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Original Research Article

A Hospital Based Study to Determine the Bacterial Profile of Pneumonia Cases and its Antibiogram

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Abstract

Aim: This study aimed to determine the bacterial profile of pneumonia cases and its antibiogram.

Methods: The present study was conducted at Department of microbiology, AIIMS, Patna, Bihar, India for a period of 12 months and 100 patients meeting all the inclusion criteria enrolled for the study. Institutional ethical committee clearance was obtained and written consent taken from the parent/ guardian in case of a minor before the beginning of the study.

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. Out of 75 samples which yielded causative agents, 60 yielded single isolate and 15 yielded double isolates. Amongst the bacteria isolated 9 (12%) were Gram positive and 66 (88%) were Gram negative. 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 (27, 40.90%) followed by Pseudomonas species (20, 30.30%), Acinetobacter (9, 13.63%), E. coli (9, 13.63%) and Providencia spp (1, 1.51%). Among the Enterobacteriaceae, Extended Spectrum Beta-Lactamase (ESBL) production was noted in 7 of the 46 organisms, the most common being Klebsiella spp (3) followed by E. coli (2).

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.

Keywords: Antibiogram, Bacterial Profile, MRSA, Pneumonia.

Introduction

Tracheobronchial secretions are produced by mucous glands and goblet cells of the tracheobronchial tree. [1] These secretions are not only involved in the protection of the respiratory tract but are also responsible for the exchange of heat and water during breathing. [2] Respiratory infections are associated with high morbidity and mortality, especially in critically ill patients. [3] Such patients are usually maintained using invasive devices which themselves tend to be a major

reservoir for hospital-acquired infections. [4,5] About 15% of hospital acquired infections (HAIs) are caused by ventilator-associated pneumonia (VAP). This is the second-most-common HAI having the highest morbidity and mortality. [6]

Bloodstream infections (BSIs), which range from self-limiting bacteraemia to an outright life-threatening septicaemia, are some of the most common healthcare-associated infections globally. [7] Bacteraemia is simply described as the presence of viable bacteria in the blood, while septicaemia connotes systemic manifestations caused by bacteria or their toxins in blood. Septicaemia constitutes a significant cause of morbidity and mortality, requiring prompt assessment, diagnosis, and antibiotic treatment. The risk factors for BSIs include the use of healthcare devices such as: peripheral and central venous catheters on patients; age (elderly patient, neonates); and premorbid medical conditions of patients, such as diabetes mellitus, malignancies, renal failure, burns, and prior hospitalisation. [8] The mortality rate from bloodstream infections ranges from 4.0% to 41.5% depending on severity, age, sex, and other risk factors. Infections due to antibiotic-resistant strains of bacteria present with a significantly higher morbidity and mortality. [9] Blood culture remains the gold standard in the laboratory diagnosis and identification of bloodstream pathogens; however, bacteria are not isolated in many cases of BSI. [10]

Numerous bacteria have been associated with causation of BSIs including Gram-negative bacteria: Escherichia coli, Pseudomonas species (spp.), Klebsiella spp., Serratia spp., Salmonella spp. and Enterobacter spp.; and Gram-positive bacteria: Staphylococcus spp., Streptococcus spp. And Enterococcus spp. [11,12] However, recent findings suggested an upsurge in BSIs caused by multidrug-resistant bacteria, including the members of the Enterobacteriaceae family and other Gram-negative bacteria, such as Klebsiella spp., Pseudomonas spp., Acinetobacter spp. and Citrobacter spp., most of which are extended spectrum beta-lactamase (ESBL) producers, and also some Gram-positive bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and the vancomycin-resistant enterococci. [13,14] The carbapenem antibiotics remain the antibiotic agents of choice in the management of emerging ESBL-producing Gram-negative bacteria, while vancomycin is the mainstay in treatment of MRSA. [15] However, of particular concern is the recent emergence of increasing resistance of some Gram negative bacteria to carbapenems through the production of enzymes, carbapenemases. [16]

This study aimed to determine the bacterial profile of pneumonia cases and its antibiogram.

Materials and Methods

The present study was conducted at department of microbiology, AIIMS, Patna, Bihar, India for a period of 12 months and 100 patients meeting all the inclusion criteria enrolled for the study. 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.

Included 100 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.

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.

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. [17,18]

3. 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).

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.

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. [19]

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. [20]

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. 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 (30mg) 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. [21]

Results

Table 1: Age and gender distribution of patients and Culture results

Age groups	**N%**
< 10	7 (7)
10-19	5 (5)
20-29	6 (6)
30-39	10 (10)
40-49	7 (7)
50-59	10 (10)
60-69	30 (30)
>70	25 (25)
Gender	
Male	70 (70)
Female	30 (30)
Culture results	
Pathogenic growth	75 (75)
Commensals	25 (25)

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. In the commensals isolated, the most common were alpha-hemolytic streptococci. Out of 75 samples which yielded causative agents, 60 yielded single isolate and 15 yielded double isolates. Amongst the bacteria isolated 9 (12%) were Gram positive and 66 (88%) were Gram negative.

Table 2: 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=66	Acinetobacter	9	13.63
	E.coli	9	13.63
	Klebsiella spp	27	40.90
	Providencia spp	1	1.51
	Pseudomonas	20	30.30

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 (27, 40.90%) followed by Pseudomonas species (20, 30.30%), Acinetobacter (9, 13.63%), E. coli (9, 13.63%) and Providencia spp (1, 1.51%).

Table 3: ESBL producers in Gram negative bacteria

Bacteria	ESBL		Total
	Producer	Non-Producer	
Acinetobacter	1	8	9
E.coli	2	7	9
Klebsiella spp	3	24	27
Providencia spp	1	0	1
Total	7	39	46

Among the Enterobacteriaceae, Extended Spectrum Beta-Lactamase (ESBL) production was noted in 7 of the 46 organisms, the most common being Klebsiella spp (3) followed by E.coli (2).

Table 4: Associated co-morbid conditions

Co-morbid conditions	No of cases	Percent
Diabetes Mellitus	16	16
Hypertension	13	13
Ischemic heart disease	1	1
Scoliosis	1	1
Asthma	2	2
Epilepsy	1	1
Idiopathic Thrombocytic Purpura	1	1

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

Discussion

The resistance to conventional antibiotics is severely increasing in bacteria in clinical and nonclinical settings. [22] The rate of nosocomial infection is also steadily mounting in the patients admitted in the ICU due to excessive invasive procedures performed including artificial ventilator support. [23] This constantly emerging resistance is a serious situation implying the need for new regulations for the cautious use of antibiotics and refining the conditions of hospitals to prevent further exacerbation of resistance shown by the bacteria. 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, alcoholism, 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. [24]

The predominant age group in our study is 60-69 years (30%) which is higher than the study done by Sandeep Kumar Jain et al. where the mean age group was 52.36. [24] It is well documented that pneumonia incidence rises sharply with extremes of age. [25] In the present study, 75 (75%) cultures grown were pathogenic isolates and 25 (25%) 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. [26]

Gram negative bacilli (66, 88%) out-numbered the Gram positive cocci (9, 12%) 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%). [27] 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%). [27] 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%). [24]

In the present study, ESBL production was seen in 7 isolates and 39 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.28 This implies that the organism is suggestive of multidrug resistance. 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 metallo-beta-lactamase. [28]

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. Further studies should closely examine the administration of initial therapy in pneumonia patients.

References

1. Florey H, Carleton H, Wells AQ. Mucus secretion in the trachea. British journal of experimental pathology. 1932 Jun;13(3):269.
2. Yeager Jr H: Tracheobronchial secretions. Am J Med. 1971, 50:493-509.
3. Anusha N, Madhu KP, Arun BJ, Vidyasagar B. Microbiological profile and sensitivity pattern of endotracheal secretions in mechanically ventilated patients in the ICU. J Evid Based Healthc. 2014; 1:1177-84.
4. Safdar N, Crnich CJ, Maki DG. Nosocomial infections in the intensive care unit associated with invasive medical devices. Current Infectious Disease Reports. 2001 Nov;3(6):487-95.
5. National Nosocomial Infections Surveillance (NNIS) system report, data summary from Jan 1992-April 2000. Am J Infect Control. 2000, 28: 429.
6. Fiel S. Guidelines and critical pathways for severe hospital-acquired pneumonia. Chest. 2001 Feb 1;119(2): 412S-8S.
7. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. Journal of clinical microbiology. 2003 Aug;41 (8):3655-60.
8. Aliyu S, Cohen B, Liu J, Larson E. Prevalence and risk factors for bloodstream infection present on hospital admission. Journal of Infection Prevention. 2018 Jan;19(1) :37-42.
9. Christaki E, Giamarellos-Bourboulis EJ. The complex pathogenesis of bacteremia: from antimicrobial clearance mechanisms to the genetic background of the host. Virulence. 2014 Jan 1;5(1):57-65.
10. Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis?

International journal of pediatrics. 2012 Jan 26;2012.
11. Ahmed D, Nahid MA, Sami AB, Halim F, Akter N, Sadique T, Rana MS, Elahi MS, Rahman MM. Bacterial etiology of bloodstream infections and antimicrobial resistance in Dhaka, Bangladesh, 2005–2014. Antimicrobial resistance & infection control. 2017 Dec;6(1):1-1.
12. Rolston KV, Yadegarynia D, Kontoyiannis DP, Raad II, Ho DH. The spectrum of Gram-positive bloodstream infections in patients with hematologic malignancies, and the in vitro activity of various quinolones against Gram-positive bacteria isolated from cancer patients. Int J Infect Dis. 2006;10(3):223–230.
13. Vanitha RN, Kannan G, Venkata NM, Vishwakanth D, Nagesh VR, Yogitha M, Venkata SM, Palani T. A retrospective study on blood stream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital. Int J Pharm Pharm Sci. 2012; 4(1):543-8.
14. Ndugulile F, Jureen R, Harthug S, Urassa W, Langeland N. Extended Spectrum β-Lactamases among Gram-negative bacteria of nosocomial origin from an Intensive Care Unit of a tertiary health facility in Tanzania. BMC infectious Diseases. 2005 Dec; 5(1):1-6.
15. Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME. Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum β-lactamases: a systematic review and meta-analysis. Journal of antimicrobial chemotherapy. 2012 Dec 1;67(12):2793-803.
16. Codjoe FS, Donkor ES. Carbapenem resistance: a review. Medical Sciences. 2017 Dec 21;6(1):1.
17. Lahti E, Peltola V, Waris M, Virkki R, Rantakokko-Jalava K, Jalava J, et al. Induced sputum in the diagnosis of childhood community-acquired pneumonia. Thorax. 2009; 64 (3):252–7.
18. Grant LR, Hammitt LL, Murdoch DR, O'Brien KL, Scott JA. Procedures for Collection of Induced Sputum Specimens from Children. Clin Infect Dis. 2012;54(suppl_2): S1 40–5.
19. Elorriaga G, Pineda DR. Basic concepts on Community Acquired Bacterial Pneumonia in Paediatrics. Paediatr Infect Dis. 2016;1(1):3.
20. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. vol. 31. New Delhi: Elsevier; 2007. p. 131–49.
21. M100 – S24 performance standards for Antimicrobial Susceptibility Testing; 24th informational supplement-2014, CLSI. Wayne, PA 19087 USA. PA.
22. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet infectious diseases. 2010 Sep 1;10(9): 597-602.
23. Pattanayak C, Patanaik SK, Datta PP, Panda P. A study on antibiotic sensitivity pattern of bacterial isolates in the intensive care unit of a tertiary care hospital in Eastern India. Int J Basic Clin Pharmacol. 2013 Mar; 2(2): 153-9.
24. Kumar S, Jain S, Trikha S. Study of Clinical, Radiological and Bacteriological Profile of Community Acquired Pneumonia in Hospitalised Patients of Gajra Raja Medical College. Int J Sci Study. 2014;2(6):96–100.
25. Vilar J, Domingo ML, Soto C, Cogollos J. Radiology of bacterial

pneumonia. Eur J Radiol. 2004; 51 (2):102–13.
26. García-Vázquez E, Marcos MA, Mensa J, de Roux A, Puig J, Font C, Francisco G, Torres A. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Archives of internal medicine. 2004 Sep 13;164(16):1807-11.
27. Vasuki V. Bacteriological profile of hospital acquired pneumonia in a tertiary care teaching hospital, South India. Int J Tech Res Appl. 2016; 4(2):338–44.
28. Kaur M. Occurrence of the CTX-M, SHV and the TEM Genes Among the Extended Spectrum b-Lactamase Producing Isolates of Enterobacter riaceae in a Tertiary Care Hospital of North India. J Clin Diagn Res. 2013; 7(4):642–5.
29. Tamubango Kitoko H. Accouchement prématuré aux cliniques universitaires de Lubumbashi de 2011-2019: fréquence et prise en charge. Journal of Medical Research and Health Sciences, 2023; 6(2), 2457–2470.