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Original Research Article

An observation study to determine the bacteriological profile and antibiogram in cases of pneumonia

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Abstract

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

Methods: This study was carried out in the Department of Microbiology, Madhubani medical college Madhubani, Bihar, India, for 1 year 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. 100 patients were included in this study.

Results: Most of the samples collected were sputum (77, 77%) and 7(7%) were Endotracheal tube tips. Out of 100 samples which yielded causative agents, 83 yielded single isolate and 17 yielded double isolates. Amongst the bacteria isolated, 77(77%) were Gram positive and 23(23%) were Gram negative. Staphylococcus aureus (8,8%), Coagulase negative Staphylococcus(8,8%) and Pneumococci(2,2%) were the common Gram positive bacteria isolated. Among the Gram negative bacteria, the commonest organism isolated was Klebsiella species (35,35%) followed by Pseudomonas species (20, 29.4%), Acinetobacter (9,13.2%), E.coli (9,13.2%) and Providencia spp (25,25%). (Table 3). Methicillin resistance was seen in all 8 cases of Saphylococcus aureus isolates (100%) and in 8 case of CONS (8%). 82, 82%), Norfloxacin, Levofloxacin and Meropenam (72, 72% each). E.coli was sensitive to Amikacin (88,88%), Aztreonam (77, 77%), Nitrofurentoin, Netilmicin and Meropenam (66, 66% each).

Conclusion: According to this study, most of the organisms are resistant to 3rd generation Cephalosporins. Further studies should closely examine the administration of initial therapy in pneumonia patients.

Keywords: gram positive, gram negative, antibiogram, pneumonia

Introduction

Uses of invasive drugs and therapeutic methods have saved many lives but on the other hand it has caused life threatening consequences due to severe, persistent, resistant infections. These invasive therapeutic and diagnostic methods the may lead to nosocomial infections particularly in Intensive Care Units'(ICU) and Critical Care

Units'(CCU). There is documented evidence that hospital personnel and environment are the microbial source, and prolonged hospital stay& overuse of antimicrobial agents has led to multidrug resistance of these microbes.[1] The tracheostomized patients are colonized mostly by gram negative bacteria which may cause either tracheobronchitis or bronchopneumonia.[2] Lower respiratory tract infections (LRTI) are most common bacterial infections among patients in neurological intensive care units, resulting in high overall mortality, which may range from 22% to 71%. [3] There is a dire need of epidemiological studies for ventilated patients, to know the local microbial flora and their antibiotic profiles for rational use of antibiotics. Hence, this study was undertaken to determine the prevalence of pathogenic bacteria in respiratory secretions of ventilated patients and their antibiotic susceptibility patterns.[4]

Material and methods

This study was carried out in the Department of Microbiology, Madhubani medical college Madhubani, Bihar, India for 1 year.100 patients were included in this study.

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.

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.

Methodology

Sample collection

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.

Induced sputum collection: was done in pediatric patients (who cannot bring out sputum) using 3%-5% hypertonic saline in nebulizer.[5,6]

Endotracheal tube tip/ endotracheal aspiration: collected in mechanically ventilated patients following standard guidelines. In case of Endotracheal tube aspiration, colony count of 105cfu/ml was taken as diagnostic culture threshold.

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).

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. 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 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.[7] 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. [8] 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. [9]Antibiotic discs tested for Gram negative bacilli (Entero bacteriaceae) were: Aminkacin, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenam, Meropenam,Cefipim-e, Netilmicin, Levofloxacin, Norfloxacin, Nitrofurantoin,Cotrimoxazole,Piperacillin+Tazobactum,Tetracycline,Ceftazidime,Ceftazidi-me+Clavulanic acid, Cefotoxime,Cefotoxime+Clavulanic acid, Aztreonam. Antibiotic discs tested for non entero bacteriaceae were: Aminkacin,Ceftriaxone,Ciprofloxacin,Genta-micin,Imipenam,Meropenam,Cefipime,Netil-micin,Ofloxacin,Cotrimoxazole,Piperacillin+Tazobactum,Ceftazidime,Ceftazidime+Clavulanic acid, Cefotoxime, Cefotoxime+Clavu-lanic 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. [9]

Results

Out of the total 100 patients meeting all the inclusion criteria enrolled for the study, 70(70%) were male and 30(30%) were female. All age groups were considered for the study but most of them 30(30%) were between 60-69 years (Table 1). Most of the samples collected were sputum (77, 77%) and 7(7%) were Endotracheal tube tips.

Table 1: Age distribution of patients

Age	Number	%
Below 10	7	7
10-20	4	4
20-30	5	5
30-40	9	9
40-50	8	8
50-60	11	11
60-70	30	30
>70	26	26
Total	100	100

In the commensals isolated, the most common were alpha-hemolytic streptococci. Out of 100 samples which yielded causative agents, 83 yielded single isolate and 17 yielded double isolates. Amongst the bacteria isolated, 77(77%) were Gram positive and 23(23%) were Gram negative. (Table 2)

Table 2: Culture results

Culture	No. of cases	%
Pathogenic growth	77	77
Commensals	23	23

Staphylococcus aureus (8, 8%), Coagulase negative Staphylococcus(8, 8%) and Pneumococci (2, 2%) were the common Gram positive bacteria isolated. Among the Gram negative bacteria, the commonest organism isolated was Klebsiella species (35,35%) followed by Pseudomonas species (20, 29.4%), Acinetobacter (9,13.2%), E.coli (9,13.2%) and Providencia spp (25,25%). (Table 3).

Table 3: Gram positive and Gram negative Bacteria isolated

Bacteria isolated	Organism	Frequency	Percent
Gram Positive (N=28)	Pneumococci	2	2
	Staph aureus	8	8
	Staph CONS	8	8
	Acinetobacter	10	10
Gram Negative (N=72)	E.coli	10	10
	Klebsiella spp	35	35
	Providencia spp	2	2
	Pseudomonas	25	25

Table 4: ESBL producers in Gram negative bacteria

Bacteria	Producer	Non- producer	Total
Acinetobacter	1	9	9
E.coli	1	8	9
Klebsiella spp	2	28	31
Providencia spp	1	0	1
Total	5	45	50

Table 5: Associated co-morbid conditions

Co-morbid conditions	No of cases	%
Diabetes Melitus	34	34
Hypertension	30	30
Ischemic heart disease	8	8
Scoliosis	8	8
Asthama	4	4
Epilepsy	8	8
Idiopathic Thrombocytes Purpura	8	8

Methicillin resistance was seen in all 8 cases of Saphylococcus aureus isolates (100%) and in 8 case of CONS (8%). Acinetobacter was sensitive to Aztreonam (88, 88%), Meropenam, Imipenam, Netilmicin and Ofloxacin (55, 55% each). 85(85%) Pseudomonas isolates were sensitive to Amikacin, Netilmicin and Imipenam followed by Gentamicin (80, 80%) and Meropenam

(80, 80%). 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 (100, 100%), Gentamicin (100, 100%) and Linezolid (100, 100%) Coagulase negative Staphylococcus was most sensitive to Amikacin, Cefotaxime, Gentamicin, Linezolid and Tetracyclin (3% each). Pneumococci were sensitive to Optochin, Tetracyclin, Linezolid, Amikacin, Gentamicin (100% each). E.coli was sensitive to Amikacin (88,88%), Aztreonam (77, 77%), Nitrofurentoin, Netilmicin and Meropenam (66, 66% 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)

Discussion

Health care associated infections (HCAI) continue to be a major cause of morbidity and device associated infections contributes a maximum towards Healthcare Associated Infections. The mechanically ventilated and tracheostomized patients are colonized with bacteria of either endogenous or exogenous origin which might end up in Ventilator Associated Tracheobronchitis or Ventilator Associated Pneumonia. [1] Bypassing of the upper respiratory tract and imperfect functioning of mucociliary escalator (due to insertion of tube in trachea) impair the immune system. Besides, leakage of secretion around the tube and opening of the binding site for gram negative bacteria may have cause high rate of colonization. [2]

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%). [10]

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. [11]

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%). [10]

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%). [12]

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. 13 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.[13,14]

Conclusion

According to this study, most of the organisms are resistant to 3rd generation Cephalosporins. Further studies should closely examine the administration of initial therapy in pneumonia patients.

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