. FTIR-ATR spectroscopy offers the opportunity for direct colony analysis without the need for pretreatment; in this respect, it provides significant advantages over classical methods in terms of time, labor, and cost.

In the data obtained in this study, minimal differences in nucleic acid sequences or concentrations between different serotypes caused limited changes in the 1600-1300 nm range. This suggests that nucleic acid-based differentiation is less pronounced in this wavelength range.

The main structural element that is decisive in the serotyping of *S. pneumoniae* is the capsular polysaccharides on the cell surface. These polysaccharides produce characteristic absorption bands in FTIR spectra, particularly in the 1200-950 cm^{-1} wavenumber range. This region is known as the "carbohydrate region," where C-O-C glycosidic bonds and C-O ring vibrations are dominant. Different serotypes possess capsular polysaccharides composed of structurally distinct numbers and types of monosaccharide units. This structural diversity creates distinct differences in the intensity, position, and number of peaks observed in the spectrum. In particular, some serotypes carry more branched and complex capsules, while others are simpler in structure; this creates unique "chemical fingerprints" that provide diagnostic discrimination in FTIR analysis. Thus, the unique polysaccharide composition of each serotype produces a distinctive profile in the FTIR spectrum, providing a reliable basis for the serotyping process.

The spectral differences observed in both sample preparation methods used during the study were minimal and did not provide sufficient resolution to create a discriminating spectral library for serotyping purposes. This indicates that the sensitivity of the current FTIR-ATR system is insufficient for serotype distinctions at this level. The components of the FTIR-ATR device that come into direct contact with the sample surface provide spectral information. However, in some *S. pneumoniae* serotypes, the capsule structure may be thin, loose, or excessively thick. This results in insufficient capsular material contacting the ATR crystal, leading to a low signal. Capsular polysaccharides that differ between serotypes can cause overlapping peaks in FTIR spectra when they are based on structurally similar monosaccharide subunits. This can

make spectral discrimination difficult. The heterogeneous nature of bacterial colonies can cause different serotypes to show similarities in their FTIR signals. In particular, insufficient standardization and variation in biomass density can reduce the quality of the spectral signal. For all these reasons, sufficient resolution may not have been achieved to create a spectral library.

Based on all these results, it is evident that future studies should be conducted with more different serotypes and a larger number of samples to overcome the limitations of this study and develop a more sensitive and discriminative method. In this regard, studies should be conducted by adding a pre-treatment step to the samples to enable a more accurate analysis of the polysaccharide structural differences that are indicative of serotype differentiation.

In the study, the spectral data of each isolate was obtained by repeating the measurement three times consecutively. As clearly seen in these spectra, the spectra obtained in three separate analyses for each serotype show overlap. This scientifically confirms that the FTIR-ATR methodology used has a high level of repeatability and that measurement consistency is successfully achieved.

Conclusion

When all the data obtained were evaluated comparatively, it was seen that although the FTIR-ATR device offers cost-effective and rapid analysis methods, it is not specific enough to develop a serotyping method by creating a *S. pneumoniae* serotype library.

However, the sensitivity of the method can be increased by increasing the capsule density of streptococci. For this purpose, pre-processing techniques (e.g., enzymatic cell wall removal, capsule extraction) can be applied to the sample. This allows capsule-derived signals to become dominant in the spectrum.

Another approach for this purpose could be the use of artificial neural networks, Support Vector Machines (SVM), or deep learning-based classifiers rather than simple statistical approaches. These methods will provide an advantage in terms of serotyping as they will achieve superior success in distinguishing even minimal variations within the spectrum.

Furthermore, datasets containing biological replicates for each serotype will increase the reliability of classification. Therefore, this method shows promise for distinguishing *S. pneumoniae* from other bacterial species by increasing the number of serotypes analyzed.

Various studies in the world literature have also highlighted both the potential and limitations of FTIR-ATR spectroscopy in pneumococcal serotyping. For example, in one study, Sahu et al. aimed to evaluate the potential of FTIR microscopy to distinguish between the capsular proteins of *S. pneumoniae* and identify different serotypes (22). They suggested that the spectral fingerprints of different serotypes likely stem from differences in capsule composition among *S. pneumoniae* strains. They noted that shifts in absorption peaks indicate differences in carbohydrate content among various serotypes. However, they emphasized the limitations of the method they found at the end of the study and stated that the reproducibility of their work should be demonstrated with more data.

In another study, Vaz et al. evaluated FTIR spectroscopy as an alternative method for serotyping capsulated *S. pneumoniae* strains (14). According to the results of the study, they determined that the spectral region that best revealed serotype differences was between 1185-900 cm^{-1}. The results showed that it was possible to distinguish serotypes belonging to the same serogroup, even though the spectral differences were not very pronounced. In parallel with the data obtained by Vaz et al., the 1185-900 cm^{-1} region covered the carbohydrate and nucleic acid region that we identified in our study, yielding results consistent with the literature.

Consistent with this and similar studies in the literature, our findings indicate that although FTIR-ATR is promising, improved statistical approaches (such as multivariate statistical analysis methods like principal component analysis and partial least squares discriminant analysis or machine learning models such as SVM, artificial neural networks & deep learning, random forests etc.) and expanded spectral databases (high-quality representative samples, standardized sample preparation and measurement protocols, data normalization and preprocessing etc.).

Ethics Committe Approval: Since no primary biological samples were used in this study, no ethics committee approval is required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - GÖ, AK; Design - GÖ, AK; Supervision - SNO, AK; Resource - NUS, SNO, AK; Data Collection and/or Processing - GÖ, EAB, ANY, FT, AB; Analysis and/or Interpretation - GÖ, EAB, ANY, FT, AB; Literature Search - GÖ, EAB, ANY, FT, AB; Writing - GÖ, AK; Critical Review - GÖ, EAB, SNO, AK.
Conflict of Interest: All authors declare that they have no conflict of interest.
Financial Disclosure: The authors declared that this study has received no financial support.

References

1. *Zhou ML, Wang ZR, Li YB, Kudinha T, Wang J, Wang Y, et al. Rapid identification of Streptococcus pneumoniae serotypes by cpsB gene-based sequetyping combined with multiplex PCR. J Microbiol Immunol Infect 2022;55(5):870-9. https://doi.org/10.1016/j.jmii.2021.11.004*

2. *Lynch JP 3rd, Zhanel GG. Streptococcus pneumoniae: Epidemiology, risk factors, and strategies for prevention. Semin Respir Crit Care Med 2009;30(2):189-209. https://doi.org/10.1055/s-0029-1202938*
3. *Henriques-Normark B, Tuomanen EI. The pneumococcus: epidemiology, microbiology, and pathogenesis. Cold Spring Harb Perspect Med 2013;3(7):a010215. https://doi.org/10.1101/cshperspect.a010215*
4. *O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet 2009;374(9693):893-902. https://doi.org/10.1016/S0140-6736(09)61204-6*
5. *Manna S, Werren JP, Ortika BD, Bellich B, Pell CL, Nikolaou E, et al. Streptococcus pneumoniae serotype 33G: genetic, serological, and structural analysis of a new capsule type. Microbiol Spectr 2024;12(1):e0357923. https://doi.org/10.1128/spectrum.03579-23*
6. *Ganaie F, Saad JS, McGee L, van Tonder AJ, Bentley SD, Lo SW, et al. A new pneumococcal capsule type, 36, and revision of the capsule nomenclature system to include recently discovered types and subtypes. Clin Infect Dis 2020;70(5):798-805.*
7. *Burckhardt I, Zimmermann S. Susceptibility testing of bacteria using maldi-tof mass spectrometry. Front Microbiol 2018;9:1744. https://doi.org/10.3389/fmicb.2018.01744*
8. *Vasconcelos TM, Rodrigues LS, Krul D, Barbosa SDC, Siqueira AC, Almeida SCG, et al. Fourier transform infrared spectroscopy for Streptococcus pneumoniae capsular serotype classification in pediatric patients with invasive infections. Front Microbiol 2024;15:1497377. https://doi.org/10.3389/fmicb.2024.1497377*
9. *Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet 2011;378(9807):1962-73. https://doi.org/10.1016/S0140-6736(10)62225-8*
10. *Burckhardt I, Sebastian K, Mauder N, Kostrzewa M, Burckhardt F, Zimmermann S. Analysis of Streptococcus pneumoniae using Fourier-transformed infrared spectroscopy allows prediction of capsular serotype. Eur J Clin Microbiol Infect Dis 2019;38(10):1883-90. https://doi.org/10.1007/s10096-019-03622-y*
11. *Jürgen Schmitt, Hans-Curt Flemming. FTIR-spectroscopy in microbial and material analysis. International Biodeterioration & Biodegradation 1998;41(1). https://doi.org/10.1016/S0964-8305(98)80002-4*
12. *Passaris I, Mauder N, Kostrzewa M, Burckhardt I, Zimmermann S, van Sorge NM, et al. Validation of Fourier Transform Infrared Spectroscopy for Serotyping of Streptococcus pneumoniae. J Clin Microbiol 2022;60(7):e0032522. https://doi.org/10.1128/jcm.00325-22*
13. *Romero CT, Moreira NK, da Cunha GR, Mott MP, Dias C, Barth AL, et al. Evaluation of Fourier-Transform Infrared Spectroscopy with IR Biotyper® system for Streptococcus pneumoniae serotyping. Eur J Clin Microbiol Infect Dis 2025;44(8):1967-76. https://doi.org/10.1007/s10096-025-05162-0*
14. *Vaz M, Meirinhos-Soares L, Sousa CC, Ramirez M, Melo-Cristino J, Lopes JA. Serotype discrimination of encapsulated Streptococcus pneumoniae strains by Fourier-transform infrared spectroscopy and chemometrics. J Microbiol Methods 2013;93(2):102-7. https://doi.org/10.1016/j.mimet.2013.02.008*
15. *Naumann D, Helm D, Labischinski H. Microbiological characterizations by FT-IR spectroscopy. Nature 1991;351(6321):81-2. https://doi.org/10.1038/351081a0*
16. *Lemmer K, Naumann D, Raddatz B, Tintelnot K. Molecular typing of Cryptococcus neoformans by PCR fingerprinting, in comparison with serotyping and Fourier transform infrared-spectroscopy-based phenotyping. Med Mycol 2004;42(2):135-47. https://doi.org/10.1080/13693780310001624565*
17. *Luck JN, Tettelin H, Orihuela CJ. Sugar-coated killer: Serotype 3 pneumococcal disease. Front Cell Infect Microbiol 2020;10:613287. https://doi.org/10.3389/fcimb.2020.613287*
18. *Ricketson LJ, Vanderkooi OG, Wood ML, Leal J, Kellner JD. Clinical features and outcomes of serotype 19A invasive pneumococcal disease in Calgary, Alberta. Can J Infect Dis Med Microbiol 2014;25(2):e71-5. https://doi.org/10.1155/2014/196748*
19. *Pınar A, Şener B, Ustaçelebi Ş. Serotype prediction for frequently isolated serotypes of streptococcus pneumoniae by heteroduplex analysis and modification of this technique to real-time fluorometric nucleic acid detection system. Turkiye Klinikleri Journal of Medical Sciences 2010;30(5):1487-92. https://doi.org/10.5336/medsci.2009-15243*
20. *Dhoubhadel BG, Suzuki M, Ishifuji T, Yaegashi M, Asoh N, Ishida M, et al. High prevalence of multiple serotypes of pneumococci in patients with pneumonia and their associated risk factors. Thorax 2022;77(11):1121-30. https://doi.org/10.1136/thoraxjnl-2021-217979*
21. *Kassem A, Abbas L, Coutinho O, Opara S, Najaf H, Kasperek D, et al. Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Front Microbiol 2023;14:1304081. https://doi.org/10.3389/fmicb.2023.1304081*
22. *Sahu RK, Mordechai S, Pesakhov S, Dagan R, Porat N. Use of FTIR spectroscopy to distinguish between capsular types and capsular quantities in Streptococcus pneumoniae. Biopolymers 2006;83(4):434-42. https://doi.org/10.1002/bip.20576*