Section: Miscellaneous

Original Research Article

MICROBIOLOGICAL AND CYTOLOGICAL PROFILE OF BRONCHOALVEOLAR LAVAGE FLUID IN PEDIATRIC RESPIRATORY INFECTIONS: A STUDY FROM KARVEER, KOLHAPUR

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ABSTRACT

Background: Pediatric respiratory infections remain a leading cause of morbidity and mortality, particularly in developing countries. Bronchoalveolar lavage fluid (BALF) provides a reliable specimen for identifying lower respiratory tract pathogens when noninvasive samples are difficult to obtain. The above study was conducted to evaluate the microbiological and cytological profile of BALF in pediatric patients with respiratory infections and to assess antimicrobial susceptibility patterns in a tertiary care center in Karveer, Kolhapur.

Materials and Methods: A prospective observational study was conducted on 100 pediatric patients undergoing bronchoscopy with BAL. BALF samples were subjected to direct microscopy, cytological examination, bacterial and fungal cultures, mycobacterial testing, and antimicrobial susceptibility testing using CLSI guidelines.

Results: Of 100 BAL samples, 64% yielded bacterial growth, 2% each yielded Mycobacterium tuberculosis and Aspergillus species, and 32% showed no growth. Gram-negative bacilli accounted for 50% of isolates, and gram-positive cocci for 14%. The most common isolate was Klebsiella pneumoniae (26.56%), followed by Pseudomonas aeruginosa (21.87%) and Acinetobacter spp. (18.75%). Among gram-positive cocci, MRSA (10.93%) predominated over MSSA (9.37%), with one isolate of Streptococcus pneumoniae. Cytologically, neutrophil-predominant inflammation was observed in 64% of cases, correlating with bacterial infection. Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter spp. demonstrated 100% sensitivity to colistin and polymyxin B, with variable sensitivity to carbapenems and aminoglycosides. MRSA showed 100% sensitivity to linezolid, vancomycin, and teicoplanin.

Conclusion: BALF proved to be a valuable diagnostic tool for identifying pathogens and guiding targeted therapy in pediatric respiratory infections. The predominance of multidrug-resistant gram-negative organisms underscores the need for regular institutional antibiograms and region-specific antibiotic policies to optimize empiric treatment and combat antimicrobial resistance.

Keywords: Pediatric flexible bronchoscopy, Broncho alveolar lavage, pneumonia, cystic fibrosis, cytology.

INTRODUCTION

Bacterial pneumonia are very common in children. Worldwide, approximately 156 million children under 5 years of age present with pneumonia annually, with an estimated two million cases resulting in death—95% of which occur in developing countries. Neonates are at the greatest

risk of mortality from pneumonia, as their normal lung defenses are not yet fully developed, leading to increased susceptibility to infection.[1]

In adults, sputum samples are considered a valuable, noninvasive alternative for bacterial detection, but obtaining sputum from children is often difficult to obtain. Consequently, most pediatric studies rely on samples from the upper respiratory tract or blood. However, the Upper Respiratory Tract is frequently colonized by normal commensal organisms or bacteria, making it difficult to distinguish commensal organisms from acute pathogens.[2]

Moreover, although blood is normally sterile and blood cultures are highly specific, their sensitivity in childhood community-acquired pneumonia is low.[2]

Bronchoalveolar lavage fluid, on the other hand, offers a more direct and reliable method for pathogen identification in pediatric respiratory infections, bypassing the limitations associated with sputum and upper respiratory tract samples.[3]

Flexible bronchoscopy with Broncho-alveolar lavage (BAL) is an option when noninvasive lower respiratory tract samples(sputum) cannot be obtained or for selected patients who do not respond to antibiotic treatment. BAL fluid has the advantage of being suitable for multiple detection methods, including direct microscopy, aerobic bacterial culture, fungal culture, TB diagnosis and polymerase chain reaction.[3]

Bronchoalveolar lavage fluid provides a more accurate representation of lower respiratory tract microbiology in pediatric patients. It is done with a flexible bronchoscope. BAL is done in various conditions like persistent pneumonia, recurrent pneumonia, ventilator associated pneumonia, suspected tuberculosis, immunodeficiency and bronchiectasis.[4]

The above study aims to delineate the microbiological profile of bronchoalveolar lavage fluid in pediatric respiratory infections within this region, contributing critical data to local and global epidemiological surveillance. The insights gained from the study are anticipated to refine diagnostic approaches and therapeutic interventions for pediatric patients suffering from respiratory infections in this area.[5]

This direct sampling minimizes contamination from upper respiratory tract flora, providing a more accurate representation of the pathogens causing lower respiratory tract infections.[5]

Consequently, BALF is considered a superior specimen for identifying pathogens in severe pulmonary infections, often regarded as the gold standard in clinical practice due to its high detection rate.[6]

While BAL fluid analysis can detect pathogens and cellular patterns, its findings should be interpreted alongside clinical and radiologic evidence to enhance diagnostic precision.[7]

MATERIALS AND METHODS

The above study was conducted at Dr. D.Y. Patil Hospital and Research Institute, Kolhapur for a period of 18 months (January 2024-June2025). Ethical clearance was obtained before beginning the study.100 specimens were collected based on the inclusion and exclusion criteria.

Inclusion criteria

Pediatric patients with respiratory diseases undergoing bronchoalveolar lavage were included. These conditions encompassed recurrent pneumonia, persistent pneumonia, suspected tuberculosis, and suspected immune deficiency in children.

Exclusion criteria

Children with congenital anomalies, such as laryngomalacia or subglottic cysts with evidence of infection, were excluded. BALF samples from pediatric patients meeting the inclusion criteria were meticulously collected following established aseptic protocols and expert consensus guidelines to ensure sample integrity and prevent contamination.[6]

Bronchoalveolar lavage fluid was collected from pediatric patients after obtaining written informed consent. Patients were thoroughly informed about the bronchoscopy procedure, including its associated risks and benefits. A total of 100 BAL specimens were collected aseptically by a pediatric pulmonologist using standard procedures and immediately sent to the microbiology laboratory for processing. Direct smears were prepared and subjected to Gram staining for the detection of bacteria and yeast-like cells, and Ziehl-Neelsen staining was performed for acid-fast bacilli.

From the pathology laboratory, additional smears were prepared for Hematoxylin and Eosin (H&E) and Papanicolaou (Pap) staining for cytomorphological evaluation. In clinically suspected cases of tuberculosis or when suggestive cytological features were present, Ziehl–Neelsen staining was performed. Special stains such as Gomori Methenamine Silver (GMS) and Periodic Acid–Schiff (PAS) were used for the identification of fungal organisms. Wherever adequate material was available, cell blocks were prepared and subjected to appropriate histochemical staining for further evaluation.

Culture and identification: BAL fluid specimens were cultured on blood agar, MacConkey agar, chocolate agar, and Sabouraud dextrose agar for bacterial and fungal identification. Blood and MacConkey agar plates were incubated aerobically at 37°C for 18–24 h, chocolate agar plates at 37°C in 5% CO2 for 24–48 h, and Sabouraud dextrose agar at 25°C for up to 3 weeks. For Bacterial culture, a Colony Forming Unit count of 10 4ml (i.e. 10 colonies) is considered significant.[8] Bacterial isolates were identified to the species level based on colony morphology, Gram staining, and biochemical tests (indole, methyl red, Voges- Proskauer, triple sugar iron, citrate, and urea hydrolysis.[9]

Antimicrobial susceptibility testing (AST): Antibiotic Sensitivity Testing was performed for bacterial isolates using the conventional Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines, antibiotics were selected based on organism identification. Interpretation of inhibition zone diameters was performed according to CLSI M100, 2024.[9,10]

For suspected fungal infections, 10% potassium hydroxide mounts were prepared to demonstrate fungal elements, and fungal cultures were initiated. KOH Preparation was done for demonstration of fungal elements and fungal culture was done.

In suspected TB infections, samples were sent for CBNAAT (Gene X pert) as well and TB culture by MGIT method.

Cell blocks were prepared from BAL specimens using the plasma–thrombin method. Cell block sections were stained with Hematoxylin and Eosin (H&E) for detailed cytomorphological and architectural evaluation. Special stains including Ziehl–Neelsen, Periodic Acid–Schiff (PAS), and Gomori Methenamine Silver (GMS) were performed on cell block sections wherever indicated to aid in the identification of mycobacterial and fungal organisms.

RESULTS

Table 1: Distribution of organisms from BAL

Type of organisms	No. of Isolates	Percentage
Gram Negative Bacilli (GNB)	50	50
Gram Positive Cocci (GPC)	14	14
Aspergillus species	2	2
Mycobacterium tuberculosis	2	2
No growth	32	32
Total	**100**	**100**

Among 100 BAL samples processed, 64 samples showed bacterial growth (50 samples isolated Gram Negative Bacilli and 14 samples isolated Gram Positive Cocci), 32 samples showed no growth, 2 samples isolated Mycobacterium tuberculosis and Aspergillus spp. were isolated from 2 samples. These findings underscore the diverse microbiological landscape observed in pediatric respiratory infections within the Karveer, Kolhapur region, encompassing bacterial, fungal and mycobacterial etiologies.

Table 2: Percentage yield of each organism among total bacterial isolates

Organism Isolated	No. of Isolates	Percentage
Klebsiella pneumoniae	17	26.56
Pseudomonas aeruginosa	14	21.87
Acinetobacter spp.	12	18.75
Methicillin Resistant Staphylococcus Aureus(MRSA)	7	10.93
Escherichia coli (E.coli)	6	9.37
Methicillin Sensitive Staphylococcus Aureus (MSSA)	6	9.37
Burkholderia cepacia	1	1.56
Streptococcus pneumoniae	1	1.56
Grand Total	**64**	**100**

Most common isolate in this study was Klebsiella pneumonia in 17 samples (26.56%), 2nd most common Pseudomonas aeruginosa in 14 samples (21.87 %) followed by Acinetobacter spp. in 12 samples (18.75%).

E. coli was isolated in 6 samples and Burkholderia cepacia in 1 sample.

Among gram positive cocci, MRSA was the most isolate in 7 sample followed by MSSA in 6 samples. One sample isolated was Streptococcus pneumoniae. Among Gram-positive cocci, MRSA accounted for 10.93% and MSSA for 9.37% of isolates, highlighting the persistent challenge of staphylococcal infections.

Table 3: Cytological findings in the BAL

Cytological/ Pathological Findings	Probable Etiology	Number of Cases	Percentage
Predominant neutrophilic inflammation	Acute bacterial infection (GNB/GPC)	64	64
Eosinophil-rich inflammatory infiltrate	Allergic / fungal etiology (e.g., Aspergillosis)	2	2
Necrotic background with lymphohistiocytic infiltrate	Granulomatous inflammation (suspected tuberculosis)	2	2
Fungal elements identified on PAS/GMS	Fungal infection (Aspergillus species)	2	2
Acid-fast bacilli on Ziehl–Neelsen stain	Mycobacterium tuberculosis	2	2
Adequate sample with inflammatory cells, no identifiable organisms	Inflammatory / non-specific	28	28
Total		**100**	**100**

The most common cytological finding in the above study was neutrophil-predominant inflammation (64%) as in Selimovic et al,[11] suggestive of acute bacterial infection, correlating with the predominance of Gram-negative and Gram-positive bacterial isolates on microbiological culture.

Eosinophil-rich inflammatory infiltrate (2%) was observed in cases showing fungal elements on PAS and GMS stains, correlating with microbiologically confirmed Aspergillus species.

Granulomatous inflammation with necrotic background (2%) demonstrated positivity for acid-fast bacilli on Ziehl–Neelsen staining, correlating with cases of Mycobacterium tuberculosis detected by CBNAAT and MGIT culture.

Cell block evaluation and the use of special stains (ZN, PAS, and GMS) improved organism detection and provided confirmatory pathological evidence in selected cases.

Adequate BAL samples showing inflammatory cells without identifiable organisms (28%) were categorized as non-specific inflammatory pathology, correlating with cases showing no growth on culture.

Table 4: Antibiotic sensitivity pattern of isolates among Gram Negative Bacilli

Antibiotics	Acinetobacter spp.	Burkholderia cepacia	E. coli	Klebsiella pneumoniae	Pseudomonas aeruginosa
Cotrimoxazole	66.70	100.00	33.30	70.60	-
Doxycycline	75.00	-	16.7	76.5	-
Ciprofloxacin	50.00	100.00	33.30	41.20	64.30
Levofloxacin	83.30	100.00	66.70	64.70	71.40
Piperacillin/Tazobactam	50.00	100.00	66.70	76.5	57.10
Gentamicin	50.00	-	66.70	88.20	57.10
Amikacin	66.70	-	83.30	58.80	50.00
Imipenem	41.70	100.00	66.70	70.60	57.10
Meropenem	58.30	100.00	66.70	82.4	78.6
Colistin	100.00	0.00	100.00	100.00	100.00

In above study, Klebsiella pneumoniae was found to be sensitive to colistin and polymyxin-B (100%) followed by gentamycin (88%), and meropenem (82%), PIT and CFS each 76.5% followed by imipenem (70%).

Pseudomonas aeruginosa was sensitive to colistin and polymyxin- B (100%) followed by cefepime, ceftazidime and meropenem. (each 78.6%.)

In above study, Acinetobacter spp. was found to be 100 % sensitive to colistin, polymyxin-B followed by levofloxacin (83%).

Single isolate of Burkholederia was 100 % sensitive to cotrimoxazole, ciprofloxacin, levofloxacin, piperacillin – tazobactam, cefepime, ceftazidime, imipenem and meropenem.

Table 5: Antibiotic sensitivity pattern of isolates (GPC)

Antibiotics	MRSA	MSSA	Streptococcus pneumoniae
Clindamycin	71.40	83.30	100.00
Linezolid	100.00	100.00	100.00
Vancomycin	100.00	100.00	100.00
Teicoplanin	100.00	100.00	100.00
Ciprofloxacin	28.60	0.00	-
Levofloxacin	42.90	33.30	100.00
Clarithromycin	85.70	83.30	100.00
Azithromycin	85.70	83.30	100.00
Gentamicin	85.70	100.00	-
Doxycycline	100.00	83.30	0.00
Cotrimoxazole	85.70	83.30	0.00
Cefoxitin	0.00	100.00	-

MRSA was 100 % sensitive to linezolid, vancomycin, teicoplanin and doxycycline followed by cotrimoxazole, azithromycin and clarithromycin (each 85.7 %)

MRSA was 100 % sensitive to linezolid vancomycin, teicoplanin followed by clindamycin cotrimoxazole, doxycycline, azithromycin, and clarithromycin (each 83.3 %).

Single isolate of Streptococcus pneumoniae is sensitive to linezolid, vancomycin, teicoplanin, clindamycin, azithromycin and clarithromycin and levofloxacin (100%)

DISCUSSION

The most common organism isolated in the above study was Klebsiella pneumoniae (26.56%), followed by Pseudomonas aeruginosa (21.87%) and Acinetobacter spp. (18.75%). These findings were in concordance with the study conducted by Tejaswini et al,[9] which reported Klebsiella pneumoniae (36%) and Acinetobacter spp. (18%) as the predominant isolates. Similarly, Mohd. Sohail et al,[1] reported Klebsiella pneumoniae in 23% of isolates, followed by Pseudomonas aeruginosa (18%) and Escherichia coli (12%).

Klebsiella pneumoniae showed 100% sensitivity to colistin and polymyxin B, followed by meropenem (93%), gentamicin (93%), piperacillin–tazobactam and cefoperazone–sulbactam (87% each), imipenem (75%), and levofloxacin (64%). Pseudomonas aeruginosa demonstrated 100% sensitivity to colistin and polymyxin B, followed by cefepime and

ceftazidime (78% each) and meropenem (76%). These findings were consistent with the study by Mohd. Sohail et al,[1] which also reported 100% sensitivity of Klebsiella pneumoniae and Pseudomonas aeruginosa to colistin.

In the study, Acinetobacter spp. exhibited 100% sensitivity to colistin, which was in concordance with the findings of Mohd. Sohail et al,[1] Other studies have similarly identified Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii as predominant pathogens in bronchoalveolar lavage fluid, particularly in cases of severe pneumonia.[12]

Among the total 14 gram-positive cocci isolates, 7 were MRSA (50%), 6 were MSSA, and 1 was Streptococcus pneumoniae. This distribution was consistent with the study conducted by Adhikari et al,[13] which reported an MRSA prevalence of 52%.

CLINICAL IMPLICATIONS

BAL culture gives exact organism causing infection and its antimicrobial sensitivity which helps treating pediatrician to give specific targeted treatment. Isolating some specific organisms can help to work up patient for specific disease e.g. isolation of pseudomonas in BAL directs work up of cystic fibrosis, aspergillus species isolation in BAL directs work up of Primary Immunodeficiency Diseases, Pneumocystis jiroveci in HIV. BAL can be used for diagnosis of TB.

CONCLUSION

There is a risk of emergence of MDR pathogens with inadequate, inappropriate antibiotic treatment. To initiate an empiric antimicrobial therapy, we should have the knowledge of microbial flora of the locality and their sensitivity and resistance patterns; such information needs to be analyzed periodically and institution based antibiotic policies formed from time to time and made available to all consultants treating infectious diseases. Hospital antibiograms are an important component of detecting and monitoring trends in antimicrobial resistance. It would be ideal, through multicenter studies, to generate nationwide or more appropriately region-specific antibiograms.

REFERENCES

1. Mohd Suhail Lone, Dr. Arifat Jabeen, Dr. Junaid Ahmad and Dr. Kaiser Wani. Microbiological profile of bronchoalveolar lavage in patients with nosocomial pneumonia in neonatal intensive care unit : Int. J. Adv. Res. 10(02), 1363-1366.
2. Iris De Schutter et al. Microbiology of Bronchoalveolar Lavage Fluid in Children with Acute Nonresponding or Recurrent Community-Acquired Pneumonia: Identification of Non-typeable Haemophilus influenzae as a Major Pathogen. Microbiology of CAP in Children d CID 2011:52
3. Wang, L., Lu, S., Guo, Y., Liu, J., Wu, P., & Yang, S. (2023). Comparative study of diagnostic efficacy of sputum and bronchoalveolar lavage fluid specimens in community-acquired pneumonia children treated with fiberoptic bronchoscopy. BMC Infectious Diseases, 23(1). https://doi.org/10.1186/s12879-023-08522-3
4. Pediatric Respiratory Medicine; 1st Edition 2019, D. Vijaysekaran
5. Shao, J., Hassouna, A., Wang, Y., Zhang, R., Zhen, L., Li, R., Chen, M., Liu, C., Wang, X., Zhang, M., Wang, P., Yuan, S., Chen, J., & Lu, J. (2021). Next-generation sequencing as an advanced supplementary tool for the diagnosis of pathogens in lower respiratory tract infections: An observational trial in Xi'an, China. Biomedical Reports, 16(2). https://doi.org/10.3892/br.2021.1497
6. Yu, M., Li, M., & Sun, H. (2025). Dynamic analysis of the epidemiology and pathogen distribution of bronchoalveolar lavage fluid in children with severe pulmonary infection: a retrospective study. The Italian Journal of Pediatrics, 51(1). https://doi.org/10.1186/s13052-025-01859-2
7. Marguet C, Jouen-Boedes F, Dean TP, Warner JO. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis. American journal of respiratory and critical care medicine. 1999 May 1;159 (5):1533-40.
8. Indian Council of Medical Research. (2019). Standard operating procedures: Bacteriology
9. Tejaswini Olambe, Seema Agrawal. Trends in the Microbial Profile of Bronchoalveolar Lavage Samples from Patients with Lower Respiratory Tract Infections. J Med Microbiol Infect Dis, 2025; 13 (2): 127-133.
10. CLSI M100 (2024) 34th edition guidelines
11. Selimovic A, Mujicic E, Milisic S, Pejicic T, Rancic M, Mesihovic-Dinarevic S, Lukic-Bilela L, Moro M. The Significance of Bronchoalveolar Lavage Fluid Cytology in Diagnosing Lung Infiltrates in Children. Medical archives. 2016 Jan 31;70(1):27.
12. Zhang, C., Liu, T., Wang, Y., Chen, W., Liu, J., Tao, J., Zhu, X., Zhang, Z., Ming, M., Wang, M., Lü, G., & Yan, G. (2022). Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage Fluid from Children with Severe Pneumonia in Pediatric Intensive Care Unit. Research Square (Research Square). https://doi.org/10.21203/rs.3.rs-1781582/v1
13. Adhikari et al. Bacterial etiology of bronchoalveolar lavage fluid in tertiary care patients and antibiogram of the isolates. Journal of Institute of Science and Technology, 26(1), 99-106 (2021).