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Original Article

Pre- & post-vaccine trends in pneumococcal serotypes & antimicrobial resistance patterns

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***Background & objectives*: The Pneumococcal vaccines were introduced under the Universal Immunization Programme (UIP) in 2021 in India. Drawing from the collective experience of various nations, it is anticipated that there will be a substantial shift in serotype patterns following the introduction of this vaccine. The available data is limited to years until 2018 when the vaccine was introduced in only five States. The present study was carried out to estimate the changes in serotypes and antimicrobial resistance patterns pre- and post-vaccine introduction from a tertiary care centre.**

***Methods*: All isolates from various clinical specimens in the pre-vaccine era (January 2015-July 2021, except for 2019) and post-vaccine era (August 2021- March 2023) were included. Antimicrobial susceptibility was tested using disc diffusion or VITEK2, and serotyping was performed using the Quellung test (post-vaccine introduction) or sequential multiplex PCR (pre-vaccine introduction). The Chi-square or Fisher exact test was used to identify associations between antimicrobial resistance and serotypes. The z-test for proportions was used to identify significant changes in serotype frequencies between the pre- and post-vaccine era; $P<0.05$ was considered as the level of significance.**

***Results*: Overall, the resistance rates increased for most of the antibiotics in the post-vaccine era, and there was no significant increase in the non-vaccine serotypes. The proportion of serotypes 19F and 15B/C increased, and serotypes 23F and 14 reduced in the post-vaccine era. The majority of the 19F and 19A isolates (89.7% and 80%, respectively) were multidrug resistant in the post-vaccine era.**

***Interpretation & conclusions*: Introducing pneumococcal vaccination reduced the burden of many vaccine serotypes, while the burden of non-vaccine serotypes slightly increased. Most of the vaccine serotypes (like 19F and 19A) that persisted in the post-vaccine era were drug resistant.**

Key words Antimicrobial resistance - PCV13 - serotype trends - *Streptococcus pneumoniae* - vaccine

Streptococcus pneumoniae is a major human pathogen causing both invasive and non-invasive infections. Globally, the evolution and spread of antibiotic-resistant clones have been reported, and

multidrug-resistant pneumococci are not rare. There are more than 100 identified pneumococcal serotypes based on their polysaccharide capsules[1]. The capsules are among the most successfully targeted antigens in pneumococcal vaccines, while other vaccine targets are currently being explored[2]. The vaccines include a set of common serotypes identified across the globe, and based on this, PCV13 (Pfizer) and pneumosil (Serum Institute India) are intended for clinical use. India currently employs these vaccines in its national immunization programme initiated in 2021[3]. While PCV13 includes serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, pneumosil is a vaccine prepared specifically based on serotype prevalence data from India and includes serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, and 23F.

From our centre, we previously published the serotypes common in the pre-vaccine era (2014-17) using sequential multiplex PCR approach recommended by Centre for Disease Control (CDC)[4]. We identified a significant association of antimicrobial resistance with the serotypes and high vaccine coverage among invasive isolates. Most of the multidrug resistant isolates (80%) were covered by the PCV13, and serogroup 6 was commonly associated with macrolide resistance.

Based on the experience of other countries, the serotype distribution tends to change after the introduction of vaccines, and serotypes 19F, 19A and 23F included in the PCV13 had reduced susceptibility to penicillin and ceftriaxone in the post-vaccine era[2,5-7]. Therefore, it is necessary to continuously monitor the serotypes and antimicrobial resistance (AMR) of pneumococcus to understand the long-term efficacy of vaccines, identify changes in antimicrobial resistance patterns, emergence of multidrug resistant serotypes, and to aid in the inclusion of other serotypes in vaccines that are prevalent in the country. The present study was carried out to identify the antimicrobial resistance and serotype patterns in the post-vaccination era and compare them with that of pre-vaccination era including the isolates from 2014-17 to identify possible serotype shifts and antimicrobial resistance rates among these serotypes.

Materials & Methods

Study design: A descriptive study was carried out in the department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, during two different study periods: 2015-18 and 2020-23. The pneumococcal isolates from various clinical specimens sent to the department of Microbiology for bacteriological investigations were included. The study was approved by the Institute Ethics Committee for Human Studies (for the period 2015-18) and waived for ethical review (for the period 2020-23) as it did not involve interaction with human participants. The study participants' demographic data were collected using structured proforma in a de-identified manner.

Bacterial isolates: The isolates were initially tested by optochin susceptibility and confirmed using *lytA-cpsA* PCR during 2015-2018 as per the previously published protocol[8,9] or MALDI-TOF MS (Bruker, USA) during 2020-2023 following manufacturer instructions.

Antibiotic susceptibility testing and serotyping: For all the isolates from 2015-18, antibiotic susceptibility was tested using Kirby-Bauer disc diffusion (HiMedia Labs Pvt. Ltd.) and E-test (for penicillin alone) using commercial strips (bioMérieux) following standard procedures. The serotypes were inferred using sequential multiplex PCR (smPCR) using CDC-recommended primers[10]. Each PCR reaction was carried out in 25 µl reaction volumes with 2.5 µl of purified DNA template. The serogroup 6 isolates were further identified into respective serotypes by a three-step PCR (the results were validated on a subset of isolates using Quellung test)[4,11]. Since 2020, all isolates were tested using VITEK2 (bioMérieux) and the Quellung reaction (Statens Serum Institut) was used to identify the serotypes. The susceptibility results were interpreted according to CLSI 2023 guidelines[12]. The isolates were tested for susceptibility to penicillin (PEN) and ceftriaxone (CRO; only for 2020-23 isolates), vancomycin (VAN), erythromycin (ERY), tetracycline (TET), levofloxacin (LEV), trimethoprim-sulphamethoxazole or cotrimoxazole (SXT), chloramphenicol (CHL; only for 2020-23 isolates), clindamycin (CLN) and linezolid (LNZ).

Definitions: The study participants were classified into different age groups based on specific ranges. Those isolates recovered from sterile body fluids were termed 'invasive isolates' (INV), while those from non-sterile sites were 'non-invasive isolates' (NON-INV). Any isolate not positive for any serotype in smPCR or Quellung reaction was termed as non-typable (NT). The isolates that were typed at serogroup level and may possess one of the vaccine serotypes were classified as

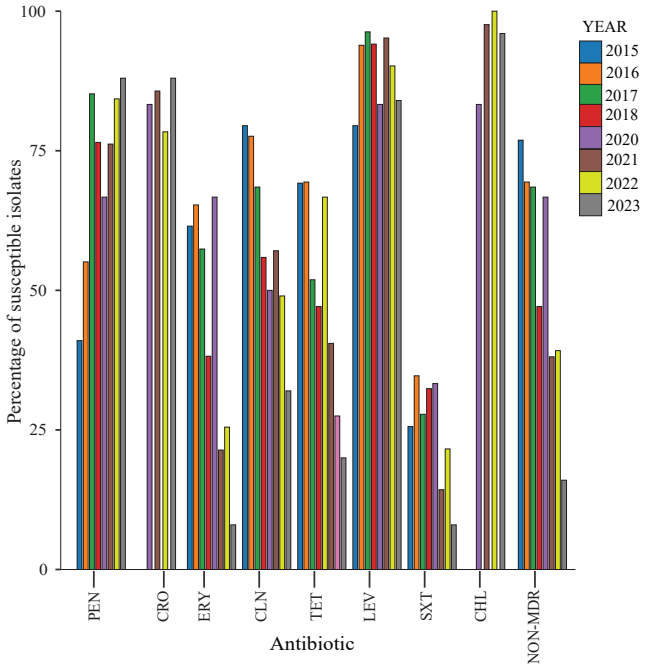

Fig. 1. Antimicrobial susceptibility rates for the tested antibiotics. All isolates are susceptible to vancomycin and linezolid, and hence not shown. The total number of isolates tested for each antibiotic is given in the Supplementary Table VI. Very few isolates (6) were tested in the year 2020. Interpretations for beta-lactam resistance was according to CLSI 2023 guidelines. PEN, penicillin; ERY, erythromycin; CLN, clindamycin; TET, tetracycline; LEV, levofloxacin; SXT, trimethoprim-sulphamethoxazole; CHL, chloramphenicol; CRO, ceftriaxone, MDR, multidrug resistance.

'vaccine serogroup' (VSG), while those isolates that were successfully serotyped and covered under vaccine were classified as 'vaccine serotypes' (VST). As both 6A and 6B were included in pneumosil and PCV13, isolates identified as belonging to either serotype were considered as VST. Any isolate not susceptible to at least three classes of antibiotics were grouped as multi-drug resistant (MDR). The vaccine was introduced in the States of Tamil Nadu and Puducherry on July 13, 2021, and this date was used to classify an isolate from the pre- or post-vaccine era[13].

Statistical analyses: All the data was expressed as percentages (for categorical data) and median with interquartile ranges (IQR) [for penicillin- minimum inhibitory concentration (MIC) values]. For identifying the level of MIC creep of penicillin, the median penicillin MIC in the year 2015 was chosen as the index year. The association between various categorical variables was assessed using Fisher Exact test or Chi-Square test as applicable, and if necessary, *P* values were corrected for multiple testing using Bonferroni method. For comparing the serotype proportions, z-test for proportions was used. All analyses were carried out at 95% confidence interval (CI), and a two-tailed *P* value of 0.05 was chosen as the cutoff for statistical significance. The statistical analyses were carried out using R 4.3.3 using customized scripts available at GitHub repository (*https://github.com/sreerampeela/GPSC_India_project/blob/main/data_analysis_r.r*).

Results

Demographics: A total of 300 pneumococcal isolates were collected, of which 58.7 per cent were from the 2015-18. Most of the isolates were from male participants (61.7%), and study participants' ages ranged from one month to 82 yr (mean age & standard deviation 35.8 ± 22.9 yr). The participants were predominantly adults aged between 19 and 45 yr (102/300; 34%) and 15.7 per cent (47/300) were children ≤5 yrs. The full demographic details are presented in the Supplementary Table I. Of these 300 isolates, 28.7 per cent (86/300) were from patients diagnosed with an invasive disease. Among the invasive isolates, blood was the most frequent source (61.6%; 53/86), and all the isolates were from non-meningeal infections, while 19 (6.3%) were from CSF specimens (Supplementary Table II).

Antimicrobial resistance: The non-susceptibility to cotrimoxazole (Fig. 1) was predominant among the isolates (73.5%; 208/283) followed by erythromycin (56.8%; 168/296) and tetracycline (51.7%; 155/300). None of the isolates had inducible clindamycin resistance, and non-susceptibility to levofloxacin was low. The median MIC of penicillin (Fig. 2) for the index year (2015) was 0.016 µg/ml (IQR: 0.016 - 0.056 µg/ml), and the MIC values increased over the subsequent years ($P<0.001$). Overall, 139 (46.3%) isolates were identified as multidrug resistant. Decreasing trends were observed for erythromycin, tetracycline and cotrimoxazole (SXT) across the study years, while the rates of multidrug resistance increased (Fig. 1). Among the isolates from the pre-vaccine era, the AMR rates were significantly lower except for ceftriaxone (Supplementary Fig. 1).

Among the 19 isolates from CSF, four (21.1%) were susceptible to penicillin, while two of the three isolates were susceptible to ceftriaxone. All the CSF isolates were susceptible to levofloxacin, while susceptibility rates were higher for the antibiotics clindamycin (57.9%) and tetracycline (57.9%). Resistance was common for the antibiotics erythromycin (57.9%) and cotrimoxazole (72.2%) among these isolates.

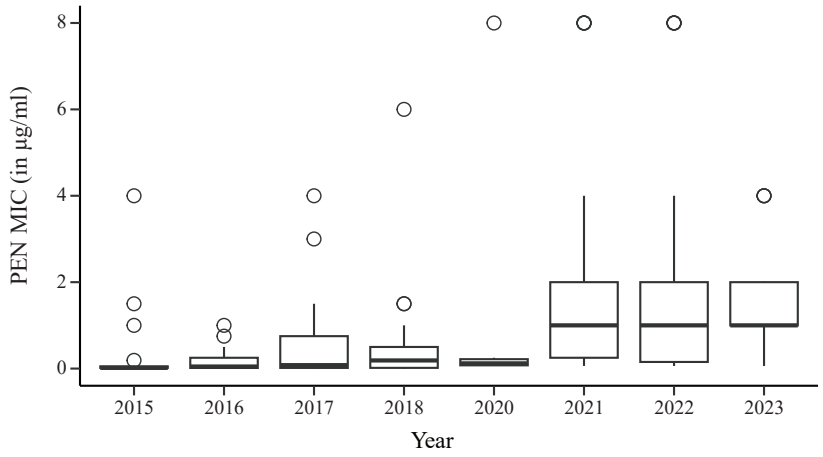

Fig. 2. Box plot showing increase in the median MIC values of penicillin across the study years. MIC, minimum inhibitory concentration.

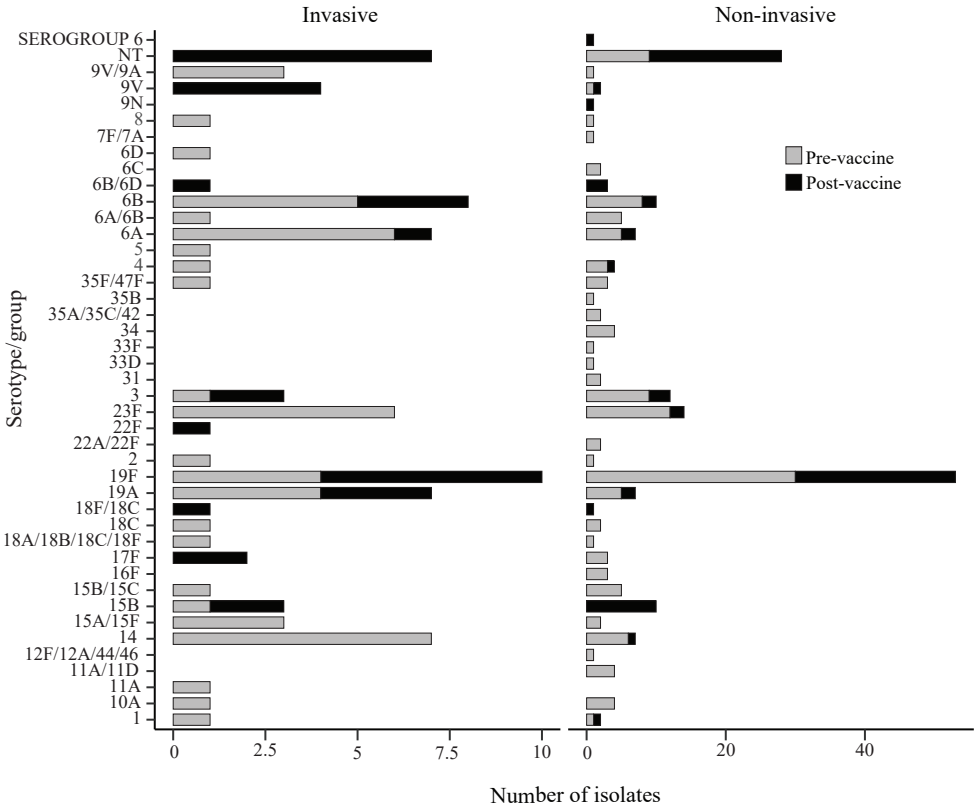

Fig. 3. Serotype patterns of invasive and non-invasive disease isolates in the pre and post-vaccination introduction.

Serotypes: About 73.7 per cent (221/300) isolates were typed at serotype level, 32 (10.7%) isolates were non-typable, and 3 (1%) isolates had unidentified serotypes (by Quellung reaction). The predominant serotypes (Fig. 3) were 19F (63/300; 21%) followed by 23F (20/300; 6.7%), 6B (18/300; 6%) and 3 (15/300; 5%). Six isolates were identified as belonging to either 6A/6B. Most of the non-typable and unidentified isolates were from non-invasive specimens. Serotype 19F was negatively associated with invasive disease (odds ratio: 0.4; 95% CI: 0.19 – 0.8; *P*=0.01). Serotype/group 15B/15C was less frequent in the invasive isolates (4/86; 4.7%) when compared with non-invasive isolates (15/199; 7.5%), though the results were not statistically significant (*P*=0.47).

*Serotypes and AMR***:** Among the vaccine serotypes, the resistance rates were higher for all antibiotics except

Table I. Percentage of PCV13 and 15B serotypes among the study isolates (n=300) before and after the introduction of vaccine

Serotype	Pre-vaccination (n=194)	Post-vaccination (n=106)	Adjusted *P* value[$]
1	1	0.9	1
3	5.2	4.7	1
4	2.1	0.9	1
5	0.5	0	NA[#]
6A	5.7	2.8	1
6B	6.7	4.7	1
7F	0[*]	0[*]	NA[#]
9V	0.5	4.7	0.14
14	6.7	0.9	0.25
18C	1.5[*]	0	NA[#]
19A	4.6	4.7	1
19F	17.5	27.4	0.44
23F	9.3	1.9	0.15
15B	0.5[*]	11.3	0.001

Serotype 15B was included as it was the predominant non-vaccine serotype. [$]z-test for two population proportions (*P* values adjusted using Bonferroni method). [*]More isolates were identified at serogroup level, but only those that were serotyped were included; [#]Not calculated if one of the columns was zero; NA, not applicable

chloramphenicol and levofloxacin (Supplementary Fig. 2 & 3). The association between drug resistance and various vaccines (PCV13 and Pneumosil) are shown in Supplementary tables III & IV, respectively. Serotype-specific temporal trends in AMR were assessed only for the commonest serotypes where at least 10 isolates were assigned to it. Serotype 19F was associated with multi-drug resistance post-vaccine introduction (odds ratio: 10.54, 95% CI: 2.85, 51.2, adjusted $P<0.001$) and negatively associated with chloramphenicol resistance (odds ratio = 0.0382, 95% CI: 0.001754, 0.221, adjusted $P<0.001$). For the other antibiotics and serotype combinations, no significant differences in the non-susceptibility rates across the two periods were noticed (Supplementary Table V).

Serotype trends before and after vaccine introduction: Among the 300 total isolates, 185 and 80 isolates were identified as any vaccine serotype or serogroup after excluding the isolates that were non-typable and unidentified as any serotype tested. Among the serotypes covered under the PCV13 vaccine, 19F (17.5% in pre-vaccine era and 27.4 per cent in post-vaccine era; $P=0.44$) and 9V (0.5% in pre-vaccine era and 4.7% in post-vaccine era; $P=0.14$) serotypes increased after the vaccine was introduced, while the serotypes 6A, 6B, 14 and 23F reduced significantly (Table I). Other vaccine serotypes 4 and 18 were reduced in the later years. However, the reduction was not statistically significant. Serotypes 19A, 3 and 1 were not significantly altered. Apart from the vaccine serotypes, the prevalence of non-vaccine serotype 15B increased significantly post-vaccination ($P=0.001$), especially among the non-invasive isolates. Most of the other non-vaccine serotypes reduced post vaccination, but were comparatively rare even during the pre-vaccination era (Fig. 3).

Among the invasive and non-invasive isolates, no significant differences among the vaccine coverages were noted across pre- and post-vaccine eras (Supplementary Table VI). Among the two vaccines, PCV13 had slightly higher coverage when compared with pneumosil (Table II). Overall, the proportion of PCV13 and pneumosil-covered serotypes did not vary significantly across the study period. However, there was a decrease in the proportion of vaccine serotypes in the later years (Table II). Comparing vaccine coverage among young children ≤5 yr before and after vaccine introduction, no significant differences in PCV13 and pneumosil coverages were observed for both invasive and non-invasive isolates (Table III).

Discussion

India introduced pneumococcal vaccine in its immunization program in 2017 (in select States) and nation-wide in 2021[3]. Currently two formulations of conjugate vaccine are in clinical use in India - PCV13

Table II. Proportion of PCV13 and pneumosil serotypes in the pre-vaccination and post-vaccination era

Vaccine type	Pre-vaccination (n=167), n (%)	Post-vaccination (n=98), n (%)	Total (n=265), n (%)	Odds ratio
PCV13	120 (65.9)	62 (34.1)	182 (68.7)	1.48 (0.87, 2.5)
Non-PCV13	47 (56.7)	36 (43.4)	83 (31.3)	
Pneumosil	103 (64.8)	56 (35.2)	159 (60)	1.206 (0.72, 2)
Non-pneumosil	64 (60.4)	42 (39.6)	106 (40)	

95% Confidence intervals for the Odds ratio are shown in parentheses. The non-typable isolates were excluded for calculating proportions

Table III. Vaccine coverage among children ≤5 yr before (PRE) and after (POST) vaccine introduction

Age ≤5 yr		PCV13; n (%)	Non-PCV13; n (%)	Pneumosil; n (%)	Non-pneumosil; n (%)
Invasive (n=27)	PRE	13 (72.2)	5 (27.8)	12 (66.7)	6 (33.3)
	POST	4 (44.4)	5 (55.6)	4 (44.4)	5 (55.6)
Non-invasive (n=20)	PRE	12 (92.3)	1 (7.7)	11 (84.6)	2 (15.4)
	POST	4 (57.1)	3 (42.9)	3 (42.9)	4 (57.1)

and pneumosil[3]. Although the vaccines are intended for children and old adults with high risk of mortality due to invasive pneumococcal diseases, there is a decrease in vaccine serotypes causing pneumococcal disease even in the non-targeted adult population[14-16]. Based on a time-series analysis in Malawi, the prevalence of PCV13-invasive disease decreased by 74 per cent in children less than five years and 47 per cent among adolescents and adults[16]. Similarly, in the USA, the incidence of pneumococcal invasive pneumococcal disease (IPD) in adults due to PCV13 serotypes reduced by 74 per cent even when vaccines were not indicated[14]. Such studies highlighted the herd immunity acquired post-pneumococcal vaccination. Across the globe, the lower-valent vaccines were introduced initially and later expanded to include higher-valent vaccines[17]. The Indian pneumococcal vaccination is in its early stages, and therefore it is important to assess its efficacy in reducing pneumococcal infections, especially the invasive infections. The present study was thus an attempt to understand the changes in temporal trends of serotypes pre- and post-vaccine introduction, and to understand the trends of antimicrobial resistance during these phases. As India currently employs PCV13 or pneumosil in its immunization programmes and pneumosil being a recently introduced vaccine, our discussion is limited to PCV13 alone when comparing with other countries.

Overall vaccine serotype trends: Overall, there was a non-significant reduction in the prevalence of PCV13 or pneumosil-covered serotypes. In the pre-vaccine era, the overall proportion of PCV13 serotypes was 71.9 per cent, while in the post-vaccine era, it reduced to 63.3 per cent, though the reduction was not statistically significant (Table II). However, in the study period 2015-18, the sequential multiplex PCR approach could not resolve the serotypes 9V/9A, and the serogroup 9V/9A was identified with a proportion of 2.1%. Even if the rates of serogroup change pre- and post-vaccine (4.7%) are analyzed, the increase was not statistically significant. Among the non-vaccine serotypes, a significant increase in serotype 15B was noted in the post-vaccine era (Fig. 3). Using smPCR, this serotype was identified as a serogroup 15B/15C, and if these isolates were included for analyzing the trends, the rates of isolation were 3.6 per cent (7/194) and 11.3 per cent (12/106) in the pre and post-vaccine eras, respectively.

As pneumococcal vaccine is primarily intended to prevent IPD in children < 5 yr, we separately assessed vaccine coverage across the two study periods in this population (Table III). There was an increase in the prevalence of non-vaccine serotypes for both invasive and non-invasive infections in this age group. However, the results were not statistically significant, presumably due to the low sample size in this age group.

Globally, significant serotype changes were noted after vaccine introduction. In Japan, the prevalence of PCV13 serotypes post-vaccination reduced significantly (31.5 to 5.6%), with a concomitant increase in non-vaccine serotypes (68.5 to 95.4%)[18]. Serotype replacement in children was predominantly done by serotypes 15A and 35B. In Europe, the incidence rates of non-vaccine serotypes increased after the introduction of PCV13. Based on a recent review and meta-analysis, the serotype 19F increased among adults in the post-vaccination era, and in children, it reduced[19]. In the same study, it was also noted that the 19F serotype remained one of the predominant serotypes. Similar finding was observed in our study where serotype 19F persisted at a high frequency even after vaccine introduction.

*Trends in serotypes 15B/C, 19A and 19F***:** In a study from Korea, the serotypes 19A, 19F and 23F were isolated in the patients with invasive pneumococcal disease at a higher rate in the post-vaccination era[7]. These isolates were identified to be drug resistant, and an overall reduction in penicillin and ceftriaxone susceptibility rate was observed. In the present study, we tried to evaluate the pre- and post-vaccination

changes in antibiotic resistance of 19F and 19A isolates, along with other common serotypes. When compared with the pre-vaccination era, the multidrug resistant isolates increased almost two-fold for both 19F and 19A isolates. Based on the Indian data available in the GPS project, there was an increase in the isolation of 19F isolates post-vaccination (27.7%) when compared with the pre-vaccination era (13.2%)[20]. It must be noted that the year of introduction of PCV into immunization was considered as 2017 where PCV13 was introduced in five states that did not include either Tamil Nadu or Puducherry. Globally, based on the GPS data of more than 20,000 isolates, 6.5 per cent of the 3667 and 3.1 per cent of the 2239 isolates from pre and post vaccine eras were of serotype 19F[21]. Therefore, globally, it appears that serotype 19F reduced significantly in the post-vaccine era.

Based on our findings, we can assume the following: (*i*) Decreased prevalence of serotypes 1, 3, 4, 6A, 6B, 14, 18C and 23F; (*ii*) Persistence of serotypes 19F and 19A and increased drug resistance among these isolates; (*iii*) Emergence of single non-vaccine serotype 15B post-vaccination with a three-fold increase compared to the pre-vaccination era.

The present study is the first study from India to analyze and understand the changing trends of serotypes after the introduction of the vaccine in the universal immunization schedule in 2021. Some of the limitations of the present study were: (*i*) the inability to completely serotype isolates in 2015-18; (*ii*) some isolates in 2015-18 were not tested for antibiotics like penicillin; (*iii*) while none of the isolates were tested for ceftriaxone and chloramphenicol; (*iv*) the lack of information on vaccination status even among the pediatric patients; and (*v*) the genetic mechanisms underlying the drug resistance were not identified. Nevertheless, the study identified significant changes in serotype patterns during the early years of the introduction of vaccines, and might inform the future vaccination strategies.

Although the results were based on a short-term post-vaccination, our results were similar to those observed globally as discussed above. It would, therefore, be interesting to identify the clonal relationships among these isolates with marked temporal trends. Such clonal relationships using the genome sequence data may highlight the evolution and acquisition of antimicrobial resistance genes *via* horizontal gene transfer. For example, GPSC10 (Global Pneumococcal Sequence Clusters) was the genotype commonly associated with non-vaccine serotypes and increased drug resistance across the world[22,23]. The present study, therefore, aids in understanding the changing trends of serotype and antibiotic resistance patterns of pneumococcus, with focus on pre- and post-pneumococcal vaccine introduction in the Universal Immunization Programme (UIP) of India. Such surveillance can form the basis of improved treatment regimes and prevention practices at the clinical front.

Overall, within a few years of vaccine introduction, the prevalence of vaccine serotypes reduced and that of non-vaccine types increased. There was a significant increase in the non-vaccine serotype 15B in the post-vaccine era. At the same time, 19F and 19A continued to be isolated at a high rate and had high levels of antimicrobial resistance. Continued surveillance is, therefore, necessary to understand the effects of vaccine introduction on the serotype trends and shifts in antibiotic resistance patterns.

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***Conflicts of Interest*:** None.

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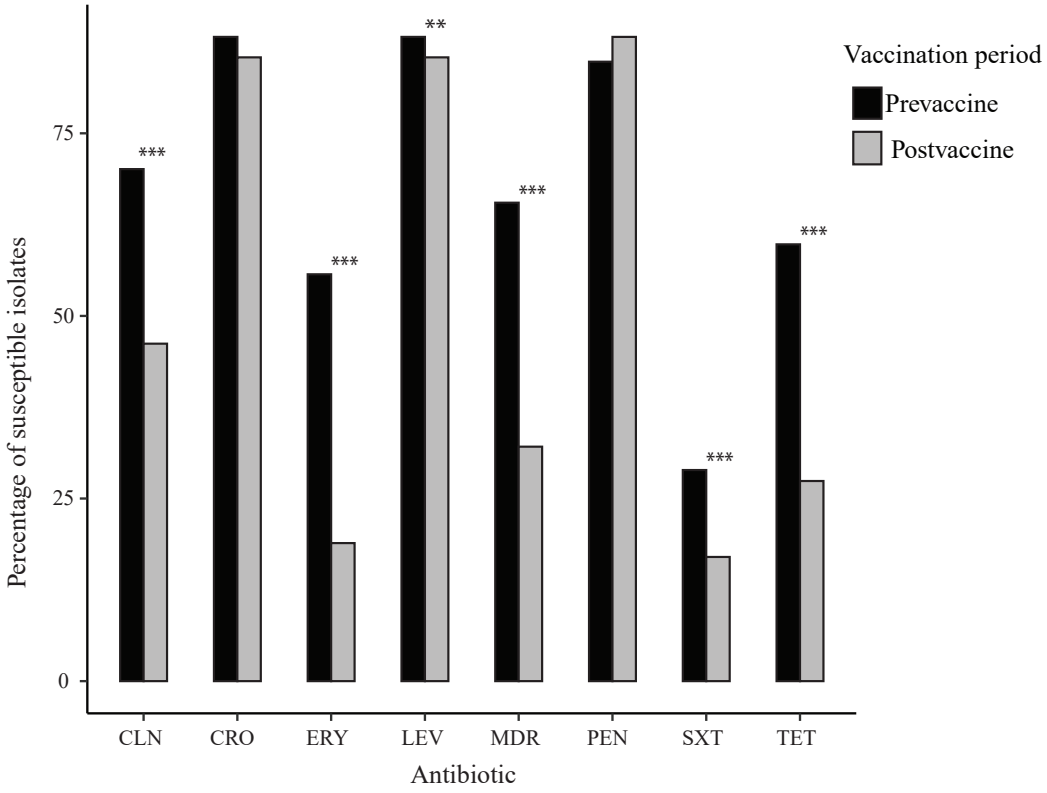
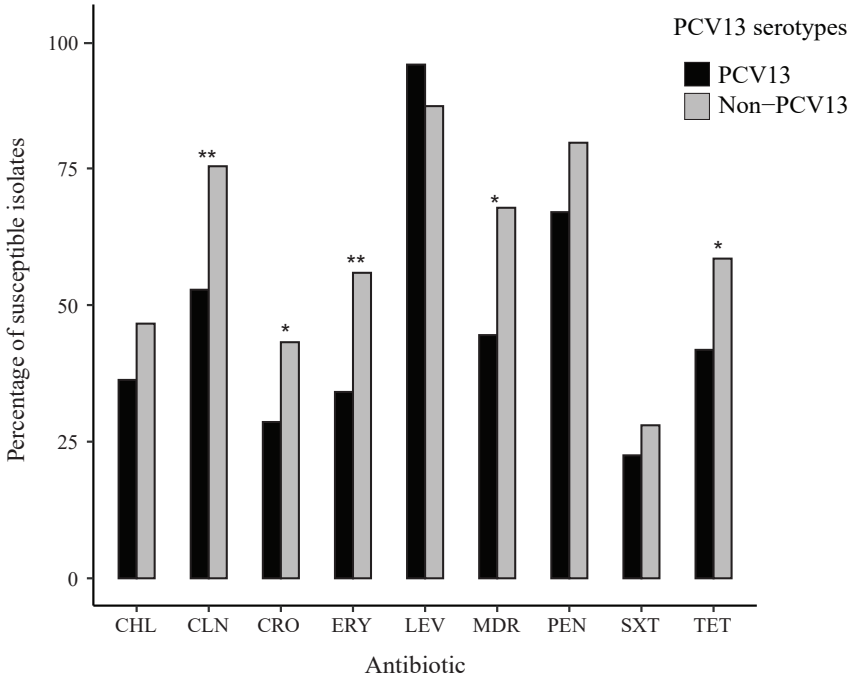
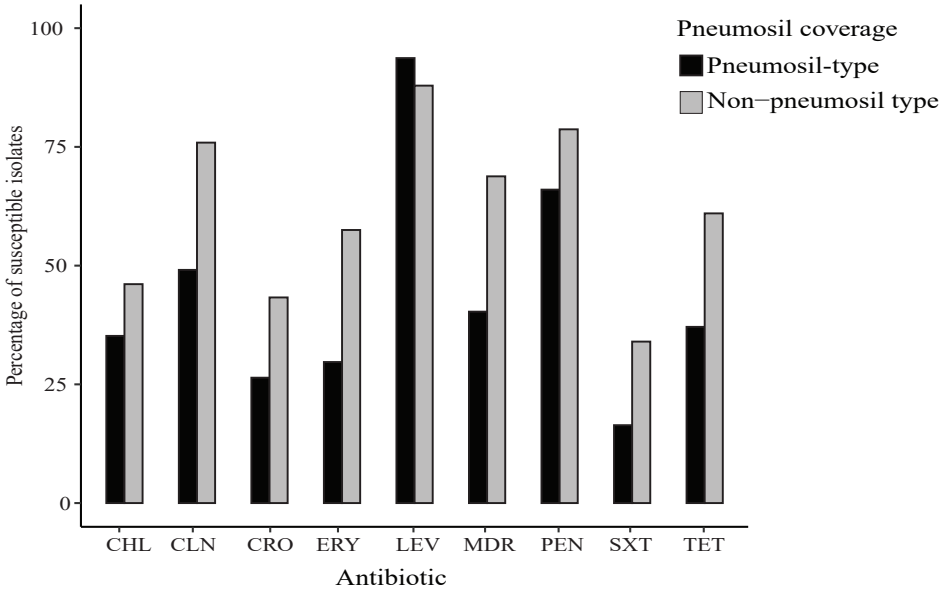


Supplementary Fig. 1. Susceptibility patterns of all isolates in the pre and post-vaccination era. PEN, penicillin; CRO, ceftriaxone; ERY, erythromycin; TET, tetracycline; LEV, levofloxacin; SXT, trimethoprim-sulphamethoxazole, CHL, chloramphenicol; CLN, clindamycin (*P* ***<0.001, **<0.01).



Supplementary Fig. 2. Susceptibility rates of isolates belonging to pneumosil-covered (P.Sil) and not covered (Not P.Sil) serotypes. $P^{*}<0.05$, $^{**}<0.01$ comparing susceptibility rates among PCV13 and non-PCV13 serotypes



Supplementary Fig. 3. Susceptibility rates of isolates belonging to PCV13-covered and not covered serotypes.

Supplementary Table I. Demographic details of study participants

Charachterstic	Labels	Total study participants; n (%)
Gender	Female	115 (38.3)
	Male	185 (61.7)
Age (yr)	≤5	47 (15.7)
	6-18	16 (5.3)
	19-45	102 (34)
	46-60	74 (24.7)
	>60	43 (14.3)

Supplementary Table II. Clinical specimens from where the isolates were collected

Sample type	Isloate specimens; n (%)
Respiratory	181 (60.3)
Blood	53 (17.7)
Cerebrospinal fluid (CSF)	19 (6.3)
Ocular samples	12 (4)
Ophthalmic samples	9 (3)
Pleural fluid	9 (3)
Ascitic fluid	4 (1.3)
Others	13 (4.3)
Total	300

Respiratory specimens like sputum, endotracheal aspirate, *etc*., were grouped as 'Respiratory', rare samples like pus were grouped as 'Others'

Supplementary Table III. Susceptibility and non-susceptibility rates of isolates belonging to pneumosil-covered and not covered serotypes

Antibiotic	PCV13	Non-PCV13	Total	Adj *P* value	Odds ratio
Penicillin				0.06	0.29
Susceptible	122 (56.5)	94 (43.5)	216		
Non-susceptible	27 (81.8)	6 (18.2)	33		
Total	149	100	249		
Ceftriaxone					
Susceptible	52 (50.5)	51 (49.5)	103	0.16	0.22
Non-susceptible	14 (82.4)	3 (17.6)	17		
Total	66	54	120		
Erythromycin					
Susceptible	62 (48.4)	66 (51.6)	128	0.003	0.41
Non-susceptible	117 (69.6)	51 (30.4)	168		
Total	173	117	296		
Clindamycin					
Susceptible	96 (51.9)	89 (48.1)	185	<0.001	0.35
Non-susceptible	83 (75.5)	27 (24.5)	110		
Total	179	116	295		
Tetracycline					
Susceptible	76 (52.4)	69 (47.6)	145	0.058	0.51
Non-susceptible	106 (68.4)	49 (31.6)	155		
Total	182	118	300		
Levofloxacin					
Susceptible	171 (62.6)	102 (37.4)	273	0.2	3.34
Non-susceptible	6 (33.3)	12 (66.7)	18		
Total	177	114	291		
Cotrimoxazole					
Susceptible	41 (54.7)	34 (45.3)	75	1	0.741
Non-susceptible	130 (62.5)	78 (37.5)	208		
Total	171	112	283		
Chloramphenicol					
Susceptible	66 (54.6)	55 (45.4)	121	1	0.602
Non-susceptible	2 (66.7)	1 (33.3)	3		
Total	68	56	124		
Multidrug resistant					
Yes	101 (72.7)	38 (27.3)	139	<0.001	0.38
No	81 (50.3)	80 (49.7)	161		
Total	182	118	300		

Raw *P* value from Fisher exact test was adjusted using Bonferroni method. Comparisons were between susceptibility rates of different antibiotics in PCV13 and non-PCV13 serotypes. Adj, adjusted

Supplementary Table IV. Susceptibility and non-susceptibility rates of isolates belonging to pneumosil-covered and not covered serotypes. Raw *P* value from Fisher exact test was adjusted using Bonferroni method

Antibiotic	Pneumosil	Non-pneumosil	Total	Adj *P* value	Odds ratio
Penicillin				0.04*	0.304
Susceptible	105 (48.6)	111 (51.4)	216		
Non-susceptible	25 (75.8)	8 (24.2)	33		
Total	130	119	249		
Ceftriaxone					
Susceptible	42 (40)	61 (60)	105	0.07	0.215
Non-susceptible	13 (76.5)	4 (23.5)	17		
Total	55	65			
Erythromycin					
Susceptible	47 (36.7)	81 (63.3)	128	<0.001*	0.3154
Non-susceptible	109 (64.9)	59 (35.1)	168		
Total	156	140	296		
Clindamycin					
Susceptible	78 (42.2)	107 (57.8)	185	<0.001*	0.3
Non-susceptible	78 (70.9)	32 (29.1)	110		
Total	156	139	295		
Tetracycline					
Susceptible	59 (40.7)	86 (59.3)	145	<0.001*	0.379
Non-susceptible	100 (64.5)	55 (35.5)	155		
Total	159	141	300		
Levofloxacin					
Susceptible	149 (54.6)	124 (45.4)	273	0.27	3.113
Non-susceptible	5 (27.8)	13 (72.2)	18		
Total	154	137	291		
Cotrimoxazole					
Susceptible	26 (34.7)	49 (65.3)	75	0.005*	0.376
Non-susceptible	122 (58.7)	86 (41.3)	208		
Total	148	135	283		
Chloramphenicol					
Susceptible	56 (46.3)	65 (53.7)	121	1	1.716
Non-susceptible	1 (33.3)	2 (66.7)	3		
Total	57	67	124		
Multidrug resistant					
Yes	Yes	95 (68.4)	44 (31.6)	<0.001*	0.307
No	No	64 (39.8)	97 (60.2)		
Total	Total	159	141		

Comparisons were between susceptibility rates of different antibiotics in serotypes covered/not-covered in pneumosil vaccine

Supplementary Table V. Association of predominant serotypes with drug resistance in pre (PRE) and post-vaccine (POST) eras

Serotype	Antibiotic	POST_NS	PRE_NS	POST_SUS	PRE_SUS	Odds ratio	Raw *P*	Adj *P*
14	MDR	1	6	0	7	1.886	0.74	1
15B	MDR	10	0	2	1	6.99	0.230769	1
19A	MDR	4	4	1	5	4.452109	0.300699	1
19F	MDR	26	15	3	19	10.53725	0.000173	0.012432
23F	MDR	1	13	1	5	0.405998	0.521053	1
3	MDR	2	0	3	10	9.2	0.16	1
6A	MDR	2	2	1	9	7.295443	0.175824	1
6B	MDR	4	6	1	7	4.295561	0.313725	1
14	PEN	0	5	1	13	0	1	1
15B	PEN	0	1	12	0	0	0.076923	1
19A	PEN	2	3	3	8	1.712047	1	1
19F	PEN	12	11	17	30	1.906676	0.301525	1
23F	PEN	0	8	2	14	0	0.536232	1
3	PEN	0	2	5	10	0	1	1
6A	PEN	1	2	2	11	2.546789	0.489286	1
6B	PEN	0	6	5	12	0	0.27249	1
14	CRO	0	13	1	13	0	1	1
15B	CRO	0	0	12	1	0	1	1
19A	CRO	0	9	5	9	0	0.115665	1
19F	CRO	11	33	19	34	0.599675	0.277364	1
23F	CRO	0	16	2	18	0	0.492063	1
3	CRO	0	10	5	10	0	0.061265	1
6A	CRO	1	10	2	11	0.56343	1	1
6B	CRO	2	13	3	13	0.675379	1	1
14	LEV	0	0	1	13	0	1	1
15B	LEV	4	0	8	1	0.86	0.94	1
19A	LEV	0	0	5	9	0	1	1
19F	LEV	3	1	27	34	3.705294	0.327699	1
23F	LEV	0	1	2	18	0	1	1
3	LEV	0	0	5	10	0	1	1
6A	LEV	0	1	3	11	0	1	1
6B	LEV	1	1	4	12	2.792848	0.490196	1
14	ERY	1	7	0	6	1.414	0.86	1
15B	ERY	10	0	2	1	3.74	0.39	1
19A	ERY	4	5	1	4	2.953851	0.58042	1
19F	ERY	27	19	3	15	6.890793	0.004532	0.326332
23F	ERY	2	14	1	4	0.588562	1	1
3	ERY	4	0	1	10	41.61	0.008	0.576
6A	ERY	3	6	0	5	3.74	0.39	1
6B	ERY	4	7	1	6	3.215424	0.595588	1
14	CLN	0	4	1	9	0	1	1
15B	CLN	8	0	4	1	3.1	0.56	1

Contd...

Serotype	Antibiotic	POST_NS	PRE_NS	POST_SUS	PRE_SUS	Odds ratio	Raw *P*	Adj *P*
19A	CLN	4	4	1	5	4.452109	0.300699	1
19F	CLN	21	13	9	21	3.686465	0.013555	0.975951
23F	CLN	2	14	0	4	0.94	0.95	1
3	CLN	3	0	2	10	18.75	0.042	1
6A	CLN	1	0	2	11	7.66	0.31	1
6B	CLN	4	7	1	6	3.215424	0.595588	1
14	TET	1	8	0	5	1.05	0.97	1
15B	TET	11	0	1	1	13.27	0.22	1
19A	TET	4	6	1	3	1.907544	1	1
19F	TET	26	21	3	13	5.227818	0.018753	1
23F	TET	2	9	0	9	3.08	0.482	1
3	TET	2	1	3	9	5.206688	0.241758	1
6A	TET	2	2	1	9	7.295443	0.175824	1
6B	TET	4	5	1	8	5.759094	0.294118	1
14	CHL	0	13	1	13	0	1	1
15B	CHL	1	0	11	1	0.2	0.457	1
19A	CHL	0	9	5	9	0	0.115665	1
19F	CHL	0	33	29	34	0	2.84E-07	2.04E-05
23F	CHL	0	16	2	18	0	0.492063	1
3	CHL	0	10	5	10	0	0.061265	1
6A	CHL	1	10	2	11	0.56343	1	1
6B	CHL	0	13	5	13	0	0.058001	1
14	SXT	1	12	0	3	0.453	0.69	1
15B	SXT	12	1	0	0	0	1	1
19A	SXT	5	7	0	3	3.24	0.449	1
19F	SXT	28	25	1	9	9.780892	0.015749	1
23F	SXT	1	16	1	4	0.271157	0.411255	1
3	SXT	1	1	4	9	2.121357	1	1
6A	SXT	3	7	0	6	3.86	0.37	1
6B	SXT	4	9	1	5	2.137445	1	1

Raw *P* value from Fisher exact test was adjusted using Bonferroni method. POST_NS, non-susceptible in post-vaccine era; POST_S, susceptible in post-vaccine era; PRE_S, susceptible in pre-vaccine era; PRE_NS, non-susceptible in pre-vaccine era. Odds ratio for drug resistance *vs.* vaccine era was computed for each major serotype and antibiotic

Supplementary Table VI. Vaccine coverages among invasive and non-invasive isolates in the pre (PRE) and post-vaccine (POST) eras

Overall vaccine coverages	PCV13	Non-PCV13	Pneumosil	Non-pneumosil
Invasive				
PRE	38	15	35	18
POST	19	14	17	16
Non-invasive				
PRE	87	54	73	68
POST	38	35	34	39