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Evaluation Of Antimicrobial Susceptibility Pattern And Trends In Resistance Rate Of Streptococcus Pneumonia Isolated From Clinically Significant Specimens At Tertiary Care Hospital In North Mumbai

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Abstract

Aim: To observe the antimicrobial susceptibility pattern and trends in resistance rate of *Streptococcus pneumonia* isolated from clinically significant specimens at tertiary care hospital.

Materials & Methods: A study was conducted retrospectively at BhaktiVedanta Hospital & Research center from January 2018 to February 2020. Clinical specimens derived from patients were processed for routine bacteriological culture. *S.pneumoniae* identification and susceptibility testing were done by automated Vitek2 compact system. The data obtained were analysed for antimicrobial resistance pattern.

Results: Total thirty six clinically significant *S.pneumoniae* isolates were obtained from patients. Resistance to two antibiotics was observed in 20 (55.55%) of isolates and one isolate was found to be multi drug resistant. 2 (5.55%) isolates showed intermediate resistance to ceftriaxone. Penicillin G resistance observed in 2(5.55%) meningeal isolates. The susceptibility of vancomycin was 100%. The resistance rate of levofloxacin, cotrimoxazole and erythromycin were 1 (2.77%), 19 (52.77%), 20 (55.55%) respectively.

Conclusion: Emerging resistance strains of *S.pneumoniae* is a matter of concern. Continuous monitoring and evaluation of resistance pattern required to guide about empirical treatment and for preventing transmission of multidrug resistant strains in community. Restricted use of antibiotics must be emphasized.

Keywords: Streptococcus pneumoniae, Antimicrobial agents, Resistance

Introduction

Streptococcus pneumoniae is a gram positive coccus belongs to family Streptococcaceae. It is a predominant pathogen of community-acquired infections like bacterial pneumonia, meningitis and otitis media but can also causes severe sepsis. According to data available from the World Health Organization (WHO), annual mortality under 5 years old children due to *S. pneumoniae* infection is close to half a million worldwide and majorly belonging to developing countries. [1] In countries like India, burden of pneumococcal disease is even high; especially meningitis and pneumonia were found to be the commonest clinical conditions accounting for 39% and 24.3% of total invasive pneumococcal diseases, respectively. [2]

Usually, nasopharyngeal colonization of *S. pneumoniae* precedes pneumococcal disease. In young children, asymptomatic nasopharyngeal carriage is widely prevalent in young and has been related to spread of the pathogen. [3] Over the last few years, several studies in India reported high nasopharyngeal colonization and antibiotic resistant strains. [4, 5, 6]

Till 1980s, *S. pneumoniae* were highly susceptible to all classes of antibiotics. Later on, increasing trend of antibiotic resistance has been observed. [7] High level resistance to penicillin emerged and even resistance to other antibiotics like clindamycin, erythromycin, tetracycline, and ciprofloxacin. At present resistant strains of *S.pneumoniae* including multidrug resistant strains are being reported worldwide. [8]

There is a need to produce an antibiogram of *S.pneumoniae* on regular basis. Emerging resistant strains needs to be controlled, especially in invasive pneumococcal diseases as that can ultimately lead to treatment failures, prolonging hospitalization and increase in morbidity and mortality. So, aim of the present study is to analyse the pattern of antimicrobial susceptibility and resistance rate of *S.pneumoniae* at tertiary care hospital.

Materials And Methods

A retrospective study was conducted at 200-bedded tertiary-care hospital which caters approximately 20000 annual admissions in a catchment area of 20 lakh. The hospital has robust database system featuring centralized electronic medical and laboratory record with unique identification numbers.

Clinical specimens for bacteriological culture and antibiotic susceptibility testing were collected from patients suspected of bacterial infection. All collected specimens processed from the period of January 2018 to February 2020 that grew *Streptococcus pneumonia* were included in the study. Using existed data entry system in the microbiology laboratory, socio demographic variables, types of specimens and microbiological data of the patients were noted and evaluated.

Specimens collected from patients suspected for pneumococcal disease were eye swab, sputum, ET secretion, cerebrospinal fluid and blood cultures. Corneal scrapings were collected by ophthalmologist and inoculated directly on sheep blood agar plate. All specimens except cerebrospinal fluid and blood culture were transported in ice packs from collection room and immediately delivered to microbiology laboratory for further processing. Samples were plated on Sheep Blood agar (Biosmart, Mumbai) and Mac-Conkey agar. Chocolate agar used as additional enriched media. Streak plate culture method done using Hiflex loop of 2 mm diameter (HiMedia Labs, Mumbai). Optochin disc was placed at the junction of first and second quadrant of streaking lines. After sample inoculation, all agar plates were kept in incubator for 24-48 hrs at 37°C. Chocolate agar plate were kept in candle jar and incubated to create anaerobic environment. All inoculated plates were screened for significant growth at 24hr and 48hr. Organisms grown on plate that showed characteristics like alpha haemolysis and optochin sensitive were subcultured and gram staining was done. The isolate assumed to be *S.pneumoniae* if gram positive cocci in pairs, lanceolate in shape were seen in stained slides. Further identification of *S.pneumoniae* and its antibiotic susceptibility testing were done by Vitek2 compact automated system (BioMerieux, Mary l'Etoile, France).

The antibiotics tested were Penicillin G, Ceftriaxone, Clindamycin, Vancomycin, Erythromycin, Levofloxacin and Cotrimoxazole. *E.coli* ATCC 25922 and *S.aureus* ATCC 25923 were used as quality control strains. All ATCC strains were procured from Microbiologics, USA. The antibiotic susceptibility results were divided into resistant, intermediate or sensitive as per the Clinical & Laboratory Standards Institute (CLSI) 2019 guidelines. [9] Data obtained were analysed using SPSS statistical software. The study was conducted after ethical clearance obtained from institutional ethics committee.

Results

Study conducted for time period of two years from January 2018 to March 2020, total 36 isolates of *Streptococcus pnuemoniae* obtained. Out of 36 isolates, 14 (38.88%) were females and 22 (61.12%) were males. Age wise distribution showed 16 (44.45%) patients were more than 60 years followed by 12 (33.33%) patients with 40-60 years of age and 5 (13.88%) isolates from infants with less than 12 months age. [Table 1]

In present study, clinical specimens received from both outdoor and indoor patients were processed. Out of 36 total isolates, 22 (61.12%) were obtained from indoor patients and 14 (38.88%) were from outdoor. [Table 2]

Amongst 36 isolates, 14 (38.88%) were blood cultures, followed by corneal scrapping 9 (25%), sputum 7 (19.44%), CSF 3(8.33%), ET secretion 2

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(5.55%) and eye swab 1(2.77%). Majority of specimens were obtained from IPD patients. [Table 3]

Pattern of antimicrobial susceptibility for all *S.pneumoniae* isolates were noted. Out of total 36, 21 (58.33 %) isolates were resistant to only one antibiotic tested. Resistance against two antibiotics was observed in 20 (55.55%) of isolates and one isolate was found to be multi drug resistant. 2 (5.55%) isolates showed intermediate resistance to ceftriaxone. Penicillin G resistance observed in 2(5.55%) meningeal isolates. The susceptibility of vancomycin was 100 %. The resistance rate of levofloxacin, cotrimoxazole and erythromycin were 1 (2.77%), 19 (52.77%), 20 (55.55%) respectively. [Table 4]

Discussion

Worldwide pneumococcal diseases are a major public health concern. Though preventable but the main obstacles in effective management are risk factors, limited antimicrobial susceptibility data and emergence of antibiotic resistance. Besides, other risk factors like extremes of age, comorbid conditions, and varied serotypes further affect treatment. Total 93 serotypes of *S.pneumoniae* exits, which is characterized by distinct polysaccharide capsule. Koul PA *et al* reported most common serotypes found in India were 1, 3, 5, 19F, 8, 14, 23F, 4, 19A and 6B. [10] All pneumococcal serotypes differ in their biological behaviour due to variations in colonizing and tissue invasiveness.

It has been observed that pneumococcal diseases were more prevalent in older adults. Vulnerability increases with advancing age as immunity decreases. Moreover, compared to young adults, the older population tends to have one or more chronic comorbidities that increase the severity of disease. An underlying chronic illness led to weaker immune status and thus favors the growth of the pathogen. [11] Present study showed 44.45% patients were more than 60 years of age followed by 33.33% patients of 40-60 years age and 13.88% isolated from infants with less than 12 months age. One of the reasons might be lack of awareness about defined immunization schedule for the older population in India. Imai K *et al* noted that adult population aged 50–64 years along with medical conditions have higher risk of pneumococcal disease as compared to adults aged ≥ 65 years without any condition. [12] Hence, emphasis on adult immunization needed along with robust guidelines.

Management of pneumococcal diseases differs on basis of source of specimen collection. In present study, clinical specimens received from both outdoor and indoor patients were processed. Zhang W *et al* reported more usage of penicillins, macrolides and cephalosporins for outpatient department, while second and third generation cephalosporins were more commonly used for admitted patients, even fourth generation cephalosporins and carbapenems were also administered. [13] Out of 36 total isolates, 22 (61.12%) were obtained from indoor patients and 14 (38.88%) were from outdoor. Differences in prescription pattern can led to varied resistance among *S.pneumoniae* isolates. This might cause treatment failures. More indoor patients highlights that management of *S.pneumoniae* needed hospitalisation as treatment failures increased due to resistance. Lyu S *et al* highlighted the significance of antibiotic usage and quoted that the empirical antibiotic program should be different between the inpatients and outpatients. [14] Hence, separate antibiogram for community acquired infections should be emphasized.

It was observed in previous studies that recovery of *S.pneumoniae* from respiratory specimens, cerebrospinal fluid and blood cultures is more. Other specimens when submitted for microbiological analysis rarely grew *S.pneumoniae*. In present study, 14(38.8%) isolates were obtained from blood cultures followed by 9(25%) corneal scrapings. Parmar P *et al* reported that 33.3% of bacterial keratitis due to by *S.pneumoniae*. [15] In era of multi drug resistance, patients visiting at tertiary care hospitals with complaints of ophthalmic infections were already prescribed antibiotic course by general practitioners. So, isolation of *S.pneumoniae* becomes extremely difficult. Though in pres ent study, it was observed that majority of patients with keratitis were started on antibiotics after sample collection. Hence, it's significant to note that the recovery of *S.pneumoniae* in culture is highly affected by antibiotics. It's always a good practice to send corneal scrapings for bacterial culture and antimicrobial susceptibility testing before starting antibiotic.

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As far as treatment of pneumococcal infection is concern, choice of antibiotic, dosage and duration varies according to the site of infection. Furthermore, minimum inhibitory concentration of antimicrobials against *S.pneumoniae* differs too. The breakpoints formulated for treatment of pneumococcal meningeal infections may not be applicable to non-meningeal infections. CLSI had clearly defined separate susceptibility break points for pneumococcal meningeal and non-meningeal infections. These break points were derived based on reviews on microbiological, pharmacokinetics or pharmacodynamics and clinical outcome data. But resistance increasing due to overuse, indiscriminate use and starting empirical treatment without antimicrobial susceptibility testing. Such increased prevalence of resistant strains has complicated the treatment.Many surveillance programs worldwide indicated that the proportion of drug resistant *S.pneumoniae* isolates will be increasing continually. [16] On the contrary, increased vaccination and sufficient supply of antimicrobial agents has been observed, but emerging antimicrobial resistance is still a major issue. Few reports from India showed resistance in *S. pneumoniae*. Lalitha *et al* [17] noted upsurgence of intermediate sensitivity during surveillance for resistance of *S. pneumoniae*, whereas Goyal *et al* [18] reported 2.3% resistance. On more study from South India done by Kanungo *et al* [19] reported low level resistance while Song JH *et al* [20] reported total resistance in *S.pneumoniae* around 35.1%. Cherazard R *et al* concluded that high prevalence of resistance was with beta-lactams antibiotics. Amongst them, resistance rate of penicillins was 13.8-41.8%, cephalosporins was <1-29.9%, macrolides was 20-40%, clindamycin was 21.8%, TMP-SMX was 25-45% and tetracycline was 25.9%. [21]

Amongst all available antimicrobials, global concern is penicillin resistance in *S. pneumoniae*. Compared to other countries, total resistance for penicillin is lesser in India but intermediate resistant strains were observed. In present study, rate of penicillin resistance was 2 (5.55%). Resistance rate was low and reason might be due shorter study period, type of methodology used and smaller sample size. 4.6% of intermediate resistance to penicillin was observed in Lalitha *et al* [17] study while Goyal *et al* [18] reported 15.2% intermediate resistance and 2.3% penicillin resistance. The differences in resistance pattern might be due strains from different geographical areas with high genetic diversity. Studies also reported that strains with penicillin resistance can have genes responsible for developing resistance to other group of antibiotics. [18] Hence, judicious use of penicillin for pneumococcal infections is essential as intermediately resistant strains results in greater spread of resistance and higher rate of treatment failure.

Resistance to cefotaxime amongst meningeal isolates has increased tremendously over a period of time. Multiple studies have shown increase in resistance to third generation cephalosporins. Present study showed 2 (5.55%) strains with intermediate resistance to ceftriaxone. The current pattern indicated upcoming resistance to ceftriaxone. Study conducted by Shahriar M *et al* [22] reported 9.8% resistance. While Catalan *et al* [23] noted treatment failures in cases of intermediately resistant strains. Reason might be usage of ceftriaxone as an empirical antibiotic without performing antimicrobial susceptibility testing. For non-meningeal isolates, resistance was noted against trimethoprim-sulphamethoxazole. Many studies reported that resistance rate to trimethoprim-sulphamethoxazole had increased significantly from 21.8% to 61.7%. [18, 19, 24]

The broad spectrum bactericidal activity of fluoroquinolones had promoted its irrational usage worldwide. Jones ME *et al* [25] reported the increasing trend for quinolone resistance. Chawla *et al* [24] observed 14% resistance to ciprofloxacin. Cherazard R *et al* [21] study concluded that low level resistance observed against fluoroquinolones but increasing gradually. In present study, only one strain with intermediate resistance was reported. Resistance to fluoroquinolones were lower as compared to studies from other Asian countries. [26]

After the emergence of penicillin resistant isolates, trend of increased macrolide usage was observed. But the prevalence is variable according to geography and in India resistance was found to be 32% (19% to 47%). [27] High prevalence of erythromycin resistant strains led to the conclusion that resistance might rise in countries where the drug is used widely. In present study, erythromycin showed 20 (55.5%) resistance. Hence, antibiotics should be prescribed carefully and

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after antimicrobial susceptibility testing even for non-meningeal isolates.

Recently, multi drug resistant pneumococcal strains are reported worldwide. Multi drug resistant strains are defined if more than 3 antibiotics are resistant. First MDR strain was isolated in 1977 at Johannesburg. [28] Afterwards many studies and reports were published worldwide. As per the current report, MDR strains predominantly belong to the serogroups 6, 9, 14, 23, 26 and 27. [29] Major reasons of MDR strains are prolonged nasopharyngeal carriage and reacquisition that increases the chances of exposure to antibiotics and particular selection of serogroups to antibiotic resistance, in hospital settings. In present study, MDR reported in 1 (2.77%) isolate from blood culture. Out of 36, 20(55.5%) isolates were resistant to two antibiotics. Lalitha M *et al* [17] reported only one MDR strain of *S. pneumonia* from India. Though the prevalence is low but is a matter of concern as it will increase the burden on health sector as well as for patinets.

For prevention of pneumococcal diseases, vaccines are available but serotype switching and replacement are significant causes of vaccine ineffectiveness.[30] For high risk elderly population, vaccines are recommended and advocated globally but their usage is suboptimal in many regions and is dependant on clinician's awareness of guidelines. Despite this, vaccination should be regarded as a key factor in preventive strategies.

Present study highlighted that resistance observed in different group of antibiotics. Hence, antibiotic for empirical treatment should be chosen wisely. Penicillin was considered to be drug of choice in the treatment of life threatening pneumococcal infections but reconsideration of empirical antibiotic is required. It is recommended that antimicrobial susceptibility testing should be done prior to administration of antibiotic.

Limitations of present study was smaller sample size. Only phenotypic detection of *S.pneumoniae* was done, as all isolated strains were preserved on chocolate agar slants, so revival of majority isolates failed. Serotyping should be done to correlate the results in better and effective way. Present study provided information on antimicrobial susceptibility of *Streptococcus pneumonia* to guide the physicians for starting empirical treatment. The clinical implication of antibiotic resistance was not evaluated. Hence clinical correlation should be done for better understanding of the management. Data was limited to a particular region, therefore it neither reflect the epidemiology of pathogen nor the burden of antimicrobial resistance pattern across the country. Furthermore, a robust evidence for effectiveness and safety of pneumococcal vaccines in Indian population is needed for reducing the incidence pneumococcal disease and for prevention of multidrug resistance crisis.

Conclusion

Antimicrobial resistance in *S.pneumoniae* and its community spread is a matter of concern. Strengthening vaccination strategies might play a significant role in prevention of pneumococcal infection especially in older age patients. Continuous monitoring, timely intervention and a restriction in antibiotic usage are required to keep a check on its resistance pattern and for development of an effective treatment plan. Supportive molecular tests should be done for epidemiological studies to accurately identify the resistant isolates.

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Table 1 Age and gender wise distribution of *Streptococcus pnuemoniae* isolated from clinical specimens during study period of January 2018 to March 2020

Age	**Male**	**Female**	**Total**
<=12months	3(8.34)	2(5.56)	5(13.88)
1-20 years	-	2(5.56)	2(5.56)
20-40 years	-	1 (2.78)	1 (2.78)
40-60 years	6 (16.67)	6 (16.67)	12 (33.33)
>=60 years	12 (33.34)	4 (11.12)	16 (44.45)
Total	22 (61.12)	14 (38.88)	36 (100)

* Values in parenthesis are percentage.

Table 2 Distribution of Streptococcus pneumoniae isolates based on location of collection

Location	**Number of isolates (%)**
OPD	14 (38.88)
IPD	22 (61.12)

Table 3 Distribution of Streptococcus pneumoniae based on various clinical specimens of outpatients and inpatients

Specimens	**OPD**	**IPD**	**Total**

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Eye swab	1 (2.77)	-	1 (2.77)
Corneal scrapping	9 (25)	-	9 (25)
Sputum	3 (8.33)	4 (11.11)	7(19.44)
Et secretion	-	2 (5.55)	2 (5.55)
CSF	-	3 (8.33)	3 (8.33)
Blood culture	1 (2.77)	13 (36.11)	14 (38.88)
Total	14 (38.88)	22 (61.12)	36(100)

*OPD - Out Patient Department; IPD - Indoor Patient.

* Values in parenthesis are percentage

Table 4 Antimicrobial susceptibility patterns of S.pneumoniae isolates during study period of January 2018 to March 2020 (n=36)

Antibiotics		**Sensitive**	**Intermediate**	**Resistant**
Penicillin G	Meningitis	-	-	2
	Non meningitis	34	-	-
Clindamycin		28	-	8
Cotrimoxazole		17	-	19
Erythromycin		16	-	20
Vancomycin		36	-	-
Levofloxacin		35	-	1
Ceftriaxone	Meningitis	1	1	
	Non meningitis	33	1	-

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