Original Article

Bacteriological Analysis of Bronchoalveolar Lavage Fluid in Patients with Respiratory Tract Infections

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Abstract

Background and Aim: Bacterial respiratory infections are most commonly causes of illness for all age group patients in ICU. Most of the patients suffer from urosepsis, postoperative disease and lower respiratory infection when admitted in ICU's. Broncho alveolar lavage (BAL) is an ideal sample that allows the recovery of pathogens cellular and noncellular components from the epithelial surface of lower respiratory tract. This study was performed to detect pathogenic organisms by microscopy of BAL fluid and isolate and identify various bacteria and fungi from BAL fluid in culture and analyze their antibiogram.

Material and Methods: The cross-sectional prospective study was conducted in the Department of Microbiology, tertiary care teaching hospital, India. The study included 200 BAL samples taken from all consecutive patients referred with suspicion of pneumonia. Bronchial wash was done with the help of fibreoptic bronchoscope under local anaesthesia. All BAL samples were cultured on three bacteriological media agar plates using a sterile 4mm nichrome loop (0.01ml), and incubated at 37 C for 72 hours for quantitative bacterial culture using standard laboratory techniques. Bacterial isolates were identified by performing standard microbiological procedures such as study of colony morphology, Gram staining and standard biochemical tests.

Results: Out of the total 200 samples, 120 (60%) were from males, and 80 (40%) were female patients. The predominant GNB was Klebsiella pneumoniae 45 (61%), followed by Pseudomonas aeruginosa 22(30%), Esch. coli 6(8%) and the fungal isolate was Aspergillus niger 5(1%). Klebsiella & Pseudomonas were highly sensitive to amikacin, piperacillin-tazobactam, imipenem, gentamycin, followed by tobramycin.

Conclusion: Results of the present study demonstrate the high incidence of gram-negative isolates. The study also suggests that regular antimicrobial sensitivity monitoring should be done as most isolates are highly resistant to cephalosporin and other commonly used antimicrobials.

Key Words: Amikacin, Broncho alveolar lavage, Gram-Negative Bacteria, Klebsiella Pneumoniae,

Introduction

Bacterial respiratory infections are most commonly causes of illness for all age group patients, which cured with invasive mechanical ventilation in ICU.[1] It is systemic pathway via which body acquired fresh oxygen and remove carbon dioxide. The respiratory tract systems are classified in the following parts upper and lower respiratory tracts.[2]

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Upper respiratory tract structure consist of nose, nasal passages, paranasal sinuses, the pharynx and the part of larynx above the vocal folds. The commonly occurring URTI are common cold, tonsillitis, pharyngitis, laryngitis, rhino sinusitis and otitis media.[3] Lower respiratory tract structure includes larynx, vocal folds, trachea, bronchi, bronchioles and alveoli. The most commonly occurring respiratory tract infection are tracheitis, bronchiolitis and pneumonia. It can have severe effects which may lead to hospitalization and loss of life.[4]

Pulmonary infections may be defined as those infections presenting with symptoms such as cough with expectoration, dyspnea, wheeze, chest pain/ discomfort to potentially life-threatening infections usually for period ranging from 1-3 weeks.[5,6] Most common causes of infections in these patients are viruses and bacteria. Most frequent bacteria involved in exacerbations include Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis.[7] The bacteriological profile of pulmonary infections varies within the same country due to differences in the frequency of use of antibiotics, environmental factors, and ventilation in the critically ill patients. Also, increasing variety of emerging pathogens provide challenges for the microbiology laboratory.[8] In these patients the high mortality rate of these infections is attributed, in most part, to bacterial etiological agents as well as to the lack of prompt and appropriate access to treatment. Effective antimicrobial therapy depends on the identification of the etiologic agent. It is therefore necessary to obtain the appropriate material for bacteriological diagnosis. The advent of bronchoscopy and quantitative invasive techniques like Bronchoalveolar lavage has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections.[9]

Broncho alveolar lavage (BAL) is an ideal sample that allows the recovery of pathogens cellular and noncellular components from the epithelial surface of lower respiratory tract.[10] It is increasingly utilized as diagnostic tool though in the past it remained as investigative and research tool. Early diagnosis and proper choice of antimicrobials is crucial for management of these patients and as the sputum culture yields diagnosis in fewer than 50% of patients with pulmonary infections. Further if the sputum culture report is inconclusive or the patient shows little response to the antibiotics reported as sensitive the situation gets complicated.

This study was performed to detect pathogenic organisms by microscopy of BAL fluid and isolate and identify various bacteria and fungi from BAL fluid in culture and analyze their antibiogram.

Material and Methods

The cross-sectional prospective study was conducted in the Department of Microbiology, tertiary care teaching hospital, India. The study was carried out for the duration of 1 year. The study included 200 BAL samples taken from all consecutive patients referred with suspicion of pneumonia. **Ethical Approval was taken from ethical committee of the Medical College, Hospital and Research centre, Ahmedabad, Gujarat.**

Inclusion criteria

Patients with progressive infiltrates on chest roentgenogram 48 hours or more after ICU admission with or without ventilatory support along with fever, purulent secretions, patients in whom clinical examination and routine laboratory findings could not clinch the diagnosis and patients not responding to empirical treatment were included in the study.

Exclusion criteria

Patients with Pulmonary Tuberculosis, Chronic Kidney Disease, Pulmonary oedema, recent cardiac manifestations were excluded from the study.

Bronchial wash was done with the help of fibreoptic bronchoscope under local anaesthesia (transtracheal). Around 10-30 mL of sterile normal saline was instilled into the infected lung lobe/ bronchopulmonary segments. Instilled saline was suctioned back and collected into sterile containers. Initial microscopic examination consisted of wet mount and Gram staining to observe the presence of pus cells and epithelial cells, bacteria. Bronchial secretions with less than 10^3 CFU/ml were regarded as commensals or contaminants and were excluded from the study. Collected samples of 200 patients were sent to microbiology laboratory immediately for further processing.

All BAL samples were cultured on three bacteriological media (Nutrient, Chocolate and MacConkey's) agar plates using a sterile 4mm nichrome loop (0.01ml), and incubated at 37 C for 72 hours for quantitative bacterial culture using standard laboratory techniques. Sample was also inoculated in brain heart infusion broth. For growth positive plates, the colony forming units was calculated.[11]

Bacterial isolates were identified by performing standard microbiological procedures such as study of colony morphology, Gram staining and standard biochemical tests.[12] Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and on Blood agar for fastidious organisms. After incubation at 37°C for 18-24 hours, the results were read and interpreted as per CLSI guidelines.[13]

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

Out of the total 200 samples, 120 (60%) were from males, and 80 (40%) were female patients. The majority of cases were in the age group 51-60 years, followed by the age group 61-70 yrs. The least number of patients belonged to the age group between 18-30 yrs. Out of 200 samples processed, 78 were positive for growth on culture. Most of the isolates were Gramnegative bacteria (GNB). Among 78 isolates, 73 were GNB, and 5 were fungal isolates. The predominant GNB was Klebsiella pneumoniae 45 (61%), followed by Pseudomonas aeruginosa 22(30%), Esch. coli 6(8%) and the fungal isolate was Aspergillus niger 5(1%). The highest numbers of isolates were in the age group of 51-60yrs followed by 61-70yrs, and the least number of isolates was in the age group of 18-30yrs.

Klebsiella & Pseudomonas were highly sensitive to amikacin, piperacillin-tazobactam, imipenem, gentamycin, followed by tobramycin. They showed resistance to cephalosporins and slightly resistant to ciprofloxacin also.

Table 1: Age and Gender wise distribution of subjects

Age Group	Male	Female	Total
15-30	11	8	19
31-40	2	24	26
41-50	23	9	32
51-60	56	21	77
61-70	28	18	46
Total	120	80	200

Table 2: Spectrum of bacterial isolates from BAL fluid

Isolates	Number	Percentage
Klebsiella	45	57.69
Pseudomonas	22	28.20
Esch.coli	6	7.69
Aspergillus niger	5	6.4
Total	78	100

Discussion

Chronic respiratory diseases represent an important health challenge, both in developing and developed countries because of their frequency and economic impact. Respiratory tract infections are the second most common cause of hospital acquired infections. The etiological agents of Lower respiratory tract infections and their susceptibility patterns vary from area to area and these are a major cause of mortality and morbidity across the globe. Also, clinical findings alone may not be sufficient for definitive diagnosis. A variety of invasive and non-invasive tests have been proposed as guides for diagnosis and treatment. Bronchoalveolar lavage provides a very useful tool for diagnosing lower respiratory tract infections.

The present study was conducted to determine the bacterial etiology in patients with respiratory infections with the perspective of evaluating BAL fluid, which provides a handy diagnostic tool. The present study showed positive BAL cultures in 78 samples out of 200 samples collected. Of these, GNB were the predominant organisms, being 73 and 5 were fungal growths. These results are similar to the studies done by Goel et al[14], Barsanth et al[15]. The maximum numbers of positive cases were between the age group 51-60yrs followed by 61-70yrs. This may be due to more respiratory cases observed with

increasing age which lowers host defense.[16] The rate of isolation was higher in males than in females shown in Table 1, which was on par with the findings of Shah et al.[17] Birasen Behera et al.[18]

In this study, among culture-positive GNB cases, Klebsiella pneumoniae was the commonest bacterial isolate followed by Pseudomonas and E. coli. A resembling study was done by Khatun Mst and Shamsuzzaman S.M. et al.[19] percentage of Acinetobacter baumanni were 34.21%, Pseudomonas aeruginosa 15.79%, Klebsiella spp. 23.68%, Citrobacter spp. 2.63%, Enterobacter spp. 2.63%, Staphylococcus aureus 7.89%, Staphylococcus epidermidis 2.63%, Moraxella catarrhalis 5.26%, Proteus spp. 5.26%.

In our study, all GNB isolates were susceptible to amikacin followed by piperacillin-tazobactam, gentamycin, tobramycin, and imipenem while resistant to cephalosporins. High degree of resistance against all the generations of cephalosporins was seen among the gram-negative isolates. The reason for such high a percentage of beta lactam-resistant organisms could be the frequent use of cephalosporins in the empirical antibiotic regimens. Similar findings were observed by Regha IR et al. [20] Goel et al., [14] Barsanti et al.[15] Olugbue V. Onouha et al.[21]

In our study, most gram-negative isolates were sensitive to amikacin, piperacillin, tazobactam, followed by imipenem. Therefore it can be one of the best combinations for treating infections induced by gram-negative bacilli, which are similar to the study reported by Olugbue V. Onouha Set al.[21] In such cases of highly resistant strains to most of the frequently used broad spectrum antibiotics, Colistin/ Polymyxin B remains the last option for treatment. As such all health care personnel should be trained in proper hygiene techniques and aseptic precautions for all therapeutic and diagnostic procedures done, which can go a long way in preventing nosocomial infections to an extent.

The increasing antibiotic resistance problem, mainly due to wide spread and irrational use of antimicrobial agents in hospitals and community is of great concern, especially in developing countries. Hence it is very necessary that robust measures be adopted. A combined clinical, microbiological and infection control approach which include proper diagnosis, appropriate specimen collection, strict antimicrobial stewardship and hospital infection control should be adopted and stringently implemented.

Conclusion

Results of the present study demonstrate the high incidence of gram-negative isolates. The study also suggests that regular antimicrobial sensitivity monitoring should be done as most isolates are highly resistant to cephalosporin and other commonly used antimicrobials. Proper identification of the probable pathogens and their antibiotic susceptibility pattern can help our health professionals to choose the right antibiotic therapy and improve the outcome.

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Informed Consent was taken from all the participants.

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Conflict of Interest: None declared

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