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Bacteriological Profile Of Community Acquired Lower Respiratory Tract Infections and IgM Antibody Detection for Mycoplasma Pneumoniae and Chlamydia Pneumoniae

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Original Research Article

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Abstract: Community acquired lower respiratory tract infections are common cause of acute illness in adults. The present study was undertaken to identify the etiological agents of community acquired lower respiratory tract infections. Methods 132 patients with symptoms of community acquired lower respiratory tract infection were included in study. Sputum culture and serological studies for the detection of *Mycoplasma pneumoniae* and *Chlamydiaepneumoniae* specific IgM antibodies by enzyme linked immunosorbent assay were done. Results: Rate of isolation from sputum culture, was 53.7%.Serology for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*IgM antibodies were 9.1% and 2.2% respectively. Organisms isolated were *Klebsiellapneumoniae* 21.1%, optochin resistant *Streptococcus pneumoniae* 16.6%, followed by *Staphylococcus aureus, Escherichia coli, Pseudomonas* and *Moraxella*. Gram negative organisms were sensitive to third generation cephalosporins. 17% were ESBL producers. *Streptococcus pneumoniae* was sensitive to ampicillin and erythromycin. Conclusion: Prevalence of optochin resistant *Streptococcus pneumoniae* with capsule was 16.6%. As phenotypic identification of *Streptococcus pneumoniae* is difficult based on optochin sensitivity, bile solubility and inulin fermentation tests, further evaluation of optochin resistant capsular *Streptococcus pneumoniae* by latex agglutination, PCR is recommended for their detection. Since seropositivity for *Mycoplasma pneumonia* is high, it is recommended to screen for these organisms also in culture negative pneumonia cases.
Keywords: Pneumonia, sputum culture, mycoplasma pneumoniae, Chlamydia pneumoniae, ELISA.

INTRODUCTION

Community acquired lower respiratory tract (CALRTI) is a common cause of acute illness in adults. The spectrum of disease ranges from a mild mucosal colonization, acute bronchitis or acute exacerbation of chronic bronchitis to overwhelming parenchymal infection with patient presenting with severe community acquired pneumonia (CAP). Although majority of LRTIs are self limiting viral infections, CAP is often bacterial disease with substantial morbidity.

Community acquired pneumonia remains a common and serious illness with significant morbidity and mortality despite the availability of potent antibiotics [1]. Pneumonia is a microbial infection involving the terminal airways and alveoli of the lung. Pneumonia results in more than 500000 hospital admissions annually in adults and ranks as the sixth leading cause of death in United States. The problem is much greater in developing countries. Though definite statistics are lacking pneumonia remains a leading cause of death in India [2].

The increase in resistant respiratory pathogens to antibiotics has complicated the use of empirical treatment with traditional agents .Prevalent flora and antimicrobial resistance pattern may vary from place to place depending upon the antibiotic usage in that area. Thus, there is a great need for local resistance prevalence data in order to guide empirical prescription and to identify areas in which medical need for new agents is greater [3]. The present study would, therefore, be required for effective management of community acquired lower respiratory tract infections

The present study was undertaken to identify the bacteriological causes of community acquired lower respiratory tract infection ,to detect the seroprevalence of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in community acquired lower respiratory tract infections

and to study the antibiotic susceptibility pattern including extended spectrum beta lactamases in organisms isolated.

MATERIALS AND METHODS

The present study was conducted from January to October at department of microbiology at tertiary care hospital. The study group included 132 patients attending outpatient department or admitted with at least two of the following symptoms-fever, cough, production of purulent sputum, breathing difficulty, chest pain, laboratory finding-leucocytosis (WBC > 10,000/cumm) and new infiltrate in chest radiograph. Inpatients samples were collected within 24hrs of admission. Demographic and clinical data were recorded using a structured proforma. Samples collected were sputum for culture and sensitivity and serum for IgM antibody detection of *Chlamydia pneumonia* and *Mycoplasma pneumonia*.

Sputum gram stain was done and sample accepted according to Barletts scoring. It was then subjected to culture by inoculating on Blood agar, Chocolate agar, and Mac Conkey agar. Plates were incubated at 37^0C for 18-24 hrs in candle jar. Capsular demonstration for *Streptococcus pneumococci* was done by dry India ink method [4].

All the isolates were identified by standard biochemical reactions [4]. Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method as per CLSI guidelines. Antibiotics used were ampicillin(10µg), gentamicin (10 µg), cefotaxime(30 µg) ceftazidime(30 µg), cephalexin(30 µg), ciprofloxacin(5 µg), cotrimoxazole(25 µg) tetracycline(30 µg), using standard strains *Klebsiellapneumoniae* ATCC 25922, *Escherichia coli* ATCC 700603 and *Streptococcus pneumoniae* ATCC 49619 as controls. Gram negative organisms resistant to third generation cephalosporins were confirmed by double disc synergy test and disc potentiation test methods for ESBL production as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Double disc synergy test:Inoculum was standardized with 0.5MC Farlands and swabbed on to a 90mm Muller- Hinton agar plate. A susceptibility disk, containing Amoxycillin/clavulanate was placed in the centre of the plate and discs of ceftazidime, cefotaxime were placed 20mm (centre to centre) from the Amoxycillin/clavulanate disc. The presence of distinctive enhancement of the inhibition zone towards the Amoxycillin/clavulanate disc was, considered as positive for ESBL production.

Disk potentiation test**:** In this test a pair of discs containing cephalosporin with and without clavulanic acid was placed on opposite sides of the same inoculated plate. The test organism was regarded as an ESBL producer if the zone of inhibition around the combination disk was atleast 5mm larger than that of the cephalosporin alone.

Serum samples were subjected to ELISA (Euroimmun) according to manufactures instructions to detect IgM antibodies against *Chlamydia pneumoniae and Mycoplasma pneumonia*

RESULTS

The common age group affected was 46-55 yrs. In the study group male predominance was seen. The male to female ratio was 2:1.

Cough, fever, chest pain and dyspnoea were common symptoms. Smoking and alcoholism were common associated risk factors.

Table 1: Age wise distribution of cases (n=132)

Age	No	%
15-25	22	16.6
26-35	20	15.15
36-45	20	15.15
46-55	34	25.75
56-65	29	21.96
>65	7	5.3

Table 2: Sex wise distribution of cases (n=132)

Sex	No	%
Males	88	66.6
Females	44	33.3

Table 3: Associated risk factors (n=132)

Risk factor	No	%
Smoking	59	44.6
Alcohol	50	37.8
Diabetes mellitus	12	9.0

COPD (asthma)	15	11.3

A total of 132 patients were screened for pathogenic organisms, 71 patients were sputum culture positive(53.7%).The major pathogen isolated was *Klebsiellapneumoniae*21.1% followed by optochinresistant *Streptococcus pneumoniae*16.6%, *Staphylococcus* 8.3%, *Escherichia coli* 3.7%, *Pseudomonas* 2.5%, *Moraxella catarrhalis* 2.5%.

Table 4: Isolates in sputum culture n=132

Isolate	No	%
Klebsiella	28	21.1
Streptococcus pneumoniae	22	16.6
Staphylococcus aureus	10	8.3
Escherichia coli	5	3.7
Pseudomonas	3	2.5
Moraxella catarhalis	3	2.5
Total	71	53.7

Gram negative organisms identified were more susceptible to third generation cephalosporins .*Streptococcus pneumoniae* was found to be sensitive to erythromycin, ampicillin, third generation cephalosporins17.8% of *Klebsiellapneumoniae* were ESBL producers. 50% of the *Staphylococcus aureus* isolates were resistant to methicillin. 87 persons were tested for prevalence of IgM antibodies of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* and detection rates were 9.1% and 2.2% respectively.

Table 5: IgM antibody detection of atypical pathogens by ELISA (n=87)

Pathogen	No (n=87)	%
Chlamydia pneumoniae	2	2.29
Mycoplasma pneumoniae	8	9.1

DISCUSSION

Our study consisted of 132 patients out of which 65.9% were out patients and 34.1% were inpatients. Males were found to be more commonly affected than females. The male to female ratio was 2:1. This correlated with studies conducted in India by Oberai et al(1.5:l)[1], and in Spainby Sopena et al(2.5:1)[5].

Patients ranged in age from 16 –80 yrs. The most affected age group was 45- 65 yrs. The maximum age was 80yrs and the minimum age 16 yrs. The mean age was 55.2 which correlated with other studies who found the mean age to be 56.9 by Reechaipichitkul W et al [6].The most common identified risk factor was smoking 44.6% which correlated with study done by Shah BA who reported smoking as risk factor in 65% [7].

Sputum culture was positive in 53.7%. Similar observations were reported by Chawla. K et al as 50.9% [8]and 42% by Arancibia F et al [8].The most common isolated pathogen was *Klebsiellapneumoniae* 21.1% followed by optochin resistant *Streptococcus pneumoniae* 16.6%, *Staphylococcus aureus* 8.3%. It correlated with the study conducted in India by Bansal.S[2], who found the isolation of *Klebsiellapneumoniae* 22.6%. Though *Streptococcus pneumoniae* have been reported as the commonest organisms causing community acquired pneumonia, Indian studies over the last three decades have reported higher incidence of Gram negative organisms among culture positive pneumonia [1]. Gram negative bacteria are a common cause of CAP in some Asian studies and are predominant cause in Malaysia and Singapore. Increased incidence of *Klebsiellapneumoniae* CAP in Asian countries may reflect the effects of different environmental conditions on transmission, or possibly an increased frequency of host factors such as abnormal nutritional status, comorbidities or genetic background that may favour*Klebsiellapneumoniae* infection [10]. In our study *Klebsiellapneumoniae* was the major pathogen. This can be due to our hospital being a tertiary referral hospital we receive community acquired pneumonia patients with wide range of severity, many of them carrying multiple co morbidities. These patients might have been exposed to antibiotics for treatment of respiratory or non respiratory tract infections.

Capsulated optochin resistant *Streptococcus pneumoniae* were 16.6%. These were identified based on morphology and dry India ink method of capsular staining, optochin sensitivity, bile solubility and inulin fermentation test. Optochin susceptibility and encapsulation are the phenotypic characteristics that are the most frequently used to differentiate between *Streptococcus pneumoniae* and other streptococci. Optochin resistant *Streptococcus pneumoniae* strains are

being isolated more frequently but overlooked since in many laboratories primary isolation relies on optochinsusceptibility [11]. Even though the phenotypic identification of typical pneumococci is unambiguous, the existence of optochin resistant isolates may increasingly cause problems in clinical bacteriological laboratories. Optochin resistance result from mutations in H+ ATPase jeopardizing the detection and correct identification of pneumococci. It was concluded that optochin sensitive α hemolytic Streptococcus is almost always a pneumococcus, for optochin resistant isolates the presence or absence of capsule should be verified by quellung reaction or counterimmunoelectrophoresis method or colony morphology [11].

Moraxella catarrhalis was isolated in 3 cases (2.5%), 1 case was diabetic. Isolation rate correlated with Diane C Halstead et al 1-2% [12], and Lim WS et al 2% [13]. Adults with chronic lung disease have higher rates of *Moraxella catarrhalis*respiratory tract colonization when compared to healthy adults. It occurs predominantly in winter months and patients having underlying cardiopulmonary disease, COPD, bronchiectasis and immunosuppression.[14]

Table 6: Antibiotic susceptibility pattern of isolated microorganisms

Organism	Ap	Cp	Cz	Cx	Ci	Co	G	Er	Te	Ox	Vm	Pt	I
Streptococcus pneumoniae(22)	72.7	40	100	100	100	0	-	82	27.2	--	_	_	_
Klebsiellapneumoniae (28)	0	39.28	75	78	57.1	28.5	64.2	-	-	-	-	-	-
Staphylococcus aureus (10)	50	60	100	100	80	40	-	50	50	50	100	-	-
Escherichia coli (5)	40	20	80	80	60	20	80	-	-	-	-	-	-
Pseudomonas aeruginosa (3)	-	33	66.5	-	66.5	-	33	-	-	-	-	100	100
Moraxella catarralis 3	66.6	100	100	100	66.6	33.3	-	-	-	-	-	-	--

Ap-ampicillin, Cp- cephalexin, Cz—ceftazidime, Cx- cefotaxime, Co- cotrimoxazole, G- gentamycin, Er- erythromycin, Te- tetracycline, Ox- oxacillin, Vm- vancomycin, Pt- piperacillintazobactum, I- imepenem

Klebsiellapneumoniae was highly sensitive to cefotaxime 78% ceftazidime 75%, gentamycin 64.%, ciprofloxacin 57.1% and resistant to ampicillin, cotrimoxazole 71.5%. Similar observations were reported by Chin Yow Wen et al [15], Okesola AO et al [16] andLoh LC et al [17]. The prevalence rate of ESBLs was 17% similar to studies by Rammaert B et al 17% [18]. Loh LC et al [19] and Babay HA et al [20].

Streptococcus pneumoniae was 100% sensitive to cefotaxime, ceftazidime, ciprofloxacin, 72.7% to ampicillin, 82% to erythromycin and resistant to tetracycline 72.8% , cotrimoxazole 100% similar to studies conducted byOkesola AO et al[16], Diane C Halstead et al [12],Capoor MR et al [21]. The prevalence rate of MRSA was 50% which correlated with Diane C Halstead et al 50% [12], Lim WS et al 2% [13].

The concept of "presumptive antimicrobial therapy" could replace that of "empiric antimicrobial therapy"; based on common pathogens, known susceptibility patterns and host factors in any given region. Local studies should be carried out to elucidate the mechanisms of resistance of different pathogens. Judicious use of antimicrobials is essential to prevent the emergence of resistant bacteria.[3]

IgM antibodies detection for *Mycoplasma pneumoniae* was 9.1% similar to study from Koreaby Lee SJ et al 8.6% [22] and 11.3% by MukeshChoudhary et al. India [23]. IgM antibodies detection of *Chlamydia pneumoniae* was 2.2% in our study similar to study conducted by Kumar KR et al 1.1% from India [24]. All these patients were sputum culture negative for bacteria.

The frequency and importance of atypical pathogens have significant implications for therapy. These organisms are intrinsically resistant to all beta-lactam agents and must be treated with a macrolide, a fluoroquinolone, or a tetracycline. Many atypical pneumonia cases mimic typical pneumonia in clinical features and laboratory findings. Thus, all the suspected cases of pneumonia should be screened for both atypical and typical pathogens and more than one test has to done for isolation of organisms for better diagnosis. Hence, appropriate serological investigation and prompt treatment are important to reduce complications and mortality. [25]

CONCLUSION

Klebsiellapneumoniae was the most common organism isolated. Prevalence of optochin resistant *Streptococcus pneumoniae* with capsule was 16.6%. As phenotypic identification of *Streptococcus pneumoniae* is difficult based on optochin sensitivity, further evaluation of optochin resistant capsular *Streptococcus pneumoniae* by latex agglutination and PCR is recommended. Since seropositivity for *Mycoplasma pneumonia* is high, it is recommended to screen for these organisms also in culture negative pneumonia cases. Because of the high incidence of resistant pattern of the

organisms, judicious use of antibiotics based on antibiotic susceptibility pattern is recommended.

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