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

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SM and PS designed the study, wrote the protocol and wrote the first draft of the manuscript managed the analyses of the study. Author PS performed the statistical analysis and managed the literature searches. Author VL managed the analyses of the study and infrastructure support. All authors read and approved the final manuscript.

Short Research Article

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 $36 \pm 1°C$ in 5% CO_2 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

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below between 0-10 years and 17.4% were collected from adults above 60 years of age.
The most prevalent source was blood (45.34%), followed by Sputum (20.93%), CSF (12.79%), Pus (n= 9.3%) throat (4.76%), ear (3.5%), nasal (2.3%) and eye (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 comparision to extrapulmonary isolates.

1. 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. The *Streptococcus pneumoniae* human respiratory bacterial pathogen is a gram positive, catalase negative facultative anaerobic member of genus Streptococcus that grows as lancet shaped diplococci and in short chains. The isolates in our study were α haemolytic in nature no beta haemolytic *Streptococcus pneumoniae* was isolated. 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 from India were uniformly susceptible to Penicillin. However, since late 1995 strains of *Streptococcus pneumoniae* with resistance to Penicillin have been observed in world. Many researchers [5,9,13,15] 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 our 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.

2. MATERIALS AND METHODS

We did retrospective study from January 2016 to December 2017 on 86 isolates of *Streptococcus pneumoniae* was conducted at the *Dr. Lal Path labs,* Delhi in India among clinically diagnosed cases 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 5% sheep blood agar plates (BioMerieux, India) and incubated at 36± 1°C for 24 to 48 hours in 5% CO_2 incubator.

Streptococcus pneumoniae isolates were analysed and identified using standardized laboratory procedures including colony morphology on blood agar, the presence of alpha hemolysis, the optochin sensitivity test (Hi-media, Mumbai) and all the isolates were identified on MALDI TOF-MS (Bruker Daltonics) with a probability score of > 2.0.

The antimicrobial susceptibility was evaluated by VITEK® 2 using susceptibility card (AST ST01, BioMerieux, India) as per as CLSI M100-S-28 which included 12 antibiotics named Penicillin, Clindamycin, Ceftriaxone, Cefotaxime Chloramphenicol, Co-trimoxazole, Erythromycin, Levofloxacin, Linezolid, Tetracycline, Moxifloxacin, Vancomycin.

3. RESULTS

Bacterial resistance to antibiotics is an increasing problem in many parts of the world including India.

Among 86 isolates from pulmonary (21%) and extra pulmonary (79%) specimens were analysed for their respective antibiotic resistance

pattern. In our study male predominance was seen with 73% isolates while in female it was isolated in 27% cases. Children of age group 0-10 years had the largest number of isolation while least affected age group was 21-30 years (Fig. 1).

The most prevalent source of *Streptococcus pneumoniae* was largely isolated from 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%).

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 revealed following results of the 86 isolates 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, Fig. 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; Fig. 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 resistance whereas 40 were fully resistant.

In our study more alarming was the finding that about 22.1% Penicillin resistant *Streptococcus pneumoniae* isolates showed multiple 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). Extra pulmonary isolates also showed high resistance in Co-trimoxazole (60.3%), Erythromycin (57.3%), and Tetracycline 52.9% (Table 2). Cephalosporins showed high resistance in pulmonary isolates in comparision 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 (Fig. 3).

4. 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 comparision 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].
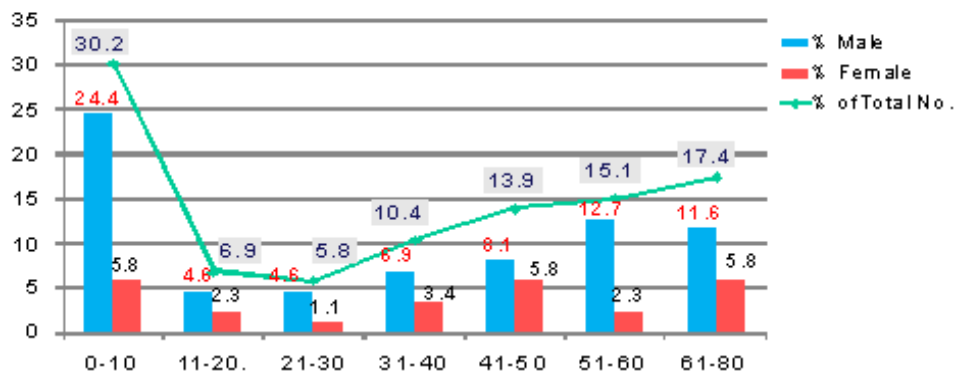

Fig. 1. Percentage of age wise distribution of *Streptococcus pneumoniae* isolates in pulmonary and extra pulmonary specimen

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Table 1. Antibiotic resistance patterns of *Streptococcus pneumoniae* isolated in Dr Lal Path Labs, Delhi from 1 January 2016 to 31 December 2017

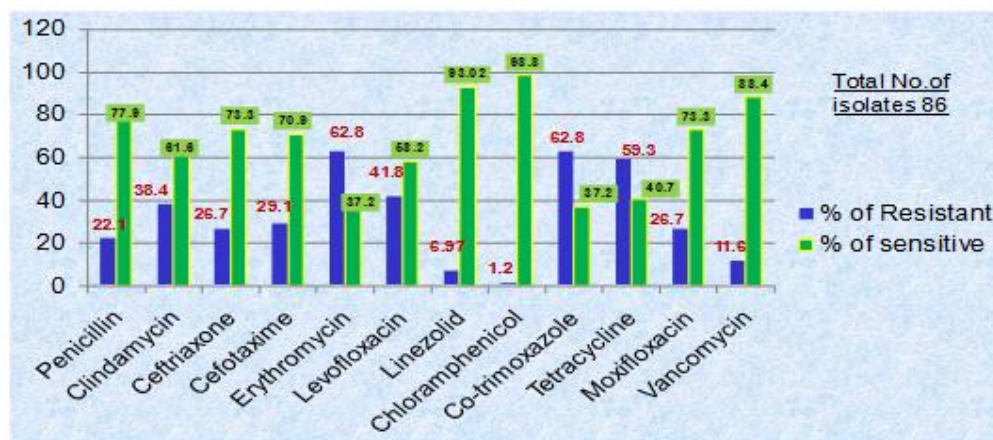| *Streptococcus pneumoniae* (n=86)			
Antibiotics	Resistant no. (%) (n=86)	Intermediate no. (%) (n=86)	Sensitive no. (%) (n=86)
Penicillin	12 (13.95)	7 (8.1)	67 (77.9)
Clindamycin	30 (34.8)	3 (3.4)	53 (61.6)
Ceftriaxone	20 (23.3)	3 (3.4)	63 (73.3)
Cefotaxime	22 (25.6)	3 (3.4)	61 (70.9)
Chloramphenicol	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)

Fig. 2. Percentage of antibiotic resistance patterns of *Streptococcus pneumonia*

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% [1,5,9,15].

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 show 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 comparision 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 [11,12,18,]. 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].

Table 2. Comparision 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

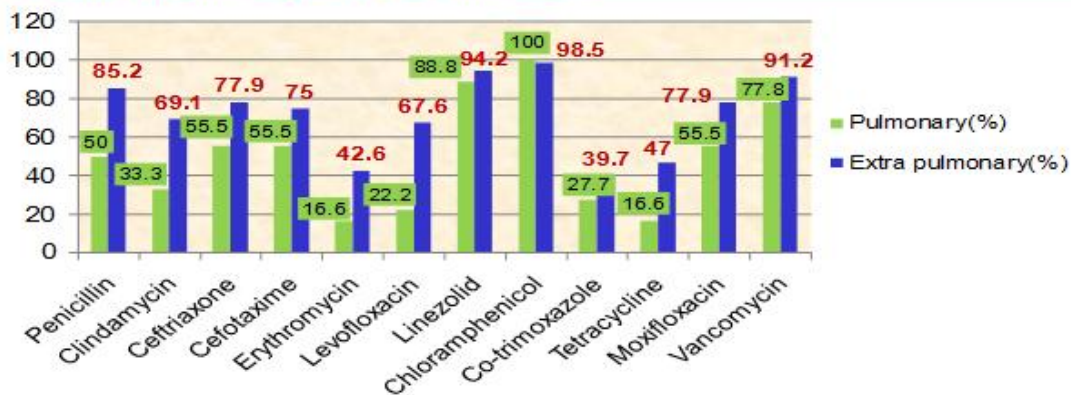| *Streptococcus pneumoniae* isolates from pulmonary specimen (n=18) and extra pulmonary specimen (n=68)				
Antibiotics	Pulmonary Resistance (%)	Intermediate resistance (%)	Extrapulmonary Resistance (%)	Intermediate resistance (%)
Penicillin	33.3	16.6	8.8	5.8
Clindamycin	66.6	0	26.5	4.4
Ceftriaxone	38.8	5.5	19.1	2.9
Cefotaxime	44.4	0	20.6	4.4
Chloramphenicol	0	0	1.4	0
Co-trimoxazole	55.5	16.6	44.1	16.1
Erythromycin	83.3	0	57.3	0
Levofloxacin	77.7	0	26.5	5.8
Linezolid	11.1	0	5.8	0
Tetracycline	83.3	0	52.9	0
Moxifloxacin	44.4	0	19.11	2.9
Vancomycin	22.2	0	8.8	0

Fig. 3. Comparision of antibiotic sensitivity pattern in isolates of *Streptococcus pneumoniae* among 18 pulmonary and 68 extra pulmonary specimen

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 [5,10,13,]. So the powerful approach needed to managing these infections to best treat all *Streptococcus pneumoniae* infections due to resistant strains [9].

The oxazolidinones, 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 pneumonia* 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 [2,7,16,17].

5. 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.

CONSENT

As per international standard informed and written participant and parental consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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