ANTIMICROBIAL RESISTANCE IN INVASIVE AND COLONISING STREPTOCOCCUS PNEUMONIAE IN NORTH INDIA

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Abstract

The present study was done to detect the antibiotic resistance in \textit{S. pneumoniae}. One hundred twenty \textit{S. pneumoniae} isolates from clinical specimens and 50 from nasopharyngeal sites were subjected to antimicrobial susceptibility testing by Kirby Bauer disk diffusion method and minimum inhibitory concentration (MIC) determination for penicillin and cefotaxime non-susceptible isolates. A total of 22 isolates (18.3\%) from clinical sites and eight (16\%) from nasopharyngeal sites showed decreased susceptibility to penicillin by oxacillin disk diffusion test. MICs of 26 of these resistant strains ranged from 0.12-1 \(\mu\)g/mL (intermediate resistance) by broth dilution and E test. Only four isolates, two from sputum and two from nasopharyngeal swabs, showed MIC of 2 \(\mu\)g/mL (complete resistance). However, MIC of two cefotaxime resistant isolates (by disk diffusion) was in the susceptible range (0.5 \(\mu\)g/mL). Highest antimicrobial resistance was seen to cotrimoxazole (55.2\%) and tetracycline (61.2\%). Antimicrobial resistance to cotrimoxazole and tetracycline was much more in clinical isolates than colonizing isolates. Multi-drug resistant phenotype was detected in 76.9\% (20 of 26) of isolates that were intermediately sensitive to penicillin and 50\% (2 of 4) of penicillin resistant isolates (co-resistant to tetracycline and cotrimoxazole). Routine screening for antibiotic susceptibility is recommended for clinical isolates of pneumococci. Strains with reduced susceptibility to penicillin should be subjected to MIC determination to detect relative resistance or true resistance as such strains are associated with increased virulence. The choice of antibiotics should be guided by the prevalence of local resistance patterns of pneumococci.

Key words: Multidrug resistance, nasopharyngeal isolates, penicillin resistance, Streptococcus pneumoniae

\textit{Streptococcus pneumoniae} is the leading cause of morbidity and mortality worldwide especially among extremes of age and people with underlying disease. \textsuperscript{1,2} Penicillin has been the drug of choice for treatment of pneumococcal infections but the increasing number of reports of penicillin resistant pneumococci (PRP) throughout the world makes it essential to determine the prevalence of PRP regionally.\textsuperscript{1,2} Moreover the PRP has been reported to harbour resistance to other antimicrobial classes making the treatment much more difficult.\textsuperscript{2}

Although data on penicillin and other antimicrobial resistance in \textit{S. pneumoniae} is meagre from India, it appears that penicillin resistant and multi drug resistant (MDR) \textit{S. pneumoniae} were rarely being isolated until mid 1990s.\textsuperscript{3,4} This was probably due to the fact that most of the clinical laboratories were not routinely testing pneumococcus for antimicrobial susceptibility assuming 100\% susceptibility to most of the antibiotics. With the increase in case fatality rates due to \textit{S. pneumoniae} and worldwide emergence of drug resistant pneumococci it becomes imperative to do continuous surveillance of antimicrobial resistance patterns with emphasis on PRP to recognize the potential hazards associated with \textit{S. pneumoniae} infections.

This study was undertaken to determine the antimicrobial susceptibility patterns and penicillin resistant rates of \textit{S. pneumoniae} in invasive and colonizing isolates as antimicrobial resistance is not confined to hospitals but also exists in the community.\textsuperscript{9}

Materials and Methods

The study was conducted in the Department of Microbiology, University College of Medical Sciences and GTB Hospital. One hundred seventy isolates of \textit{S. pneumoniae} from various clinical specimens and 400 nasopharyngeal swabs were identified and confirmed by standard biochemical reactions from April 1999 to April 2002.\textsuperscript{10}

Antimicrobial susceptibility testing

Disc diffusion method

Pneumococci were screened for penicillin resistance (oxacillin 1 \(\mu\)g, Oxoid) by Kirby Bauer disc diffusion method as per NCCLS guidelines (presently CLSI).\textsuperscript{11} Other antimicrobials tested by disc diffusion method were erythromycin (15 \(\mu\)g), cotrimoxazole (25 \(\mu\)g), chloromycetin (30 \(\mu\)g), tetracycline (30 \(\mu\)g), ciprofloxacin (5 \(\mu\)g), cefotaxime (30 \(\mu\)c). All discs were obtained from Hi-media, Mumbai, India. Zone diameter interpretative standards were defined...
according to NCCLS CLSI guidelines for antibiotics other than cefotaxime and ciprofloxacin which were interpreted as for other haemolytic streptococci. Strains showing a zone diameter ≤ 19 mm around oxacillin disc were tested for minimum inhibitory concentrations (MICs) by broth dilution method and by E test strip. All the isolates with cefotaxime zone diameter ≤ 25 were tested for MIC by broth dilution method.

**Broth dilution method**

Inoculum was prepared by suspending growth obtained from 5% sheep blood agar plates in 0.9% saline to a turbidity equivalent of 0.5 McFarland standard. The suspension was further diluted with in 15 min to give a final inoculum density of 5x 10^5 CFU/mL in each tube containing 1 mL of drug diluted in Mueller Hinton broth with 5% lysed sheep blood. The following concentration of penicillin and cefotaxime were used: 0.03 µg/mL, 0.06 µg/mL, 0.12 µg/mL, 0.25 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL and 4 µg/mL. The test tubes were incubated at 35°C in ambient air for 20 to 24h prior to determination of MIC. MIC of ≤ 0.06 µg/mL defined pneumococci susceptible to penicillin, MIC of 0.12-1 µg/mL defined intermediate or relative resistance and MIC ≥ 2 µg/mL defined resistance to penicillin. For cefotaxime susceptibility breakpoint was ≤ 0.5 µg/mL, intermediate when 1µg/mL and resistance when MIC was ≥ 2 µg/mL.

**E test**

MIC was also determined for penicillin by a standard E test strip (AB Biodisc, Sweden) as recommended for the oxacillin non-susceptible isolates.

**Results**

One hundred twenty isolates of *S. pneumoniae* were obtained from clinical specimens (blood, CSF, pleural fluid, ascitic fluid, ear swab, eye swab and sputum) and 50 from nasopharynx of healthy school (5-8 years) and college students (17-20 years). Isolation of *S. pneumoniae* from various sites is shown in Table 1.

**Table 1: Source of isolation of S. pneumoniae**

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>10</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>18</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>8</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>4</td>
</tr>
<tr>
<td>Ear</td>
<td>14</td>
</tr>
<tr>
<td>Eye</td>
<td>16</td>
</tr>
<tr>
<td>Sputum</td>
<td>50</td>
</tr>
<tr>
<td>Nasopharyngeal swabs</td>
<td></td>
</tr>
<tr>
<td>Healthy college students (n=200)</td>
<td>11</td>
</tr>
<tr>
<td>Healthy School students (n=200)</td>
<td>39</td>
</tr>
</tbody>
</table>

**Disc diffusion assay**

A total of 22 isolates (18.3%) from clinical specimens and eight isolates (16%) from nasopharyngeal sites showed decreased susceptibility to penicillin by oxacillin disc diffusion test. Resistance to cefotaxime and tetracycline was detected in 61.7 and 76.7% of isolates from clinical specimens and in 40 and 24% of nasopharyngeal isolates respectively. Twenty of 26 (76.9%) isolates were intermediate sensitive to penicillin and 50% (2 of 4) of penicillin resistant isolates were also resistant to tetracycline and cotrimoxazole. Resistance to chloramphenicol and cefotaxime (by disc diffusion method) was seen in 11.7 and 1.7% of clinical isolates respectively with no resistance to these antimicrobials in colonizers. No resistance was seen to erythromycin and ciprofloxacin. Resistance to only one drug was seen in 30 clinical isolates and eight nasopharyngeal isolates. Resistance to two or more drugs was seen in 70 clinical isolates and 14 nasopharyngeal isolates (Table 2).

**Minimum inhibitory concentration**

MIC of 26 penicillin resistant strains by oxacillin screening test ranged from 0.12-1 µg/ml by broth dilution and E test. Only four isolates, two from sputum and two from nasopharyngeal swabs showed MIC = 2 µg/mL. MIC of two cefotaxime resistant isolates (by disc diffusion) was 0.5 µg/mL each by broth dilution method.

**Discussion**

The increase in the incidence and the spread of antibiotic resistant pneumococci has placed a great emphasis on prompt and accurate recognition of resistant pneumococcal isolates by clinical microbiology laboratory. The surveillance of alteration in antibiotic susceptibilities due to time is important in recognizing the potential hazards associated with *S. pneumoniae* infections. Antimicrobial resistance in *S. pneumoniae* is not confined to hospital but also exists in community and it becomes important to identify antimicrobial resistance patterns in both the community and the hospitals. Antimicrobial resistance patterns of these community strains is largely unknown and recently the India CLEN community antimicrobial resistance study group has undertaken to analyse antimicrobial resistance pattern and trends over time in both rural and urban communities during a three-year period.

Though very few studies are available from India on nasopharyngeal colonisation by *S. pneumoniae*, all have reported a highly variable rate (24.3 to 81%) of colonisation with a higher colonisation rate among infants which gradually decreases with age. In the present study, colonisation with *S. pneumoniae* was found in 12.5% of healthy students, with 19.5% among school children (5-8 years) and 5.5% in college students (17-20 years).

Oxacillin disc test has been considered cost-effective.
to screen isolates in areas with very low penicillin resistant rates despite its inability to distinguish intermediate resistant strains or strains that demonstrate borderline susceptibility to penicillin. Further MIC testing with penicillin and alternative agents in oxacillin resistant strains is a much logical approach in resource poor settings. In the present study, 18.3% of clinical isolates and 16% of nasopharyngeal isolates were resistant to penicillin by oxacillin disc diffusion method. Fortunately, determination of MIC of these strains by broth dilution and E test revealed that 86.7% of isolates had MIC between 0.12-1 µg/mL (intermediate resistance). Only four isolates had complete resistance to penicillin (MIC=2 µg/mL). In an earlier study from the same institute high level penicillin resistance was reported in 8% and intermediate resistance in 14% of 340 S. pneumoniae isolates by disc diffusion method (MIC determination was not done). Evidence indicates that high dose penicillin may be used to treat penicillin intermediate pneumococcal infections outside the CNS but clinical experience has shown that penicillin is not an adequate regimen for treatment of penicillin intermediate and resistant strains. Analysis of studies from India reveals that intermediate resistance to penicillin in pneumococcus is much more prevalent than complete resistance but continued surveillance is a must as at any time high resistance to penicillin can appear in S. pneumoniae. However in United States the intermediate resistant rates are generally lower and the relative prevalence of complete resistance is higher.

Extended spectrum cephalosporins have been successfully used for the treatment of strains intermittently sensitive or resistant to penicillin in serious infections provided MIC is \( \leq 0.03 \mu g/mL \) for cefotaxime and cefotaxime. Resistance to cefotaxime and cefotaxime has been reported to be very low from India. In the present study, no resistance was detected for cefotaxime by MIC determination although two strains from sputum showed resistance with disc diffusion method. Resistance to tetracycline and cotrimoxazole was quite high in both the clinical and nasopharyngeal isolates possibly due to the wider use of these antibiotics in the hospital as well as the community because of the dose convenience, cost-effectiveness and easy availability over the counter. Resistance to chloramphenicol was seen in 11.7% of clinical isolates while other Indian studies have shown a low prevalence of chloramphenicol resistance. However, an earlier study from our institute showed higher resistance to chloramphenicol (28%). No resistance was encountered for erythromycin in the present study similar to other studies where erythromycin resistance has varied from 0 to 4.2%. Though, erythromycin is widely used drug for the treatment of acute respiratory infections, it has escaped resistance in our strains and thus can still be used for empirical treatment.

Multidrug resistant S. pneumoniae, defined as resistant to penicillin and two or more non β lactam agents such as macrolides, cotrimoxazole or tetracycline, are increasingly being reported from many parts of the globe. Penicillin susceptibility is an important marker for the presence or absence of a multidrug resistant phenotype. Strains with
reduced susceptibility to penicillin are usually cross-resistant to other antibiotics but in our study such cross-resistance was seen only with tetracycline and cotrimoxazole. The multidrug resistant phenotype occurred in 76.9% (20 of 26) and 50% (2 of 4) of penicillin intermediate and resistant isolates respectively as against other Indian studies where multi drug resistance phenotype has been reported only once.7

The increasing penicillin and multidrug resistance of S. pneumoniae worldwide has important clinical implications. Routine screening for antibiotic susceptibility, judicious use of antibiotics, continued surveillance and attention to pneumococcal vaccination is advocated to inhibit the constant increase in resistance in pneumococci.

References


Source of Support: Nil, Conflict of Interest: None declared.

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