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Original Research Article

Bacteriological profile and antibiogram in cases of pneumonia attending to tertiary care hospital

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ABSTRACT

Background: Causative bacteria differ in different regions and this knowledge is necessary for formulation of local antibacterial guidelines. Diagnosing the microbial etiology of pneumonia is challenging because the site of infection (lung tissue) is not easily accessible for specimen collection and contamination by upper respiratory tract secretions. This study aimed to determine the bacterial profile of pneumonia cases and its antibiogram.

Materials and Methods: A cross sectional, descriptive study was done at the Department of Microbiology involving 84 patients.Samples collected were sputum, induced sputum in children and endotracheal tube tip in mechanically ventilated patients following standard guidelines. All samples were processed within 2 hours of collection and subjected to Gram staining, incubated on Blood, Chocolate and Mac Conkey agar. Antibiotic susceptibility patterns, Methicillin resistance for Staphylococcus aureus and ESBL production among Gram negative bacteria was confirmed.

Results: Out of 84 cases studied, pathogenic growth was seen in 65(77.4%) and commensals were isolated in 19(22.6%) samples. The most common Gram negative bacteria was Klebsiella species(29, 42.7%) followed by Pseudomonas(20, 29.4%). Most common Gram positive bacteria was Staphylococcus aureus(4, 44.4%) and CONS(4, 44.4%). Staphylococcus aureus showed 100% resistance to Methicillin. Out of 20 Pseudomonas isolates, 6 were ESBL producers. Out of the remaining 48 Gram negative bacteria, 7 were ESBL producers.

Conclusion: Incidence of pneumonia has increased due to lack of early diagnosis and multidrug resistance. The incidence of Gram negative bacteria has also increased tremendously. According to this study, most of the organisms are resistant to 3rd generation Cephalosporins.

1. Introduction

Pneumonia, an inflammatory condition due to infection of the pulmonary parenchyma is often misdiagnosed and underestimated despite being the cause of significant morbidity and mortality.[1]

In India, as per 2015 data, pneumonia was responsible for 15% of all deaths in children under the age of 5 years accounting to 9, 22,000 deaths.[2]

Typical symptoms and signs include a varying severity and combination of productive or dry cough, chest pain, breathlessness, fever, chills, rigors, delirium, pulmonary infiltrates and consolidation on chest X-ray. Risk factors are age, gender, nature of work, environment and frequent prescription of antibiotics.[3] It affects all age groups but particularly common at the extremes of age. Most cases are spread by droplet infection.

Pneumonia is due to infections caused primarily by bacteria or viruses and less commonly by fungi and parasites. Streptococcus pneumoniae remains the most common infecting agent. The others include Hemophilus influenzae (31%), Respiratory syncitical virus, and influenza. In infants < 3 months, E. coli was the commonest organism (50%) followed by Klebsiella

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(25%).[4]

Community acquired pneumonia (CAP) refers to acute infection of lung parenchyma in patients not hospitalized for 14 or more days before presentation. Nosocomial pneumonia is an episode of pneumonia contracted by patients after 48 hours of admission. It is one of the most frequent nosocomial infections (30-33% of cases) among combined medical-surgical intensive care units.[5] Ventilator Associated Pneumonia (VAP) is a special type of nosocomial infection, affecting 8%-28% of patients receiving mechanical ventilation.[6] Increasing multidrug resistance in Gram negative bacteria due to Extended Spectrum β- Lactamase (ESBL) production, particularly in Actinobacter baumanii, Pseudomonas aeruginosa and Klebsiella pneumoniae and Methicillin resistance in Staph aureus has led to serious clinical problems.[7] Risk factors for VAP are shock and altered sensorium on admission, reintubations (if done multiple times), central line insertions, transfusions, feeding on ventilator, use of intravenous steroids, transport out of ICU.[8]

Causative bacteria differ in different regions and this knowledge is necessary for formulation of local antibacterial guidelines. Problems faced in India are necessity of radiograph, sputum collection, quality of sputum and culture, delay in presentation of the patients to hospital and lack of compliance to treatment, lack of workup for viral and atypical agents, urinary diagnostic tests, and problem of multidrug resistant bacterial pathogens.

Pneumonia is a significant problem in developing countries, and confirmation of microbial etiology is important for individual, as well as public health.

Diagnosing the microbial etiology of pneumonia is challenging because the site of infection (lung tissue) is not easily accessible for specimen collection and difficulty in obtaining samples without contamination by upper respiratory tract secretions.

Hence there is need for early diagnosis of pneumonia and improved access to care so that morbidity and mortality can be reduced to a large extent. We undertook this study to help clinicians identify the most common causative bacteria in our geographical area. Therefore, more effective and appropriate antibiotics can be prescribed which would also reduce multi drug resistance among bacteria.

The objective of this study was to determine the bacterial profile of pneumonia cases and its antibiotic susceptibility pattern. It also aimed to determine Methicillin resistance among Staph aureus isolates and ESBL production in Gram negative bacilli isolates.

2. Materials and Methods

Institutional ethical committee clearance was obtained and written consent taken from the patients or parent/ guardian in case of a minor before the beginning of the study.

2.1. Study design

A cross-sectional, descriptive study was done at the Department of Microbiology attached to our tertiary care hospital for a period of 2 months.

2.2. Study participants

Included 84 different patients of all age groups clinically diagnosed with primary or secondary pneumonia attending the OPD, Department of Pediatrics/ General Medicine/ Respiratory medicine or admitted to the Pediatric-ICU/ Medicine-ICU referred to the Department of Microbiology of our hospital. Complete history of onset, duration, progression of symptoms, past associated illnesses and other demographic details were collected from the patient or the attender.

2.3. Inclusion criteria

Clinically diagnosed cases of pneumonia (symptomatic), patients who developed symptoms of pneumonia after 48 hours of admission to the hospital and patients who developed symptoms 48 hours after being administered on the ventilator.

2.4. Exclusion criteria

Patients already on antibiotic treatment, patients with other lower respiratory infections like Bronchitis, Bronchiectasis, emphysema, hydropneumothorax, and patients clinically diagnosed with active Tuberculosis, HIV.

2.5. Sample collection

1. Sputum: Thick mucopurulent sputum collected in sterile screw capped container. Strict instructions about rinsing mouth with water and to expectorate after deep cough directly into sterile container were given to the patient.
2. Induced sputum collection: was done in pediatric patients (who cannot bring out sputum) using 3%-5% hypertonic saline in nebulizer.[9,10]
3. Endotracheal tube tip/ endotracheal aspiration: collected in mechanically ventilated patients following standard guidelines. In case of Endotracheal tube aspiration, colony count of $\geq$ 105cfu/ml was taken as diagnostic culture threshold.[11]

Induced sputum, endotracheal aspiration and endotracheal tube tip collection processes were done by a well-trained, skilled person following standard procedures (under the supervision of a pediatrician in required cases).

2.6. Sample processing

All samples collected were processed in Microbiology laboratory within 2 hours. Samples containing more of

saliva according to lab findings were rejected. All samples were subjected to Gram staining and culture.

i) Gram staining- to look for the presence of pus cells, epithelial cells and bacteria. The presence of <10 squamous cells and >25 PMN per low field, or $\geq$ 10 leucocytes for every squamous epithelial cell is indicative of high quality of expectorated sputum samples in adults. Hence, sputum samples showing less than above mentioned cell count were not included for culture as it is suggestive of oropharyngeal contamination.[2]

ii) Culture: Every sample was inoculated onto Blood agar, Chocolate agar and Mac Conkey agar plates and incubated aerobically at 37°C for 18-24 hours. Chocolate agar plate was incubated in candle jar at 37°C for obtaining good growth of pneumococci if any.

Growth obtained was identified based on colony morphology and standard biochemical reactions.[12]

Antibiotic susceptibility patters to various antibiotics was studied by Kirby-Bauer disc diffusion method on Muller Hinton Agar using Mc Farland's 0.5 turbidity standard for the inoculum.[13] Antibiotic discs tested for Gram negative bacilli (Entero bacteriaceae) were: Aminkacin, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenam, Meropenam, Cefipime, Netilmicin, Levofloxacin, Norfloxacin, Nitrofurantoin, Cotrimoxazole, Piperacillin+Tazobactum, Tetracycline, Ceftazidime, Ceftazidime+Clavulanic acid, Cefotoxime, Cefotoxime+Clavulanic acid, Aztreonam. Antibiotic discs tested for non entero bacteriaceae were: Aminkacin, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenam, Meropenam, Cefipime, Netilmicin, Ofloxacin, Cotrimoxazole, Piperacillin+Tazobactum, Ceftazidime, Ceftazidime+Clavulanic acid, Cefotoxime, Cefotoxime+Clavulanic acid, Aztreonam. Antibiotic discs tested for Gram positive cocci were: Ampicillin, Amoxicillin+Clavulanic acid, Amikacin, Cefoxitin, Ceftriaxone, Ciprofloxacin, Erythromycin, Gentamicin, Tetracycline, Linezolid, Cotrimoxazole.

Methicillin resistance for Staphylococcus aureus was detected using Cifoxitin (30μg) disc.

ESBL production among Gram negative bacteria was confirmed using the cephalosporin and cephalosporin/ clavulanic acid (cefotaxime and cefotaxime plus clavulanic acid, ceftazidime and ceftazidime plus clavulanic acid) combination disc test following clinical laboratory standard institute (CLSI) guidelines.[13]

3. Results

Out of the total 84 patients meeting all the inclusion criteria enrolled for the study, 60(71.4%) were male and 24(28.6%) were female. All age groups were considered for the study but most of them 25(29.76%) were between 60-69 years (Table 1).

Most of the samples collected were sputum (77, 91.7%) and 7(8.3%) were Endotracheal tube tips.

Table 1: Age distribution of patients

Age (In Yrs)	Frequency	Percent
< 10	6	7.14
10-19	4	4.76
20-29	5	5.95
30-39	8	9.52
40-49	6	7.14
50-59	9	10.72
60-69	25	29.76
$\geq$ 70	21	25
Total	84	100.0

In the commensals isolated, the most common were alpha-hemolytic streptococci. Out of 65 samples which yielded causative agents, 53 yielded single isolate and 12 yielded double isolates. Amongst the bacteria isolated, 9(11.7%) were Gram positive and 68(88.3%) were Gram negative. (Table 2)

Table 2: Culture results

Culture	No. of cases	Percent
Pathogenic growth	65	77.4
Commensals	19	22.6

Staphylococcus aureus (4,44.4%), Coagulase negative Staphylococcus(4, 44.4%) and Pneumococci(1, 11.2%) were the common Gram positive bacteria isolated. Among the Gram negative bacteria, the commonest organism isolated was Klebsiella species (29,42.7%) followed by Pseudomonas species (20, 29.4%), Acinetobacter (9, 13.2%), E.coli (9,13.2%) and Providencia spp (1, 1.5%). (Table 3). Methicillin resistance was seen in all 4 cases of Saphylococcus aureus isolates (100%) and in 1 case of CONS (25%).

Table 3: Gram positive and Gram negative Bacteria isolated

	Organism	Frequency	Percent
Gram Positive (N=9)	Pneumococci	1	11.2
	Staph aureus	4	44.4
	Staph CONS	4	44.4
Gram Negative (N=68)	Acinetobacter	9	13.2
	E.coli	9	13.2
	Klebsiella spp	29	42.7
	Providencia spp	1	1.5
	Pseudomonas	20	29.4

Among the Enterobacteriaceae, Extended Spectrum Beta-Lactamase (ESBL) production was noted in 7 of the 48 organisms, the most common being Klebsiella spp (3) followed by E.coli (2) (Table 4). Out of 20 isolates of pseudomonas spp, 6 were ESBL producers and 14 of them were non producers.

Among the Gram negative bacteria, Klebsiella spp were sensitive to Aztreonam (26, 89.6%), Gentamicin

Table 4: ESBL producers in Gram negative bacteria

Bacteria	ESBL		Total
	Producer	Non-producer	
Acinetobacter	1	8	9
E.coli	2	7	9
Klebsiella spp	3	26	29
Providencia spp	1	0	1
Total	7	41	48

(24, 82.8%), Norfloxacin, Levofloxacin and Meropenam (21, 72.4% each). E.coli was sensitive to Amikacin (8,88.9%), Aztreonam (7, 77.8%), Nitrofurentoin, Netilmicin and Meropenam (6, 66.7% each) (Table 5). Acinetobacter was sensitive to Aztreonam (8, 88.9%), Meropenam, Imipenam, Netilmicin and Ofloxacin (5, 55.6% each). 17 (85.0%) Pseudomonas isolates were sensitive to Amikacin, Netilmicin and Imipenam followed by Gentamicin (16, 80.0%) and Meropenam (16, 80.0%). Providencia spp was sensitive to Amikacin, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenam, Cefipime, Netilmicin, Levofloxacin, Nitrofurantoin, Cotrimoxazole and Tetracycline.

Among the Gram positive bacteria, Staphylococcus aureus was sensitive to Amikacin (4, 100%), Gentamicin (4, 100%) and Linezolid (4, 100%) Coagulase negative Staphylococcus was most sensitive to Amikacin, Cefotaxime, Gentamicin, Linezolid and Tetracyclin (3.75% each). Pneumococci was sensitive to Optochin, Tetracyclin, Linezolid, Amikacin, Gentamicin (100% each).

Out of all the cases taken for the study, few patients had other co-morbid conditions like Diabetes, Hypertension, Bronchial asthma, Epilepsy, etc. (Table 5)

Table 5: Associated co-morbid conditions

Co-morbid conditions	No of cases	Percent
Diabetes Melitus	14	16
Hypertension	12	13.8
Ischemic heart disease	1	1.1
Scoliosis	1	1.1
Asthama	2	2.3
Epilepsy	1	1.1
Idiopathic Thrombocytic Purpura	1	1.1

4. Discussion

Pneumonia is one of the leading causes of morbidity and mortality especially in developing countries like India. In this study, 84 patients were studied and majority of them were male, more than double the number of female cases. This could be attributed to the well-established fact that majority of predisposing factors like cigarette smoking, alocoholism, COPD, coronary artery disease, etc are more common and predominant in middle aged and elderly men. This is in accordance to an earlier study done by Sandeep Kumar Jain et al.[14]

The predominant age group in our study is 60-69 years (29.76%) which is higher than the study done by Sandeep Kumar Jain et al. where the mean age group was 52.36.[14] It is well documented that pneumonia incidence rises sharply with extremes of age.[15]

In the present study, 65(77.4%) cultures grown were pathogenic isolates and 19(22.6%) were commensals even though Gram staining was highly specific and showed plenty of pus cells along with bacteria. The samples were still considered for the study as they exceeded the Gram stain threshold values by an ample number. In a study done by Garcı E et al. in 2017, good quality sputum could be obtained only in 14.4% of all patients which is far below the percentage of samples obtained in the current study.[16]

Gram negative bacilli (68, 90.7%) out numbered the Gram positive cocci (9, 11.7%) in the bacteriological profile which was similar to the results obtained in a South Indian study by Vasuki V in which commonest organism isolated was Klebsiella(48.2%), Pseudomonas(15.3%), E.coli(8.4%), Acinetobacter(7.7%).[17]

Causal role of CONS in pneumonia is not well established. Out of the 4 cases from whom CONS was isolated in our study, one patient was diabetic and another was a neonate. These cases were considered due to lowered immunity. Whereas causal role could not be explained in the other 2 cases.

Methicillin resistance was seen in all 4 isolates of Staphylococcus aureus and showed sensitivity to Amikacin, Gentamicin and Linezolid in contrast to a study done by Abdulhakeem O Althaqafi et al in which all 93 MRSA isolates were sensitive to Linezolid, Vaccomycin and Teicoplanin.[18]

Many Klebsiella spp were sensitive to Aztreonam, Gentamicin, Meropenam, Norfloxacin which is in contrast to the study done by Vasuki V in which all Klebsiella isolates were sensitive to Imipenam (100%).[17]

Pneumococci was isolated in only one sample which is in complete contrast to the study done by Sandeep Kumar Jain et al. in which Streptococcus pneumoniae was the commonest pathogen (20, 36.4%).[14]

In the present study, ESBL production was seen in 7 isolates and 41 isolates were non-producers of ESBL which is comparitively lower than the study done by Maninder Kaur and Aruna Aggarwal in which 45%(299) of the isolates were found to be ESBL producers.[19] This implies that the organism is suggestive of multidrug resistance. Out of 20 Pseudomonas spp isolated, 6 were ESBL producers and 14 were non- producers. Even though the phenotypic method used in our study for detection of ESBL production is also adopted by many researchers, it may not be a legitimate method in case of Pseudomonas as they are intrinsically resistant and therefore have other mechanisms

of ESBL production. Multidrug resistance could be due to co-production of metallobetalactamase.

5. Conclusion

Incidence of pneumonia has increased due to lack of early diagnosis and multidrug resistance. The incidence of Gram negative bacteria as an etiological factor has also increased tremendously. According to this study, most of the organisms are resistant to 3^{rd} generation Cephalosporins. Further studies should closely examine the administration of initial therapy in pneumonia patients.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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