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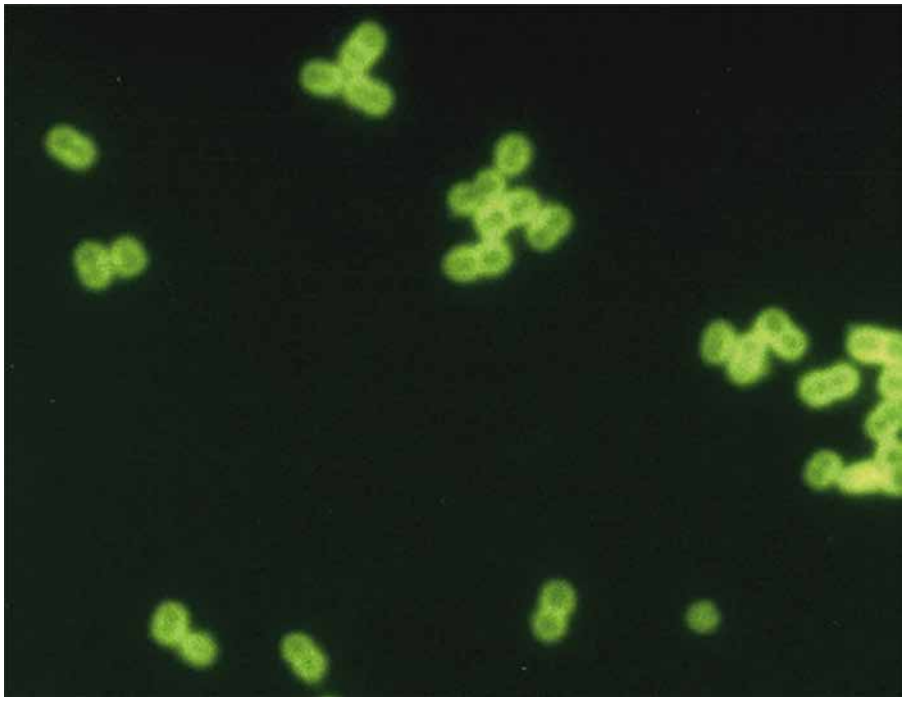
Pneumococcal disease, caused by *Streptococcus pneumoniae* remains a serious health issue. Here, Carolyn Moonen and Markus Meyer examine how recent technological advances in identification of bacterial isolates and their serotypes are improving disease surveillance and treatment.

Despite decades of research, vaccination programmes and improved clinical management, pneumococcal disease continues to pose a serious threat to global public health. The disease, caused by *Streptococcus pneumoniae*, can range from mild infections, such as otitis media and sinusitis, to severe and potentially life-threatening infections, including meningitis and pneumonia, and is a particular threat to young children, the elderly and immunocompromised individuals. Pneumococcal meningitis is fatal in around 15% of children, while a further 25% face long-term disabilities such as brain damage, hearing loss and seizures. Alongside this, 40,000 people in the UK are hospitalised with pneumococcal pneumonia annually, with a mortality rate of approximately 20% despite antibiotic treatment.[1]

Against this backdrop, effective surveillance of *S. pneumoniae* is a key component to limit infection and protect public health. Accurate and timely identification of bacterial isolates and their serotypes is essential for tracking transmission and evaluating vaccine performance. Traditional microbiological methods, while reliable, can be time-consuming and resource-intensive, revealing the need for newer phenotype-based approaches, including Fourier-transform infrared (FTIR) spectroscopy, that can differentiate and classify strains at sub-species level and deliver information more rapidly.



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Streptococcus pneumoniae in cerebrospinal fluid (fluorescent antibody [FA] staining).

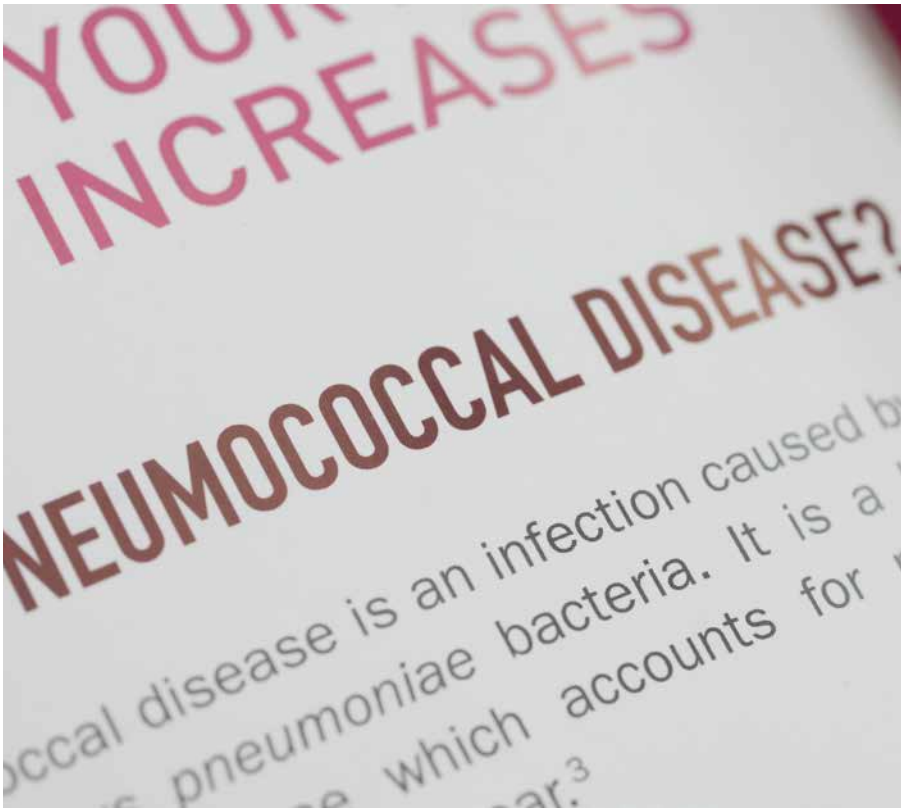
Challenge of serotype diversity

S. pneumoniae is a common bacterium that is transmitted person-to-person via respiratory droplets and frequently colonises the mucosal surfaces of the nasopharynx, often without causing disease. Between 40-50% of healthy children and 20-30% of healthy adults carry the bacteria.[2] However, nasopharyngeal colonisation can be a precursor to the development of both localised infections and invasive pneumococcal disease. In such cases, *S. pneumoniae* is disseminated beyond the upper respiratory tract, either by aspiration, bacteraemia or local spread. Although localised infections, such as otitis media and sinusitis, are usually mild and treatable, they can progress to life-threatening invasive diseases, including pneumonia and meningitis.

The main virulence factor for

S. pneumoniae is the polysaccharide capsule as it interferes with host opsonophagocytic clearance mechanisms, making it the primary target of many pneumococcal vaccines. As there are over 100 known polysaccharide serotypes and protection is strictly serotype-specific, developing effective vaccination strategies can be challenging. Pneumococcal conjugate vaccines (PCVs) which immunise against specific serotypes are typically used to protect against *S. pneumoniae* infections, and ongoing research looks to expand formulations to offer broader serotype coverage. For example, the PCV10 vaccine, approved by the European Medicines Agency (EMA) in March 2009, targets the 10 most common disease-causing serotypes, while the latest PCV vaccine (PCV21) covers 21 different strains, with a PCV24 vaccine currently in development.

Continuous surveillance of strain and serotype diversity is therefore essential to ensure that vaccine formulations target the most prevalent disease-causing serotypes, conferring protection to those immunised and limiting the transmission of threatening invasive disease.



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Traditional methods

Culture-based workflows, biochemical testing and antimicrobial susceptibility testing (AST) are well established methods for the identification of bacteria at species-level and can be used to perform basic phenotyping. Although they are highly reliable, these methods are also time and labour intensive. It can take up to 72 hours to obtain results using culture-based workflows, which can significantly delay the treatment of severe infections that demand fast response times. Furthermore, workflows can be lengthy and require manual intervention at several key stages, which is a particular challenge for reference laboratories handling large workloads.

Lacking the ability to perform serotyping and strain-level characterisation, these methods are often used alongside molecular and immunological approaches. Molecular techniques, such as polymerase chain reaction (PCR)-based serotyping and whole-genome sequencing (WGS), offer rapid and sensitive microbial analysis but are costly due to expensive reagents and equipment. They also rely on large infrastructure, including specialised hardware and bioinformatics systems, which must have the capacity to manage high-throughput workflows.

The historical gold standard for *S. pneumoniae* serotyping is the Quellung reaction (Neufeld test): a method that uses the immunological properties of capsular epitopes to identify pneumococcal capsular types. The technique allows for visual confirmation of the presence of bacterial capsules and provides highly specific results. However, it is costly, time-consuming and dependent upon skilled staff with extensive experience and training. A rapid, standardised and more cost-effective method that can differentiate below the species level is needed to support routine public health *S. pneumoniae* surveillance.

Complementary phenotypic platforms

Mass spectrometry (MS) profiling used alongside FTIR spectroscopy provides comprehensive microbial identification at both species and strain level.

Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) MS generates unique proteomic spectral fingerprints of unknown microorganisms which are then matched against a large reference library to determine the identity of the species. Samples are processed within minutes, enabling rapid identification from a pure culture. Offering reproducible and cost-effective analysis, MALDI-TOF MS is well established in routine microbiology workflows.

Following species confirmation with MALDI-TOF MS, FTIR spectroscopy can be used to differentiate below the species level, resolving differences among strains, isolates or clones. The technique analyses the molecular vibrations caused by the absorption of infrared light across various cellular components, including carbohydrates, lipids and proteins. As different chemical structures vibrate at different wavenumber regions, it provides information about the full range of diagnostic molecules present in the sample. The carbohydrate

MALDI-TOF mass spectrometry generates unique proteomic spectral fingerprints of unknown microorganisms which are then matched against a large reference library to determine the identity of the species

region is especially useful for bacterial typing as it reflects the variation in cell-wall polysaccharides and capsular composition, supporting the classification of microorganisms such as *S. pneumoniae*.

Analysis using FTIR spectroscopy follows a simple three-step workflow. First, the pure culture is transferred to a vial, and the bacterial suspension is prepared. The sample is then spotted on to a plate which is dried before being inserted into the instrument for analysis. Automated bioinformatics software measures and analyses the IR spectra generated. An individual spot can be processed in around a minute, meaning an entire batch of up to 30 isolates can be harvested, prepared and analysed in about three hours.

FTIR spectroscopy supports efficient and effective infection control, outbreak investigation and vaccine development. The speed of analysis enables clinicians to quickly determine and contain bacterial infections, while the ability to investigate unusual patterns in bacterial samples means that outbreaks can be detected and prevented at an early stage. Real-time strain typing using FTIR spectroscopy provides a better understanding of the causes of infection, with precise serotype identification allowing for the development of more targeted vaccination strategies. Together, MALDI-TOF MS technology and FTIR spectroscopy facilitate rapid species identification and strain-level differentiation for bacterial surveillance.

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Case example: Pneumococcal disease surveillance in Brazil

In Brazil, pneumococcal disease is a major public health concern, with pneumonia caused by *S. pneumoniae* accounting for around 30% of hospitalisations.[3] The Brazilian National Immunization Program (PNI) introduced the PCV-10 vaccine for children under the age of two in 2010 and provides the PPV-23 vaccine to high-risk patients with underlying conditions.[4] As the PNI looks to expand towards higher-valency vaccines, continuous and comprehensive serotype surveillance is crucial to track replacement and vaccine escape.

The Instituto Adolfo Lutz in São Paulo, Brazil, a national reference laboratory for bacterial meningitis and invasive pneumococcal disease, has integrated IR spectral analysis for fast and effective routine pneumococcal surveillance in Brazil. The laboratory has developed and validated an IR-based *S. pneumoniae* classifier which has improved the cost-efficiency and turnaround time of serotype identification in the epidemiological surveillance of invasive *S. pneumoniae* isolates. With the ability to

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quickly discriminate at strain level, FTIR spectroscopy and dedicated classifiers can be vital components in surveillance.

As dominant serotypes vary over time and previously rare serotypes can emerge and increase, the development of the classifier began with a prevalence survey of *S. pneumoniae* serotypes in Brazil from 2022 to 2024. The survey revealed the 12 most prevalent serotypes which together accounted for more than 71.4% of invasive isolates. These serotypes were then confirmed using either WGS or the Quellung reaction before being incorporated into the classifier.

The *S. pneumoniae* classifier was trained to achieve optimal performance using a sample preparation technique which has been optimised by the Instituto Adolfo Lutz according to its needs. A sample set of invasive *S. pneumoniae* isolates was analysed using FTIR spectroscopy, generating 1,110 spectra and achieving complete accuracy across the 12 serotypes. Subsequent linear discriminant analysis of the spectra yielded clear separation between serotypes, indicating that the newly developed *S. pneumoniae* classifier has strong discriminatory power at the serotype level.

Following development of the classifier, the laboratory performed comprehensive validation using a set of 265 isolates. The set comprised both trained serotypes (included in the classifier) and non-trained serotypes (excluded from the classifier). Validation demonstrated overall concordance of 99.6% with only one discordant result, emphasising the high performance of the classifier.

Using validation results, the reference laboratory conducted a reliability ranking to measure the extent to which unknown isolates fit into the classifier model. Serotypes with a score above 80 show high reliability and can be used with confidence, while those above 60 display moderate reliability and require a confirmatory test. The majority of results attained a score of at least 80, supporting the implementation of the *S. pneumoniae* classifier into routine use in a public health setting.

The Instituto Adolfo Lutz's successful development of a 12-serotype classifier reflecting the most prevalent pneumococcal serotypes in Brazil demonstrates the suitability of IR-based classifiers for routine surveillance workflows, particularly as validation findings indicate that FTIR spectroscopy generates robust and highly reliable results. These developments raise important questions about how best to deploy an expanded toolkit of microbiological techniques, spanning classical serology, genomics, and IR-based phenotyping, to support rapid outbreak response and long-term vaccine strategy.

Moving towards integrated surveillance

Integrating rapid phenotyping methods, such as FTIR spectroscopy, into national and global networks could support faster and more comprehensive bacterial surveillance. IR-based strain-level typing complements species-level MALDI-TOF MS and molecular methods, such as PCR and WGS, to create a tiered, multi-layer approach which can accelerate outbreak detection, track infection transmission and monitor serotype shifts post-vaccination. Low-cost and easy to deploy, phenotypic platforms are especially well suited to bacterial surveillance in resource-limited reference laboratory settings.

Improved bacterial surveillance in turn facilitates the development of more effective vaccination strategies. With the ability to perform serotype-level analysis, researchers can better evaluate and refine vaccine coverage and effectiveness, as well as detect non-vaccine-type replacement and emerging resistant clones at an early stage.

Evolving serotype and strain landscapes, such as those associated with pneumococcal disease, are an ongoing public health threat and demand flexible, fast and scalable laboratory tools that can keep pace with the latest developments. The use of IR-based strain-typing, as evidenced by the Brazilian *S. pneumoniae* classifier, can help bridge the gap between traditional microbiology and genomic sequencing and could play a crucial role in combating invasive infectious diseases like pneumococcal disease. PP

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