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RESEARCH ARTICLE

DRUG RESISTANT STREPTOCOCCUS PNEUMONIAE IN LOWER ASSAM: A HOSPITAL BASED STUDY

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ABSTRACT

Introduction: Drug resistant Streptococcus pneumoniae (DRSP) is a common problem-afflicting world over. Delay in isolation of pathogens and rapidly evolving drug resistance globally are making the effective management of condition like CAP, especially in developing countries, very challenging. Empirical therapy, based on knowledge of local drug resistance pattern is the mainstay. This study was a preliminary work in DRSP from CAP subjects. **Aim:** Identification of common agents of our CAP subjects and to study the pattern of drug resistant isolates. **Methods:** Semi quantitative culture method was employed on sputum sample followed by drug sensitivity testing based on disc diffusion technique. **Results:** Adult CAP was found to be more common in middle-aged to elderly male with Strpetococcus pneumoniae in more than one fourth of the subjects. Beta lactam resistance in Pneumococci was high and drug resistance in other agents were found to be of moderate to high level. **Conclusion:** DRSP is a menace and it needs to be contained urgently. A larger study with more intensive experimental component is the need of the hour.

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INTRODUCTION

Common identifiable isolates of adult Community-acquired pneumonia (CAP) can vary with factors like geographical locations, age of the patients, clinical profile of the patients, co-morbid conditions etc. However, *Streptococcus pneumoniae* is considered the principal pathogen of CAP world-wide and a considerable focus on its recent observation of fast acquisition of drug resistance against many commonly employed drug to treat CAP empirically. It is more so due to heavy reliance on empirical therapy of CAP cases due to time and expertise necessary for accurate sensitivity determination in clinical setting, especially in underdeveloped regions (American Thoracic Society, 2001). Emergence of high rates of antimicrobial resistance has complicated the empiric management of CAP patients. Drug Resistance *S. pneumoniae* (DRSP) has been the focus of numerous recent studies, due to its high virulence and extraordinary rise in antibiotic resistance level in relatively short period (American Thoracic Society, 2001).

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Some studies carried out in India indicate existence and increasing threat of drug resistant strains of pneumococci, especially in respiratory tract infections (Kanungo, 2002 and Kanungo, 2001). Unfortunately, to the best of our knowledge, so far there is no published study on CAP or CAP associated DRSP or other drug resistance from North Eastern part of India.

Objective

To study drug resistance pattern and risk factor analysis of of Streptococcus pneumoniae isolates from adult community-acquired pneumonia cases attending a tertiary care hospital in lower Assam.

MATERIAL AND METHODS

About 94 clinically and/or radiologically diagnosed (as per definition of ATS) subjects of CAP visiting Gauhati Medical College during October 2005 to September 2006 were included in this study (American Thoracic Society, 2001). Inclusion criteria were as per ATS guidelines (American Thoracic Society, 2001). Sputum samples were collected as per standard guidelines, preferably before antibiotic administration (Duguid, 1989).

Table 1. Age and sex distribution

Age group in years	Male	Female	Total	Percentage
20-29	8	3	11	11.70
30-39	13	5	18	19.15
40-49	20	7	27	28.72
50-59	14	5	19	20.21
60-69	10	2	12	12.77
70-79	5	2	7	7.46
Total: (%)	70(74.47)	24 (25.53)	94(100)	100

Table 2. Severity of illness

Age group in years	Mild CAP (Outdoor treated)	Moderate CAP (Indoor treated)	Severe CAP (ICU treated)	Total
20-29	9	1	1	11
30-39	16	2	0	18
40-49	18	7	2	27
50-59	11	6	2	19
60-69	6	5	1	12
70-79	4	3	0	7
Total	64 (68.09%)	24(25.53%)	6 (6.38%)	94 (100%)

Table 3. Culture result, growth pattern and isolates

Culture results & growth pattern	Samples: culture positive			Samples: culture negative: no (%)	Total
	Monomicrobial: no (%)	Polymicrobial: no (%)	Total (%)		
	53 (56.38)	2(2.13)	55(58.51)	39(41.49)	94(100)
Organism isolated	53 (92.98)	4 (7.02)	57 (100)		

Table 4. Organisms isolated in culture positive samples

Organism isolated	Number of isolate from monomicrobial growth	Number of isolate from polymicrobial growth	Total	
			Number	Percentage out of 94 subjects
Streptococcus pneumoniae	24	0	24	25.53%

Table 5. Isolation of S pneumoniae in 3 different grades of illness severity

Organism / Severity	S. pneumoniae	Percentage (p-value)
Outdoor cases (mild CAP) /64	18	28.12 (0.0432)
Indoor cases (moderate CAP)/24	4	16.67 (0.983)
ICU cases (Severe CAP)/6	2	33.33 (0.977)
Total	24	25.53

(Abbreviation used: - SP=*S.pneumoniae*)

Semiquantitative culture technique was adopted (Duguid, 1989). Suitability of Sputum samples (for culture) was checked as per Murray-Washington criteria defined elsewhere (Murray, 1975). Selected samples were homogenized by use of dithiothreitol (Mucasol) (NHS Standards Unit, 2000), Homogenized samples were subjected to culture by standard semi quantitative culture method (Duguid, 1989). 0.005 ml each of this (representing 0.000025 ml of original unhomogenized sputum sample) was inoculated into 4 different culture media {Blood agar, MacConkey agar, Chocolate agar and CVNG agar (Crystal violet, Nalidixic acid, gentamicin blood agar – selective for pneumococci)}. Blood agar and MacConkey agar plates were incubated aerobically at 37^{o} C overnight while CVNG agar (with Optochin disc) and Chocolate agar were incubated with 5-10% CO_2 under similar environment (Duguid, 1989 and Murray, 1975). After incubation, presence of 25 or more colonies of the same agent (in any plate) implied presence of 10^6 or more of this agent per ml of original sputum, indirectly suggesting a pathogenic role. Any growth lesser than this was dis-regarded as commensal/contaminant (Duguid, 1989).

Identification and antibiotic susceptibility of the isolates were performed as per standard guidelines (Clinical and Laboratory Standards Institute, 2011 and Collee, 1989).

RESULTS

Table 1 outlined below shows the general clinic-epidemiological features of 94 subjects included in the study. Table 3 shows that 58.51% samples yielded significant growth with 53 samples mono-microbial, while 2 samples yielded double bacterial isolates. Total isolates recovered were 57 (53 & 4). Table 4 depicts : *Streptococcus pneumoniae* exclusively growing in mono-microbial isolation only and it grew in 25.53% samples (24/94). Table 5 shows pneumococci associated CAP may be more associated with outdoor setting (significant statistically) than in indoor or severe cases. Table 6 shows risk factors and co-morbidities associations with Pneumococci. None of them seems to be significant. Table 7 shows 75% isolates to be Oxacillin (1mcg disc) resistance – indicating a probable PBP2a related resistance with epidemiological significance.

Table 6. Showing pneumococci isolation with reference to comorbid illness/ risk factors

Organisms / Risk factors	*S P* (%, p value)
Smoking	16 (43.2, 0.8679)
Alcoholism	6 (33.3, 0.4040)
Diabetes	0
Old age	0
Chronic lung disease	2 (25, 0.2502)
Previous hospitalization	14 (50,0.2276)
Prior antibiotic exposure	19 (38.8, 0.2444)
Precedent viral fever	0

Table 7. *Streptococcus pneumoniae* drug resistance pattern

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Oxacillin 1 µg	6 (25.0%) 18.75		18 (75.0%)
Chloramphenicol	14 (58.33%)	1 (4.17%)	9 (37.55%)
Tetracycline	5 (20.83%)	12 (50.0%)	7 (29.17%)
Erythromycin	15 (62.5%)	2 (8.33%	7 (29.17%)
Clindamycin	20 (83.33 %)	1 (4.17%)	3 (12.5%)
Linezolid	24 (100.0%)	0	0
Ciprofloxacin	8 (33.33%)	10 (41.67%)	6 (25.0%)
Gatifloxacin	15 (62.5%)	6 (25.0%)	3 (12.5%)
Levofloxacin	10 (41.67%)	10 (41.67%)	4 (16.67%)
Moxifloxacin	23 (95.83%)	1 (4.17%)	0
Ofloxacin	8 (33.33%)	7 (29.17%)	9 (37.5%)
Amoxyclav	13 (54.17%)	3 (12.5%)	8 (33.33%)
Co-trimoxazole	0	0	24 (100.0%)
Vancomycin	24 (100%)	0	0

Table 8. Risk factors for β-lactam resistant*S pneumoniae* causing CAP

Risk factors	β-lactam resistant*Streptococcus pneumoniae* (n=18)	Percentage	*p* value
β-lactam therapy in last 3 months	10	55.56	0.0097
Old age	4	22.22	0.2058
Alcoholism	4	22.22	0.7716
Multiple medical comorbidities	0	0	

About 25% isolates were Ciprofloxacin resistant (37.5% for ofloxacin) while near about 30% isolates were resistant to Macrolides. Amoxyclav resistance was depicted by one third of isolates. However Vancomycin and Linezolid were sensitive in all isolates. Table 8 depicts β-lactam therapy in last 3 months was significantly associated with β-lactam resistant *Streptococcus pneumoniae* isolates.

DISCUSSION

Pneumonia is increasingly recognized as a serious issue among older patients and those with comorbidity (American Thoracic Society, 2001). Although not much new antibiotics are in pipeline to tackle this ailment, fast evolution of bacterial resistance here a reality staring at us now. Many respiratory pathogens have become resistant to widely used antimicrobials including *Streptococcus pneumonia* (American Thoracic Society, 2001). The subjects in this study were between 20 to 75 years with a highest prevalence in 40-49 year age group (28.72%). This observation was similar to study by Bansal *et al*. patient older than 40 years found to be more predisposed to development of CAP (Bansal, 2004). 58.51% samples showed growth of isolates, which was similar to isolation rate of Sopena *et al.* at 58% (Sopena, 1999). The present study showed the dominance of *Streptococcus pneumoniae* (25.53% of subjects) and significantly associated with outdoor (mild cases) subjects. Ishida et al (23.0%) & Ruiz et al noted similar finding (29.0%) while Peñafiel et al (10.5%) had lower rate though Bansal et al. (35.8%), Lim et al. (48.0%) and Jokinen et al. (41.0%) all had higher rate (Bansal, 2004; Ishida, 1998; Jokinen, 2001; Lim, 2001 and Song, 2004). 75% of the pneumococci isolates were found to be resistant to β-lactam antibiotics. Song *et al.* found 52.4% pneumococcus with reduced susceptibly to penicillin (Song, 2006). Kanungo *et al.*found non-susceptibility at 11.6% (Kanungo, 2002). Another study Kanungo et al found 7.3% of isolates to be intermediately resistant to penicillin (Kanungo, 2001). Among many known factors of penicillin resistant Pneumococcus, only β-lactam therapy during last 3 months was found to be statistically significant (p value 0.0097). 62.5% of *Streptococcus pneumoniae* isolates were sensitive to Erythromycin. Amongst the fluoroquinolones, Moxifloxacin was sensitive in 95.83% of isolates while 41.67% isolates were sensitive to Levofloxacin. Ciprofloxacin sensitivitiy was observed in 33.33% isolates. This resistance was high compared to other studies worldwide {e.g. Song et al. (11.8%)}.[16] Increasing and indiscriminate use of drugs like Ciprofloxacin could be an explanation of such high rate of resistance observed in this study.

Conclusion

Streptococcus pneumoniae is an important pathogen and it is more so in mild CAP cases where hospitalization is not

required. The findings, of large proportion of β- antibiotic resistant *Streptococcus pneumoniae* as well as detection of resistance against other common use drugs is really alarming. A wider study with variety of samples and molecular methods may give a better picture of the situation.

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