***Short Research Article***

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


## Abstract

Aims and objectives: Our study were 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℃ in 8% CO2 incubator. Identification & Antibiotic susceptibility testing were also done using VITEK® 2 GP card. / *S. pneumoniae* susceptibility card (AST ST01 Card –BioMerieux, India).

**Results:** A total 86 isolates from pulmonary (21%) and extra pulmonary (79%) specimens were analysed for their antibiotic resistance pattern.

30% isolates were found between 0-10 years and 17.4% after 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 VI we found strains were most susceptible to Chloramphenicol (98.8%), Linezolid (93%) and Vancomycin (88.37%). However most resistance was 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 ie: 30% (0-10 y) & 17.4% (60-80y) which correlates well with other findings and we found resistance in Erythromycin (62.8%), Tetracycline (59.3%), Co-trimoxazole (62.8%) and Penicillin resistance were (22.1%) which is concordance with other studies.

Alarming thing found is the emergence of resistance in Vancomycin (11.62%) and Linezolid (6.97%) in India.



**Keywords :** Streptococcus pneumonia, Vancomycin, Linezolid, pulmonary, extra pulmonary


## Introduction:

**T**he 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. [9]

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.[15, 5,13] 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 (11from 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).

Columbia 5% sheep Blood agar (BioMerieux) plates after incubation for 24 to 48 hours at 36± 1℃ in 8% CO2 incubator. Identification & Antibiotic susceptibility testing were also done using VITEK® 2 GP card. / *Streptococcus pneumoniae* susceptibility card (AST ST01 Card –BioMerieux, India).

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 in children aged between 0-10 years, 6.9% (6) in aged 11-20 years and 17.4% after 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%).

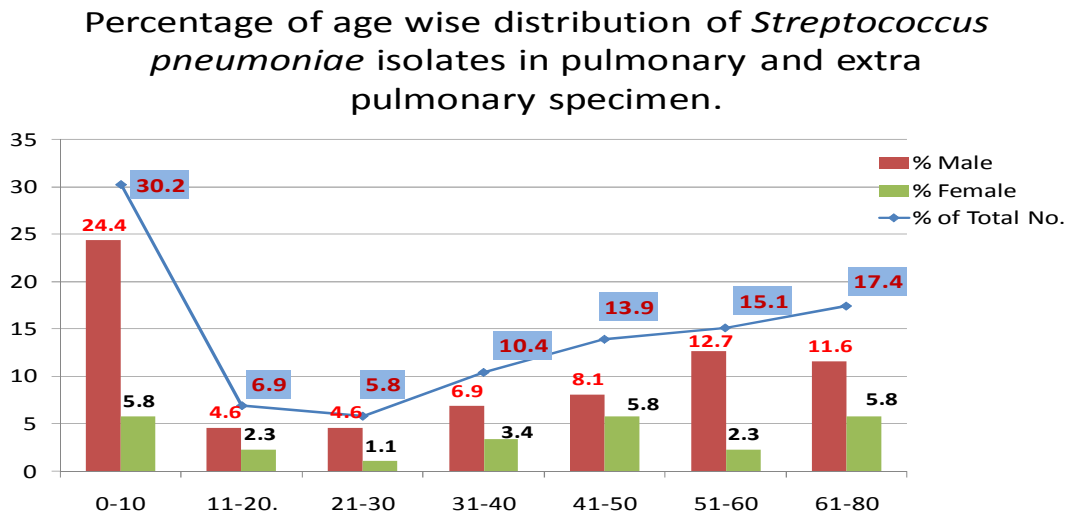


**Figure 1: Percentage of age wise distribution of *Streptococcus pneumoniae* isolates in pulmonary and extra pulmonary specimen**

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 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).**

Resistant of drugs in Extra pulmonary isolates to high in Co-trimoxazole (60.3%), Erythromycin (57.3%), Tetracycline 52.9% **(Table 2; Figure 3).** Ceftriaxone, Cefotaxime and Clindamycin showed high resistance in pulmonary isolates in comparision with 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)** |



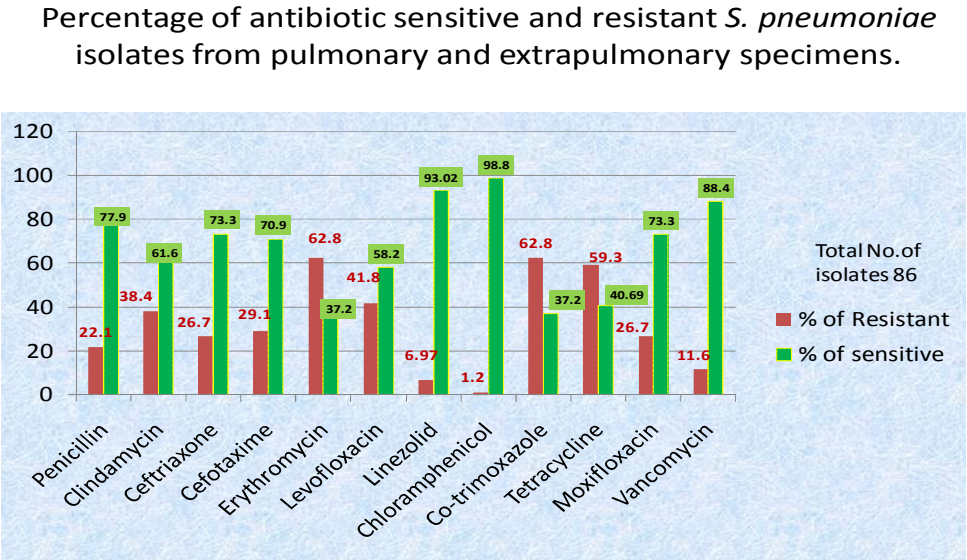


**Figure 2: Percentage of antibiotic sensitive and resistant *S. pneumoniae* isolates from pulmonary and extrapulmonary specimens.**

**Table2. 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 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) |

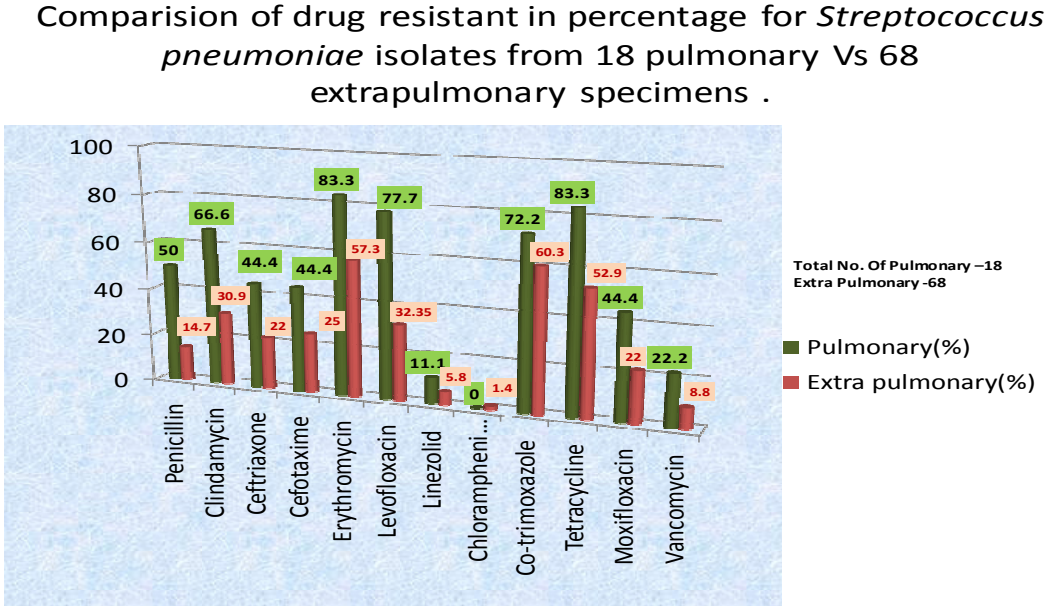


**Figure 3: Comparision of drug resistant in percentage for *Streptococcus pneumoniae* isolates from 18 pulmonary Vs 68 extrapulmonary specimens.**

**Table3. Comparision 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)** |

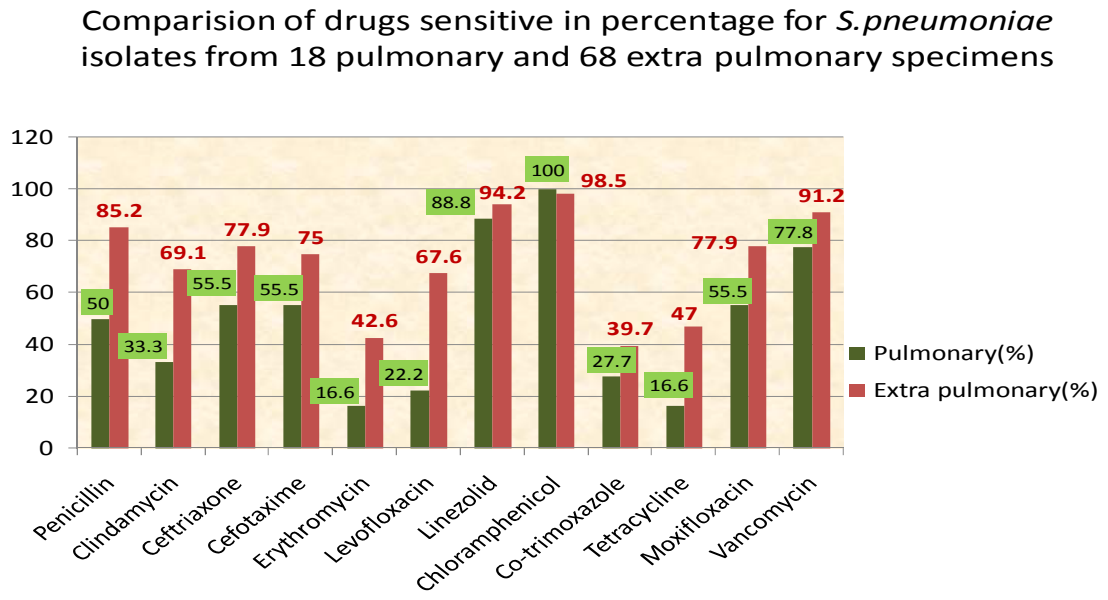


**Figure 4: Comparision of drugs sensitive in percentage for *S.pneumoniae* isolates from 18 pulmonary and 68 extra pulmonary specimens.**

**Discussion:** Antibiotic resistance among *Streptococcus pneumoniae* continues to evolve and this threatens to affordable management. *Streptococcus pneumoniae* will continue to be a leading public health and clinical problem for future.

In our 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. [18, 8, 12, 4]

Prior to 1995 all strains of *Streptococcus pneumoniae* isolated India were uniformly susceptible to penicillin. In our study penicillin (22.1%) were a similar observation to that from an earlier report [18,8] from south India. worldwide with some countries in the Asian continent reporting up to 70% resistance to penicillin (Jones et al.,2010; Daka et al.,2011; and Tsai et al.,2013;). Penicillin generally was the antibiotic of choice but rapid development and spread of Penicillin resistant (50%) in pulmonary infection in comparision with (14.7) in extra pulmonary infection. We found age of (0-10y) all isolates of *Streptococcus pneumoniae* were found sensitive to Penicillin except three.

Around 85 to 90% of Antibiotics consumption occurs in the treating respiratory tract infection. WHO guidelines recommended the use of cost effective treatment of antibiotic currently recommended for younger children aged 2-5 years with non severe Co-trimoxizole and Amoxicillin for three days and for severe pneumonia Ampicillin or Penicillin and Gentamicin are recommended as first line drug for treatment Ceftriaxone should be used as second line treatment when first line treatment fails, we found high Co-trimoxazole resistance (72.2%) resistant in pulmonary infection and (60.3%) resistant in extra pulmonary infection. Our study demonstrated that the rates of Erythromycin (62.8%) and Cotrimoxazole (62.8%) resistant among Streptococcus pneumonia in Delhi, India remained much higher than isolates from united states where nonsusceptibility to these two drugs was (37%) and (33%) respectively [5]and similar to Taiwan were Erythromycin (92%) and Co-trimoxazole (70%) respectively.[15]

Levofloxacin the active isomer of Ofloxacin, has excellent invitroactivity against Penicillin resistant pneumococci.[3] Although resistance of pneumococci to Levofloxacin increased and finding were not similar to from other countries (Tsai et al., 2013). The arrival of resistant pneumococci was, however, given the degree of irrational use of third generation cephalosporins and other newer antimicrobials. The emergence of drug resistant *S. pneumoniae* to important antibiotics such as cephalosporins and macrolides is becoming increasingly severe in our study and problem of global concern that has made treatment of disease more difficult.[14] Our retrospective study demonstrates the burden of antibiotics in pulmonary and extra pulmonary infections.

Based on our study we found commonly used antibiotic showed high resistance to following drugs Erythromycin (83.3%), Tetracycline (83.3%) and Levofloxacin (77.7%) in pulmonary infection comparision with in extra pulmonary followed in Erythromycin (57.3%), Tetracycline (52.9%) and Levofloxacin (32.4%).

Several reports of treatment related to *S. pneumoniae* isolates the combination of Vancomycin plus Ceftriaxone or Cefotaxime was synergistic and superior for treatment of children. [(11,18,12)] Vancomycin is the antibiotic of last resort, its resistance represents a new health risk we found that the Vancomycin (11.6%), Ceftriaxone (26.7%), Cefotaxime (29.04%) resistant respectively in pulmonary and extra pulmonary infection. In our study were a similar observation to that from earlier report of Asia and all over world.[15,13,10]

The oxazolidones, represented by Linezolid are new class of antibiotic with unique structure and good activity against gram positive [6]. In our study (11.1%) resistant were found in pulmonary sites and (5.8%) resistant in extra pulmonary sites so the powerful approach needed to managing these infections to best treat all *S. pneumoniae* infections due to resistant strains.

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.

In a country where quacks are able to prescribe allopathic medicines, antibiotics are prescribed by most practitioners do not have access to good investigation facilities, and a policy on the use of antibiotics is almost totally lacking, choice of antibiotics or duration of antibiotic therapy is incorrect in 30% to 50% of cases in the world.[7,17,16.] Thus management of drug resistance Streptococcus pneumoniae continues to change and increased needed multidisciplinary approach involving clinicians, pharmacists and microbiologists.

At this time Chloramphenicol were the standard empirical agents for pulmonary and extra pulmonary infection for the treatment of Streptococcus pneumonia and considered as an acceptable alternative agent to complete therapy if penicillin and other multidrug resistant.

**Conclusion:** Pneumonia has a great burden of morbidity and mortality in developing countries, which results in economic and social pressures on families and the country as a whole. Therefore, very few reports of penicillin-resistant pneumococci from India, present no details on the susceptibility profile to other classes of antibiotics. We report a multidrug-resistant isolates of *Streptococcus pneumoniae* in Delhi, India, and its susceptibility pattern.

We found most of resistant isolates from pulmonary site than extra pulmonary sites.

Prescription of antibiotic against pneumococcal infection should be judiciously followed and reported as per CLSI M100-S-28 document. Selective antimicrobial reporting for pneumococci should be followed to arrest further resistant of antibiotics.

Disclaimer: - This manuscript was presented in a Conference as a e-Poster

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