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Tracking the evolution of emerging serotypes and antibiotic resistance patterns in *Streptococcus pneumoniae* among Indian adults using high-throughput genome sequencing

Geetha Nagaraj[1*], Varun Shamanna[1,2], Harshitha Gangaiah Krishnappa[1], Vandana Govindan[1], Mettingal Ramakrishnan Shincy[1] and Ravikumar Kadahalli Lingegowda[1]

Abstract

Background *Streptococcus pneumoniae* is a major cause of respiratory infections, particularly affecting children and the elderly. However, data on pneumococcal disease among Indian adults remain limited. This study investigated the epidemiology of *S. pneumoniae* from invasive and non-invasive sources in Indian adults using whole-genome sequencing (WGS).

Methods A prospective study was undertaken in five hospitals of India between 2022 and 2023, including 254 *S. pneumoniae* isolates, 126 from invasive and 128 from non-invasive specimens. WGS was performed using the Illumina platform to determine serotypes, multi locus sequence types (STs), lineages, antimicrobial resistance (AMR), and virulence profiles. Antimicrobial susceptibility was assessed using the Vitek-2 system.

Results A total of 37 serotypes, 53 Global Pneumococcal Sequence Clusters (GPSCs), and 128 STs (including 39 novel STs) were identified. Predominant serotypes included 19 F, 19 A, and 9 V, with GPSC1, GPSC10, and GPSC6 being the most common lineages. Vaccine coverage was estimated at 64% for PCV13 and 72% for PPSV23. Multidrug resistance (MDR) was observed in 70% of isolates, mainly among GPSC1, 10, and 6. Virulence genes were widely distributed, and pilus genes were more common in non-invasive isolates. Phylogenetic analysis showed GPSC1, 10, and 6 as dominant in both invasive and non-invasive sources.

Conclusion The high prevalence of non-vaccine lineages, elevated MDR, and large number of novel STs reflect ongoing pneumococcal evolution in India, likely driven by recombination and capsular switching. These dynamics may reduce vaccine effectiveness. Continuous genomic surveillance is crucial to inform vaccine strategies and control pneumococcal disease in Indian adults.

Keywords Serotypes, *S. pneumoniae*, India, WGS, AMR, GPSC

*Correspondence:
Geetha Nagaraj
geetha.ndri@gmail.com

[1]Central Research Laboratory, KIMS, Bengaluru, India
[2]Department of Biotechnology, NMAM Institute of Technology, Nitte, Udupi, India

Background

Streptococcus pneumoniae has a complex relationship with the human host, residing as an adapted commensal on the mucosal surface of the upper airways to enable transmission, yet is also capable of causing severe disease by invading sterile sites such as the middle ear spaces, bloodstream, and meninges when conducive bacterial and host factors align [1]. Globally, these diseases place a considerable clinical and economic burden on patients and society, with adults experiencing a substantial burden [2, 3]. The incidence, prevalence, and mortality of pneumococcal diseases vary significantly, both in different countries and over time [4]. Approximately 14.5 million cases of severe pneumococcal diseases are reported globally each year, resulting in an estimated 826,000 deaths [5]. India bears a significant burden of pneumococcal disease, accounting for 23% of the global pneumonia burden, with fatality rates ranging from 14 to 30% [6]. Pneumococcal infection susceptibility increases with age, preexisting chronic illness and immune deficiencies, with annual incidence rates of pneumococcal disease in India increasing from 13.9% in 18–44-year-olds to 31.3% in individuals older than 60 years [7, 15].

Pneumococci are genetically variable, with over 100 distinct polysaccharide capsule types, which play a critical role in virulence by interfering with host opsonophagocytic clearance mechanisms [8]. The chemical makeup of polysaccharides is crucial because only a subset of pneumococcal serotypes accounts for the most invasive infections. In Indian adults, the distribution of serotypes is influenced by factors including population density, geographical area, antibiotic usage, and vaccination coverage. Densely populated nations like India often face a substantial burden of infections and exhibit a wide diversity of serotypes [9]. Studies conducted in India have identified serotypes 19 F, 19 A, 9 V, 6B, 15 A/B, 11 A, 23 F, 8, 3, 9 N, and 10 A as predominant in adults [10–14]. Immunization with pneumococcal vaccines plays a crucial role in preventing disease and improving the quality of life of at-risk populations. The pneumococcal vaccines currently accessible for adult use in India include PCV13 (a 13-valent pneumococcal conjugate vaccine) and PPSV23 (a 23-valent pneumococcal polysaccharide vaccine) [15]. Pneumococcal vaccination is included in India's National Immunization Program for children; however, no such program currently exists for adults. Nonetheless, the Nation Against Pneumonia Expert Panel Opinion (NAP-EXPO) and the Geriatric Society of India recommend the administration of PCV13 and/or PPSV23 for elderly individuals (≥ 65 years) and high-risk adults aged 19–64 years [16, 41].

One of the greatest threats posed by *Streptococcus pneumoniae* is its increasing resistance to commonly used antibiotics in India, making it difficult to treat, posing a concerning threat [9, 17]. The primary drivers of resistance are the overuse of antibiotics, self-medication, poor infection control practices, the ability of pneumococcus to undergo serotype replacement and capsular switching, and the horizontal transmission of antibiotic resistance genes [18]. Limited disease burden data, coupled with the emergence of antibiotic resistance and risk factors such as age, alcohol intake, smoking status, air pollution, and comorbid conditions, pose obstacles to the effective management of this disease in older adults [19]. Current data are essential not only for clinical management but also for assessing evolutionary antimicrobial resistance (AMR) trends and prioritizing efforts to reduce resistance; nonetheless, there is limited information from the adult Indian population.

Understanding the diversity and population structure of pathogenic pneumococci is currently hampered by limited representative genomic data from Indian adults [14]. The introduction of high-throughput sequencing technologies has accelerated the process of identifying, characterizing, serotyping and AMR profiling *S. pneumoniae*, increasing the speed, cost-effectiveness, and precision of the process [20]. Embracing whole-genome sequencing (WGS) as a surveillance tool has enhanced our ability to monitor and control pneumococcal diseases, thereby safeguarding public health [21]. This study aimed to assess the distribution of serotypes, antimicrobial resistance patterns, and vaccine coverage among *S. pneumoniae* isolates from invasive and non-invasive sites using high-throughput sequencing. It also sought to investigate the molecular characteristics of the isolates, including molecular epidemiology, in silico AMR profiling, phylogenetic relationships, and lineage distribution.

Materials & methods

Study design

We conducted a laboratory-based, prospective, cross-sectional investigation to isolate *Streptococcus pneumoniae* from clinical specimens obtained from adult patients (aged ≥ 18 years) between 2022 and 2023 in five tertiary care hospitals located across different regions of India. Clinical specimens submitted to the Department of Microbiology for routine bacteriological analysis were screened, and all consecutive, culture-positive cases of *S. pneumoniae* were considered for inclusion. Only one isolate per patient was analyzed to avoid duplication.

Specimens were categorized into two types. Invasive pneumococcal disease cases were defined as isolates recovered from normally sterile sites, including blood, cerebrospinal fluid (CSF), and pleural fluid. Isolates obtained from non-sterile sites such as the respiratory tract, eye and ear swabs, sputum, pus, nasopharyngeal, and urine specimens, were considered as non-invasive [60]. Patients who tested positive for *S. pneumoniae* and

provided informed consent were included. Isolates were excluded only if species identification was inconclusive or associated metadata were incomplete. This approach aimed to minimize selection bias and ensure that the sample represented a broad cross-section of pneumococcal strains circulating among Indian adults. Demographic and clinical characteristics are summarized in Table 1.

Microbiological methods

Samples from clinical specimens were cultured on 5% sheep blood agar (Chromogenic Life Sciences, Hyderabad, India) plates and incubated for 18–24 h at 37 °C and 5% CO_2. Isolation of *S. pneumoniae* was confirmed by colony morphology, alpha hemolysis, optochin sensitivity test and bile solubility test. Positive isolates were frozen at −80 °C in a Skimmed Milk-Trypticase Soy-Glucose-Glycerol (STGG) medium until further characterization and transported to the Central Research Laboratory, KIMS, Bangalore for further characterization and whole genome sequencing.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *S. pneumoniae* isolates was performed using the Vitek 2 Compact System with AST03 cards (bioMérieux, Marcy-l'Étoile, France) at the Central Research Laboratory, KIMS, Bangalore. The antibiotic panel included penicillin, erythromycin, co-trimoxazole, cefotaxime, ceftriaxone, levofloxacin, clindamycin, tetracycline, and linezolid. Results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2023 M100-Ed33 guidelines. Isolates resistant to three or more classes of antimicrobial agents were defined as multidrug resistant (MDR) [22].

Table 1 Demographic and clinical characteristics and specimen types of the 254 *S.pneumoniae* isolates

Characteristics	No. of Isolates	%
Total	254	100
Invasive isolates	126	49.6
Non-invasive isolates	128	50.4
Gender		
Male	155	61.0
Female	99	39.0
Age (Years)		
18–30	45	17.7
31–40	31	12.2
41–50	54	21.3
51–64	71	28.0
>65	53	20.9
Specimen type		
A. Invasive		
Blood	91	35.8
CSF	14	5.5
Pleural fluid	21	3.9
B. Non-invasive		
Sputum	96	37.8
Ear swab	4	1.6
Others*	28	12.9

*Others – Pus, Urine, Endotracheal aspirate (ETA), Nasopharyngeal (NP) swab

Whole genome sequencing and bioinformatic analysis

Genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany). Library preparation was performed with the NEBNext® UltraTM II FS DNA Library Prep Kit for Illumina (New England Biolabs, UK). Library quality was assessed using an Agilent Tapestation (Santa Clara, CA, USA), and sequencing was carried out on the Illumina MiSeq platform (Illumina, San Diego, California, United States of America) with 250 bp paired-end reads.

Sequence data were analyzed using the GPS pipeline v1.0.0-rc7 [23, 28], which included quality assessment, de novo assembly using Shovill [24], and taxonomic assignment. Samples were evaluated using specific quality control criteria: total base count ≥ 38 million base pairs, mean sequencing depth ≥ 20X, ≥ 60% reads mapped to *S. pneumoniae* using Kraken 2, ≤ 2% reads mapped to top non-*Streptococcus* genus, ≥ 60% reference genome (ATCC 700669) coverage, ≤ 220 non-cluster heterozygous single nucleotide polymorphisms (SNPs), ≤ 500 contigs, and assembled genome size between 1.9 and 2.3 Mb.

Serotyping was predicted using Seroba [25], multilocus sequence typing (MLST) using the mlst tool [26], and Global Pneumococcal Sequence Cluster (GPSC) assignment via popPUNK [27]. Antimicrobial susceptibility against 20 commonly used antibiotics was detected using CDC PBP AMR Predictor [61] and ARIBA v2.14.6 [62]. Virulence genes were predicted using ARIBA v2.14.7 [29] against the VFDB database [30]. Genome annotation was performed using Bakta v1.9.3 [31], and pangenome analysis was conducted using Panaroo v1.3.4 [32] in strict mode with core alignment enabled. Single Nucleotide polymorphism (SNPs) were filtered using snp-sites v2.5.1 [33], and a maximum likelihood phylogenetic tree was constructed with 1000 bootstrap replicates using IQ-TREE v2.2.6 [34]. The tree was midpoint-rooted and visualized using Microreact [35].

Statistical data analysis

Descriptive statistical analyses were conducted using GraphPad Prism version 7 (GraphPad Software, San Diego, CA, USA). Associations between variables were tested using two-tailed Fisher's exact test, and odds ratios (ORs) were calculated. A P-value < 0.05 and OR > 1 were considered statistically significant. Data visualization was performed using the ggplot2 package [36], and data parsing was conducted using the tidyverse package [37] in RStudio v2023.12.0. All scripts used for analysis are

available at https://github.com/varunshamanna/Indian-adult-SPN-Seroypes.

RESULTS

Demographic and clinical characteristics of the isolates

The study analyzed 254 *Streptococcus pneumoniae* isolates from adults ≥ 18 years comprising 126 invasive and 128 non-invasive specimens. The demographic, specimen, and clinical profiles of the study population are summarized in Table 1.

Serotype distribution

A total of 37 serotypes were identified, with 64% coverage by PCV13, 75.6% by 20-valent Pneumococcal conjugate vaccine (PCV20) and 72% by PPSV23. The most prevalent serotypes were 19 F (16.9%, $n=43$), 19 A (8.7%, $n=22$), 9 V (7.5%, $n=19$), and 6 A and 15B (5.9% each, $n=15$ each). Among invasive isolates, 19 F (13.5%), 19 A (9.5%), and 9 V (8.7%) dominated, while non-invasive isolates featured 19 F (20.3%), 15B (8.6%), and 19 A (7.8%). Non-PPSV23 serotypes 6 A, 35B, and serogroup 24 were frequently observed in both specimen types (Fig. 1; Table 2). Statistical analysis showed no significant association between sample types and predominant serotypes (19 F $P=0.22$, 19 A $P=0.83$, 9 V $P=0.64$), (Supplementary Table 2).

Distribution of sequence types

Multilocus sequence typing revealed 128 STs, including 89 known and 39 novel (15.4%) STs, distributed across 55 clonal complexes and 18 singletons. The most common STs were ST11921 (6.3%, $n=16$), ST320 (5.1%, $n=13$), ST236 (4.7%, $n=12$), and ST13727 (4.3%, $n=11$) (Fig. 2). 9.8% of isolates belonged to internationally recognized Pneumococcal Molecular Epidemiology Network (PMEN) clones [38], such as Taiwan^{19F}–14 and Spain^{9V}–3 (Fig. 2; Table 2). STs like ST236 (19F) and ST320 (19 A), showed strong serotype specificity ($P<0.05$), while others exhibited multiple serotypes. Serotype 19 F exhibited the greatest ST diversity, comprising 12 different STs (Table 2).

Virulence factor analysis

A total of 31 known virulence genes were screened and categorized into adherence, capsule synthesis, exoenzyme, exotoxin, and immune modulation groups. Pilus-I was more prevalent in non-invasive isolates (40.6%) than invasive (35.7%, $P=0.64$), while pilus-II was equally distributed (20.6% invasive, 21.8% non-invasive). Pilus-II genes (*pitAB*, *sipA*, *srtG1*, *srtG2*) were linked to 19 F/19A (GPSC1), and pilus-I genes (*rrgC* + *srtBCD*) to 19 F/19A, 9 V/9A, and 6B (GPSC1, 6) strains, indicating lineage-specific virulence (Supplementary Fig. 1, Supplementary

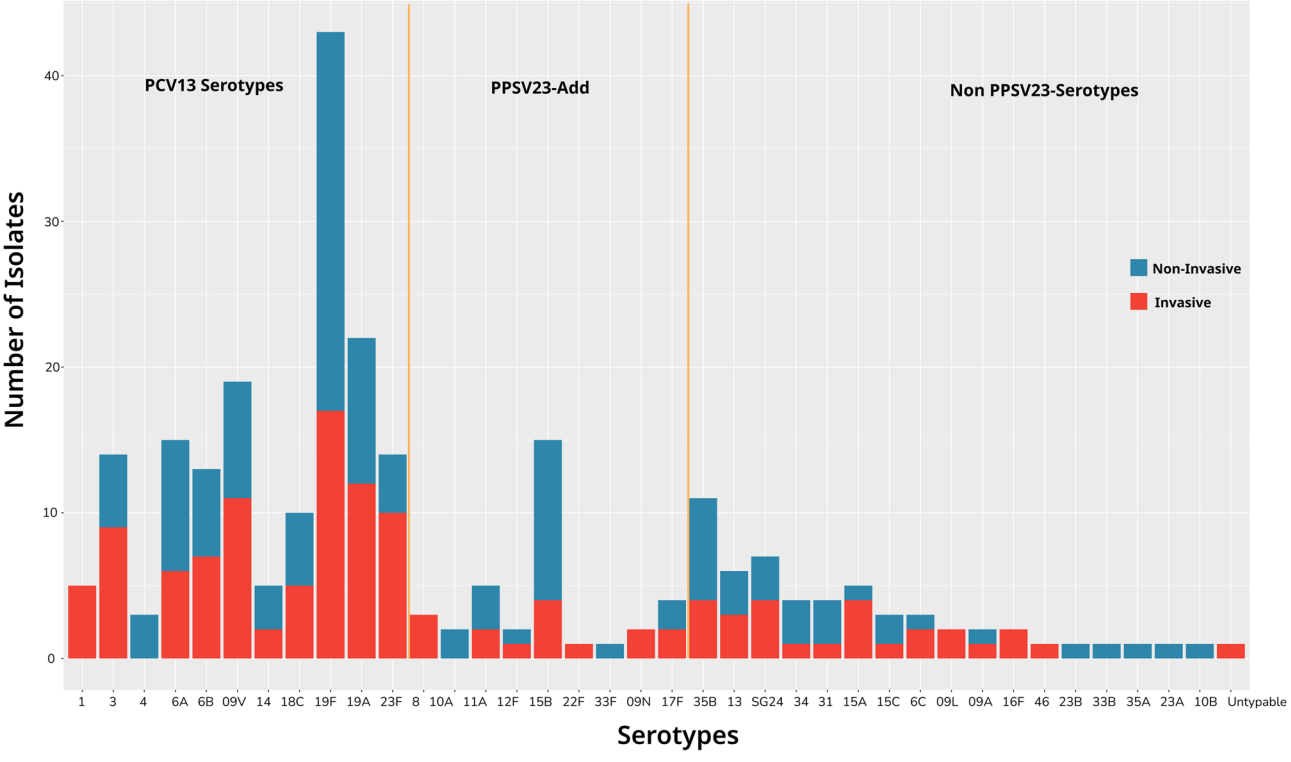

Fig. 1 Distribution of serotypes among invasive and non-invasive specimens. The bars are stratified by the number of samples in each serotype belonging to invasive and non-invasive specimens. The serotypes are divided based on their presence in either PCV13 or PPSV23 vaccine and Non-PPSV23 serotypes. The bars are stacked to total number of isolates. Blue bars – Non-Invasive, Red bars – Invasive

Table 2 Serotype distribution among invasive and non-invasive specimen types. The top 5 serotypes of frequency > = 14 are shown in the table. The top STs are shown individually and others are grouped as st_other

Serotype	Invasive (*n* = count)	Non-invasive (*n* = count)	STs (*n* = count)	No. of STs	MDR Prevalence (%)	Key Resistance (OR, *P*-Value)
19 F	17	26	ST236(*n* = 12), ST2697(*n* = 9), ST271(*n* = 7), Novel STs(*n* = 5), ST6397(*n* = 2), ST320(*n* = 2), ST_other (*n* = 6)	12	81.3	Erythromycin (4.06, < 0.01), Penicillin (3.46, 0.07)
19 A	12	10	ST320(*n* = 11), ST17264(*n* = 5), ST_other (*n* = 6)	8	81.8	Erythromycin (3.68, < 0.01), Penicillin (0.33, < 0.01)
9 V	11	8	ST11921(*n* = 15), Novel STs(*n* = 3) and ST156(*n* = 1),	3	100	Erythromycin (INF, < 0.01), MDR (INF, < 0.01)
15B	4	11	ST13727(*n* = 8), Novel STs(*n* = 4), ST7629(*n* = 2), ST1710(*n* = 1)	4	80	Levofloxacin (4.83, < 0.01)
6 A	6	9	ST473(*n* = 6), ST14518(*n* = 3), ST_Novel(*n* = 3) ST_other (*n* = 3)	6	53.3	None significant
23 F	10	4	ST15691(*n* = 3), ST_Novel(*n* = 3), ST81(*n* = 2), ST1701(*n* = 2), ST9491(*n* = 2), ST_other(*n* = 2)	7	64.2	Ceftriaxone (1.93, < 0.01)

Prevalence based on 254 isolates (2022–2023); MDR = Multidrug resistance (resistance to ≥3 antibiotic classes); OR = Odds Ratio; INF = Infinite due to zero susceptible isolates

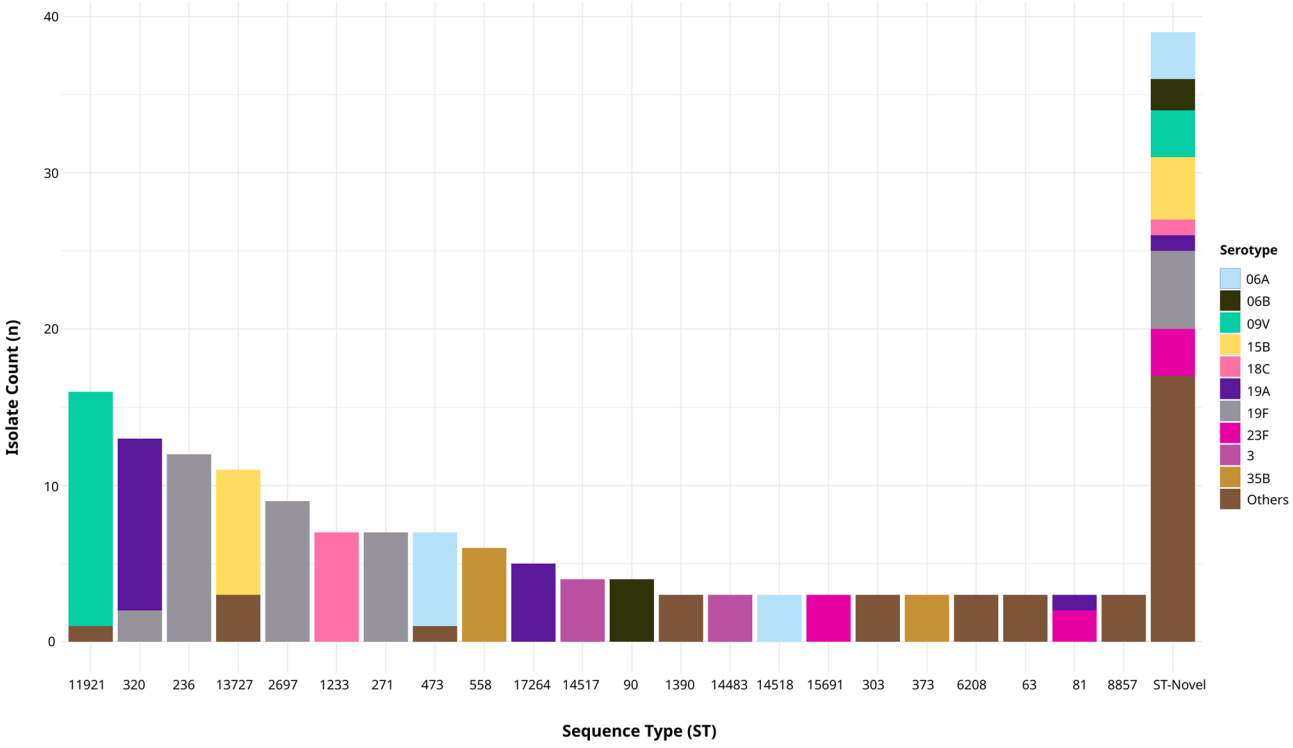

Fig. 2 Sequence type distribution of *S.pneumoniae* isolates. The bars are stratified by the number of samples in each sequence type and their association with serotype. The bars are stacked to total number of isolates

Table 3). Other genes (e.g., *cbpA*, *lytA*, *ply*) were widely present in both invasive and noninvasive strains without clone specificity.

Antimicrobial susceptibility

Antimicrobial susceptibility testing showed 92% of isolates resistant to at least one antibiotic, with 70% (*n* = 178) exhibiting MDR to ≥3 classes (Fig. 3, Supplementary Figs. 2 & 3, Supplementary Table 1). No significant differences were observed in resistance patterns between invasive and non-invasive isolates ($P > 0.07$) (Supplementary Table 4). Resistance to erythromycin harboring *ermB* and/or *mefA* genes was observed in 30% of the isolates, and 71.2% were resistant to tetracycline, all harboring *tetM* gene. Resistance to penicillin was linked to various combinations of *pbp* alleles, including new variants of *pbp1a*, *pbp2b*, *and pbp2x*. Co-resistance to erythromycin and tetracycline was high (65%). Serotype-specific key resistance data is depicted in Table 2.

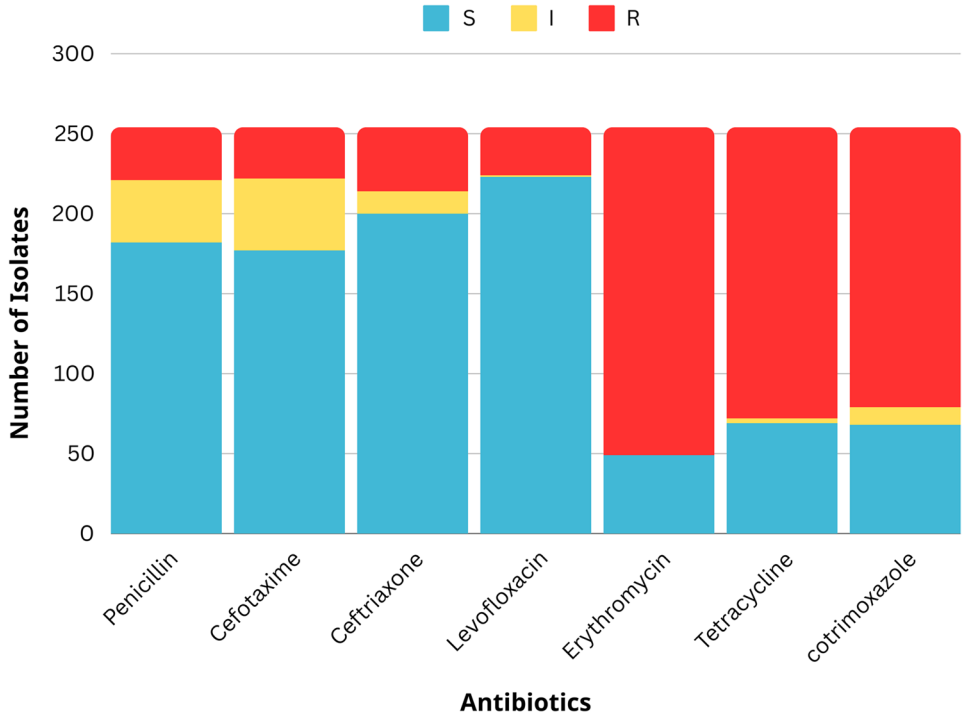

Fig. 3 Distribution of antibiotic susceptibility among clinical isolates of *Streptococcus pneumoniae*. Categorized into susceptible (S, blue), intermediate (I, yellow), and resistant (R, red). The bar graph shows the number of isolates for each susceptibility category across the different antibiotics tested

Population structure

Phylogenetic clustering using the GPSC scheme (Fig. 4) assigned isolates to 53 GPSCs, including two hybrid clusters (GPSC 904;9 and 91;756). The dominant GPSCs were GPSC1 (20%), GPSC10 (18.5%), GPSC6 (9.4%), GPSC13 (3.9%), and GPSC23 (3.5%). Among the 53 GPSCs, 29 (54%) were Vaccine type (VT) lineages, 17 (32%) were non-VT lineages, and 6 (11%) were lineages (GPSC 10, 6, 23, 9, 16, novel) with both VT and non-VT isolates. GPSC10, linked to MDR (35% of MDR isolates, $P < 0.01$) and invasive potential ($P = 0.07$), showed high serotype diversity (13 variants, 4 in PCV13, 8 in PPSV23). A novel cluster (4.3%) expressed serotypes 9 V, 3, 14, and 9 A. Diversity indices (Simpson's 1-D > 0.9) confirmed high genetic variability across serotypes, STs, clonal complex (CCs), and GPSCs, regardless of age group or specimen type (Table 3, Supplementary Tables 6, 7). MDR was particularly concentrated within GPSC1, 10, and 6, which together accounted for 48% ($n = 108/178$) of isolates ($P < 0.01$).

Discussion

Globally, *S. pneumoniae*, the leading cause of community-acquired pneumonia (CAP) in all age groups, contributes more to Lower respiratory tract infection-related deaths than all other etiologies combined. Studies have reported a high burden of pneumococcal diseases in Indians older than 50 years [12, 16, 40]. This multicentre genomic surveillance study provides critical insights into the serotype distribution, AMR patterns, and population structure of *Streptococcus pneumoniae* isolated from Indian adults. While pneumococcal research in India has largely focused on pediatric populations, data on adult pneumococcal disease remain sparse. Our findings help bridge this knowledge gap and highlight the need to address pneumococcal disease burden in Indian adults—especially given the high rates of MDR and the predominance of non-vaccine serotypes.

The most common serotypes identified in our study—19 F, 19 A, 9 V, 6 A, and 15B—align with previous reports from India [11, 13, 59], suggesting consistent circulation of these serotypes among adult populations. The estimated vaccine coverage of 64% for PCV13 and 72% for PPSV23 is moderate and indicates substantial gaps in protection, particularly against emerging non-vaccine serotypes such as 15B, 35B, and serogroup 24. The frequent isolation of 15B and 35B from invasive sources is of concern, as these serotypes have been associated with high case fatality rates, meningitis, and resistance to multiple antibiotics in other global reports [44, 45]. The observed distribution reflects early signs of serotype replacement, a phenomenon widely documented in post-PCV13 settings worldwide [43], where non-vaccine types (NVTs) increase in prevalence due to selective pressure exerted by conjugate vaccines.

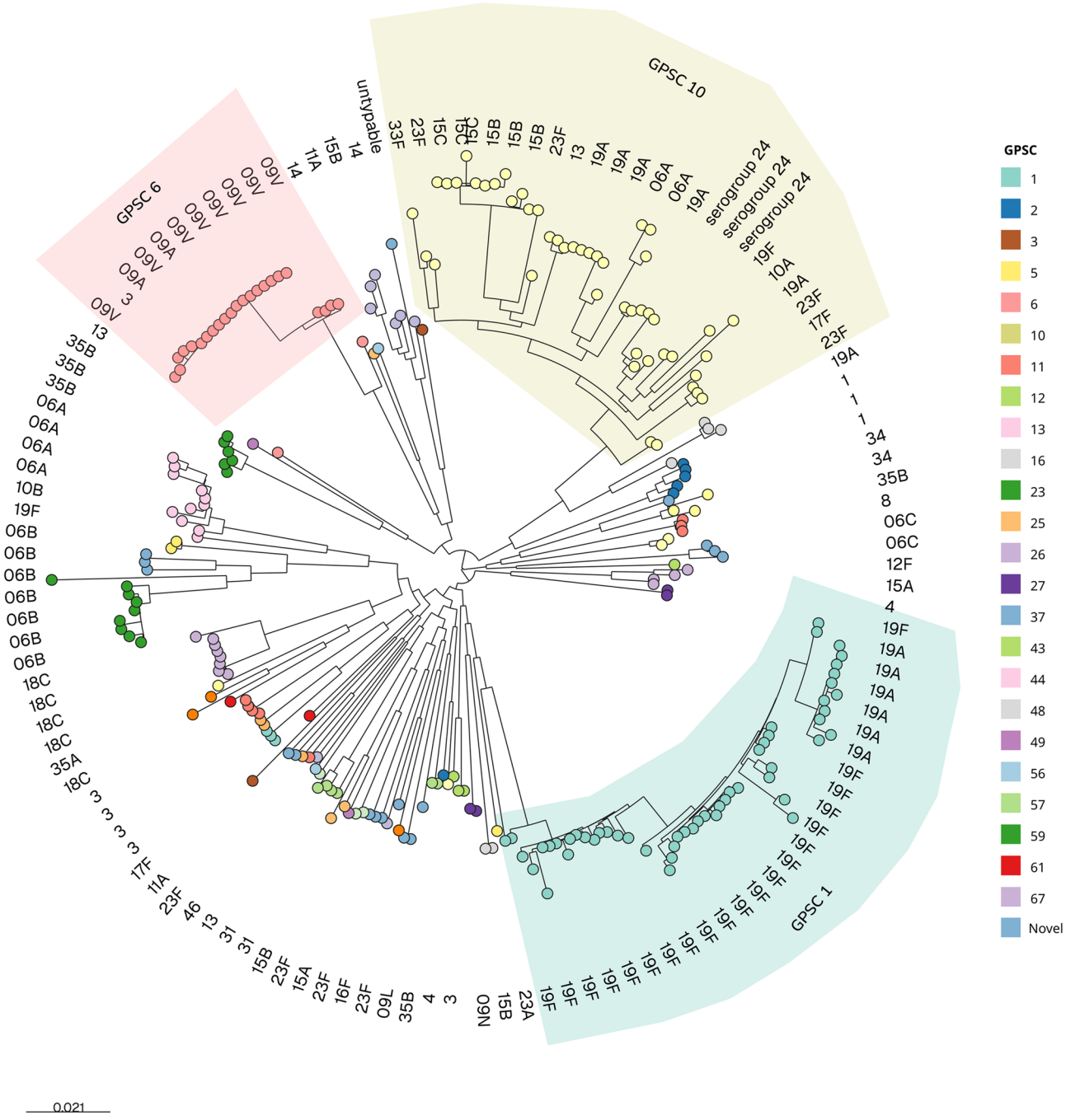

Fig. 4 Phylogenetic tree illustrating the distribution of *Streptococcus pneumoniae* serotypes and global pneumococcal sequence clusters (GPSCs) based on genome-wide analysis. The tree illustrates the genetic diversity of 234 isolates, annotated with Global Pneumococcal Sequence Clusters (GPSCs) and corresponding serotypes. Major GPSCs are shaded and labeled with node colors indicating GPSC assignments as per the legend. Clusters containing vaccine (PPSV23/PCV13) and non-vaccine serotypes are observed, with evidence of clonal expansion and serotype switching within dominant GPSCs. The scale bar represents the number of substitutions per site

High clonal diversity was evident among our isolates, with 128 distinct sequence types (including 39 novel STs) and 53 GPSCs. Predominant STs such as ST11921, ST320, and ST236 correspond to internationally recognized clones, Taiwan^{19F}–14, Spain^{9V}–3, that have also been previously detected in India [46], suggesting both local persistence and international dissemination. Apart from India, ST11921 is reported from Russia [47], Qatar [54] and China [48]. In the present study, Taiwan^{19F}–14 emerged as the prevailing strain, aligning with previous Indian studies [13, 14, 49], while the Netherland^{3}–31 clone is identified in India for the first time. Associations

Table 3 Distribution of *Streptococcus pneumoniae* isolates by gpscs, showing the number of invasive and non-invasive isolates, associated serotypes, and clonal complexes

GPSC	Invasive (*n* = Count)	Non-invasive (*n* = Count)	Serotype (*n*)	Clonal complex (*n*)
1	23	28	19 F (40), 19 A (11)	320 (43), new (5), Singleton (3)
10	18	29	15B (10), 19 A(9), SG24 (6), 23 F (5), 6 A, 13, 15 C (3 each), 19 F, 17 F (2 each), 10 A, 11 A, 22 F, 33B (1 each)	230 (28), novel (10), Singleton (8)
6	14	10	9 V (19), 3, 9 A (2 each), 14 (1)	156 (18), novel (4), Singleton (2)
Novel	7	4	23 F (3), 15 A(2), 34, 9 L, 10 A, 11 A, 16 F, Untypable	Novel (5), 6332 (2)
13	4	6	6 A (9), 10B(1)	473 (8), Novel (2)
23	4	5	6B (8), 6 A(1)	185 (4), novel (2), Singleton (2)

between STs and serotypes revealed genetic plasticity, with some STs expressing multiple capsular types. Analysis of the invasive isolates revealed a greater number of new STs which is in contrast to the observation of Verghese et al. [50], where they have reported a greater number of new STs in non-invasive isolates than in invasive. These results demonstrate a population that is constantly changing and one that has greater genetic variety.

The predominant lineages—GPSC1, GPSC10, and GPSC6—are globally recognized and known to harbor resistant and invasive clones [39]. GPSC10 stood out in our study for its broad serotype diversity, association with multidrug resistance, and a near-significant link with invasive isolates [46, 49]. These findings underscore the importance of this lineage as a high-risk clone in Indian adults. Interestingly, GPSC10 expressed both vaccine and non-vaccine serotypes, including serotype 33B, which has not been previously reported in this lineage in India. This highlights the capacity for capsular switching and the dynamic nature of pneumococcal population structure under vaccine and antibiotic pressure.

The study highlights alarming levels of AMR across both non-invasive and invasive isolates. Pneumococcal lineages GPSC1 and GPSC10 were notably associated with multidrug resistance, harboring critical resistance determinants such as *pbp* variants (for β-lactams) and *ermB/mefA* (for macrolides). These findings are consistent with earlier Indian reports [12, 51, 52], which documented rising macrolide and penicillin resistance among pneumococci, particularly in urban hospital settings. The co-occurrence of *ermB* and *tetM* genes, often associated with mobile elements like Tn1545 [53], raises concerns about the potential for horizontal transfer and stable propagation of resistance. Several studies have reported a strong association between certain *S. pneumoniae* serotypes and resistance to penicillin as well as multidrug resistance [63, 64]. Some of these multidrug-resistant serotypes appear to have a global distribution. In the present study, resistance was strongly associated with specific serotypes—particularly 19 F, 19 A, and 9 V—which have historically been linked with international multidrug-resistant clones [38, 39]. The emergence of fluoroquinolone resistance in non-vaccine serotype 15B is another key concern, as this class of drugs is widely used empirically for community-acquired pneumonia in Indian adults [55, 56, 58].

Virulence profiling showed that most pneumococcal virulence genes were uniformly present across isolates from both invasive and non-invasive sources. Although pilus-1 genes were more frequently detected in non-invasive isolates—consistent with previous studies in the pediatric population in India [49, 57]—the difference was not statistically significant. In contrast, pilus-II genes showed clear clonal associations, predominantly occurring in GPSC1 strains expressing serotypes 19 F and 19 A. This aligns with earlier reports [14, 42] linking PI-II to multidrug-resistant lineages, exemplified by GPSC1, which was also identified among our MDR isolates. The presence of virulence genes such as zmpC, implicated in epithelial barrier disruption, in both invasive and non-invasive isolates reinforces the notion that host-related factors—such as comorbidities and immune status—likely play a more important role in disease severity than pathogen genotype alone [58].

The co-circulation of multidrug-resistant non-vaccine serotypes (MDR NVTs) in India, coupled with the lack of adult vaccination programs, presents significant public health concerns. While our multicentric study provides valuable insights through comprehensive genome-wide analysis, certain limitations must be acknowledged, including the restricted sample size and limited clinical data correlation. These findings underscore the need for expanded surveillance efforts across diverse geographical regions. Future research directions should prioritize: (1) enhanced epidemiological monitoring, (2) systematic collection of clinical outcome data, and (3) rigorous evaluation of vaccine efficacy in adult populations.

Conclusion

This study provides critical insights into the circulating serotypes, genetic lineages, and antimicrobial resistance profiles of *Streptococcus pneumoniae* among adult populations in India. The concerning prevalence of MDR strains and non-vaccine serotypes highlights several key

public health imperatives: First, comprehensive nationwide surveillance encompassing all states is urgently needed to accurately monitor emerging serotypes, resistance patterns, and regional epidemiological variations. Second, the development of tailored antimicrobial stewardship programs, informed by serotype-specific resistance data, represents a crucial strategy for addressing the growing threat of antimicrobial resistance. These findings collectively emphasize the need for evidence-based interventions to guide pneumococcal disease management and prevention strategies in India.

Abbreviations

NAP-EXPO	Nation Against Pneumococcal Infections – Expert Panel Opinion
GPSC	Global Pneumococcal Sequence Cluster
MDR	Multidrug resistant
MIC	Minimum inhibitory concentrations
PCV13	13-valent pneumococcal conjugate vaccine
PPSV23	23-valent pneumococcal polysaccharide vaccine)
NVT	Nonvaccine serotypes
AMR	Antimicrobial resistance
WGS	Whole-genome sequencing
BALF	Bronchoalveolar lavage fluid
CSF	Cerebrospinal fluid
PEN	Penicillin
CRO	Ceftriaxone
CFT	Cefotaxime
ERY	Erythromycin
LEV	Levofloxacin
TET	Tetracycline
COT	Trimethoprim-sulfamethoxazole
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxy Ribo Nucleic acid
CDC	Centers for Disease Control and Prevention
VFDB	Virulence factor database
SNP	Single nucleotide polymorphism
OR	Odds Ratio
MLST	Multilocus sequence typing
CC	Clonal complexes
ST	Sequence types
VT	Vaccine Type
CAP	Community-acquired pneumonia
IPD	Invasive Pneumococcal Disease

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11587-x.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Clinical trial number

Not applicable.

Authors' contributions

R.K.L and G.N. conceptualized this study. H.G.K performed the microbiology part of the analysis and G.N did the sequencing. V.G was involved in the sample collection. V.S. performed the genomic analysis of the samples collected in this study. G.N. and V.S. were involved in statistical analysis, table generation, figure generation, and manuscript writing. M.R.S was involved in manuscript drafting. R.K.L guided the manuscript preparation and reviewed the manuscript. Funding for the study was provided through grants to R.K.L All authors read and approved the final manuscript.

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Data availability

The datasets presented in this study can be found in online repositories. All the WGS data have been submitted to ENA under the Bioproject numbers [https://www.ebi.ac.uk/ena/browser/view/PRJEB73473]. The collection of the isolates is also available on Pathogenwatch ([https://pathogen.watch/collection/z0vgi6atv1r1-indian-adult-streptococcus-pneumoniae-surveillance-study]). The Microreact link for the genomic analysis is provided ([https://microreact.org/project/71rn9zQLYxG8F2hz5yCG6k-indian-adult-streptococcus-pneumoniae-surveillance-study#67n7-default]). All the codes used in this study is available at [https://github.com/varunshamanna/Indian-adult-SPN-Seroypes].

Declarations

Ethics approval and consent to participate

The Study was approved by Kempegowda Institute of Medical Sciences independent ethics committee (Approval ID: KIMS/IEC/S06/2022 dated 22-July-2022). The study was conducted according to the guidelines and recommendations of Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from each participant or legal guardian as applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Weiser JN, Ferreira DM, Paton JC. *Streptococcus pneumoniae*: transmission, colonization and invasion. Nat Rev Microbiol. 2018;16(6):355–67. https://doi.org/10.1038/s41579-018-0001-8. PMID: 29599457; PMCID: PMC5949087.
2. Centers for Disease Control Prevention. Pneumococcal Disease: Causes and How It Spreads. (2024). Available from: https://www.cdc.gov/pneumococcal/causes/index.html#cdc_causes_risk-risk-factors. [Accessed on 12th July 2024].
3. Torres A, Blasi F, Dartois N, Akova M. Which individuals are at increased risk of Pneumococcal disease and why? Impact of COPD, asthma, smoking, diabetes, and/or chronic heart disease on community-acquired pneumonia and invasive Pneumococcal disease. Thorax. 2015;70(10):984–9. https://doi.org/10.1136/thoraxjnl-2015-206780. Epub 2015 Jul 28. PMID: 26219979; PMCID: PMC4602259.
4. Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and National estimates for 2000-15. Lancet Glob Health. 2018;6(7):e744–57. (18)30247-X. PMID: 29903376; PMCID: PMC6005122.
5. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, Lee E, Mulholland K, Levine OS, Cherian T, Hib and Pneumococcal Global Burden of Disease Study Team. 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. PMID: 19748398.
6. Farooqui H, Jit M, Heymann DL, Zodpey S. Burden of severe pneumonia, pneumococcal pneumonia and pneumonia deaths in Indian states: modelling based estimates. PLoS One. 2015;10(6):e0129191. https://doi.org/10.1371/journal.pone.0129191.
7. Bhardwaj P, Dhar R, Khullar D, et al. Role of pneumococcal vaccination as a preventative measure for At-risk and High-risk adults: an Indian narrative. J Assoc Physicians India. 2025;73(5):45–54.
8. Ganaie F, Saad JS, McGee L, van Tonder AJ, Bentley SD, Lo SW, Gladstone RA, Turner P, Keenan JD, Breiman RF, Nahm MH. A new Pneumococcal capsule type, 10D, is the 100th serotype and has a large Cps fragment from an oral Streptococcus. mBio. 2020;11(3):e00937–20. https://doi.org/10.1128/mBio.00937-20. PMID: 32430472; PMCID: PMC7240158.
9. Sharma S, Sharma M, Ray P, Chakraborti A. Antimicrobial susceptibility pattern and serotype distribution of *Streptococcus pneumoniae* isolates from a Hospital-Based study in chandigarh, North India. Cureus. 2022;14(1):e21437. https://doi.org/10.7759/cureus.21437. PMID: 35223224; PMCID: PMC8860682.
10. Molander V, Elisson C, Balaji V, Backhaus E, John J, Vargheese R, Jayaraman R, Andersson R. Invasive Pneumococcal infections in vellore, india: clinical characteristics and distribution of serotypes. BMC Infect Dis. 2013;13:532. https://doi.org/10.1186/1471-2334-13-532. PMID: 24206667; PMCID: PMC3827497.
11. Wattal C, Goel N, Byotra SP. Prevalence of pneumococcal serotypes in adults ≥ 50 years of age. Indian J Med Microbiol. 2017;35(1):95–100. https://doi.org/10.4103/ijmm.IJMM_16_132.
12. Jayaraman R, Varghese R, Kumar JL, Neeravi A, Shanmugasundaram D, Ralph R, Thomas K, Veeraraghavan B. Invasive Pneumococcal disease in Indian adults: 11 years' experience. J Microbiol Immunol Infect. 2019;52(5):736–42. Epub 2018 May 22. PMID: 29884448.
13. Varghese R, Yesudhason BL, Vimala LR, Neeravi A, Anandhan K, Baskar P, et al. Pneumococcal serotypes causing non-invasive pneumonia in adults from a South Indian tertiary care hospital and the impact of the newer conjugate vaccines. Access Microbiol. 2021;3(12):000258. https://doi.org/10.1099/acmi.0.000258.
14. Nagaraj G, Govindan V, Ganaie F, Venkatesha VT, Hawkins PA, Gladstone RA, et al. *Streptococcus pneumoniae* genomic datasets from an Indian population describing pre-vaccine evolutionary epidemiology using a whole genome sequencing approach. Microb Genom. 2021;7(9):000645. https://doi.org/10.1099/mgen.0.000645.
15. Dhar R, Ghoshal AG, Guleria R, Sharma S, Kulkarni T, Swarnakar R, et al. Clinical practice guidelines 2019: Indian consensus-based recommendations on Pneumococcal vaccination for adults. Lung India. 2020;37(Supplement):S19–29. https://doi.org/10.4103/lungindia.lungindia_272_20. PMID: 32830790; PMCID: PMC7703813.
16. Koul PA, Vora AC, Jindal SK, Ramasubramanian V, Narayanan V, Tripathi SK, et al. Expert panel opinion on adult Pneumococcal vaccination in the post-COVID era (NAP- EXPO Recommendations-2024). Lung India. 2024;41(4):307–17. https://doi.org/10.4103/lungindia.lungindia_8_24. Epub 2024 Jun 28. PMID: 38953196.
17. Chawla K, Gurung B, Mukhopadhyay C, Bairy I. Reporting emerging resistance of *Streptococcus pneumoniae* from India. J Glob Infect Dis. 2010;2(1):10–4. https://doi.org/10.4103/0974-777X.59245.
18. Cillóniz C, Garcia-Vidal C, Ceccato A, Torres A. Antimicrobial resistance among *Streptococcus pneumoniae*. In: Fong I, Shlaes D, Drlica K, editors. Antimicrobial resistance in the 21st century. Emerging infectious diseases of the 21st century. Cham: Springer; 2018. pp. 13–38. https://doi.org/10.1007/978-3-319-78538-7_2.
19. Koul PA, Chaudhari S, Chokhani R, Christopher D, Dhar R, Doshi K, et al. Pneumococcal disease burden from an Indian perspective: need for its prevention in pulmonology practice. Lung India. 2019;36(3):216–25. https://doi.org/10.4103/lungindia.lungindia_497_18.
20. Köser CU, Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. Trends Genet. 2014;30(9):401–7. https://doi.org/10.1016/j.tig.2014.07.003.
21. Kozáková J, Honskus M, Okonji Z. Implementation and use of whole genome sequencing (WGS) in the surveillance of invasive Pneumococcal disease, Czech republic, 2017–2019. Epidemiol Mikrobiol Imunol. 2020;69(3):134–41. English. PMID: 33086855.
22. CLSI. Performance standards for antimicrobial susceptibility testing, M100, 24th ed. Clinical and Laboratory Standards Institute, Wayne, PA. 2014.
23. Harry CH, Hung N, Kumar V, Dyster C, Yeats B, Metcalf Y, Li et al. A portable and scalable genomic analysis pipeline for Streptococcus pneumoniae surveillance: GPS pipeline, biorxiv 2024;11(27):625679. doi: https://doi.org/10.1101/2024.11.27.625679.
24. Torsten Seemann, Shovill, GitHub, Repository. Retrieved from 2024. https://github.com/tseemann/shovill. Accessed on Apr 16.
25. Epping L, van Tonder AJ, Gladstone RA, The Global Pneumococcal Sequencing Consortium, Bentley SD, Page AJ, et al. SeroBA: rapid high-throughput serotyping of Streptococcus pneumoniae from whole genome sequence data. Microb Genom. 2018;4(7):e000186. https://doi.org/10.1099/mgen.0.000186.
26. Tseemann T. (1991) MLST License. In: Github. Available: https://github.com/tseemann/mlst/blob/master/LICENSE.
27. Lees JA, Harris SR, Tonkin-Hill G, Gladstone RA, Lo SW, Weiser JN, et al. Fast and flexible bacterial genomic epidemiology with poppunk. Genome Res. 2019;29(2):304–16. https://doi.org/10.1101/gr.241455.118.
28. Metcalf BJ, Chochua S, Gertz RE, Li Z, Walker H, Tran T. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive Pneumococcal disease isolates recovered in the united States. Clin Microbiol Infect. 2016;22(1002):e1–8.
29. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, Keane JA, et al. Ariba: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom. 2017;3(10):e000131. https://doi.org/10.1099/mgen.0.000131.
30. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res. 2022;50(D1):D912–7. https://doi.org/10.1093/nar/gkab1107. PMID: 34850947; PMCID: PMC8728188.
31. Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, Goesmann A. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microb Genom. 2021;7(11):000685. https://doi.org/10.1099/mgen.0.000685.
32. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. Genome Biol. 2020;21(1):180. https://doi.org/10.1186/s13059-020-02090-4.
33. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. Snp-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom. 2016;2(4):e000056. https://doi.org/10.1099/mgen.0.000056.
34. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-tree 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37(5):1530–4. https://doi.org/10.1093/molbev/msaa015.
35. Argimón S, Abudahab K, Goater RJE, Fedosejev A, Bhai J, Glasner C, et al. Microreact: visualizing and sharing data for genomic epidemiology and phylogeography. Microb Genom. 2016;2(11):e000093. https://doi.org/10.1099/mgen.0.000093.
36. Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4 H. Wickham. ggplot2: elegant graphics for data analysis. Springer- New York, 2016.
37. Boehmke BC. Reshaping your data with Tidyr. In: Boehmke CB, editor. Data wrangling with R. Cham: Springer International Publishing; 2016. pp. 211–8. https://doi.org/10.1007/978-3-319-45599-0_21.
38. McGee L, McDougal L, Zhou J, Spratt BG, Tenover FC, George R, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the Pneumococcal molecular epidemiology network. J Clin Microbiol. 2001;39(7):2565–71. https://doi.org/10.1128/JCM.39.7.2565-2571.2001.
39. Lo SW, Gladstone RA, van Tonder AJ, Lees JA, du Plessis M, Benisty R, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive Pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. Lancet Infect Dis. 2019;19(7):759–69. Epub 2019 Jun 10. PMID: 31196809; PMCID: PMC7641901.
40. Ghia CJ, Dhar R, Koul PA, Rambhad G, Fletcher MA. *Streptococcus pneumoniae* as a cause of community-acquired pneumonia in Indian adolescents and adults: a systematic review and meta-analysis. Clin Med Insights Circ Respir Pulm Med. 2019;13:1179548419862790.
41. Sharma OP. Indian recommendations for vaccination in older adults. New Delhi, India: Geriatric Society of India; 2015 Available at: https://www.geriatricindia.com/publications.html.com.
42. Dzaraly N. D., Muthanna A, Mohd Desa MN, Taib NM, Masri SN, Rahman NIA et al. (2020). Pilus Islets and the Clonal Spread of Piliated Streptococcus Pneumoniae: A Review. Int. J. Med. Microbiol. 310;(7):151449.
43. Obolski U, Lourenço J, Thompson C, Thompson R, Gori A, Gupta S. Vaccination can drive an increase in frequencies of antibiotic resistance among

nonvaccine serotypes of *Streptococcus pneumoniae*. Proc Natl Acad Sci U S A. 2018;115(12):3102–7. https://doi.org/10.1073/pnas.1718712115.
44. Yamada N, Nakamoto T, Takei H, Shoji T, Takahashi K, Sato J, Takeuchi N, Ohkusu M, Ishiwada N. Two cases of bacterial meningitis due to meropenem-resistant Streptococcus pneumoniae: A threat of serotype 35B, ST 558 lineage. J Infect Chemother. 2020;26(7):745–8. Epub 2020 Mar 12. PMID: 32171658.
45. Méroc E, Fletcher MA, Hanquet G, Slack MPE, Baay M, Hayford K, et al. Systematic literature review of the epidemiological characteristics of Pneumococcal disease caused by the additional serotypes covered by the 20-valent Pneumococcal conjugate vaccine. Microorganisms. 2023;11(7):1816. https://doi.org/10.3390/microorganisms11071816.
46. Nagaraj G, Govindan V, Ganaie F, Venkatesha VT, Hawkins PA, Gladstone RA, et al. *Streptococcus pneumoniae* genomic datasets from an Indian population describing pre-vaccine evolutionary epidemiology using a whole genome sequencing approach. Microb Genom. 2021;7(9):000645. https://doi.org/10.1099/mgen.0.000645.
47. Konstantin Mironov, Central Research Institute of Epidemiology, Moscow, Russia. (2024). Streptococcus pneumoniae ST11921. PubMLST. Retrieved from. https://pubmlst.org/.
48. Lyu S, Shi W, Dong F, Xu BP, Liu G, Wang Q, et al. Serotype distribution and antimicrobial resistance of pediatric *Streptococcus pneumoniae* isolated from inpatients and outpatients at Beijing Children's Hospital. Braz J Infect Dis. 2024;28(2):103734. https://doi.org/10.1016/j.bjid.2024.103734.
49. Varghese R, Neeravi A, Jacob JJ, Vasudevan K, Kumar JL, Subramanian N, et al. Comparative genomics of invasive *Streptococcus pneumoniae* CC320/271 serotype 19F/19A before the introduction of Pneumococcal vaccine in India. Mol Biol Rep. 2021;48(4):3265–76. https://doi.org/10.1007/s11033-021-06353-6.
50. Varghese R, Neeravi A, Subramanian N, Pavithra B, Kavipriya A, Kumar JL, Girish Kumar CP, Jeyraman Y, Karthik G, Verghese VP, Veeraraghavan B. Clonal similarities and sequence-type diversity of invasive and carriage *Streptococcus pneumoniae* in India among children under 5 Years. Indian J Med Microbiol. 2019;37(3):358–362. https://doi.org/10.4103/ijmm.IJMM_19_348. PMID: 32003333.
51. Kulkarni N, Routray A, Taur S. A multicenter evaluation of overall susceptibility and antimicrobial resistance among Streptococcus pneumoniae isolates. Cureus. 2023;15(7):e41984. https://doi.org/10.7759/cureus.41984. PMID: 37593286; PMCID: PMC10427887.
52. Nagaraj S, Kalal BS, Manoharan A, Shet A. *Streptococcus pneumoniae* serotype prevalence and antibiotic resistance among young children with invasive Pneumococcal disease: experience from a tertiary care center. South India Germs. 2017;7(2):78–85. PMID: 28626738; PMCID: PMC5466826.
53. Varaldo PE, Montanari MP, Giovanetti E. Genetic elements responsible for erythromycin resistance in streptococci. Antimicrob Agents Chemother. 2009;53(2):343 – 54 53. doi: 10.1128/AAC.00781-08. Epub 2008 Nov 10. PMID: 19001115; PMCID: PMC2630629.
54. Paulina H. (2024), *Streptococcus pneumoniae* ST11921. PubMLST. Retrieved from https://pubmlst.org/.
56. Majhi A, Adhikary R, Bhattacharyya A, Mahanti S, Bishayi B. Levofloxacin-ceftriaxone combination attenuates lung inflammation in a mouse model of bacteremic pneumonia caused by multidrug-resistant *Streptococcus pneumoniae* via inhibition of cytolytic activities of Pneumolysin and autolysin. Antimicrob Agents Chemother. 2014;58(9):5164–80. https://doi.org/10.1128/AAC.03245-14.
57. Jayashree Veerasamy, Jayalakshmi Jayarajan, Demonstration of Virulence Factors in Streptococcus Pneumoniae Isolates from a Tertiary Hospital in South India [Internet]. 2017;6(2):MO16-MO19. Available from:https://www.njlm.net//back_issues.aspx?issn=0973-709x&year=2017&month=April&volume=6&issue=2&page=MO16-MO19&id=2225.
58. Muhammad S, Aleem. Robert Sexton; Jagadish Akella, Pneumonia in an Immunocompromised Patient. In StatPearls. 2023. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK557843/#:~:text=Immunocompromised%20patients%20have%20an%20impaired,Neutropenic.
59. Varun Shamanna G, Nagaraj MR, Shincy KL, Ravikumar. Tracking the Evolution and Antibiotic Resistance Patterns of Streptococcus pneumoniae in Indian Adult Populations through High-Throughput Genome Sequencing, Internal J Infect Diseases, 2025;152. Supplement. https://doi.org/10.1016/j.ijid.2024.107405.
60. Higgs C, Kumar LS, Stevens K, Strachan J, Korman T, Horan K, Daniel D, Russell M, McDevitt CA, Sherry NL, Stinear TP, Howden BP, Gorrie CL. Comparison of contemporary invasive and non-invasive *Streptococcus pneumoniae* isolates reveals new insights into Circulating anti-microbial resistance determinants. Antimicrob Agents Chemother. 2023;67(11):e0078523. https://doi.org/10.1128/aac.00785-23. Epub 2023 Oct 12. PMID: 37823632; PMCID: PMC10649040.
61. Metcalf BJ, Chochua S, Gertz RE Jr., Li Z, Walker H, et al. Using whole genome sequencing to identify resistance determinants and predict antimicrobial resistance phenotypes for year 2015 invasive Pneumococcal disease isolates recovered in the united States. Clin Microbiol Infect. 2016;22:1002. e1-1002 e8.
62. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom. 2017;3:e000131.
63. CLSI. 2014. Performance standards for antimicrobial susceptibility testing, M100, 24th ed. Clinical and Laboratory Standards Institute, Wayne, PA. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: Twenty-forth informational supplement. 950 West Valley road, suite 2500, wayne, PA 19087, USA. Clinical and Laboratory Standards Institute; 2014.
64. Gareia-Suarez Mdel M, Villaverde R, CaldeviUa AF, Méndez FJ, Vázquez F. Serotype distribution and antimicrobial resistance of invasive and non-invasive Pneumococceal isolates in asturias, Spain. Jpn J Infect Dis. 2006;59:299–305.

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