e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 14 (1); 59-63

Original Research Article

Multidrug Resistance among Penicillin Resistant Pneumococcus Isolated from Respiratory Specimens

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Received: 18-10-2023 / Revised: 21-11-2023 / Accepted: 26-12-2023
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Conflict of interest: Nil

Abstract:

Introduction: Diseases caused by Streptococcus pneumoniae are the cause of global concern. S. pneumoniae is common causative agent of pneumonia, bacteremia and meningitis which lead to morbidity and mortality, even after being treated in hospital. S. pneumoniae shows resistance for multiple antimicrobial drugs as penicillin, clindamycin, erythromycin, clarithromycin, tetracycline, chloroamphenicol, trimethoprim/sulfamethoxazole. Present study is conducted to find distribution of multidrug resistance among penicillin resistant pneumococci isolated from respiratory specimen.

Aims & Objectives: To find distribution of multidrug resistance among penicillin resistant pneumococcal strains isolated from respiratory specimen with age distribution.

Material & Methods: The study was conducted in the Department of Microbiology Gajra Raja Medical College, Gwalior (M.P.) for a period of one year. Respiratory samples were collected from patients of all age groups suffering from lower respiratory tract infection and identification of pneumococci was done. Antimicrobial susceptibility testing done. Penicillin resistance and resistance for other antimicrobial drugs was screened. Data was statistically analyzed by odds ratio, p-value <0.05 was considered statistically significant.

Result: Pneumococci isolated maximum from patients under 5 years and >50 years age group. 24 isolates (48%) showed penicillin resistance. Among other antimicrobial drugs resistance was maximum for erythromycin (46%) followed by tetracycline (38%), cotrimoxazole (36%), cefotaxime (30%) and ciprofloxacin (14%). Statistically significant correlation between penicillin resistance and multidrug resistance among S. pneumoniae isolates [odds ratio 3.31, 95% confidence interval, (CI) 1.02-10.72, p value 0.045 (i.e. p<0.05)].

Conclusion: Pneumococci can cause disease at any age though more frequently at extremes of age. Multidrug resistance is common among the penicillin resistant pneumococcal isolates. Antimicrobial-susceptibility testing must be done for pneumococcal isolates.

Keywords: Pneumococci, Penicillin, Resistance, Multidrug.

Introduction

Diseases caused by Streptococcus pneumoniae (pneumococcus) are the cause of global concern. S. pneumoniae is common causative agent of pneumonia, bacteremia and meningitis which lead to morbidity and mortality, even after being treated in hospital [1]. Community acquired pneumonia caused by it is leading cause of mortality in children under 5 years of age [2].

Studies have shown that few serotypes usually cause most of pneumococcal infections and shows antibiotic resistance [1]. Serotypes even show multi drug resistance (MDR) which is defined as resistance for three or more classes of antibiotics [3]. First MDR strain was isolated in Johannesburg in 1977 from sputum of a child. Resistance for penicillin and multiple antibiotics was first recognized in serotype 6A and 19A of pneumococci in South Africa [4]. Antimicrobial resistance among pneumococci is major concern globally and the treatment of diseases caused by the bacteria gets complicated [5]. Pili and capsule are important virulence factor among pneumococci [6,7].

Various studies have shown that previous antimicrobial agents exposure is an important predisposing factor for drug resistance especially for invasive infections of pneumococci including pneumonia [4,5]. Carriage serogroups, CNS related

disorders along with MDR are independently associated with pneumococcal molecular epidemiology [8]. MDR strains belong to different serogroups/types and vary in different countries which include 6A/B, 9, 14, 15A, 15B, 15C, 7B, 23F, 19A, 19F, 24 [4, 6, 7, 9]. Penicillin resistance and MDR is more frequent in invasive pneumococcal disease than non-invasive diseases [10]. Epidemiology of Streptococcus pneumonia found high prevalence of multi drug resistance in Asian countries especially among serotype 19A [9].

MDR is higher among isolates obtained from respiratory specimens than among isolates of blood [3]. S. pneumoniae shows resistance for penicillin, clindamycin, erythromycin, clarithromycin, tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole [3,6,10,11]. Prevalence of isolates with erm (B) and mef (A) genotype and multi drug resistance is increasing among pneumococci [12].

Pneumococcal vaccines have reduced the incidence of pneumococcal disease among children, but it leads to emergence of new serotypes as 8, 10A, 12F, 15A, and 24F. Several studies showed serotype 15A is resistant for multiple antimicrobial drugs [13].

Present study is conducted to find distribution of multidrug resistance among known penicillin resistant pneumococci isolated from respiratory specimen.

Aims & Objectives

- To find age distribution of patients with pneumococci isolated from their respiratory specimen.
- To find distribution of multidrug resistance among penicillin resistant pneumococcal strains isolated from respiratory specimen.

Materials and Methods

Study design- Prospective

The study was conducted in the Department of Microbiology Gajra Raja Medical College, Gwalior (M.P.) for a period of one year in 2016- 2017.

- Respiratory samples were collected from patients of all age group suffering from lower respiratory infection.
- Identification and confirmation of pneumococci was done based on colony characteristics, gram staining and biochemical reactions as per standard protocol.
- Antimicrobial susceptibility testing done by Kirby Bauer's disc diffusion method
- Penicillin resistance was screened by using oxacillin (1µg) disk followed by Minimum Inhibitory Concentration (MIC) detection by agar dilution method according to CLSI guidelines.
- Antibiotic susceptibility for other antimicrobial drugs as Tetracycline (30 µg), Cotrimoxazole (25 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg) and Erythromycin (15 µg) was carried out by the Kirby Bauer disk diffusion method on Muller-Hinton sheep blood agar (MH-SBA) and resistance against them was detected.
- Multidrug resistance was defined as intermediate resistance or resistance to penicillin plus resistant to at least three classes of antibiotics.

Statistical analysis: Data was statistically analyzed by odds ratio, p-value <0.05 was considered statistically significant.

Result

A total of 50 pneumococcal isolates were obtained from respiratory samples collected from patients suffering from lower respiratory infection.

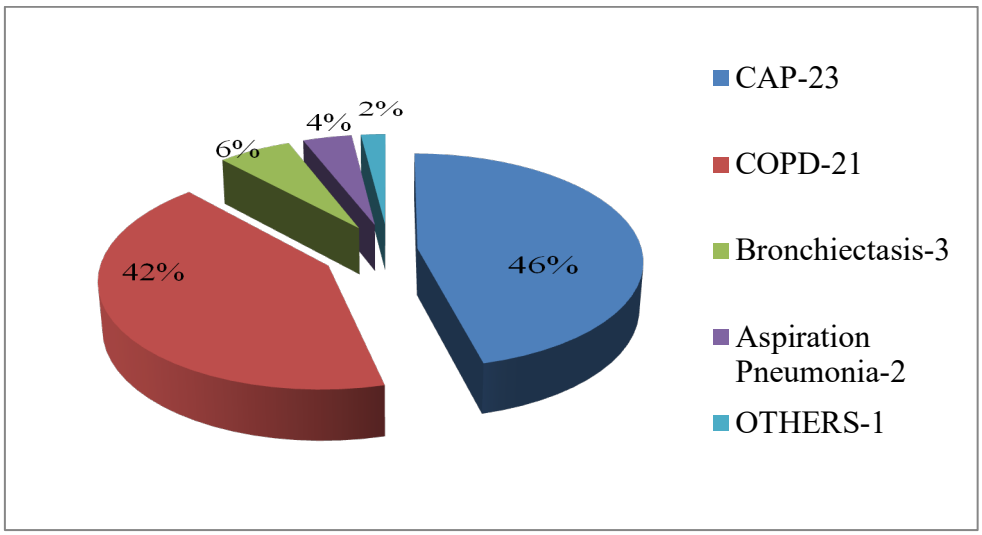

Figure 1: Distribution of diseases associated with Pneumococcal isolates

Fig. 1 shows distribution of diseases associated with Pneumococcal isolates. Pneumococci was isolated from patients of community –acquired pneumonia (CAP) 23 cases (46%) Chronic obstructive pulmonary disease (COPD) 21 cases (42%), bronchiectasis 3 cases (6%), aspiration pneumonia 2 cases (4%) and others 1 case (2%).

Table 1: Age wise distribution of pneumococcal isolates

Age group	No. of isolates
≤2 years	12 (24%)
2-5 years	3 (6%)
5-20 years	4 (8%)
20-50 years	5 (10%)
≥50 years	26 (52%)
Total	50

Table 1 shows pneumococcal isolates are 12, 3, 4, 5 and 26 in age group ≤2 years, 2-5 years, 5-20 years, 20-50 years and ≥50 years respectively.

Table 2: Resistance pattern of pneumococcal isolates for penicillin based on Minimal inhibitory concentration (MIC) value

Penicillin-susceptible	MIC ≤ 0.06 µg/mL	26 (52%)
Penicillin intermediate-resistant	MIC 0.12 – 1 µg/mL	09 (18%)
Penicillin-high resistance	MIC ≥ 2 µg/mL	15 (30%)

Table 2 shows out of total 50 pneumococcal isolates, 09 isolates (18%) showed intermediate resistance and 15 isolates (30%) high resistance to penicillin.

Table 3: Antibiotic susceptibility of pneumococcal isolates for other antibiotics by the Kirby Bauer disk diffusion method

Drugs	Resistant zone size	Isolates
Tetracycline	≤ 22 mm	19(38%)
Cotrimoxazole	≤ 15mm	18(36%)
Cefotaxime	≤ 25 mm	15(30%)
Ciprofloxacin	≤ 16 mm	07(14%)
Erythromycin	≤ 19 mm	23 (46%)

Table 3 shows resistance was maximum for erythromycin (46%) followed by tetracycline (38%), cotrimoxazole (36%), cefotaxime (30%) and ciprofloxacin (14%) among pneumococci.

Table 4: Multidrug resistance among penicillin resistant and penicillin sensitive pneumococci isolated from respiratory specimen.

	Isolates resistant for other 3 or more antimicrobial drugs (MDR)	Isolates resistant for less than 3 antimicrobial drugs	Total
Penicillin resistant isolates	17	07	24
Penicillin sensitive isolates	11	15	26

Table 4 shows out of 24 penicillin resistant pneumococcal isolates, 17 isolates were multidrug resistant while among 26 penicillin sensitive pneumococcal isolates, 11 isolates were multidrug resistant. Statistical analysis show odds ratio 3.31, 95% confidence interval, (CI) 1.02-10.72, p value 0.045 (i.e. $p<0.05$) which suggests statistically significant correlation between penicillin resistance and multidrug resistance among pneumococcal isolates obtained from respiratory specimens.

Discussion

Present study shows pneumococcal isolates are 12, 3, 4, 5 and 26 in age group ≤2 years, 2-5 years, 5-20 years, 20-50 years and ≥50 years respectively. Samples were from patients having community –acquired pneumonia (CAP) 23 cases (46%), chronic obstructive pulmonary disease (COPD) 21 cases (42%), bronchiectasis 3 cases (6%), aspiration pneumonia 2 cases (4%) and others 1 case (2%).

Study of Chawla K et al [4] found 56% patients are of >50 years of age and 34% patients having age 30-50 years with 46% cases were from community-acquired pneumonia, 40% having COPD, 8% having bronchiectasis, and 6% having aspiration pneumonia. 48.65% isolates were found among 31-60 years age group in study of Kulkarni N et al. [14] thus pneumococcal isolates are more common among children under 5 years of age and in older patients while it varies with nature of disease invasive or noninvasive and site of infection. In our study, 30% pneumococcal isolates showed resistance, 18% intermediate resistance to penicillin (total 48%) while 52% were penicillin sensitive which is similar to study of Khademi F et al. [15] which showed penicillin resistance in 46.9% cases. Study of Amari S et al [16] showed penicillin

resistance in 57.2% and 42.8% penicillin sensitive isolates while Kulkarni N et al [14] found 70% penicillin resistance, Patil S. et al [17] found 61.75% penicillin resistance and Sharma S et al [18] found 7.4% resistance, 36.8% intermediate resistance for penicillin. Study of Chawla K et al [4] showed penicillin resistance in 4% and intermediate penicillin resistance in 10% cases and study of Kim JS et al [19] showed 2.8% resistance and 11.1% intermediate resistance for penicillin G.

In this study antimicrobial drug resistance was maximum for erythromycin (46%) followed by tetracycline (38%), cotrimoxazole (36%), cefotaxime (30%) and ciprofloxacin (14%) among pneumococci. Study of Amari S et al [16] conducted among children in Morocco shows resistance for erythromycin is 17.9 % and for tetracycline is 20.9% among pneumococcal isolates from respiratory specimens. Study of Chawla et al [4] showed maximum resistance (24%) for tetracycline and cotrimoxazole followed by ciprofloxacin and erythromycin having 14% resistance for each. Study of Khademi F et al [15] showed 41.1% resistance for erythromycin, 39.9% for tetracycline, 8.3 % for ciprofloxacin, 8.3% for cefotaxime along with 63.9% for trimethoprim/sulfamethoxazole, 53.2%, for azithromycin, 31.7% for clindamycin, 20% for chloramphenicol and also for other antimicrobials among S. pneumoniae.

Study of Kim JS et al [19] showed 75%, 69.4%, 66.7%, 38.9% and 19.5% resistance for tetracycline, erythromycin, clindamycin, cotrimoxazole and cefotaxime respectively among S. pneumonia isolates from invasive specimens of nonmeningitis patients.

Study of Kulkarni N et al [14] found 80% resistance for erythromycin and 40% resistance for clindamycin. Study of Patil S. et al [17] found 73.76%, 54.41% and 7.14% resistance for tetracycline, trimethoprim/ sulphomethoxazole and cefotaxime respectively while 94.78%, 57.5% and 47.37% intermediate resistance for erythromycin, ofloxacin and levofloxacin respectively.

Study of Sharma S et al [18] found maximum 76.5% resistance with 11.8% intermediate resistance for cotrimoxazole while 36.8%, 23.5%,17.6% resistance with 14.7%, 19.1%,11.8% intermediate resistance for tetracycline, erythromycin and ciprofloxacin respectively, though the resistance vary with the non-invasive and invasive nature of infection.

Thus penicillin resistance and multidrug resistance is common among pneumococci especially for erythromycin, tetracycline, trimethoprim /sulphomethoxazole and clindamycin. So empirical therapy should be initiated keeping this in mind.

Present study shows statistically significant correlation between penicillin resistance and multidrug resistance among pneumococcal isolates obtained from respiratory specimens who are similar to study conducted by Chawla et al [4] and Amari S et al [16].

Conclusion

Pneumococci can cause disease at any age though more frequently at extremes of age. Multidrug resistance is common among the penicillin resistant pneumococcal isolates.

Antimicrobial-susceptibility testing must be done for pneumococcal isolates among all age groups, to aid clinicians to select effective antimicrobial agent for pneumococcal infections.

Limitations of study

As study was hospital based only patients seek medical care were included in study and also a small scale study so the result may not be the exact reflection of whole population.

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