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Incidence of *Streptococcus pneumoniae* in Various Invasive and Non-Invasive Specimens in A Quaternary Care Hospital with its Antibiotic Susceptibility Patterns and its Serotype Distribution

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A B S T R A C T

Pneumococcal disease burden in various body sites becomes a pressing public health matter in India. Globally, it is considered the fourth-deadliest pathogen in terms of mortality linked to or resulting from antimicrobial resistance. The true burden is yet to be fully understood, as its true magnitude remains elusive. Even though Indian research has consistently shown a high occurrence of invasive pneumococcal infections and a substantial infection pressure across all age groups, there is a scarcity of information on the pneumococcal serotypes responsible for non-invasive infections in India. Existing studies have primarily focused on invasive pneumococcal disease or a combination of both invasive and non-invasive infections, resulting in a significant knowledge gap regarding non-invasive disease, despite regular publication of data on invasive isolates. Moreover studies are mainly concentrated on pediatric and old age group. Exclusive reliance on invasive pneumococcal infections data may lead to an incomplete assessment of the total pneumococcal infection burden, as it neglects the contribution of non-invasive infections to the overall disease load. The existing reports, based on observational studies, lack laboratory confirmation and exhibit significant heterogeneity in terms of case definition, laboratory techniques, duration, and geographic scope. Consequently, there is a pressing need for up-to-date, regionally representative baseline data on serotype distribution, antibiotic resistance patterns, and vaccine coverage to inform evidence-based public health decisions. To isolate S. pneumoniae from Blood, cerebrospinal fluid, pleural fluid, other sterile body fluids, ear, eye, nasopharynx and lower respiratory tract specimens and to list reported clinical symptoms associated with pneumococcal disease and associated co-morbidities, if any. Also, to identify the prevalent serotypes and evaluate the antibiotic susceptibility patterns of the infecting strains. Clinical syndrome-based site samples are collected from patients based on routine clinical practice followed in hospital. Sterile specimens are loaded in to BACT/ALERT 3D microbial identification system and Other non-invasive specimens are examined using standard bacteriological methods. Optochin sensitive gram-positive cocci are processed for identification in Vitek 2 Compact (BioMerieux) and antimicrobial susceptibility is done by Kirby-Bauer disk diffusion method or Automated ID/AST system. Pneumococcal isolates are sub-cultured and transported to the Central Research Laboratory at KIMS Medical College, Bangalore for serotyping. During the specified period, a total of 12,180 samples were received, out of which S. pneumoniae are isolated from 53 (0.4%) samples. Among the 53 samples the majority (n=31) were obtained from invasive specimens and the remaining 22 specimens were collected from non-invasive site. The targets enrolled were categorized into six age groups, the majority being adults (52.8%), with male (66.1%) being more frequently affected. Out of 53 patients, 30 patients presented with at least one comorbidity. The most common risk factor for pneumococcal infection were respiratory disease (26.4%) and diabetes (24.5%). A total of 16 different serotypes were identified, 19F was the most common serotype in our population. 52 isolates showed antibiotic resistance to at least one of the tested antimicrobials while only one isolate was fully susceptible to all tested antimicrobials. This study provides valuable insights into the incidence, antibiotic susceptibility patterns, and serotype distribution of *Streptococcus pneumoniae*. Our findings highlight the ongoing threat of pneumococcal infections, particularly among vulnerable populations, and emphasize the need for sustained surveillance, prudent antibiotic use, and targeted vaccination strategies. Our results have significant implications for healthcare policies, vaccine development, and antimicrobial stewardship initiatives, ultimately aiming to reduce the burden of pneumococcal infections and improve patient outcomes.

Introduction

Streptococcus pneumoniae (or pneumococcus) is a gram-positive, extracellular, lanceolate shaped facultative anaerobic diplococcus. They are alpha hemolytic streptococcus which set them apart from other streptococci mainly in its morphology, bile solubility, optochin sensitivity and presence of a polysaccharide capsule. Due to the presence of over 90 immunologically and chemically unique polysaccharide capsules that envelop and shield the bacteria from phagocytosis, *S. pneumoniae* has been divided into several serotypes as determined by serology (Calix *et al.,* 2012; Park *et al.,* 2007).

Pneumococcus are part of normal inhabitants (opportunistic pathogen) of naso and oropharyngeal flora of many healthy persons. Up to 60% of healthy infants can have asymptomatic settlement of pneumococci on their nasopharynx and upper airway. It also have the ability to penetrate sterile places and cause infections ranging from mild to life-threatening (Henriques-Normark and Tuomanen, 2013; Subramanian *et al.,* 2019; Bogaert *et al.,* 2004). Infection can be generically classified as either invasive or non-invasive illness. From non-invasive conditions like sinus infection, middle ear infection and non-bacteremic pneumonia to invasive conditions like sepsis, meningitis, and bacteremic pneumonia, pneumococcal infections cover a wide spectrum. The later describes infections where the microbe is located from typical sterile body areas like the blood or the cerebrospinal fluid, meningitis and pneumonia emerged as the most common clinical conditions (Feldman and Anderson, 2014; Opavski *et al.,* 2023; Koul *et al.,* 2019).

One of the most frequent causes of Community-onset pneumonia/ CAP is pneumonia, which is the most prevalent manifestation of pneumococcal illness. While middle ear infection, sinus infection and bronchial infection are examples of less serious infections, bacteremia, septic shock, meningeal infection and osteomyelitis are quite harmful. The elderly, immunocompromised individuals, and children under two years old are particularly vulnerable to the high rates of morbidity and mortality associated with the pathogen (Cedrone *et al.,* 2023; Cillóniz *et al.,* 2018).

However, most of disease worldwide is caused by a small number of common serotypes. There are currently over a hundred distinct pneumococcus serotypes known to exist. Geographic heterogeneity has been observed in the distribution of pneumococcal serotypes worldwide (Johnson *et al.,* 2010; Hausdorff *et al.,* 2001; Sharma *et al.,* 2022).

Pneumococcal infections still face a variety of serious obstacles in identification, management, and avoidance. It can become difficult to treat due to the emergence of resistant strains to routinely used antibiotics such beta-lactams, macrolides, and tetracyclines. During the pre-antibiotic period, death rates from IPD were very high, and the global expansion of antibiotic-resistant pneumococcal strains poses a severe threat to public health. Pneumococcus is therefore considered a "priority" pathogen, necessitating immediate research into the creation of novel antibiotics (Feldman and Anderson, 2014; Subramanian *et al.,* 2019).

According to the World Health Organization (WHO), there will be around 1.6 million fatalities annually from invasive pneumococcal disease (IPD). Over half of the fatalities happen in the pediatric population, specifically those below the age of five. The majority of these fatalities take place in Asia and Africa's economically deprived regions (WHO, 2007).

Two types of pneumococcal vaccinations are being used, based on the most common serotypes causing IPD. Prior to the development of conjugated polysaccharide vaccines (PCVs), which have a stronger protective effect, particularly in risk populations like young children, the non-conjugated pneumovax 23 was introduced (O'Brien and Santosham, 2004; Subramanian *et al.,* 2019).

Since pneumococcal vaccinations lower hospitalization, mortality, and disease burden in individuals with coexisting illnesses, they are recommended by several international guidelines for the prevention of pneumococcal infections. Even with the extensive use of pneumococcal vaccinations to immunize youngsters, the most common cause of serious illnesses is still *Streptococcus pneumoniae*. An estimated 1 million children less than 5 years old die from pneumococcal illness each year, indicating the concerning global rise in the disease burden caused by *S. pneumoniae* (Koul *et al.,* 2019; Yao and Yang, 2008).

The presently licensed vaccines on the market cover only a small subset of serotypes and do not take capsular replacement into account. Sentinel surveillance has become more and more necessary due to the rise in new,

antimicrobial-resistant strains as well as the general increase in serotypes (13). In order to determine the best course of action for treating and preventing *S. pneumoniae* infections, there is a need for current base line data as regards to serotype prevalence, antibiotic resistance and vaccine coverage.

This study examined the trends in serotype-specific epidemiology and antimicrobial susceptibility as well as the vaccination coverage of the identified serotype in patients of all ages in a region.

Materials and Methods

Clinical syndrome-based site samples are collected from patients based on routine clinical practice followed in hospital (eg: Blood, CSF, Sputum etc...). Blood and sterile specimens are loaded in to BACT/ALERT 3D microbial identification system and Other non-invasive specimens are examined using standard bacteriological methods. On next day, all alpha haemolytic gram positive cocci are checked for optochin sensitivity. Optochin sensitive gram-positive cocci are processed for identification in Vitek 2 Compact (BioMerieux) and antimicrobial susceptibility is done by Kirby-Bauer disk diffusion method or Automated ID/AST system. Pneumococcal isolates sub-cultured in 5% sheep blood agar and transported in Silica sachets to the Central Research Laboratory at KIMS Medical College, Bangalore for serotyping. Pneumococci is typed by a capsular reaction test employing "Chess board Method.

Results and Discussion

During the study period, a total of 12,180 samples (8797 invasive samples [72.2%] and 3383 non-invasive samples [27.8%]) were received, out of which *S. pneumoniae* were isolated and identified using standard tests, from 53 samples (0.4%).

Incidence of Pneumococci

Incidence in Various Invasive and Non-Invasive Specimens

Of the 53 samples that tested positive, the majority (31 or 58.5%) were obtained from invasive specimens and the remaining 22 specimens (41.5%) were collected from non-invasive site, completing the profile of the 53 positive specimens.

Assessment of Incidence in Various Invasive and Non-Invasive Specimens

The data shows, out of 31 invasive isolates, accounting for 58.5% of the total, 21 (39.6%) were isolated from blood, while 10 (18.8%) were recovered from CSF. And among the remaining 22 non- invasive samples (41.5%), sputum samples yielded the highest number of pneumococcal isolates (22.6%, 12/22), while bronchial wash, ear swabs, and endotracheal aspirates each contributed 4.5% (3/22) of the total isolates. Furthermore, 1.9% of isolates were obtained from BAL samples (1/22).

Age Wise Stratification of Isolates

The targets enrolled were categorized into six age groups, with the majority being adults (52.8%, 28/53). Children aged 17 years or younger comprised 26.4% (14/53) of the total, with 64.2% (9/14) of this subgroup being ≤ 5 years of age.

Additionally, 20.7% (11/53) of the isolates were obtained from individuals in the older age group. 54.8% IPD cases (17/31) are reported from adult age group (18-49yr, 50-65yr), followed by pediatric age groups (≤ 2yr, 3-5yr, 6-17yr) accounts 29% (9/31) of total IPD cases. 11 cases of non-invasive PD (ie;50%) was identified in adult age group. In contrast, old age group accounts 27.3% (6/22) of non-invasive PD.

Gender Wise Stratification of Isolates

The majority of enrolled subjects (66.1%) were male, whereas females accounted for approximately half of the male proportion (33.9%). Among the female cases, a significant proportion (83.3%, 15/18) of positive isolates came from the adult age group, while male cases were split evenly between pediatric and adult groups (37.1%, 13/35 each).

Comparison of Pneumococcal Disease Incidence in Patients With and Without Comorbidities

Out of 53 patients, a majority of 56.6% (30 patients) presented with at least one comorbidity, while 43.4% had no known comorbidities. Furthermore, within the group of patients with comorbidities, 53.3% had multiple comorbidities, with two or more conditions.

Impact of Various Risk Factor

Among the 53 patients in our study, the most common risk factor for pneumococcal infection were observed in patients with specific underlying comorbid condition like respiratory disease (26.4%) and diabetes (24.5%). Followed by cardiovascular disease (15.1%) and usage of oral steroid (11.3%). Other risk factors included chronic liver disease (5.7%), smoking (3.8%), malignancy (7.5%) and cerebrovascular disease (7.5%).

Serotype Distribution of *Streptococcus pneumoniae*

A total of 16 different serotypes identified among the 53 isolates. 15.1% of isolates were non-typeable (NT) by quellung reaction. Most commonly isolates serotype was 19F (32.1%, 17/53). Followed by serotypes 6B/D and 9V each constitute 9.4% (5/53). Other serotypes included 1, 15B, 9N and 19A with a rate of 5.7% (n=3), 5.7% (n=3), 3.8% (n=2) and 3.8% (n=2) respectively. The least frequently isolated serotypes, each accounting for 1.9%, included 11A/C/D, 6A, 7F/A, 18F/C, 14, 23A, 3, and 4.

Age Wise Stratification of Serotypes

Among the 16 different serotypes identified, most common serotypes which causes pediatric and adult group infections are 19F, it accounts 28.7% of pediatric infection (n=4) and 39.3% of adult group infections (n=11). 14.2% of pediatric and adult isolates (each; n=2) are non-typeable by quellung reaction.

The second most common serotype isolated in pediatric group was 6B/D (21.6%), meanwhile 9V and 15B in adult group (each 10.7%). Serotypes which causes infection in old age group include 19F, 9V, 1, 19A, 6B/D and 18F/C (in order of frequency).

Serotype Distribution of *Streptococcus pneumoniae* Isolates from Different Sources

Out of 16 different serotypes identified, Overall, serotype 19F was the most prevalent serotype causes both invasive (29%; n=9/31) and non-invasive (36.4%; n=8/22) infections. In this study pneumococcal serotypes which causes IPD are 19F, 6B/D, 1, 15B, 9N, 9V, 19A, 14 and 3 (in order of frequency). 19F, 9V, 4, 6A, 6B/D, 7F/A, 18F/C, 23A and 11A/C/D are serotypes isolated from non-invasive cases in a rate of 36.4%, 13.6%, and 4.5% remaining each one. Non-typeable isolates were mostly found both in CSF and Sputum samples (37.5%, n=3/8 each). Additionally, in our study serotype 6B/D (n=3) is prevalent serotype causes IPD in children, followed by 19F (n=2), 14 and 1 (each n=1) respectively.

Likely serotype 1 (n=2) is prominent cause of IPD in old age group. In adults, non-invasive infections were primarily caused by serotypes19F (n=5), 9V (n=2), 1 each of 6A and 7F. In contrast, invasive pneumococcal disease cases in adults were predominantly attributed by serotypes 19F (n=6), 15B (n=3), 3 (n=1), 9N (n=2), 6B/D (n=1), 9V (n=1), and 19A (n=1).

Vaccine Coverage of Isolates

In the study, the observed coverage of PCV10, PCV13 and PPV23 vaccines for obtained *S. pneumoniae* serotypes was 82.2%, 84.4% and 95.5% respectively. Among the 45 different pneumococcal infection cases typed, 2.2% cases are caused by non-vaccine serotype 23A which is neither included in any licensed pneumococcal vaccine.

In contrast 14 isolates typed from pediatric infections, 2 were non-typeable and from 12 identified serotypes, 2 serotypes (11A/C/D and 23A) were not covered in neither PCV10 nor PCV13.

Antibiotic Susceptibility Patterns of the Isolates

Antibiotic susceptibility testing data reveals 52 isolates showed antibiotic resistance to at least one of the tested antimicrobials while only one isolate fully susceptible to all tested antimicrobials.

Overall non-susceptibility (including resistance and intermediate susceptibility) to penicillin was noted in 12 isolates (22.6%), 10 were resistant and 2 had intermediate susceptibility. Erythromycin resistance was predominant among the isolates, with 92.4% (n=49) showing resistance and only 7.5% (n=4) susceptible.

Susceptibility profiling of isolates shows complete sensitivity to linezolid and vancomycin (100%). The proportion of intermediate resistance was high for ceftriaxone (9.4%). 92.4% of isolates shows sensitive to levofloxacin, in contrast 7.5% isolates shows overall resistance. Resistance rates to tetracycline, cotrimoxazole and clindamycin were high at 71.1%, 67.9% and 54.7% respectively.

Comparative Analysis of Antibiotic Resistance Patterns in Invasive and Non-Invasive Pneumococcal Isolates

Analysis of antibiotic susceptibility patterns by source revealed out of 10 penicillin resistant isolates, 90% of isolates are isolated from CSF sample (n=9) and 10% obtained from blood sample (n=1). Likely intermediate resistance to ceftriaxone (n=5) is highly shown by isolates isolated from CSF specimen (80%, n=4). Isolates from all specimens are completely susceptible (100%) for linezolid and vancomycin. Among invasive strains (n=31) resistance to erythromycin, tetracycline and clindamycin was 96.8%, 90.3% and 58.1% compared to 86.4%,45.4% and 50% among non-invasive strains (n = 22 isolates).

This prospective study demonstrated a total of 53 samples positive for pneumococci among 12,180 samples (0.4%) during one year study period. According to the study conducted by Jae Soo Kim *et al.,* (2021) pneumococcal-positive rate was 0.15%. Incidence noted in the study by Deutscher *et al.,* (2011) was lower, ranging 0.05 cases/1000 women. Among the 53 positive cases, a high occurrence of pneumococci is obtained from sterile samples (58.5%) which is different from other studies. From the study conducted by López-Lacort *et al.,* (2024) evaluated the fluctuations in the occurrence of IPD and NIPD across various regions. This is due to regional differences in registration of diseases, clinical approaches and disease treatment may influence epidemiological disparities also may be due to low sample volume used in study (López-Lacort *et al.,* 2024).

This study reveals a notable finding, where the majority of isolates (n = 28, accounting for 52.8%) were sourced from adult group within age 18-65yrs, suggesting that *Streptococcus pneumoniae* infections transcend pediatric boundaries, affecting a broader demographic spectrum. Adults being left out of immunization programs may be a factor in the escalating rate of infections among this population.

This is similar to previous mention of 48.6% contribution of adult in Namrata Kulkarni *et al.,* (2023) study and study conducted by Rosemol Varghese *et al.,* (2021) 78% were between the ages of 16–60yrs. Also among pediatric group children under the age of 5 were (64.2%) identified as the high-risk group. Which is also stated in Dikshita Mazumdar *et al.,* (2023) study.

In this study observed more than half of occurrence (66.1%) of pneumococcal infection in men of all age groups, which shows similarities between the study conducted by Chand Wattal *et al.,* (2017) from North India between February 2013 and January 2015, also showed a high incidence (82%) in male (Chand Wattal *et al.,* 2017). Another study by Dong-Chul Park *et al.,* (2019) also state higher incidence of PD (69.3%) were found in males (Park *et al.,* 2019). Nearly 56.6% of patients enrolled in the study are associated with known morbidity and most among them are chronic lung (26.4%) and diabetic patients (24.5%). This finding is in concordance with the findings of other similar studies from India (Wattal *et al.,* 2017 and Parvaiz A Koul *et al.,* 2019).

In the present study results a winter peak was noted in the diagnosis of most cases. There is evidences from studies like Dikshita Mazumdar *et al.,* (2023) study. The serotype distribution in our study was led by 19F (32.1%), 6B/D and 9V (9.4% each), 1 and 15B (5.7% each), 9N (3.8%) and 19A (3.8%). Serotype 19F emerged as the dominant serotype overall which is comparable to the findings of Swati Sharma *et al.,* (2022) in Chandigarh, North India. Their study showed a similar dominance of serotype 19F, which accounted 39%, the remaining isolates were primarily composed of serotypes 6A/B/C, 1 and 10A (Swati Sharma *et al.,* 2022). It shows adult vaccination is equally crucial, while childhood immunization is widely emphasized. Unfortunately, India faces a significant challenge in achieving adequate pneumococcal vaccination coverage. In the study of Rajalakshmi Arjun *et al.,* (2020) 39 isolates that underwent serotyping, 8 could not be assigned a specific serotype and classified as non-typeable. These findings mirror those of the presence of untyped serotypes (15.1%) in our study, highlights a limitation to the scope of serotype detection. Further characterization of these untyped isolates may uncover additional serotype diversity, enhancing our understanding of the complex serotype landscape. Our study suggesting a difference in the serotype distribution between these two clinical presentations (IPD and NIPD). Dominant serotypes causing IPD in our study are 19F, 6B/D, 1, 15B, 9N, 9V, 19A,14 and 3 which almost closely matching the study's of Balaji *et al.,* (2015) and Rajalakshmi Arjun *et al.,* (2020). While 19F, 9V, 4, 6A, 6B/D, 7F/A,18F/C, 23A and 11A/C/D are NIPD serotypes in our study, a similar dominance of serotype 11A, 23A, and 35B were observed in non-invasive isolates from a report from Korea by Dong-Chul Park *et al.,* (2019).

The vaccine coverage analysis in our study revealed that PCV10, PCV13, and PPSV23 offered protection against 82.2%, 84.4% and 95.5% of the circulating strains. These findings can be correlated to the study conducted by Swati Sharma *et al.,* (2022) revealed that PPSV23 provided the broadest protection, cover's 96%, while PCV13 offered substantial coverage of 77.5%, and PCV10 provided more moderate protection (59.1%).

Table.1 Incidence of pneumococci

	Total Specimens	*Streptococcus pneumoniae* Isolates	Percentage (%)
Invasive	8797	31	0.3
Non-invasive	3383	22	0.6
Total	12,180	53	0.4

Table.2 Incidence in various invasive and non-invasive specimens

	Streptococcus pneumoniae Isolates	Percentage (%)
Invasive	31	58.5
Non-invasive	22	41.5
Total	53	

Table.3 Assessment of incidence in various invasive and non-invasive specimens

Specimens	No of Positive Cases	Percentage (%)
Blood	21	39.6
CSF	10	18.8
Sputum	12	22.6
Ear swab	3	5.7
E.T aspirates	3	5.7
BAL	1	1.9
BR wash	3	5.7
Total	53	

Table.4 Age wise stratification of isolates

Age	Positive Cases				
	Invasive Specimens (31)	(%)	Non-Invasive Specimens (22)	(%)	TOTAL
≤ 2 Years	2	6.5	3	13.6	5
3 - 5 Years	4	12.9	0	0	4
6 - 17 Years	3	9.7	2	9.1	5
18 - 49 Years	8	25.8	4	18.2	12
50 - 65 Years	9	29.0	7	31.8	16
> 65 Years	5	16.1	6	27.3	11

Table.5 Gender wise stratification of isolates

Gender	No of Positive Cases	Percentage (%)
Male	35	66.1
Female	18	33.9

Table.6 Comparison of pneumococcal disease incidence in patients with and without comorbidities

Patients	No. of Patients	Percentage (%)
With Comorbidities (With two or more comorbidity)	30 (16)	56.6 (53.3)
Without Comorbidities	23	43.4

Table.7 Impact of various risk factors

Risk factors	No. of Patients	Percentage (%)
Chronic use of steroids	6	11.3
Diabetes	13	24.5
Cardiovascular disease	8	15.1
Smokers	2	3.8
Chronic liver disease	3	5.7
Cerebrovascular disease	4	7.5
Respiratory disease	14	26.4
Renal disease	1	1.9
Malignancy	4	7.5

Table.8 Serotype distribution of *Streptococcus pneumoniae*

Serotypes	No. of isolates
NT	8
19F	17
19A	2
6B/D	5
9V	5
1	3
15B	3
9N	2
11A/C/D	1
6A	1
7F/A	1
18F/C	1
14	1
23A	1
3	1
4	1

Table.9 Age wise stratification of serotypes

Serotypes	Pediatric Group (0-17 yrs) (14/53)		Adult Group (18-65 yrs) (28/53)		Old age Group (>65yrs) (11/53)	
	No. of Isolates	(%)	No. of Isolates	(%)	No. of Isolates	(%)
NT	2	14.2	4	14.2	2	18.2
19F	4	28.7	11	39.3	2	18.2
19A	0	0	1	3.6	1	9.1
6B/D	3	21.6	1	3.6	1	9.1
9V	0	0	3	10.7	2	18.2
1	1	7.1	0	0	2	18.2
15B	0	0	3	10.7	0	0
9N	0	0	2	7.1	0	0
11A/C/D	1	7.1	0	0	0	0
6A	0	0	1	3.6	0	0
7F/A	0	0	1	3.6	0	0
18F/C	0	0	0	0	1	9.1
14	1	7.1	0	0	0	0
23A	1	7.1	0	0	0	0
3	0	0	1	3.6	0	0
4	1	7.1	0	0	0	0

Table.10 Serotype distribution of *Streptococcus pneumoniae* isolates from different sources

Serotypes (total)	CSF	Blood	Sputum	Ear Swab	E.T Aspirates	BAL	BR wash
19F - (17)	1	8	3	2	0	0	3
NT- (8)	3	1	3	0	1	0	0
19A - (2)	1	1	0	0	0	0	0
6B/D - (5)	2	2	1	0	0	0	0
9V - (5)	0	2	3	0	0	0	0
1 - (3)	0	3	0	0	0	0	0
4 - (1)	0	0	0	1	0	0	0
15B - (3)	1	2	0	0	0	0	0
9N - (2)	1	1	0	0	0	0	0
11A/C/D (1)	0	0	0	0	1	0	0
6A - (1)	0	0	0	0	0	1	0
7F/A - (1)	0	0	1	0	0	0	0
18F/C - (1)	0	0	0	0	1	0	0
14 - (1)	1	0	0	0	0	0	0
23A - (1)	0	0	1	0	0	0	0
3 - (1)	0	1	0	0	0	0	0

Table.11 Vaccine coverage of isolates

Serotypes	Frequency in isolates	Pneumococcal vaccine		
		PCV10	PCV13	PPSV23
19F	17	Present	Present	Present
19A	2	Present	Present	Present
6B/D	5	Present	Present	Present
9V	5	Present	Present	Present
1	3	Present	Present	Present
15B	3	Absent	Absent	Present
9N	2	Absent	Absent	Present
11A/C/D	1	Absent	Absent	Present
6A	1	Present	Present	Absent
7F/A	1	Present	Present	Present
18F/C	1	Present	Present	Present
14	1	Present	Present	Present
23A	1	Absent	Absent	Absent
3	1	Absent	Present	Present
4	1	Present	Present	Present
TOTAL	45	37	38	43
Percentage Covarage		82.2%	84.4%	95.5%

Table.12 Antibiotic susceptibility patterns of the isolates

Antibiotics	S	(%)	R	(%)	I	(%)
Penicillin	41	77.3	10	18.8	2	3.8
Ceftriaxone	45	84.9	3	5.7	5	9.4
Cotrimoxazole	14	26.4	36	67.9	3	5.7
Erythromycin	4	7.5	49	92.4	0	0
Tetracycline	15	28.3	38	71.7	0	0
Clindamycin	24	45.3	29	54.7	0	0
Levofloxacin	49	92.4	2	3.8	2	3.8
Linezolid	53	100	0	0	0	0
Vancomycin	53	100	0	0	0	0

Graph.1 Incidence of pneumococci

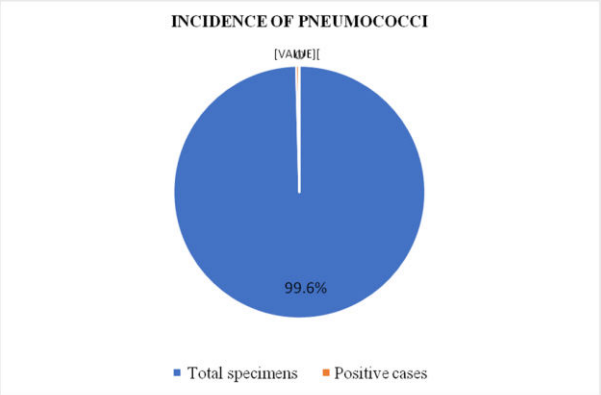


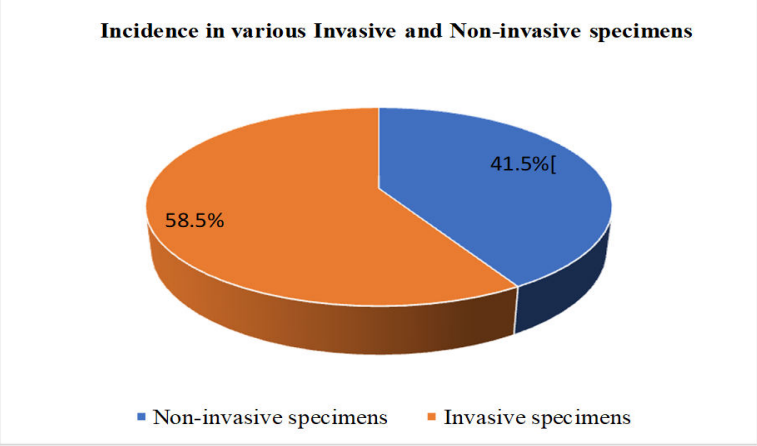
Table.13 Comparative analysis of antibiotic resistance patterns in invasive pneumococcal isolates

Antibiotics	Invasive Specimens (31)					
	Blood (21)			CSF (10)		
	S	R	I	S	R	I
Penicillin (P)	19	1	1	1	9	0
Ceftriaxone (CTR)	19	2	0	5	1	4
Cotrimoxazole (COT)	3	15	3	4	6	0
Erythromycin (E)	1	20	0	0	10	0
Tetracyclin (TE)	1	20	0	2	8	0
Clindamycin (CD)	7	14	0	6	4	0
Levofloxacin (LE)	19	1	1	10	0	0
Linezolid (LZ)	21	0	0	10	0	0
Vancomycin (VA)	21	0	0	10	0	0

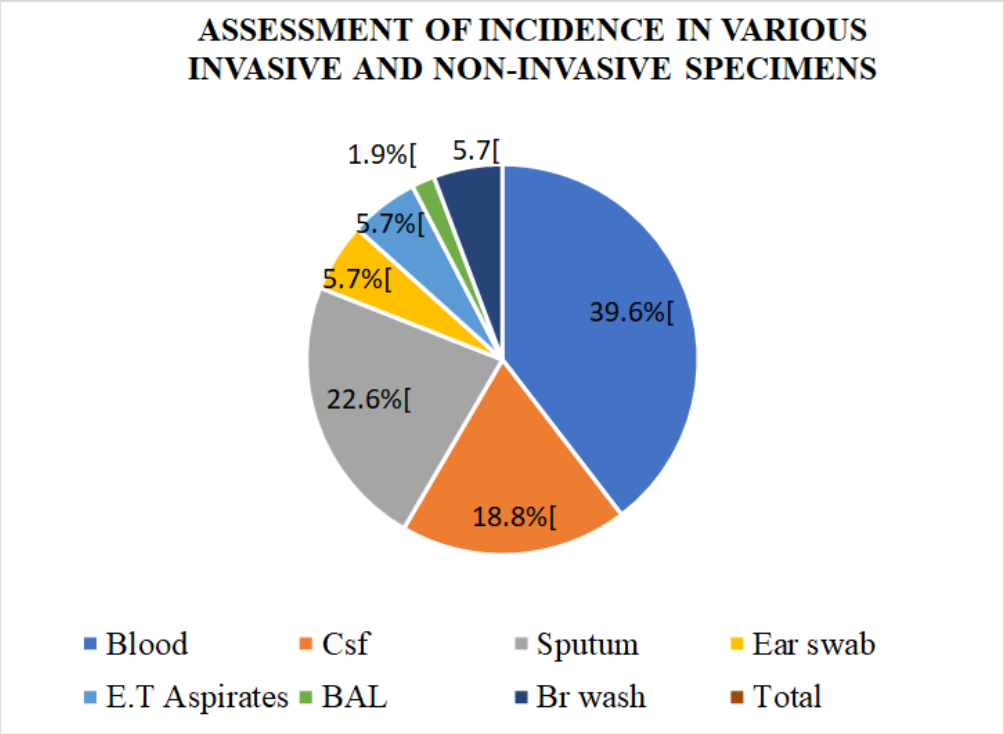
Table.14 Comparative analysis of antibiotic resistance patterns in non-invasive pneumococcal isolates

Antibiotics	Non-Invasive Specimens (22)														
	BAL (1)			Sputum (12)			E.T Aspirates (3)			BR Wash (3)			Ear Swab (3)		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
Penicillin (P)	1	0	0	12	0	0	3	0	0	2	0	1	3	0	0
Ceftriaxone (CTR)	1	0	0	12	0	0	3	0	0	2	0	1	3	0	0
Cotrimoxazole (COT)	0	1	0	5	7	0	1	2	0	0	3	0	1	2	0
Erythromycin (E)	0	1	0	2	10	0	1	2	0	0	3	0	0	3	0
Tetracycline (TE)	0	1	0	8	4	0	2	1	0	0	3	0	2	1	0
Clindamycin (CD)	1	0	0	5	7	0	3	0	0	1	2	0	1	2	0
Levofloxacin (LE)	1	0	0	12	0	0	2	1	0	3	0	0	2	0	1
Linezolid (LZ)	1	0	0	12	0	0	3	0	0	3	0	0	3	0	0
Vancomycin (VA)	1	0	0	12	0	0	3	0	0	3	0	0	3	0	0

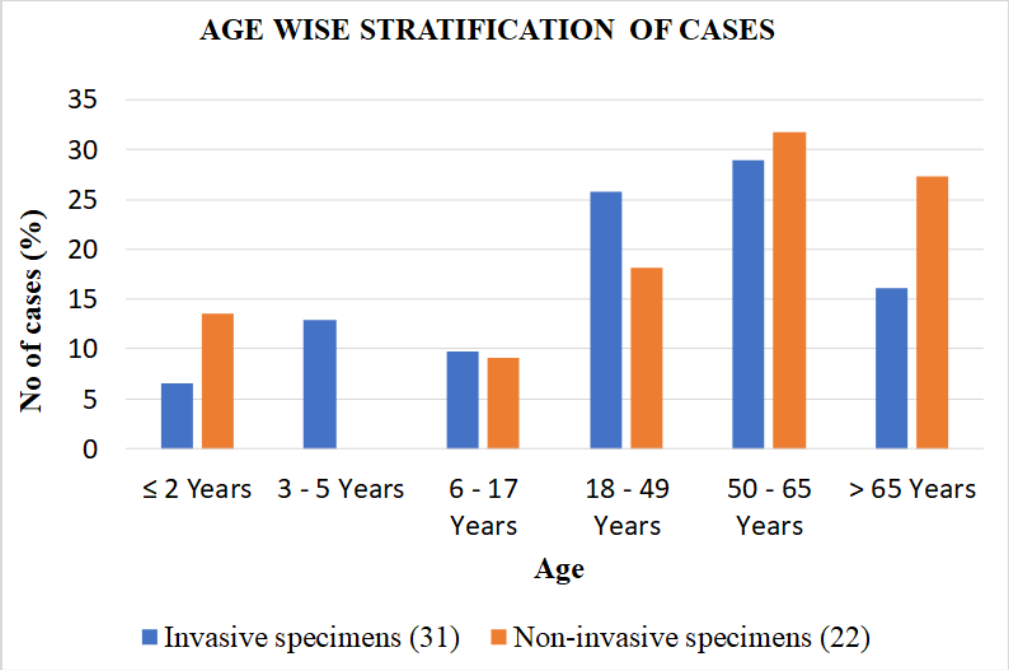
Graph.2 Incidence in various invasive and non-invasive specimens



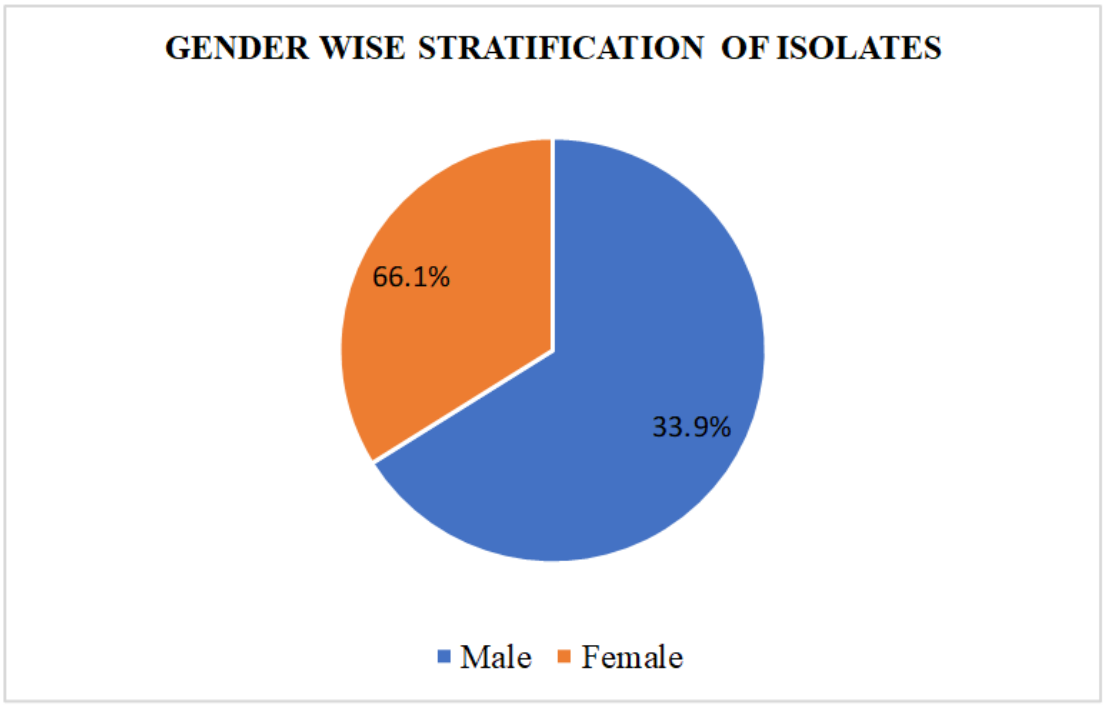
Graph.3 Assessment of incidence in various invasive and non-invasive specimens



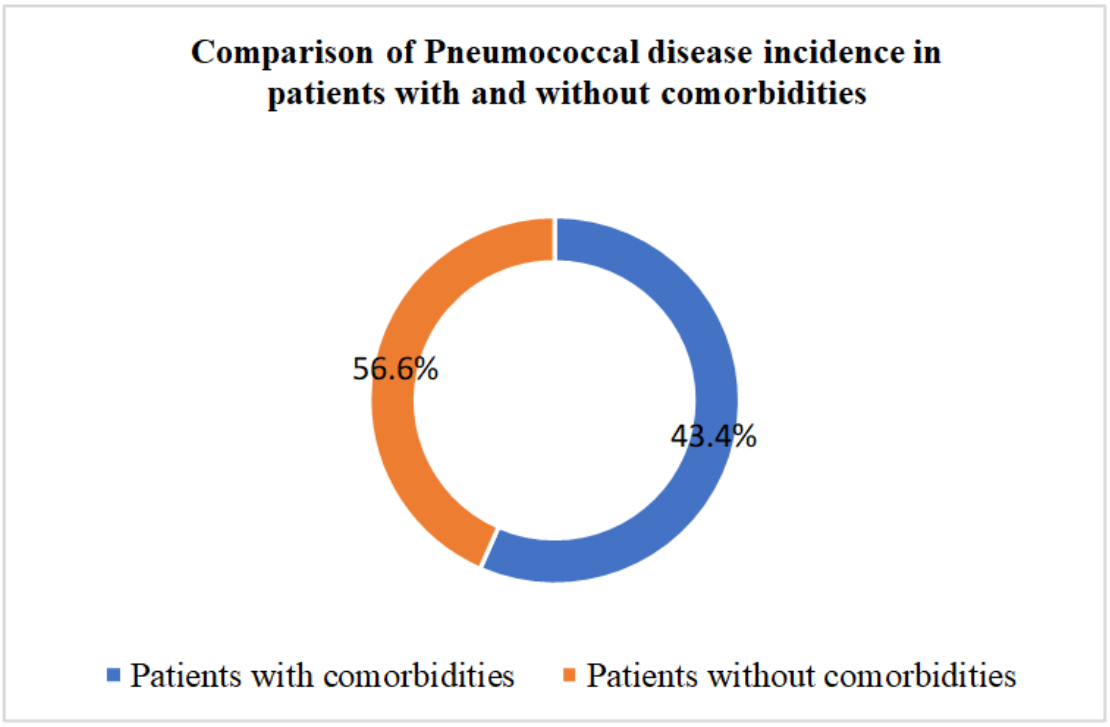
Graph.4 Age wise stratification of isolates



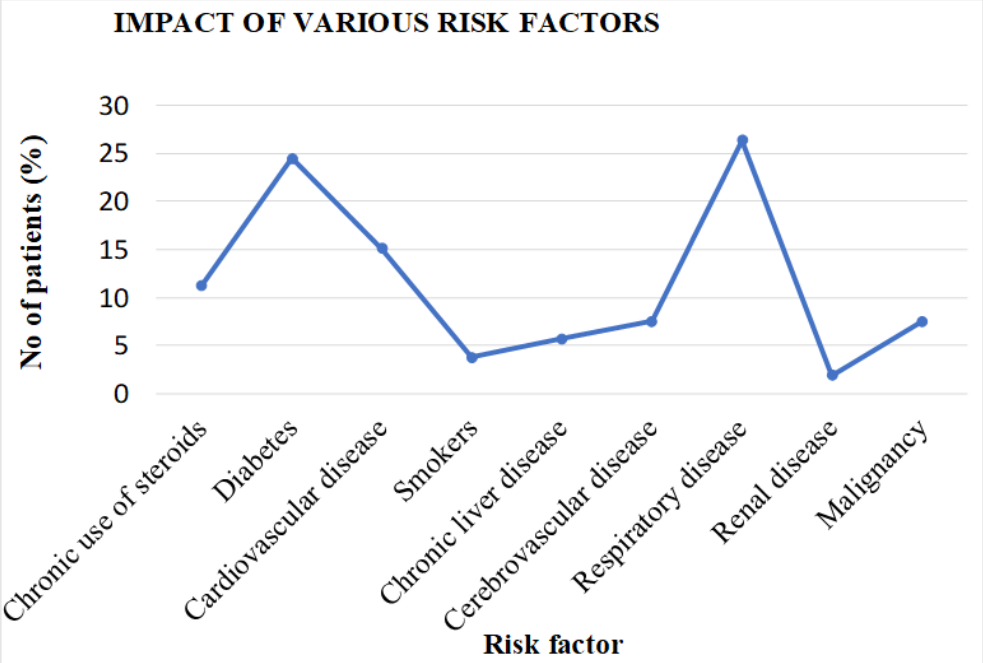
Graph.5 Gender wise stratification of isolates



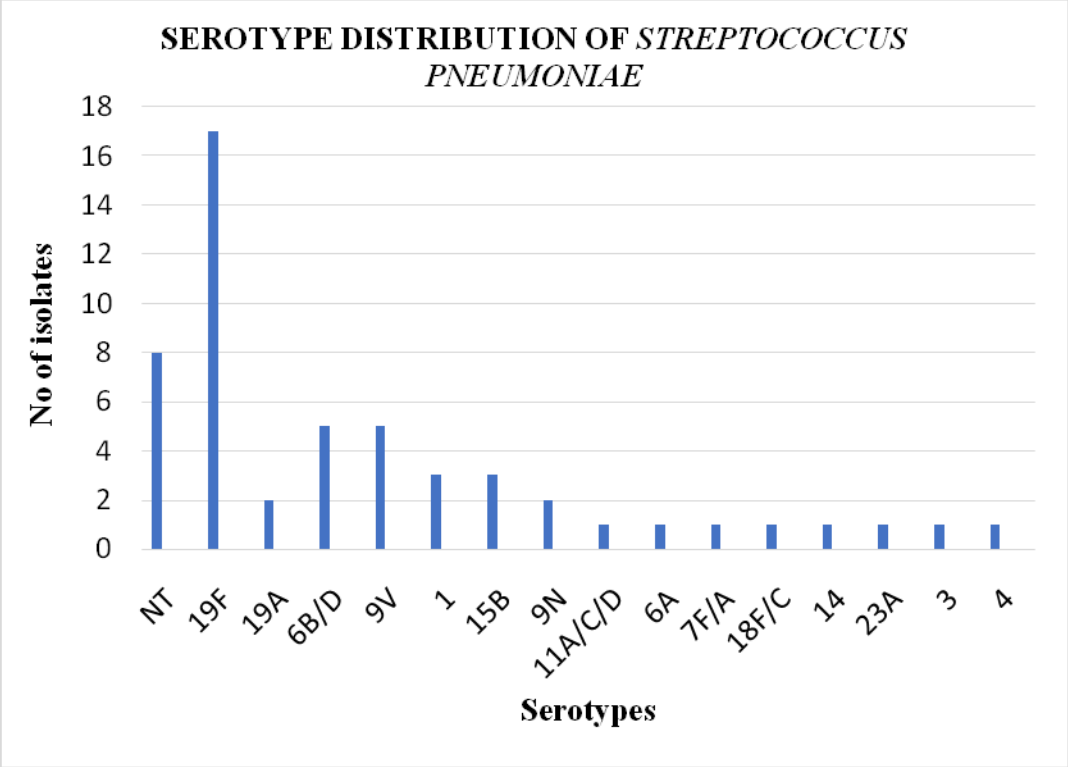
Graph.6 Comparison of pneumococcal disease incidence in patients with and without comorbidities



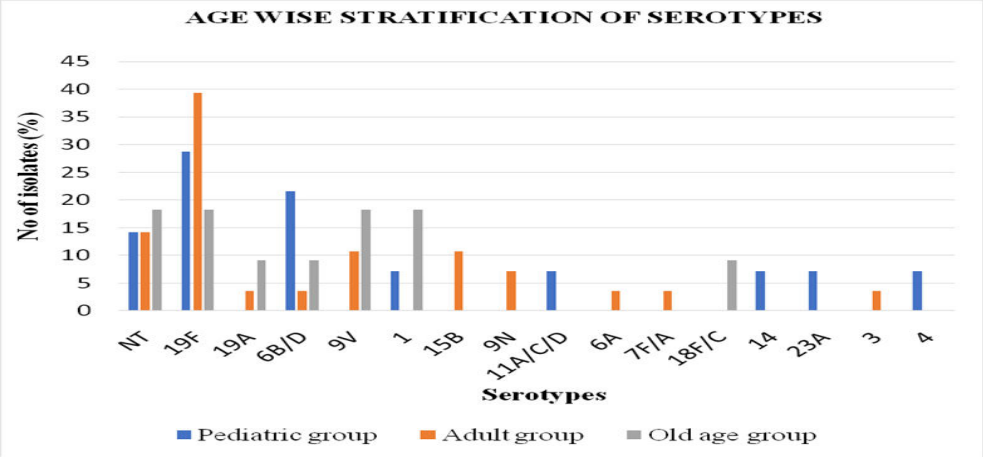
Graph.7 Impact of various risk factors



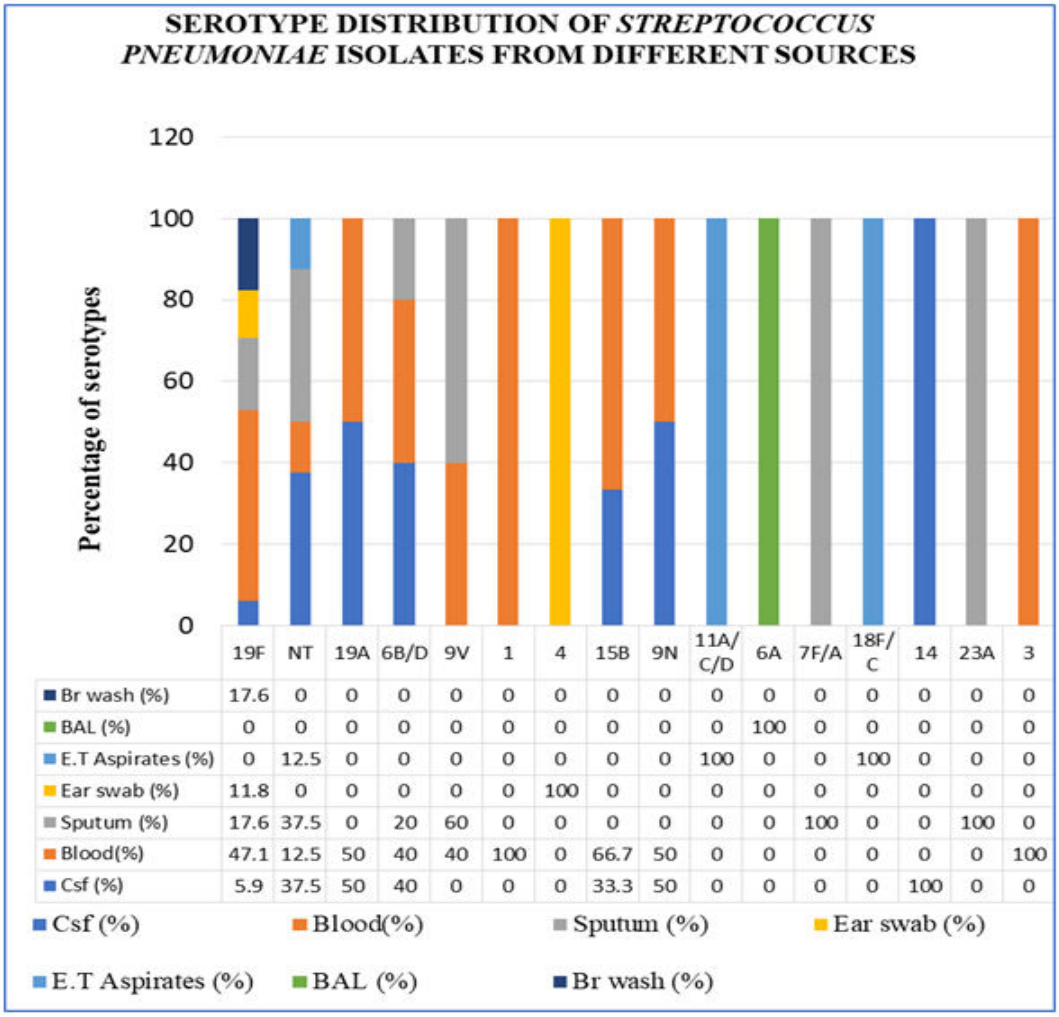
Graph.8 Serotype distribution of *Streptococcus pneumoniae*



Graph.9 Age wise stratification of serotypes

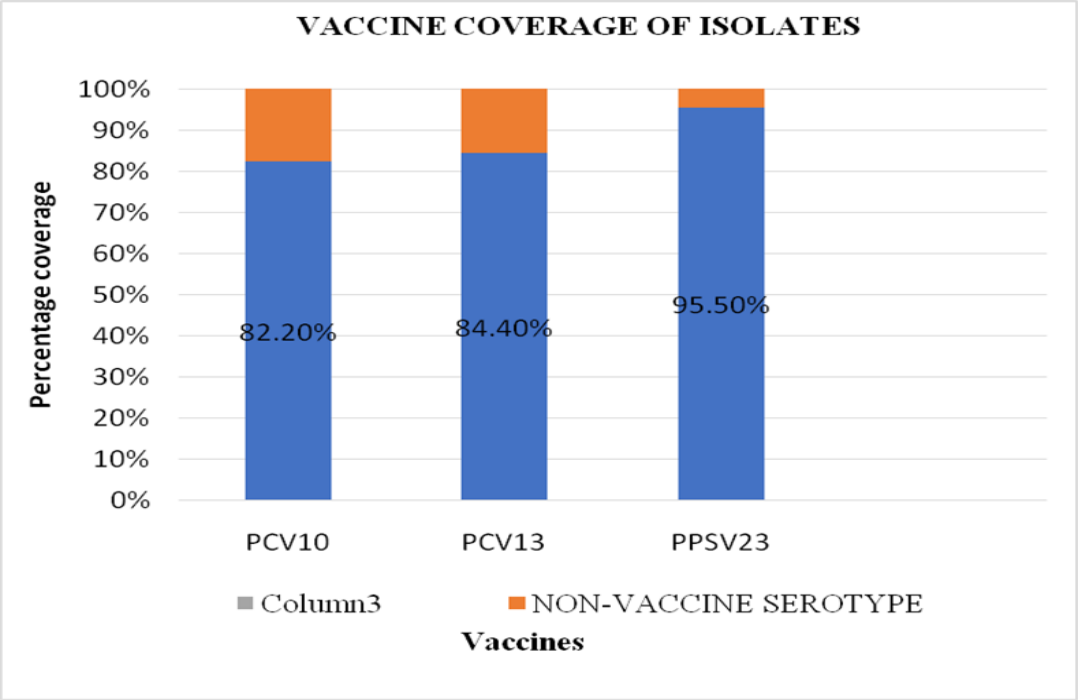


Graph.10 Serotype distribution of *Streptococcus pneumoniae* isolates from different sources

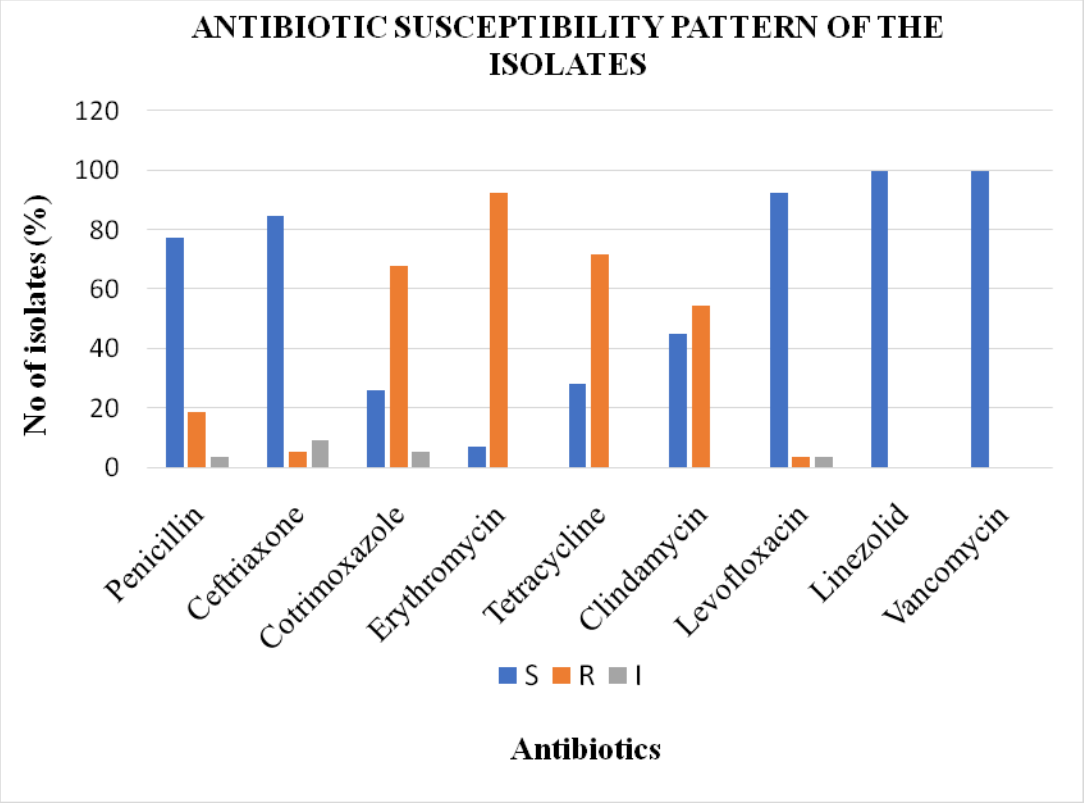


	19F	NT	19A	6B/D	9V	1	4	15B	9N	11A/C/D	6A	7F/A	18F/C	14	23A	3
Br wash (%)	17.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAL (%)	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0
E.T Aspirates (%)	0	12.5	0	0	0	0	0	0	0	100	0	0	100	0	0	0
Ear swab (%)	11.8	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0
Sputum (%)	17.6	37.5	0	20	60	0	0	0	0	0	0	100	0	0	100	0
Blood(%)	47.1	12.5	50	40	40	100	0	66.7	50	0	0	0	0	0	0	100
Csf (%)	5.9	37.5	50	40	0	0	0	33.3	50	0	0	0	0	100	0	0

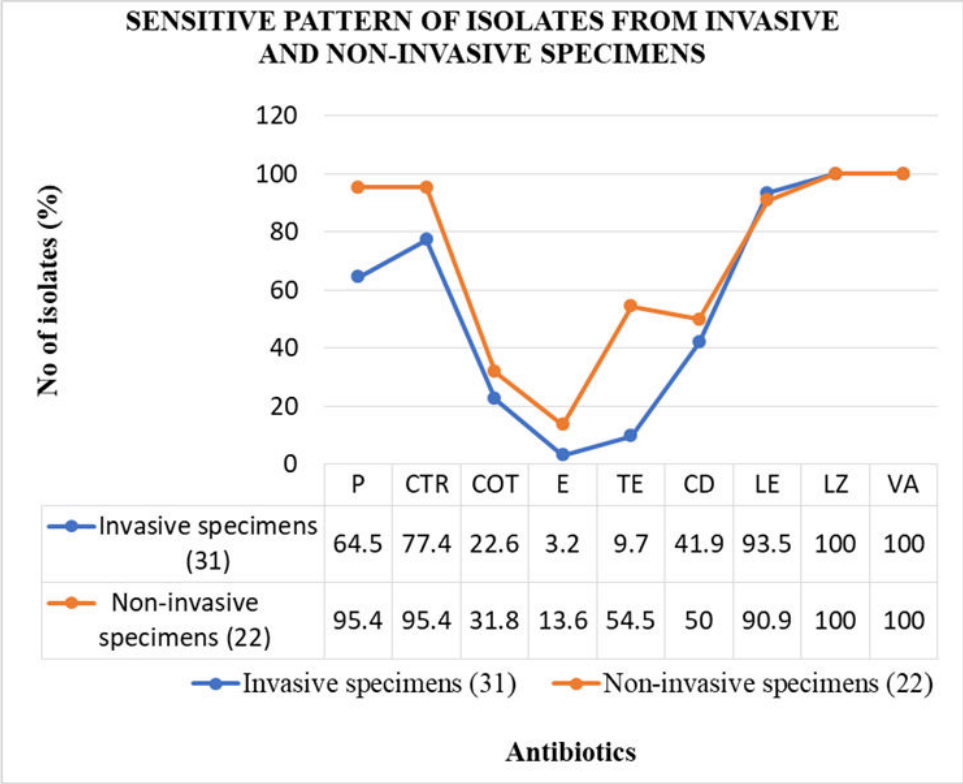
Graph.11 Vaccine coverage of isolates



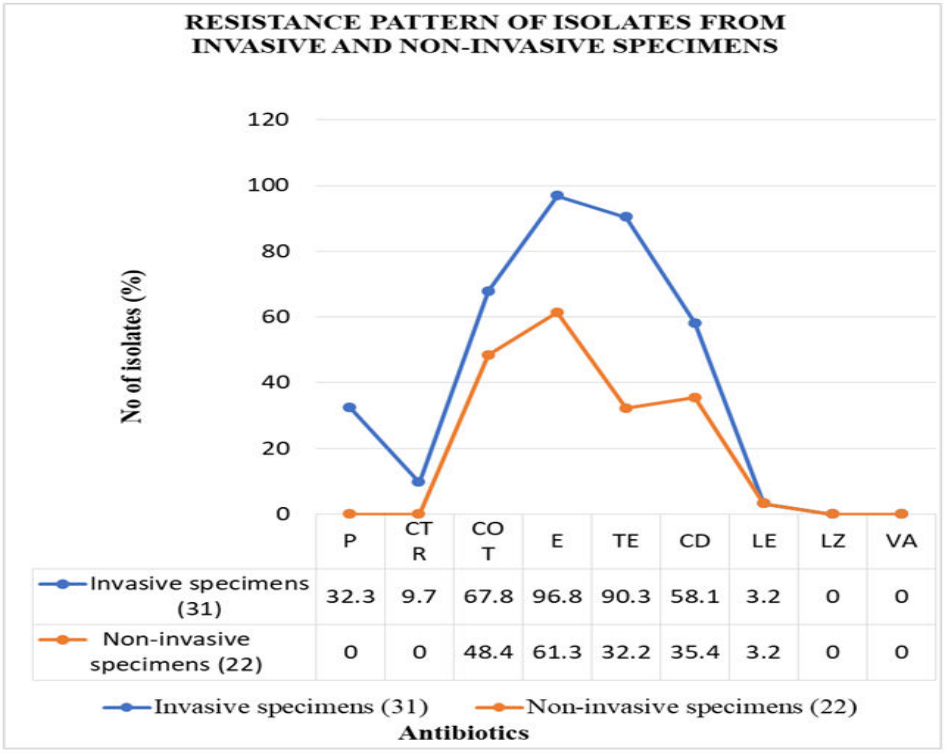
Graph.12 Antibiotic susceptibility patterns of the isolates



Graph.13 Sensitive pattern of isolates from invasive and non-invasive specimens



Graph.14 Resistance pattern of isolates from invasive and non-invasive specimens



This study shows 16.9% of isolates are non-vaccine serotype which indicates a declining pattern in the frequency of infections caused by vaccine serotypes, suggesting a successful vaccine-mediated reduction in disease burden and emerging of non-vaccine serotypes.

The data obtained in this hospital-based study demonstrate 12 isolates exhibited resistance to penicillin and notably, all of these isolates were associated with invasive infections, Furthermore, a significant proportion of meningeal IPD cases (90%). Which can be connected to study by Malik Sallam *et al.,* (2019) a notable, invasive infections displayed a significantly elevated level of resistance to penicillin and the prevalence of penicillin non-susceptibility in Jordan during the study period was found to be modest, at 10.3% (Malik Sallam *et al.,* 2019). According to Rajalakshmi Arjun *et al.,* (2020) study all isolates remained fully susceptible to vancomycin, levofloxacin, and linezolid, ensuring effective treatment choices. However, disturbingly high levels of resistance were detected for clindamycin (44%), erythromycin (74.5%), co-trimoxazole (87.3%), and tetracycline (74.5%). A more similar pattern is also observed in our study with 100% susceptibility to linezolid and vancomycin. While susceptibility of levofloxacin decreased to 92.4%. Similarly erythromycin resistance was predominant (92.4%) and increased slightly and resistance rates to tetracycline, cotrimoxazole and clindamycin with minor variation comparing the former 71.1%, 67.9% and 54.7%. In the study of Shuang Lyu *et al.,* (2024) also reported similar findings from china.

Our study revealed an high intermediate resistance of meningeal isolates to ceftriaxone, while highest intermediate resistance for oxacillin is reported by Swati Sharma *et al.,* (2022) in his study. Presence of intermediate resistance in our isolates serves as a warning sign, indicating a potential trajectory towards complete antibiotic resistance (Swati Sharma *et al.,* 2022).

Limitations of the study

Primarily this is a single-center study with small sample size and exclusively focused on culture-positive isolates, which might not capture the entire range of pneumococcal infections because asymptomatic carriers also play an important role in it.

The small sample size also precluded a more detailed analysis of serotype, strain, and antibiotic resistance differences among *S. pneumoniae* isolates. The reliance on the quellung reaction test alone restricts the identification of non-typeable isolates, as this technique may not be able to detect all serotypes.

This research aimed to study the incidence of *Streptococcus pneumoniae* in various invasive and non-invasive specimens, determine its antibiotic susceptibility patterns and identify the serotype distributed in them. By this study, it provides valuable perspectives on the real time information of dynamic nature of pneumococcal infections pooled at near locality, facilitating assessment of drug resistance and appraisal of prevalent serotypes. The study result will help alert clinicians on the trends and preventive measures. It has the potential to inform the development of improved management guideline and prioritization of policy.

During the specified timeframe, our hospital received 12,180 samples, from which 0.4% were identified as pneumococcal isolates. These isolates were predominantly associated with invasive pneumococcal diseases, primarily affecting adults.

In this study serotyping analysis revealed a predominance of serotype 19F, followed by 6A/B/C, 9V, 15B, and 1. We observed alarming resistance rates, particularly to erythromycin, as well as significant resistance to tetracycline, cotrimoxazole, and clindamycin. Our findings suggest that pneumococcal serotypes linked to vaccine coverage continue to exhibit robust antibiotic resistance.

To obtain a more precise understanding of pneumococcal epidemiology, there is a pressing need for large-scale, multi-center studies that encompass a diverse and representative sample size across the country, enabling the capture of nuanced trends and patterns.

Comprehensive pneumococcal surveillance initiatives are crucial for evaluating the pneumococcal disease burden and monitoring the impact of vaccination efforts. Moreover, these programs serve as an early warning system for healthcare authorities and the scientific community, providing valuable insights into shifts in serotype distribution.

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Author Contributions

Adila K: Conceived the original idea and designed the model and Analysed, Investigated and wrote the manuscript. Reshmi Gopalakrishnan: Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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