

Antimicrobial Resistance in isolates of *Streptococcus pneumoniae* during January 2016 to December 2017 in Dr. Lal Path Labs, Delhi.

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Abstract

Aims and objectives: The aim and objectives of this study is to assess drug resistance pattern of *Streptococcus pneumoniae* in Dr Lal Path Labs, Delhi. We did retrospective study from January 2016 to December 2017 on 86 isolates of *Streptococcus pneumoniae*.

Materials and Methods: At study sites, total 86 isolates from pulmonary and extra pulmonary grown on Columbia 5% sheep Blood agar (BioMerieux) plates after incubation for 24 to 48 hours at 36± 1 °C in 5% CO₂ incubator. Identified by MALDI TOF-MS (Bruker Daltonics) & Antibiotic susceptibility testing were also done using VITEK® 2 GP card. / *S. pneumoniae* susceptibility card (AST ST01 Card –BioMerieux, India) as per as CLSI M100-S-28.

Results: A total 86 isolates from pulmonary (21%) and extra pulmonary (79%) specimens were analysed for their antibiotic resistance pattern. 30% were from samples collected from children below between 0-10 years and 17.4% were collected from adults above 60 years of age.

The most prevalent source was blood (n =39; 45.34%), and then Sputum (n = 18; 20.93%), CSF (n=11; 12.79%), Pus (n=8; 9.3%) throat (n=4; 4.76%), ear (n=3; 3.5%), nasal (n=2; 2.3%) and eye(n=1; 1.19%).

Evaluating the antimicrobial susceptibility with 12 antibiotics we found strains were most susceptible to Chloramphenicol (98.8%), Linezolid (93%) and Vancomycin (88.37%). However most resistance were seen in Erythromycin (62.8%), Tetracycline (59.3%), Co-trimoxazole (62.8%) and Penicillin resistance were (22.1 %).

Discussion and conclusion:

In our study we found the infection is most common in extremes of age i e: 30% (0-10 y) & 17.4% (60-80y). Alarming thing found is the emergence of resistance in Vancomycin (11.62%) and Linezolid (6.97%) in India which is also reported by other studies.

We found Chloramphenicol and Linezolid are most susceptible drugs against *Streptococcus pneumoniae* whereas maximum resistance was observed for Erythromycin (62.8%), Tetracycline (59.3%), Co-trimoxazole (62.8%) and Penicillin resistance is very much present in pulmonary isolates in comparison to extrapulmonary isolates.

Keywords: *Streptococcus pneumoniae*, Vancomycin, Linezolid, pulmonary, extra pulmonary.

Introduction:

The purpose of this study is to observe the recent prevalence and to assess drug resistance pattern of *Streptococcus pneumoniae* among clinically diagnosed cases of pulmonary and extra-pulmonary infections in *Dr Lal Path Labs*, NRL, Delhi. We did retrospective study from January 2016 to December 2017 on 86 isolates of *Streptococcus pneumoniae*. The *Streptococcus pneumoniae* human respiratory bacterial pathogen is a gram positive, catalase negative facultative anaerobic organism that grows as lancet shaped diplococci and in short chains. On blood agar colonies are α haemolytic. It causes diseases in all age groups although this infection is documented to be extremely common in younger children and in older adults and is major cause of morbidity and mortality in the tropics.¹

Prior to 1995 all strains of *Streptococcus pneumoniae* isolated India were uniformly susceptible to Penicillin. However, since late 1995 strains of *Streptococcus pneumoniae* with resistance to Penicillin have been observed in world.^{5,13,15} while India has a low incidence of penicillin resistant.

Our retrospective study demonstrates the burden of resistance of antibiotics higher in pulmonary than extra pulmonary infections and we study to described the pattern of antibiotic resistance over two years with special emphasis to Vancomycin, Linezolid, Erythromycin and Cephalosporins and review existing treatment guide lines for *Streptococcus pneumoniae* isolates in India.

Materials and Methods: This study was conducted at the *Dr. Lal Path labs* situated in the Delhi in India among clinically diagnosed cases of pulmonary and extra pulmonary infections in *Dr Lal Path Labs*, NRL, Delhi, India, a total 86 cases comprising of pulmonary and extra pulmonary infections during 2 years. Most importantly 58 cases were from invasive sites (11 from CSF, 8 from Pus as well as 39 from blood), and 28 cases were from non-invasive sites (4 from throat, 18 from sputum, 3 from ear, 2 from nasal and 1 from eye).

Samples were inoculated on columbia 5% sheep blood agar (BioMerieux) plates after incubation for 24 to 48 hours at $36 \pm 1^\circ\text{C}$ in 5% CO₂ incubator. Identified by MALDI TOF-MS (Bruker Daltonics) & Antibiotic susceptibility testing were also done using VITEK® 2 GP card. / *S. pneumoniae* susceptibility card (AST ST01 Card –BioMerieux, India) as per as CLSI M100-S-28.

Evaluating the antimicrobial susceptibility with 12 antibiotics VITEK® 2 for *Streptococcus pneumoniae* using susceptibility card (AST ST01, BioMerieux),

Results: Bacterial resistance to antibiotics is an increasing problem in many parts of the world and in India. To assess drug resistance pattern of *Streptococcus pneumoniae* among clinically diagnosed cases of pulmonary and extra pulmonary infections in *Dr Lal Path Labs*, NRL, Delhi, India.

A total 86 isolates from pulmonary (21%) and extra pulmonary (79%) specimens during 2 years were analysed for their respective antibiotic resistance pattern. *Streptococcus pneumoniae* isolates were found in 73% males and 27% females patients. A total of 86 pneumococci isolates were investigated in this study of which 30% (26) of the isolates were from children aged between 0-10 years, 6.9% (6) in aged 11-20 years and 17.4% above 60 years of age (**figure.1**).

The most prevalent source was blood ($n=39$; 45.34%), followed by Sputum ($n=18$; 20.93%), CSF ($n=11$; 12.79%), Pus ($n=8$; 9.3%) throat ($n=4$; 4.76%), ear ($n=3$; 3.5%), nasal ($n=2$; 2.3%) and eye ($n=1$; 1.19%).

Percentage of age wise distribution of *Streptococcus pneumoniae* isolates in pulmonary and extra pulmonary specimen.

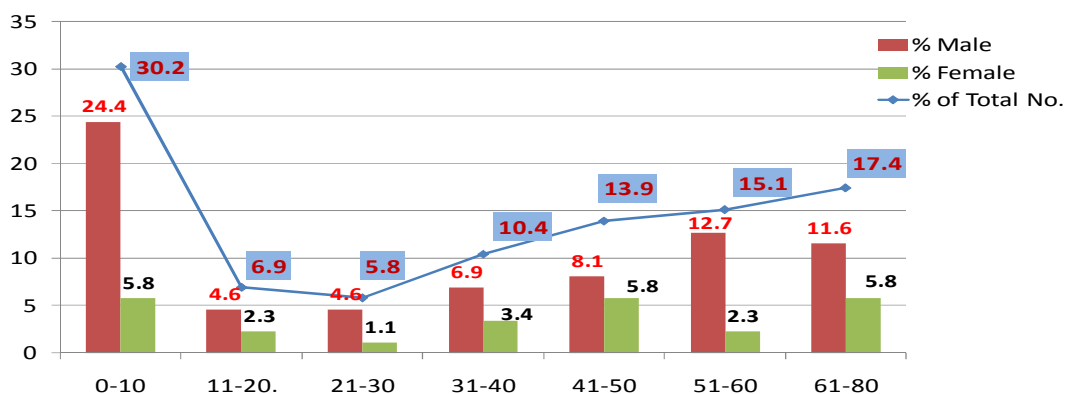


Figure 1: Percentage of age wise distribution of *Streptococcus pneumoniae* isolates in pulmonary and extra pulmonary specimen

Of the 86 isolates causing pulmonary and extra pulmonary in our study evaluating the antimicrobial susceptibility with 12 antibiotics VITEK[®] 2 for *Streptococcus pneumoniae* using susceptibility card (AST ST01, BioMerieux), we found strains were most susceptible to Chloramphenicol (98.8%), Linezolid (93%) and Vancomycin (88.37%) (**Table 1; Figure 2**).

Amongst the 19 isolates non susceptible to Penicillin, 7 isolates had intermediate susceptible where as 12 isolates were fully resistant to Penicillin (**Table 1; Figure 2**). Resistant to Erythromycin and Co-trimoxazole was found in 62.8% (54) where Erythromycin showed fully resistant among 54 isolates of Co-trimoxazole 14 had intermediate susceptibility whereas 40 were fully resistant.

Of the 86 isolates causing pulmonary and extra pulmonary infection in our study all Penicillin resistant 19 pneumococci isolates were resistant to Erythromycin, Tetracycline, Co-trimoxazole.

Amongst the 18 pulmonary isolates highly resistant to Penicillin (50%), Clindamycin (66.6%), Co-trimoxazole (72.2%), Levofloxacin (77.7%), Tetracycline and Erythromycin (83.3%) respectively (**Table 2; Figure 3**). Extra pulmonary isolates also showed high resistance in Co-trimoxazole (60.3%), Erythromycin (57.3%), and Tetracycline 52.9% (**Table 2; Figure 3**). Cephalosporins showed high resistance in pulmonary isolates in comparison to extra pulmonary isolates.

Evaluating the antibiotic susceptibility with 12 antibiotics we noted all pulmonary isolates were 100% sensitive to Chloramphenicol and in extra pulmonary 98.5% isolates were sensitive (**Table 3; Figure 4**).

Table1. Antibiotic resistance against 12 drugs of *Streptococcus pneumoniae* among 86 isolates from pulmonary and extra pulmonary specimen in *Dr Lal Path Labs*, Delhi from 1 January 2016 to 31 December 2017.

***Streptococcus pneumoniae* (n=86)**

Antibiotics	Resistant No. (%)	Intermediate No. (%)	Sensitive No. (%)
Penicillin	12 (13.95)	7 (8.1)	67 (77.9)
Clindamycin	30 (34.8)	3 (3.4)	53 (61.6)
Ceftriaxone	20 (23.3)	3 (3.4)	63 (73.3)
Cefotaxime	22 (25.6)	3 (3.4)	61 (70.9)
Chloramphenicol	1 (1.2)	0 (0)	85 (98.8)
Co-trimoxazole	40 (46.5)	14 (16.2)	32 (37.3)
Erythromycin	54 (62.7)	0 (0)	32 (37.3)
Levofloxacin	32 (37.2)	4 (4.6)	50 (58.1)
Linezolid	6 (6.97)	0 (0)	80 (93.03)
Tetracycline	51 (59.3)	0 (0)	35 (40.7)
Moxifloxacin	21 (24.4)	2 (2.3)	63 (73.3)
Vancomycin	10 (11.6)	0 (0)	76 (88.4)

Percentage of antibiotic sensitive and resistant *S. pneumoniae* isolates from pulmonary and extrapulmonary specimens.

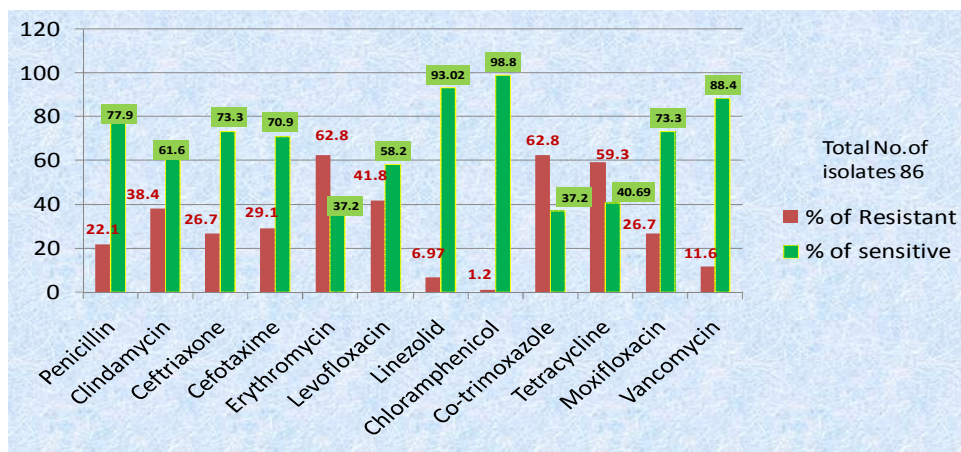


Figure 2: Percentage of antibiotic sensitive and resistant *S. pneumoniae* isolates from pulmonary and extrapulmonary specimens.

Table 2. Comparison of antibiotic resistance against 12 drugs of *Streptococcus pneumoniae* among 18 isolates from pulmonary and 68 isolates from extra pulmonary specimen in *Dr.Lal Path Labs*, Delhi from 1 January 2016 to 31 December 2017.

***Streptococcus pneumoniae* isolates from pulmonary specimen (n=18) and extra pulmonary specimen (n=68)**

Antibiotics	Pulmonary Resistant No.(%)	Intermediate No. (%)	Extra pulmonary Resistant No.(%)	Intermediate No. (%)
Penicillin	6 (33.3)	3 (16.6)	6 (8.8)	4 (5.8)
Clindamycin	12 (66.6)	0(0)	18 (26.5)	3 (4.4)
Ceftriaxone	7 (38.8)	1(5.5)	13 (19.1)	2 (2.9)
Cefotaxime	8 (44.4)	0(0)	14 (20.6)	3 (4.4)
Chloramphenicol	0 (0)	0(0)	1 (1.4)	0 (0)
Co-trimoxazole	10 (55.5)	3(16.6)	30 (44.1)	11 (16.1)
Erythromycin	15 (83.3)	0(0)	39 (57.3)	0 (0)
Levofloxacin	14 (77.7)	0(0)	18 (26.5)	4 (5.8)
Linezolid	2 (11.1)	0(0)	4 (5.8)	0 (0)
Tetracycline	15 (83.3)	0(0)	36 (52.9)	0 (0)
Moxifloxacin	8 (44.4)	0(0)	13(19.11)	2 (2.9)
Vancomycin	4 (22.2)	0(0)	6 (8.8)	0 (0)

Comparison of drug resistant in percentage for *Streptococcus pneumoniae* isolates from 18 pulmonary Vs 68 extrapulmonary specimens .

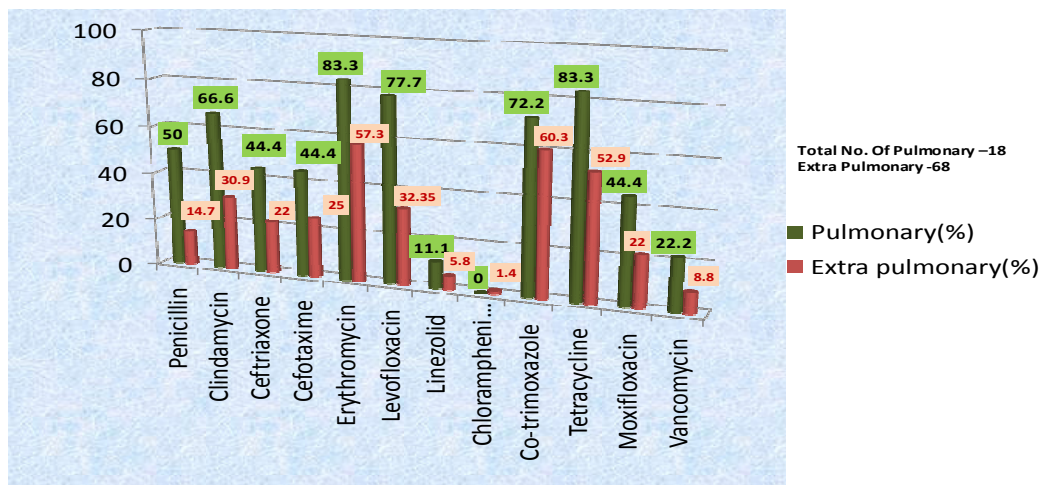


Figure 3: Comparison of drug resistant in percentage for *Streptococcus pneumoniae* isolates from 18 pulmonary Vs 68 extrapulmonary specimens.

Table3. Comparison of antibiotic sensitive against 12 drugs of *Streptococcus pneumoniae* among 18 isolates from pulmonary and 68 isolates from extra pulmonary specimen in *Dr.Lal Path Labs*, Delhi from 1 January 2016 to 31 December 2017.

Streptococcus pneumoniae isolates from pulmonary specimen (n=18) and extra pulmonary specimen (n=68)

Antibiotics	Pulmonary Sensitive No. (%)	Extra pulmonary Sensitive No. (%)
Penicillin	9 (50)	58 (85.2)
Clindamycin	6 (33.3)	47 (69.1)
Ceftriaxone	10 (55.5)	53 (77.9)
Cefotaxime	10 (55.5)	51 (75)
Chloramphenicol	18 (100)	67 (98.5)
Co-trimoxazole	5 (27.7)	27 (39.7)
Erythromycin	3 (16.6)	29 (42.6)
Levofloxacin	4 (22.2)	46 (67.6)
Linezolid	16 (88.8)	64 (94.2)
Tetracycline	3 (16.6)	32 (47)
Moxifloxacin	10 (55.5)	53 (77.9)
Vancomycin	14 (77.8)	62 (91.2)

Comparison of drugs sensitive in percentage for *S.pneumoniae* isolates from 18 pulmonary and 68 extra pulmonary specimens

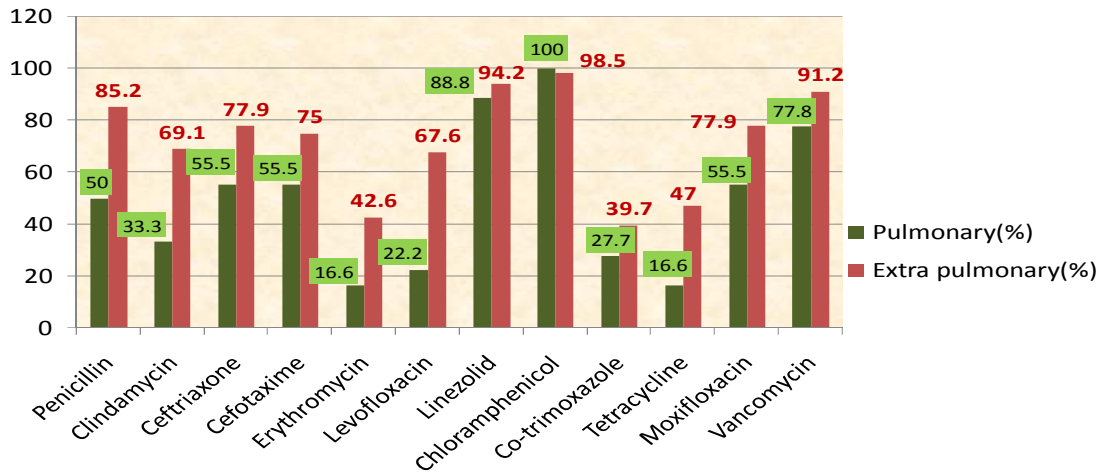


Figure 4: Comparison of drugs sensitive in percentage for *S.pneumoniae* isolates from 18 pulmonary and 68 extra pulmonary specimens.

Discussion: Antibiotic resistance among *Streptococcus pneumoniae* continues to evolve and this threatens affordable management. Fundamental to these discoveries is our ability to recognize that pulmonary isolates were higher multidrug resistant in comparison to extrapulmonary isolates.

In this study we found the infection is most common in extremes of age i.e.: 30% (0-10 y) & 17.4% (60-80y) which correlates well with other studies.^{4, 8, 12, 18}

Penicillin generally was the antibiotic of choice but rapid development and spread of Penicillin resistance observed for *Streptococcus pneumoniae* in Delhi (22.1%). In pulmonary isolates (50%) were Penicillin resistant whereas extrapulmonary isolates showed resistance of penicillin were (14.7%) similar resistance were noted from an earlier report^{18, 8} from South India. Interestingly isolates of (30%) below 10 years of age showed no Penicillin resistance. Whereas worldwide with some countries in the Asian continent mentions resistance of Penicillin up to 70%.^{1, 5, 15}

Around 85 to 90% of Antibiotics consumption occurs in the treating respiratory tract infection. Present study demonstrated that the rates of Erythromycin (62.8%) and Cotrimoxazole (62.8%) resistance among *Streptococcus pneumoniae* in Delhi, India remained much higher when compared to the data from United States Erythromycin (37%) and Co-trimoxazole (33%)⁵ and agreement with Taiwan where Erythromycin (92%) and Co-trimoxazole (70%) had shown resistance respectively.¹⁵

Our findings shows that the presence of resistance of *Streptococcus pneumoniae* to Levofloxacin, the active isomer of Ofloxacin has excellent in vitro activity against Penicillin resistant pneumococci.³ This study presents high level of resistance towards Levofloxacin (77.7%) in pulmonary infection in comparison to extra pulmonary (32.4%). More over the percentage of Levofloxacin are increased in India however the data elsewhere shown resistance which is not in agreement with our findings.¹⁵

Several reports of treatment related to *Streptococcus pneumoniae* isolates for Ceftriaxone or Cefotaxime was synergistic and superior for treatment of children.^(11,18,12) The arrival of all isolates of resistant *Streptococcus pneumoniae* to important antibiotics Ceftriaxone (26.7%) and Cefotaxime (29.04%), in pulmonary sites. These drug resistant *Streptococcus pneumoniae* boost the risk of inappropriate therapy for pulmonary sites.

This study screened the antibiotic resistant profile of Macrolide, Erythromycin (83.3%) in pulmonary infection and extra pulmonary (57.3%) is becoming increasingly severe and problem of global concern that has made treatment of disease more difficult.^{14,16,17,}

Vancomycin is the antibiotic of last resort, its resistance represents a new health risk we found that the Vancomycin (11.6%), in pulmonary and extra pulmonary infection were a similar observation to that from earlier report of Asia and all over world.^{10,13,15} so the powerful approach needed to managing these infections to best treat all *Streptococcus pneumoniae* infections due to resistant strains.

The oxazolidones, represented by Linezolid are new class of antibiotic with unique structure and good activity against gram positive according to the available reports,⁶ although there are sporadic reports of resistant isolates, Linezolid remains a very effective drug in India. The (88.8%) of sensitive of Linezolid were found in pulmonary sites and (94.2%) sensitive in extra pulmonary sites. On the other hand all these multidrug resistant isolates were susceptible to Linezolid.

Chloramphenicol sensitivity of 98.8% was noted in this study. This may be due to the fact of rare use of this antibiotic in Indian subcontinent.

Globally developed guidelines have been describing the management of most appropriate antibiotic therapy. Although differences are found in the recommendations from different regions.² Different studies have reported varied rates of resistance to commonly used antibiotics. Our study provides data for a continuous surveillance of *Streptococcus pneumoniae* isolates causing pulmonary and extra pulmonary infection and antibiotic resistance patterns in order to evaluate their possible useful development in India.

Thus management of drug resistance *Streptococcus pneumoniae* continues to change choice of therapy and increased needed multidisciplinary approach involving clinicians, pharmacists and microbiologists.^{7,16,17}

Conclusion: *Streptococcus pneumoniae* resistance across pulmonary and extrapulmonary infection is a reality. Erythromycin and Cotrimoxazole are most affected and should be avoided in treatment of *Streptococcus pneumoniae* infection. Vancomycin, Linezolid resistance is has emerging which is worrisome and once again emphasis on judiciously and selective use of antimicrobials to arrest further resistant of antibiotics.

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Ethical Approval: It is not applicable

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