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Review

Streptococcus pneumoniae: Perspectives of Clinicians and Microbiologists on the Underrecognized Threat of Pneumococcal Disease in India

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Abstract

Background: Invasive pneumococcal disease (IPD) is predominantly caused by *Streptococcus pneumoniae* and is associated with high mortality. This vaccine-preventable disease has been a significant public health concern, particularly among children < 5 years and adults > 50 years in India. Pneumococcal vaccines help prevent pneumococcal infections caused by certain strains of *S. pneumoniae*, but diagnostic challenges in India hinder disease management. **Methods**: The objective of this expert panel discussion is to deliberate upon the pathogenesis and epidemiology of *S. pneumoniae* in India, techniques for overcoming the diagnostic challenges in the isolation and detection of *S. pneumoniae*, and to explore opportunities for partnerships between clinicians and microbiologists to bridge gaps. A non-systematic, targeted literature search was conducted to identify studies supporting key themes of expert panel discussion. Eight microbiologists, one infectious disease, one pediatrics and neonatology, and one respiratory medicine specialist from different parts of India participated in the discussion. **Results**: The panelists reiterated that while culture is the gold standard, multiplex real-time polymerase chain reaction and BioFire® FilmArray® technology have been the most common diagnostic methods for the detection of *S. pneumoniae* in India, especially in tier 1 cities. **Conclusions**: This article elucidates the challenges faced by microbiologists and clinicians in isolating and detecting the pathogen, and proposes solutions to determine the true burden of pneumococcal disease in India. This information could further inform public health policy and vaccination strategies.

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Keywords: blood culture; drug resistance; real-time polymerase chain reaction; *Streptococcus pneumoniae*; whole-genome sequencing

1. Introduction

Pneumococcal diseases caused by *Streptococcus pneumoniae* can range from mild respiratory tract infections to severe, life-threatening conditions. Noninvasive *S. pneumoniae* infections—which are common—are non-life-threatening and do not spread to major organs or the bloodstream. Invasive *S. pneumoniae* infections, however, are life-threatening conditions that can cause bacteremia and/or impact major tissues such as the bone, brain, and lungs. The most common invasive pneumococcal diseases (IPDs) in India are meningitis and pneumonia, with a high fatality rate despite hospitalization [1]. Pneumococcal diseases can affect individuals of all ages, but certain populations, such as children < 5 years, older individuals, and those with compromised immune systems or underlying health conditions, are at a higher risk. The clinical presentation of pneumococcal diseases can vary, and accurate diagnosis is crucial for appropriate treatment. However, the isolation and detection of *S. pneumoniae* in India is challenging, which leads to a lower perceived burden of the disease among clinicians. This expert discussion, held in June 2023 in Chennai, India, among eight Indian microbiologists, one infectious disease expert, one pediatrics and neonatology expert, and one respiratory medicine expert, aims to:

1. Gain insights into the pathogenesis and epidemiology of *S. pneumoniae* in India;
2. Discuss the challenges in isolation and detection of *S. pneumoniae*;
3. Discuss the opportunities for collaboration between clinicians and microbiologists to bridge this gap.

2. Methods

A scientific advisory board meeting was held in June 2023 in Chennai, India. The expert panel included eight microbiologists, one infectious disease expert, one pediatrics and neonatology expert, and one respiratory medicine expert with more than 10 years of experience, who provided perspectives on pneumococcal disease due to *Streptococcus pneumoniae* (*pneumococcus*) in India.

A non-systematic, targeted literature search was conducted to define the key discussion themes on pneumococcal pathogenesis and epidemiology, diagnostic challenges, and opportunities for clinician–microbiologist collaboration to bridge this gap, for the advisory board meeting. A literature search was conducted in PubMed and Google Scholar databases using a combination of keywords related to *Streptococcus pneumoniae*, pneumococcal disease, pathogenesis, epidemiology, bacterial isolation, diagnosis, serotype analysis, molecular diagnostic methods, and vaccines. The identified literature was further organized and presented to support the advisory board discussions. The panelists were provided with the evidence summary prior to the advisory board meeting.

The key insights and opinions from the expert panel were gathered from the document meeting summary and integrated with supporting evidence from the literature review to develop this narrative expert review article.

3. Burden and Contributing Factors of *S. pneumoniae* Infections in India

Streptococcus pneumoniae (*pneumococcus*) is an important pathogen causing invasive and noninvasive infections, predominantly at both extremes of age. Although vaccines are available for prevention, the challenge in preventing *S. pneumoniae* infections is due to the presence of a large number of serotypes (>100) that vary geographically and affect

different age groups [2]. Laboratory diagnosis of *S. pneumoniae* is challenging because of its specific growth requirements and empirical therapy guidelines. All these factors lead to the underestimation of the true disease burden and the serotype prevalence of *S. pneumoniae* infections in India [1]. Although advanced molecular diagnostic tests are available, culture remains the gold standard or the most valuable diagnostic method. This is because isolation of the *S. pneumoniae* causing the infection can help identify the epidemiological profile of the infection, which includes the serotype and antimicrobial susceptibility profile (Chen et al. 2025) [3]. Hence, attempts have been made to increase the awareness of physicians and microbiologists in culturing *S. pneumoniae*, starting from the importance of methods and timing of collecting samples, immediate processing, use of appropriate culture media, and growth conditions to enhance the isolation of this bacterium. This article proposes practical recommendations to address the challenges faced by both clinicians and microbiologists, and gives alternative suggestions to enhance the isolation and diagnosis of *S. pneumoniae*.

Lower respiratory tract infections caused by *S. pneumoniae* are considered one of the leading causes of morbidity and mortality, with over two million deaths reported globally in 2016 [1]. Furthermore, India is responsible for 23% and 36% of the global and WHO regional pneumonia burden, respectively [4]. A review of evidence related to IPD from Southeast Asian countries published between 2008 and 2018 showed that approximately one in four deaths among children < 5 years of age in India was due to IPD [5]. Available evidence from India indicates a significant burden of pneumococcal disease; however, the reported estimates are derived from heterogeneous populations and vary with age, clinical syndrome, geographical settings, specimen type and surveillance methodology. Findings from studies of IPD, CAP, and non-invasive pneumococcal disease have been summarized in (Table 1).

Table 1. Summary of epidemiological evidence on pneumococcal disease relevant to India.

Reference	Population/Age Group	Region/Setting	Study Period/Surveillance Methodology	Reported Findings
Thomas et al. 2013 [6]	IPD in patients ≥ 18 years (n = 1037)	Teaching hospitals in India	Prospective surveillance between 1993–2008	Clinical syndromes associated with IPD were meningitis (34.3%) and pneumonia (33.9%). Overall case fatality of 26.4%, whereas a higher case fatality rate of 36.9% in meningitis and sepsis cases.
Varghese et al. 2021 [7]	Noninvasive bacteremic pneumonia; >16 years of age (n = 280)	South Indian tertiary care hospital	Hospital-based study in patients admitted between 2018 and 2020	Prevalence of low-invasive serotypes was more frequent (serotypes 3, 6A, 6B, 8, 19F, and 23 F in 32% of isolates) than high-invasive potential serotypes (serotypes 1, 5, and 7F).
Ghia et al. 2019 [8]	CAP in patients ≥ 12 years of age (n = 1435)	India	Systematic review and meta-analysis on studies published between 1990 and 2017	Pooled prevalence of CAP was 19%.
Ghia and Rambhad, 2022 [9]	CAP in patients ≥ 12 years of age (n = 2114)	India	Systematic review and meta-analysis on studies published between 1990 and 2021	The pooled prevalence of comorbidities and risk factors was: COPD (24.2%), hypertension (23.7%), diabetes mellitus (16%), history of smoking (51.4%), advanced age ≥ 50 years (55.8%).

CAP: Community-acquired pneumonia; COPD: chronic obstructive pulmonary disease; IPD: invasive pneumococcal disease.

Several factors, including high population density, poor living conditions, limited healthcare access, underlying health conditions, incomplete vaccination coverage, antimicrobial resistance (AMR), insufficient surveillance, and environmental factors, contribute to the epidemiological burden of *S. pneumoniae* in Indian adults (Figure 1).

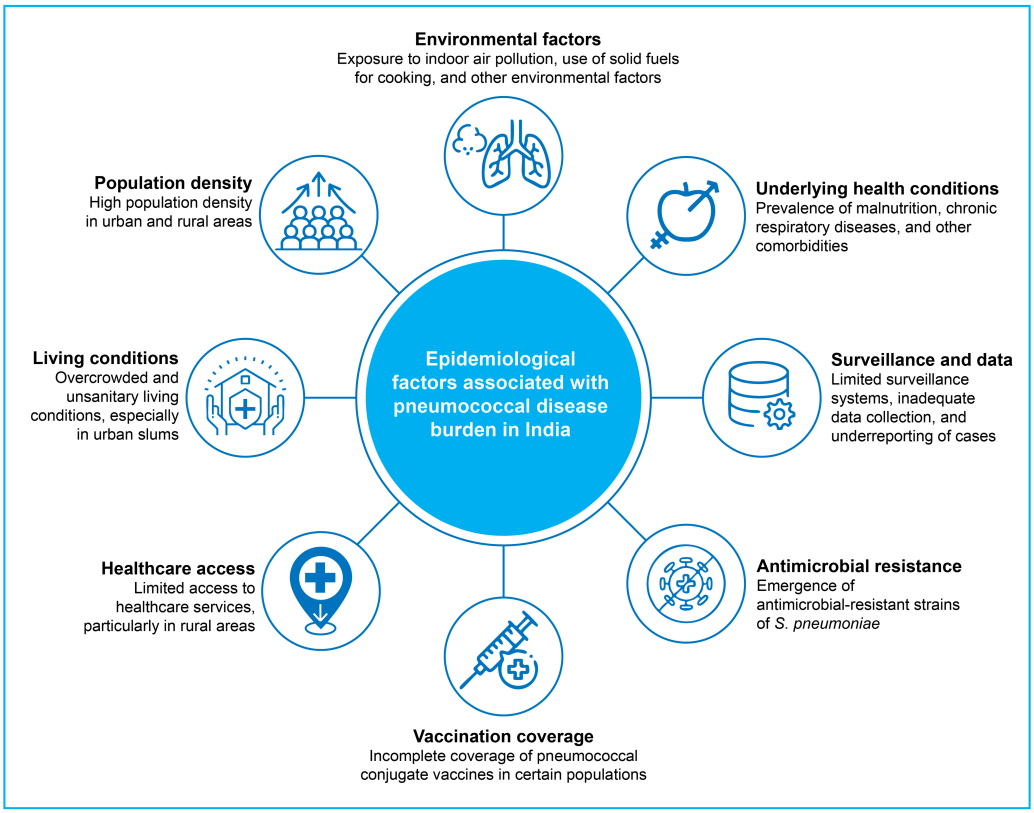


Figure 1. Epidemiological factors associated with lower perceived burden of pneumococcal disease in India.

3.1. Age

IPD has a bimodal distribution, with higher incidences in the very young and older individuals (<5 years and >50 years of age) [10]. A systematic review and meta-analysis that included Indian patients aged ≥ 12 years revealed that *S. pneumoniae* was responsible for ~19% of community-acquired pneumonia (CAP) cases [8]. An advanced age (≥50 years) was also reported as a major risk factor for CAP that accounted for 55.8% of cases in India [9]. The age-stratified incidence of pneumococcal disease in 2018 in India is reported as 31.3% in older individuals (≥60 years), 22.7% in middle-aged individuals (44–60 years), and 13.9% in young individuals (18–44 years) [11]. The case fatality rates were higher in adults ≥ 50 years; the fatality rates were 30.2% and 26.1% for the age groups of 50–60 years and >60 years, respectively [6].

3.2. Comorbidities and Immunocompromised Conditions

About 20–50% of children and 5–20% of healthy adults have pneumococcal colonization in the throat [12] that may cause opportunistic infections [13]. Several serotypes, namely, 8, 12F, 22F, 10A, 11A, 15B, and 33F, of *S. pneumoniae* are associated with more severe illness than other serotypes, with higher mortality, AMR, and impact on vulnerable populations such as those with a smoking habit, older adults, immunocompromised individuals, and those with comorbidities (such as chronic renal failure, diabetes mellitus, or asthma) [14].

In India, chronic obstructive pulmonary disease (COPD) (24.2%), hypertension (23.7%), and diabetes mellitus (16%) were the most common factors associated with an increased risk of CAP in the Indian population [9]. A multicenter prospective study (2010–2017) analyzed the mortality rate in 581 patients with IPD, aged 6–64 years, who also have underlying comorbidities. The risk factors for mortality in patients with IPD are summarized in (Table 2).

Table 2. Risk factors for mortality in patients with IPD [15].

Underlying Condition	Hazard Ratio (95% Confidence Interval)
Age (45–64 years)	3.4 (1.6–6.8)
Bacteremia with unknown focus	2.0 (1.1–3.7)
Meningitis	2.1 (1.1–4.0)
Underlying multiple non-immunocompromising condition *	2.6 (1.1–7.4)
Immunocompromising conditions related to malignancy	2.4 (1.0–5.2)

* Non-immunocompromising conditions include cerebrospinal fluid leakage, diabetes, cardiovascular diseases, lung disease, liver disease, chronic kidney disease, cerebrovascular disease, and neuropsychiatric disease.

3.3. Travelers

Pilgrimages often pose an increased risk of *S. pneumoniae* infection and transmission due to the crowded settings, especially among individuals with comorbidities. A longitudinal multicenter study from India revealed that pneumococcal carriage increased from 19% to 21.8% in Hajj pilgrims (pre- vs. post-Hajj); similarly, an increase in the multidrug-resistant strains carried by these pilgrims rose from 11% to 32% (pre- vs. post-Hajj) [16].

3.4. Lifestyle and Environmental Factors

Data from meta-analyses indicate that smoking (odds ratio, OR:2.17, 95% confidence interval, [CI]: 1.70–2.76) and alcoholism (relative risk: 1.83, 95% CI: 1.30–2.57) may elevate the risk of CAP in adults [17,18]. Overcrowding, indoor air pollution, exposure to environmental smoke, and lack of adequate access to healthcare facilities are also associated with an increase in pneumonia risk [19].

Addressing the epidemiological burden of *S. pneumoniae* in India requires a multifaceted approach, including improving healthcare infrastructure, enhancing vaccination coverage, promoting public health awareness, and addressing socioeconomic determinants that contribute to the spread of pneumococcal infections.

4. Current Diagnostic Methods for *S. pneumoniae* Isolation and Detection in India

The diagnostic approach for *S. pneumoniae* is usually guided by the clinical syndrome and specimen type. Microbiological confirmation of IPD is performed in sterile site specimens, such as blood or cerebrospinal fluid (CSF), whereas for suspected pneumococcal CAP, results from respiratory tract specimens should be interpreted along with radiological findings [20]. In contrast, detecting *S. pneumoniae* in upper respiratory tract specimens, particularly nasopharyngeal specimens, may indicate pneumococcal carriage and should therefore be interpreted in the context of clinical presentation and radiological findings [21,22]. Molecular and antigen-based diagnostic tests can complement conventional methods but may not provide an isolate for antimicrobial susceptibility testing or serotyping, which are considered critical for surveillance [23,24].

Figure 2 gives a summary of the various methods commonly used for the diagnosis of IPD and non-IPD.

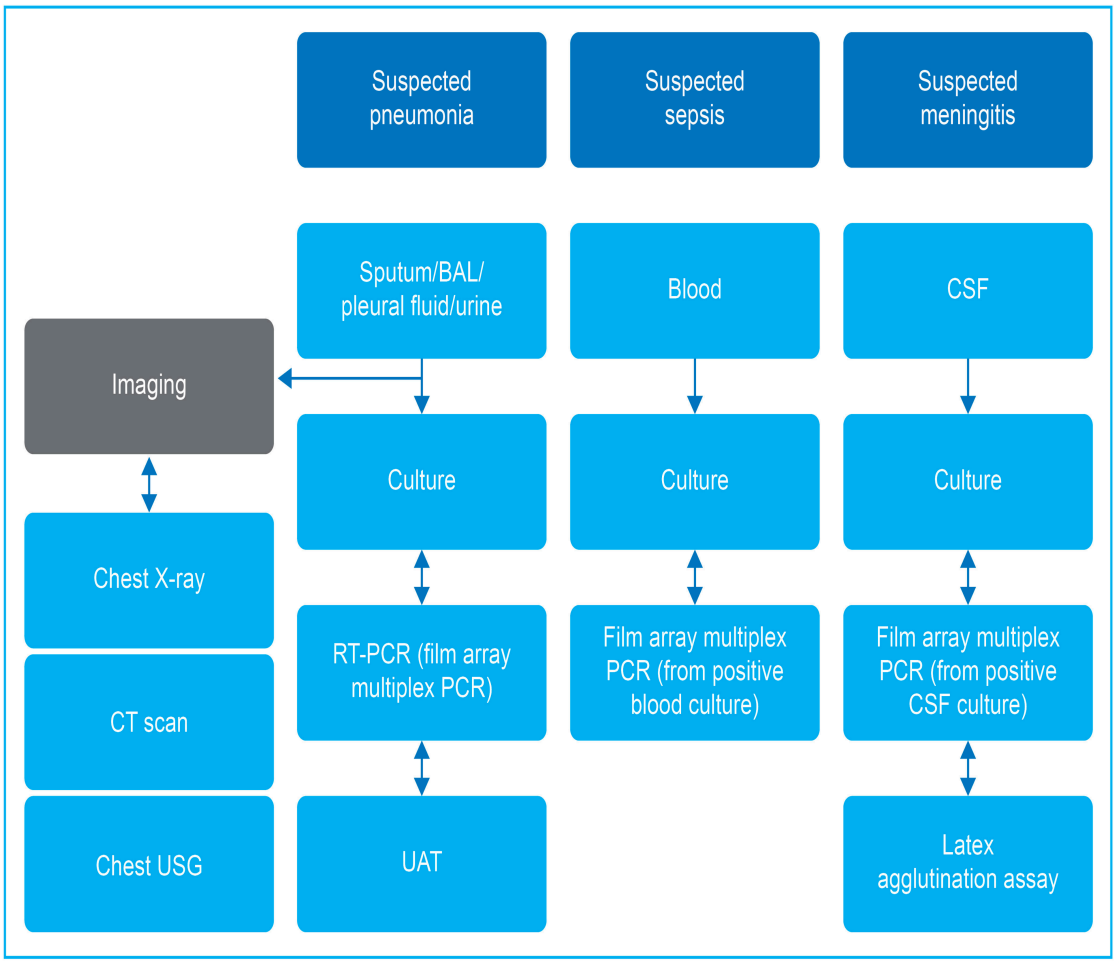


Figure 2. Summary of common diagnostic methods for invasive and non-invasive pneumococcal disease. BAL: bronchoalveolar lavage; CT: computed tomography; CSF: cerebrospinal fluid; PCR: polymerase chain reaction; RT-PCR: real-time polymerase chain reaction; UAT: urinary antigen test; USG: ultrasonography.

4.1. Isolation of S. pneumoniae

Various methods are employed to isolate *S. pneumoniae* in low-resource settings. Sputum, endotracheal aspirates, and bronchoalveolar lavage (BAL) are common respiratory specimens; however, blood may be collected for culture in suspected cases of invasive pneumonia or sepsis [20]. BAL is the gold standard for etiological diagnosis of CAP; however, it is invasive and requires specialists to obtain samples. Hence, it is usually performed solely for further evaluation of radiologic imaging in cases where patients are critically ill. Lung aspirates and sampling from sterile sites, such as CSF and pleural fluid, also aid in the diagnosis of IPD. These methods vary in invasiveness, accessibility, and accuracy. Blood culture remains the gold standard for the diagnosis of invasive pneumonia, although it requires specialized resources, and the results may vary depending on different laboratory and clinical factors. For other IPDs, culture growth of *S. pneumoniae* from blood, CSF, and other sterile fluids is diagnostic, although it is challenging. On the other hand, swab-based methods are simpler but may have lower sensitivity, because *S. pneumoniae* could be a commensal in the nasopharynx (Table 3).

Table 3. Advantages and disadvantages of specimens recommended for the diagnosis of pneumococcal disease.

Specimen	Sensitivity	Specificity	Advantages	Disadvantages	Study
Gram stain	57%	97%	Relatively simple and easy to perform	The sensitivity is affected by prior antibiotic administration	Werno et al., 2008 [20]
Blood culture			Gold standard for invasive pneumococcal disease	Requires specialized equipment and expertise	Werno et al., 2008 [20]
				Autolysis, which is a pathogen-related factor	
				Time-consuming (24–48 h)	
				Lacks sensitivity (due to laboratory and clinical factors)	
Sputum culture	93%	70%	Noninvasive	Low diagnostic yield (sensitivity)	Werno et al., 2008; Zhang et al., 2022; Musher et al., 2004 [20,25,26]
			Relatively simple procedure	Risk of contamination	
				Difficulty in obtaining samples of high quality (specimen without salivary contamination)	
				Difficult to collect from children	
				The culture sample should be collected within 24 h of receipt of treatment	
Nasopharyngeal/ oropharyngeal swab			Simple and noninvasive	Detects only carriage	
			Can be performed at the point of care	Is not usually performed unless specifically requested	
Lung aspiration	60.9–83%	100%	Used in children and individuals with disabilities	Invasive procedure, not suitable for all patients	Scott et al., 2003; Dorca et al., 1995 [27,28]
			Can provide conclusive results	Requires specialized equipment and expertise	
Other sterile sites (CSF, pleural fluid)	95–100%	100%	Targeted sampling based on clinical presentation	Invasive procedures may not be feasible	Werno et al., 2008 [20]
			Can detect infection in specific sites	Limited accessibility to specialized equipment	
				Low culture yield due to empirical therapy and autolysis	

CSF: Cerebrospinal fluid.

4.2. Detection Techniques for S. pneumoniae

Diagnosing pneumococcal disease typically involves a combination of clinical evaluation, laboratory tests, and imaging studies. Abnormal chest radiographs are commonly observed in patients with CAP, with about two-thirds of these abnormalities possibly

linked to bacterial causes, particularly *S. pneumoniae* [29]. Traditionally, the laboratory tests used for diagnosing pneumococcal pneumonia include culturing of respiratory specimens; for IPD, however, culturing of blood and sterile body fluids and latex agglutination tests are required. With the advancements in molecular methods and urinary antigen tests (UATs), the detection of pneumococcal antigens using polymerase chain reaction (PCR) and automated syndrome-based panel tests (FilmArray®) indicates that these methods are becoming essential for the diagnosis and management of pneumococcal infections, as well as for monitoring AMR patterns and conducting epidemiological surveillance [8].

The traditional culture-based technique, often considered the gold standard, involves cultivating *S. pneumoniae* on blood agar plates (Figure 3A) to highlight its colony morphology and hemolytic properties, aiding rapid identification. Commonly used culture media for the growth and isolation of *S. pneumoniae* in India include 5–7% sheep blood agar and chocolate agar plates [30].

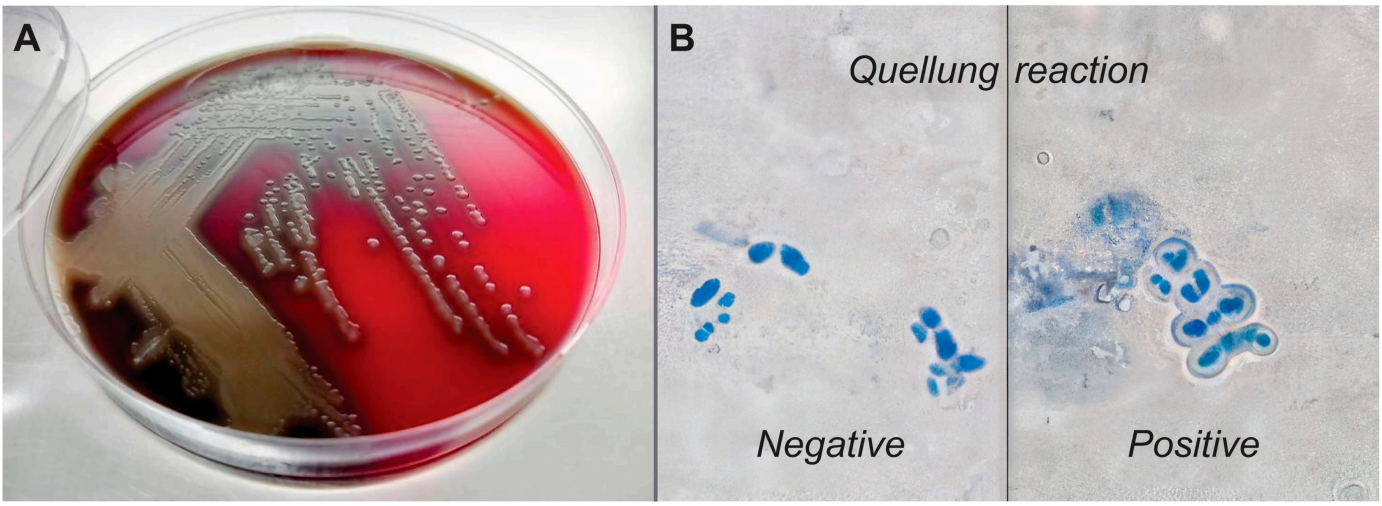


Figure 3. Culture-based identification and serotyping of *Streptococcus pneumoniae*. (**A**) Blood agar culture plate showing growth of *S. pneumoniae*. (**B**) Pneumococcal serotyping using the Quellung reaction.

The identification and confirmation of *S. pneumoniae* is based on bile solubility and optochin susceptibility tests. Among these, the bile solubility test is confirmatory as 10% of *S. pneumoniae* strains can be optochin-resistant [31].

4.3. Molecular Methods

Augmenting conventional culture with molecular methods could enhance the overall sensitivity of *S. pneumoniae* detection in sterile specimens [23]. Molecular methods, such as real-time polymerase chain reaction (RT-PCR), can be complemented with conventional culture-based methods for the identification and differentiation of *S. pneumoniae* from other *Streptococcus* spp. [31]. Additionally, detecting the serotypes of *S. pneumoniae* strains is crucial for the implementation of vaccine-based programs. Therefore, serotyping by PCR-based methods is useful in monitoring circulating strains, anticipating potential serotype replacement, and assessing vaccine effectiveness [32].

While molecular methods are considerably more reliable and rapid when performed in sterile specimens, their use in assessing nonsterile specimens, such as sputum or nasopharyngeal swabs, is limited. Qualitative PCR methods detect the presence or absence of the target pneumococcal gene, whereas quantitative PCR (qPCR) determines the bacterial load, which is important to predict invasive disease potential while enabling detection of bacteria responsible for community- and hospital-acquired lower respiratory tract infections [33,34]. Although molecular methods enhance sensitivity in the detection of multiple

serotypes of *S. pneumoniae*, they may lack specificity when pneumococcal genes are present in commensal streptococci [35]. Hence, molecular methods are generally not advised for nasopharyngeal swabs and respiratory specimens, and if performed, must be used with multiple targets and interpreted with caution. Positive PCR results should be issued after clinical correlation. PCRs on respiratory specimens have a high negative predictive value for pneumococcal disease, but a low positive predictive value [36]. Furthermore, interpretation of molecular findings depends on the specimen type and clinical context, as detection from lower respiratory tract specimens (BAL, endotracheal aspirate) may be more relevant to suspected pneumococcal pneumonia than upper respiratory specimens such as sputum [33,36]. Moreover, molecular methods are associated with higher costs, technical expertise, and limited availability in Indian settings [31]. Some commonly used methods for the detection of *S. pneumoniae* have been elaborated below.

4.3.1. RT-PCR

RT-PCRs targeting the autolysin (*lytA*) and permease genes of the pia ABC transporter (*piaB*) have been considered the gold standard in culture-independent assays for the identification of *S. pneumoniae* [37]. Several other molecular assays have also been developed to identify specific pneumococcal targets such as pneumolysin (*ply*), manganese-dependent superoxide dismutase (*sodA*), pneumococcal surface antigen A (*psaA*), and penicillin-binding protein (*pbp*) genes [31]. Multiplex PCR can simultaneously detect and quantify AMR genes and various respiratory pathogens and holds great potential for quick and accurate identification of *S. pneumoniae* strains if it can be customized to healthcare settings [31].

4.3.2. BioFire® Multiplex PCR

The polymicrobial nature of respiratory specimens makes it difficult to identify the causative pathogen, which ultimately leads to the initiation of empiric antibiotic treatments based on clinical/radiological presentation. Rapid point-of-care tests, such as the BioFire® Multiplex PCR Pneumonia Panel, can potentially differentiate between viral and bacterial pneumonia agents and also identify bacterial pathogens with selected antimicrobial resistance determinants, allowing clinicians to make appropriate and definitive treatment decisions [38]. The BioFire® FilmArray® Pneumonia Panel Plus (BFPP), a multiplex PCR assay, can be used to simultaneously detect up to 27 pneumonia-associated pathogens and 7 antibiotic-resistant targets within 1 h. Compared to standard culture-based methods, BFPP had a sensitivity of 100% and specificity of 90% [39]. However, molecular resistance-marker detection does not replace phenotypic susceptibility testing.

4.4. Rapid Diagnostic Test

For early diagnosis and treatment of fatal invasive infections, such as meningitis or sepsis, rapid diagnostics/point-of-care tests are recommended. The two assays that are currently available are latex agglutination and immunochromatography tests. The latex agglutination test can be used only on CSF samples and detects additional four pathogens causing meningitis, other than *S. pneumoniae* (namely, *Haemophilus influenzae*, *Neisseria meningitidis*, Group B Streptococcus, and *Escherichia coli* with K1 capsule). Although the test is expensive, it is invaluable in confirming bacterial meningitis in highly suspected cases with a clinical picture of the condition [40].

The pneumococcal UAT (e.g., the BinaxNOW® *Streptococcus pneumoniae* Antigen Card) is a non-culture-based, immunochromatographic test, appropriate for adults with suspected pneumococcal CAP or IPD, that identifies the C-polysaccharide antigen common to all serotypes of *S. pneumoniae*. Initially, the pneumococcal UATs were thought to have an

overall sensitivity of 70–80% and specificity of >90% compared to other conventional methods for pneumococcal pneumonia detection in adults; recent data, however, suggest that UATs may have lower sensitivities (approximately 60–65%) [20,41,42]. A positive result on a UAT allows clinicians to optimize antimicrobial therapy with good clinical outcomes [43]. The kit is also available for other important atypical pneumonia-causing pathogens such as Legionella pneumophila. UATs can be performed using either CSF or urine; however, when performed using urine, they have a higher specificity in detecting Legionella (serotype 1 only) than in detecting *S. pneumoniae*, as the latter is a commensal of the nasopharynx [24]. However, pneumonia caused by Legionella is uncommon in the Indian subcontinent.

4.5. Serotyping Methods

The Quellung method (Figure 3B) uses pooled and specific antisera directed against the capsular polysaccharides of pneumococcal cells; the clumping of the cells because of the antigen–antibody reaction is then observed under the microscope [44]. The Quellung reaction remains the gold standard for pneumococcal serotyping, is known for its high sensitivity and specificity, and continues to serve as the benchmark against which all other methods are evaluated [45]. Although this method is preferred for serotyping, it is associated with challenges such as the need for viable isolates, expertise in technique and interpretation, as well as the requirement of a large volume of antiserum per sample, which makes it more expensive than other methods [46]. Moreover, the clinical significance of seroprevalence remains more of a public health concern rather than an immediate concern for the patient's health.

4.5.1. Serotyping by RT PCR

This method is recommended by the Centers for Disease Control and Prevention (CDC) for serotyping *S. pneumoniae* directly from CSF samples or from culture isolates. This is run as a quadriplex RT-PCR, which means that each reaction set has primers for four different serotypes and will perform eight reactions. This can help to identify and differentiate between 64 serotypes, which include the 34 individual serotypes and 13 small serogroups, 4 AMR determinants, pilus targets, and penicillin susceptibility [47]. Despite these advantages, RT-PCR is expensive and requires enough sample for eight reactions, which may be challenging for testing difficult-to-obtain samples such as CSF. The pneumococcal reference laboratory, Department of Microbiology, CMC Vellore (The South East Asia regional reference laboratory for invasive bacterial disease surveillance), is equipped with all the serotyping methods and performs the serotyping for *S. pneumoniae*, H. influenzae, and N. meningitidis free of cost.

4.5.2. Latex Agglutination Test

The latex agglutination test is comparable in accuracy to the Quellung test for pneumococcal serotyping [48]. While this is an accurate method that requires minimal expertise, it is not routinely performed at all sites in India due to its expense and limited utility in identifying only a few serogroups/serotypes [49]. Table 4 highlights the advantages and disadvantages of different detection methods for *S. pneumoniae*.

Table 4. Advantages and disadvantages of the various detection methods for *S. pneumoniae* [20,24,31,33,34,40–42,46,48–51].

Detection Method	Advantages	Disadvantages	Merits
Culture	Gold standard for invasive disease	Requires expertise; time-consuming; affected by prior antibiotic use; autolysis with *S. pneumoniae*; shelf life and temperature variations can affect sheep blood agar plate quality	Confirms viable organism
RT-PCR	Rapid, does not depend on the viability of the organism, hence not affected by antibiotic use or storage	The instrument is expensive, technical expertise.	Culture-independent assay for *S. pneumoniae*
BioFire® FilmArray®	Rapid, highly accurate	The panel does not contain all relevant organisms and commensals, difficult to interpret	Identifies *S. pneumoniae* and genes associated with AMR
Pneumococcal UAT	Rapid (~15 min), noninvasive, cost-effective, not affected by prior antibiotic use	False-positive results after recent CAP (in the past 3 months) or vaccination (48 h prior to the test); lower sensitivity in children	Point-of-care option
Latex agglutination	Rapid, WHO-recommended for serotyping	Not routinely available at all sites due to high cost; limited to a few serogroups	Confirms bacterial meningitis in CSF
Quellung method	Gold standard for serotyping	Expensive, needs expertise, can be performed only on viable isolates	Used for the serotyping of pneumococci

AMR: antimicrobial resistance; CAP: community-acquired pneumonia; CSF: cerebrospinal fluid; RT-PCR: real-time polymerase chain reaction; UAT: urinary antigen test; WHO: World Health Organization.

4.5.3. Genomic Surveillance of *S. pneumoniae*

With the introduction of pneumococcal vaccines (PCVs), the prevalence of non-vaccine serotypes has increased. The most commonly observed changes post-PCV introduction are serotype replacement and capsular switching. This can be identified only by serotyping and whole-genome sequencing (WGS), which requires the isolation of *S. pneumoniae*. WGS is used to characterize the *S. pneumoniae* serotype, genotype, virulence characteristics, and AMR status [52,53]. Hence, the isolation of *S. pneumoniae* should be encouraged to understand the evolution of *S. pneumoniae* in response to vaccine-selective pressures.

5. Isolation and Detection of *S. pneumoniae*: Microbiologist's Perspective

In general, diagnosing pneumococcal pneumonia is difficult due to a lack of high-quality respiratory samples (from patients) and the inability of most tests to differentiate infection from colonization. Furthermore, in India, the rampant use of antibiotics further affects the isolation of *S. pneumoniae* from clinical samples. In a resource-limited setting like India, the diagnosis of pneumococcal disease still relies on decade-old methods with only a few laboratories using advanced techniques for *S. pneumoniae* detection. Even if the etiology cannot be proven, an attempt should be made to isolate *S. pneumoniae* for epidemiological purposes.

Table 5 depicts the challenges in the isolation and detection of *S. pneumoniae* and highlights some practical solutions. These limitations in existing diagnostic tests have hindered the accurate analysis of pneumococcal disease burden and the ability to assess the effectiveness of vaccination in India.

Table 5. Isolation and detection of *S. pneumoniae*: microbiologists' perspective.

Challenges	Solution
Clinical Practice	
If a lab does not have RT-PCR, what are the methods that can be used for *S. pneumoniae* detection and serotyping?	• For CSF samples: methods such as latex agglutination, culture, and UAT • For blood samples: culture
Limited availability of standard culture media (5% sheep blood agar)	• Blood–Columbia nalidixic acid agar and colistin–oxolinic acid blood agar may be used • Molecular methods like real-time PCR can be used
Although sputum can be a representative sample to rule out lower respiratory tract infections, it is difficult to obtain in certain demographics (young children, older adults, and individuals with disabilities). Chances of oral contamination are also higher.	• Sputum quality assessment through Bartlett's criteria • The sample should be collected before antibiotic administration
In case of mixed infection, how can *S. pneumoniae* infection be diagnosed?	• Grade the growth of colonies as scanty, moderate, and heavy • Report moderate-to-heavy growth and correlate clinically
Clinical Research	
Oropharyngeal/nasopharyngeal swabs are easier to collect; however, these have a higher chance of oral contamination.	• Can be used only for detecting nasopharyngeal carriage • Cannot diagnose the disease
Discrepancies between Quellung and molecular methods for serotype classification	In case of discrepancies between the two methods, performing WGS can give precise and effective results

CSF: cerebrospinal fluid; RT-PCR: real-time polymerase chain reaction; *S. pneumoniae*: *Streptococcus pneumoniae*; UAT: urinary antigen test.

6. Isolation and Detection of *S. pneumoniae*: Clinician's Perspective

Virus-induced CAP is becoming common, with about 50–70% of cases being noted in children < 5 years of age [54]. Consistent with findings from other studies, *S. pneumoniae* has emerged as the predominant coinfecting bacterial species in cases of influenza, contributing to 35% of coinfections [55]. Additionally, several hospital-based studies have indicated that *S. pneumoniae* was one of the most prevalent bacteria isolated from lower respiratory tract bacterial co-infections in 17.5–44% of patients infected with respiratory syncytial virus [55]. This underscores the need to address the detection and management strategies to provide comprehensive care for patients with bacterial and viral co-infections (Table 6).

Table 6. Isolation and detection of *S. pneumoniae*: clinicians' perspective.

Challenges	Solution
How can pneumonia cases be identified?	• History and physical examination • For OPD patients: X-ray, CBC, and CRP • For IPD patients: ICU setup (X ray + USG chest), intubated patients (BAL sample), CT scan of the chest • Procalcitonin as a reliable but costly diagnostic marker in appropriate clinical scenarios
Time in which the samples should reach the microbiologists for optimum yield	• For CSF samples: Less than an hour and processed immediately upon receipt • For blood samples for culture: Transport as early as possible in an automated culture medium • Use of automated blood culture bottles enhances the culture outcomes
If the sample cannot be processed immediately, how should it be stored?	• For CSF samples: In the incubator at 37 °C • For blood culture: In the incubator at 37 °C or at room temperature; do not refrigerate
What if the culture is negative	Alternatively, collect another sample and send for a rapid test, or real-time PCR
What if the culture and PCR are negative	Check whether the sample is collected before the start of empirical therapy and correlate clinically. UAT can be used, if available.
How can *S. pneumoniae*-positive samples be sent to reference laboratories?	• In case of CSF, the samples should be transported in dry ice • If the samples are isolates, then they must be sent as chocolate agar plates/slants

BAL: bronchoalveolar lavage; CBC: complete blood count; CRP: C-reactive protein; CSF: cerebrospinal fluid; CT: computed tomography; ICU: intensive care unit; IPD: inpatient department; OPD: outpatient department; PCR: polymerase chain reaction; USG: ultrasonography.

7. Changing Paradigms in the Management of *S. pneumoniae*

7.1. Rational Use of Antimicrobials/Antibiotics

AMR is a global concern that is being addressed by antimicrobial stewardship programs intending to increase awareness about AMR and suggesting steps to reduce it [56]. Recent investigations have reported an increased need for routine antibiotic sensitivity tests (ASTs) to identify an antibiotic of choice and screen for emerging multidrug-resistant (MDR) strains, as the use of broad-spectrum antibiotics remains ubiquitous worldwide. A study evaluating the attitudes of clinicians in prescribing broad-spectrum antibiotics in the management of patients with suspected hospital-acquired or ventilator-associated pneumonia from five hospitals in the UK showed that 83% of the clinicians agreed that molecular diagnostic test results decide antibiotic prescription; however, 58% deemed stopping broad-spectrum antibiotics to be a significant risk in case of negative molecular test results [57].

According to the Indian Council of Medical Research (ICMR) guidelines, pneumonia is one of the most common diseases for which antibiotics are prescribed in India. Clinical

practice guidelines that are customized based on regional data are an effective way to standardize prescribing practices. Measures also need to be taken to educate and implement such practices on a larger scale [58]. According to the 2021 AMR report by the ICMR, non-susceptibility of *S. pneumoniae* to penicillin and cefotaxime was higher in meningeal isolates than in non-meningeal isolates. The minimum inhibitory concentration (MIC) breakpoint definitions for treating pneumococcal meningeal infections are not applicable for non-meningeal infections due to differences in drug penetration into the CSF. This distinction is clinically important, as meningeal infections require lower MIC thresholds for adequate CSF drug levels. Indian surveillance data of 830 invasive pneumococcal isolates from a South Indian tertiary care hospital reported meningeal infections in 20.1% of cases, with cumulative penicillin resistance of 43.7% and cefotaxime non-susceptibility of 14.9% [59]. These findings highlight the need to interpret MIC values in the context of infection site and local AMR patterns, while also acknowledging the challenge of initiating therapy before the availability of MIC data. According to the current ICMR guidelines, combination therapy involving third-generation cephalosporins and vancomycin is recommended for such cases (evidence-based). For non-meningeal infections, penicillin and cephalosporins have been shown to be effective [60].

7.2. Role of Vaccines

India is responsible for 23% of the global pneumonia burden. The burden of CAP was highest in patients with comorbid conditions such as COPD, hypertension, diabetes mellitus, and factors (such as advanced age and smoking) [9]. CAP is also the primary cause of hospitalization, mortality, and substantial healthcare expenditures in the country. A study from North India reported an average length of hospital stay of 9.6 ($\pm$10.65) days with an average intensive care unit (ICU) and ward stay of 7 and 3 days, respectively, for patients with CAP [61].

Vaccination is a pivotal preventive tool against CAP in both children and adults. Two vaccines against *S. pneumoniae* are currently available in India, namely, the 23-valent pneumococcal polysaccharide vaccine (PPSV23) and the 13-valent pneumococcal conjugate vaccine (PCV13) (Figure 4).

While PPSV23 induces only short-term immunity, PCV13 is characterized by a T-cell-dependent response, which induces immunological memory and leads to long-lasting immunity [62]. Although numerous studies have shown the effectiveness of PPSV23 in preventing IPD, its efficacy in preventing pneumonia is not well established. A recent study has shown that although both PCV13 and PPSV23 vaccination reduced pneumonia rates significantly, only PCV13 vaccination maintained its clinical effectiveness during the 5-year follow-up period [63]. PPSV23 has been associated with an increased risk of COPD exacerbation and pneumonia episode rates 5 years after vaccination among patients > 55 years of age [64]. In the future, higher serotype pneumococcal conjugate vaccines such as PCV20 may be able to provide broader coverage, protecting children and adults from pneumococcal infection [65,66].

7.3. Limitations

The literature search was targeted and non-systematic and was conducted to inform the key discussion themes. Therefore, the possibility of selection bias cannot be excluded.

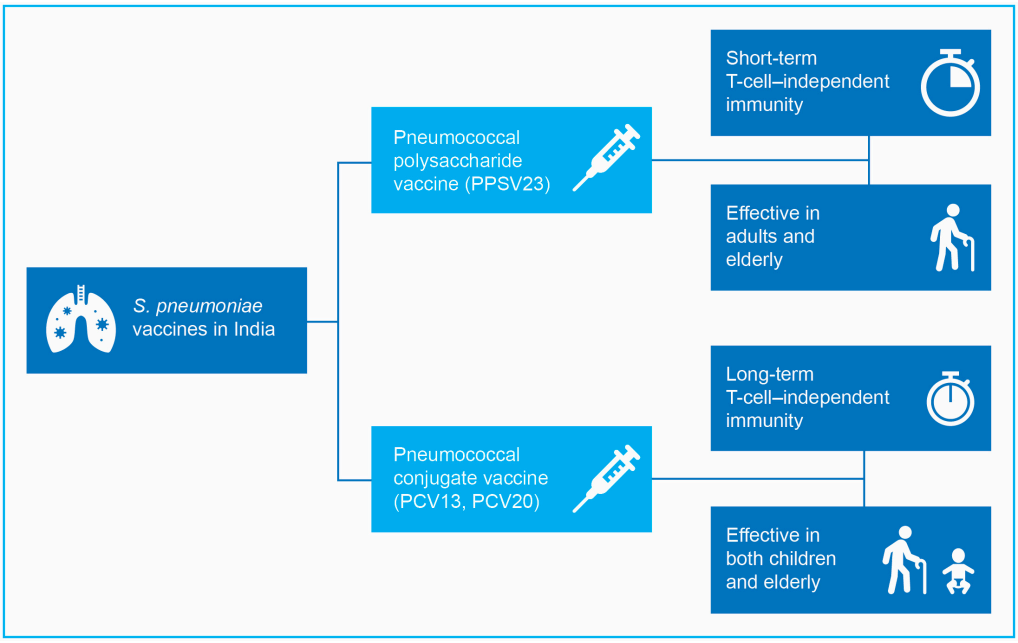


Figure 4. Pneumococcal vaccines in India. PCV13: 13-valent pneumococcal conjugate vaccine; PCV20: 20-valent pneumococcal conjugate vaccine; PPSV23: 23-valent pneumococcal polysaccharide vaccine.

8. Conclusions

Lower respiratory tract infections with *S. pneumoniae* are associated with a high level of morbidity and mortality in South Asia. The nonavailability of laboratories equipped with tests to identify the responsible pathogen, limited access to healthcare, and high burden of disease are the challenges encountered in diagnosing *S. pneumoniae* infections in developing countries. Currently available techniques, such as sputum culture and UATs, are challenging to use as they have low yields and often cannot differentiate between pathogenic and commensal *S. pneumoniae*. Improving the setting of diagnostic laboratories and highlighting the roles of physicians and microbiologists in obtaining useful samples at the right time are vital to the effective management of *S. pneumoniae* infections in India. Newer technologies using multiplex PCR, such as the BioFire® FilmArray® and QIAstat-Dx, to identify respiratory pathogens and detect AMR genes, are used only by certain laboratories for diagnosis. Therefore, high-quality data from developing countries are needed to ascertain the appropriate tools for diagnosis in resource-constrained settings. Early and accurate identification of the causative organism prompts appropriate management and prevents antibiotic abuse. This is essential to reduce AMR, which is common in developing nations. PCVs can be extremely beneficial in not only reducing the burden of the disease, but also in preventing the spread of AMR in India.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance
AST	Antibiotic sensitivity test
BAL	Bronchoalveolar lavage
BFPP	BioFire® FilmArray® Pneumonia Panel Plus
CAP	Community-acquired pneumonia
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
ICMR	Indian Council of Medical Research
ICU	Intensive care unit
IPD	Invasive pneumococcal disease
lytA	Autolysin
MDR	Multidrug-resistant
OPD	Outpatient department
OR	Odds ratio
pbp	Penicillin-binding protein
PCR	Polymerase chain reaction
PCV	Pneumococcal vaccines
PCV13	13-valent pneumococcal conjugate vaccine
piaB	Permease genes of the pia ABC transporter
ply	Pneumolysin
PPSV23	23-valent pneumococcal polysaccharide vaccine
psaA	Pneumococcal surface antigen A
RT-PCR	Real-time polymerase chain reaction
S. pneumoniae	*Streptococcus pneumoniae*
sodA	Manganese-dependent superoxide dismutase
spp.	Species
UAT	Urinary antigen test
USG	Ultrasonography
WGS	Whole-genome sequencing
WHO	World Health Organization

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