Section: Microbiology

Original Research Article

A STUDY ON PREVALENCE OF EXTENDED SPECTRUM B-LACTAMASE (ESBL) PRODUCING GRAM NEGATIVE BACTERIAL ISOLATES FROM BRONCHO ALVEOLAR LAVAGE FLUID (BAL) IN RESPIRATORY TRACT INFECTIONS AT A TERTIARY CARE CENTER.

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ABSTRACT

Background: Resistance to different groups of antimicrobials has made the proliferation of extended spectrum β-lactmase (ESBL) producing strains a serious global health concern that has complicated treatment strategies. The high proportion of ESBL producers among the Enterobacteriaceae and the complex molecular epidemiology with diverse types of ESBL genes are alarming. This study was undertaken to identify ESBL production in various bacterial isolates from the BAL (Broncho Alveolar Lavage) fluid in pulmonary infections.

Material and Methods: A total of 100 samples were processed, they were initially screened by phenotypic method (Disk diffusion method) and confirmation was done by double disk synergy test (DDST).

Results: Out of 100 isolates,51 were culture positive in this ESBL producers were 78.4% (n=40). In the present study, ESBL production was observed as Acientobacter baumannii (83.3%), Klebsiella pneumonia 78.5%, pseudomonas aeruginosa, 75%, E.coli 50% & Enterobacter species (n=1) 100% ESBL production.

Conclusion: The frequency of ESBL producing strains among clinical isolates has been steadily increasing. Advance drug resistance surveillance and molecular characteristics of ESBL isolates is necessary to guide the appropriate and judicious antibiotic use.

Keywords: BAL fluid, Pneumonia, Double disc synergy test, β-lactamase.

INTRODUCTION

ESBLs are characterized by the β-lactamases that are capable of producing bacterial resistance to penicillin, extended spectrum cephalosporins and monobactams except cephamycins and carbapenems. Beta lactamase inhibitors like clavulanic acid inhibits ESBLs.[1]

Beta-lactam drugs like penicillins, cephalosporins, carbapenems and aztreonam are common antibiotics used to combat most bacterial infections. Multiple factors are responsible for the emergence of antibiotics resistance such as their overuse, inappropriate doses, and the clinical practices concerning isolation of patients infected with multidrug resistant pathogens.[1-3] Increased use of broad spectrum antibiotics such as third generation cephalosporins has been correlated with the development of β-lactamases mediated bacterial resistance, which subsequently led to the emergence of extended spectrum beta-lactamases (ESBLs) producing organisms. At present, more than 200 ESBLs have been characterized.[3] Members of Enterobacteriaceae, especially Klebsiella species that produce ESBL have been established since 1980s.

ESBL producing strains revealed complete resistance to ampicillin and all cephalosporins used

except cefotetan. The strains isolated from the patients showed an increase in resistance rate to other non β lactam classes of antibiotics. This could be due to the load of antibiotic pressure in the hospital. When compared with patients, strains from the healthy individuals showed moderate resistance to other classes of antibiotics whereas those from the environment showed very little or no resistance. Colistin stands as the drug of choice for infections caused by multidrug resistant strains, followed by Carbapenems, amikacin and piperacillin-tazobactam for ESBL strains.[5]

Treatment of infections caused by ESBL-producing organisms with extended-spectrum cephalosporins or aztreonam may result in treatment failure even when the causative organisms appear to be susceptible to these antimicrobial agents using standard breakpoints. In addition, patients colonized or infected with ESBL-producing organisms should be placed under contact precautions to avoid cross transmission to other patients. These benefits warrant the detection of ESBL-producing organisms in clinical laboratories.[7-9] They can be found in a variety of Enterobacteriaceae species; In India, the prevalence rate varies in different institutions from 28 to 84%.[3]

ESBLs are rapidly evolving group of beta-lactamase which share the ability to hydrolyze third generation cephalosporins and aztreonam, yet are inhibited by clavulanic acid. They are derived from genes for TEM-1, TEM-2 or SHV-1 by mutations that alter the amino acid sequence around the active site of these beta-lactamases.[10]

ESBL producers are the important members of the group of antibiotic resistant pathogens that cause hospital acquired infections. Significant proportion of laboratories in India does not perform tests to detect ESBL producers.[11] Therefore, this issue is of particular concern that poses a great challenge to every laboratory as the proportion of ESBL producing Enterobacteriaceae members are growing worldwide. Present study reveals the prevalence of ESBL from BAL fluid in pulmonary infections at Vikarabad, Hyderabad, India.

MATERIALS AND METHODS

The study was carried out at the Department of Microbiology, Mahavir institute of medical sciences General and Teaching hospital, Vikarabad.

Study Period- January 2023 to June 2024. (18 Months)

Methodology

The Research protocol was approved by Institutional Review Board and Institutional Ethical Committee. After taking the consent clinical history of patient was obtained in all cases.

Subjects

Between 18 years to 70 years of age, from the inpatients (Medicine & Respiratory units) ICUs and outpatients (day care procedure patients) which comprise of both males and females.

Inclusion Criteria

Patients having at least two of the following symptoms.

1. Fever above 37°C, Cough, Production of purulent sputum, Breathing difficulty, in association with physical findings suggestive of consolidation,[6] Chest pain and Leukocytosis (W.B.C > 11,500/cumm).
2. Patients in whom clinical examination and routine laboratory findings could not clinch the diagnosis
3. Patients not responding to empirical treatment

Exclusion Criteria

1. Patients with HIV positive,
2. Patients with fungal infections,
3. Patients with cardiac diseases and those patients receiving Immunosuppressive therapy.

Sample Collection

BAL fluid samples were collected from patients suffering from LRTI after giving instructions to the patients regarding bronchoscopic procedure. Patients who were critically ill having endotracheal intubation, BAL fluid was collected using Metra`s catheter (miniBal).[12]

Bronchoscopic procedure

Site of lavage

The site of lavage depended on the localization of the abnormalities. An infection with radio graphically apparent infiltrate or suspected malignancy of the involved segment is sampled.

In patients with diffuse lung diseases the middle lobe or lingual lobes were commonly lavaged sites. Since anatomically this is the most accessible site and the fluid obtained at one site is representative of the whole lung in diffuse lung disease, using this method approximately (1.5 to 3%) of the lung (10^5 alveoli) are sampled.[13]

Fluid Used

Usually the lavage is performed using sterile saline (0.9% Nacl) preferable at 37 degrees centigrade to help prevent cough. Or saline at room temperature can also be used. The volume of saline instilled varied between 100 to 300 ml.

Fluid instillation and recovery

The fiber optic bronchoscope is wedged into a sub segmental bronchus. The fluid instilled through the bronchoscope is almost immediately recovered by applying suction (25-100) mm hg, Fluid was collected in 2 to 3 aliquots usually 2nd aliquot was preferred for microbiological examination.

Microscopy

Gram stained smears of BAL fluid were examined to access the quality of sample to detect predominant morphotypes and to differentiate gram positive from gram negative bacteria. Samples showing less than 10 squamous epithelial cells and more than 25 leucocytes or pus cells per low power field indicated good quality of the specimen. All samples were subjected for ZiehlNeelsen (Z-N) staining for acid fast bacilli.

Laboratory processing of BAL fluid

BAL fluid is processed immediately upon arrival at the laboratory and is vortexed and was checked for quality control criteria such as and is rejected if one of following criteria is observed

1. Volume less than 20 ml.
2. Presence of >10 squamous epithelial cells/low power field.
3. Presence of extensive amount of debris.

Samples under study

A total of 100 BAL fluid samples were included in the study which met the above quality control criteria.

Culture of the specimen

BAL fluid samples that were satisfactory by microscopic examination were subjected for quantitative culture using a 4 mm loop (10 micro litre). Each sample was inoculated on Blood agar, MacConkey agar and chocolate agar. For isolation of *S.* pneumoniae, 5% Sheep Blood Agar with optochin disk were incubated in 5% CO_2 rich environment using candle jar at 35°C for 24-48hrs.For isolation of H. influenzae, chocolate agar with a streak of *S. aureus* was incubated in 5% CO_2 rich environment using candle jar at 35° C for 24-72 hours.[14] In case if the isolate was suspected to be H. influenza, a lawn of the test organism is streaked onto blood agar with impregnated disks (X, V) are placed directly on the confluent inoculation, at least 4 to 5 cm apart and incubated at 35°- 37°C for 24-48 hours. The organisms will grow only around the disk that provides the appropriate factor for growth of the organism.[15]

Identification

After 24 hour incubation, the plates were observed for the following morphological characters growth, size of the colony, shape of the colony, elevation, odor, pigmentation and hemolysis. The colonies were counted for threshold. And the colonies were counted and multiplied by 100 as 0.01 ml of BAL fluid was used for inoculation and the number of CFU per ml is determined. Diagnostic threshold for BAL was taken as 10^4 CFU per ml followed by Microscopy and Gram staining.

The Bacterial isolates were further identified and speciated by using the set of relevant biochemical reactions as per standard reference. The following biochemical tests were put up for identification of Gram Positive isolates.-Catalase Test-Tube method, Coagulase test-Slide method and Tube method, Optochin Disc test, Bile solubility test, O.F Sugars (Oxidative Fermentative Sugars). Mannitol Fermentation tests.

The following tests were performed for the Gram Negative isolates- Catalase test, Hanging Drop test for Motility, Oxidase test. IMViC Reaction (Indole test, Methyl red test, VogesProskauer test, Citrate test) and Urease test. Sugar Fermentation tests using 1% primary sugars& TSI.

Detection of E.S.B.L Producing Organism

Gram negative isolates showing resistance to 2nd and 3rd Generation Cephalosporins (Ceftriaxone ≤ 25m.m, Ceftazidime- ≤22m.m, cefotaxime ≤27m.m) in diameter were selected for ESBL confirmatory test as per CLSI guidelines 2023. The ESBL phenotypic confirmatory test was done by Double Disc Synergy Test.

Double Disc Synergy Test

Inoculum was prepared and standardized using 0.5 McFarland and swabbed on to Mueller Hinton Agar plate. Antibiotic disc containing amoxicillin/Clavulanic acid 30 µg/10 µg is placed at the centre of the plate and discs containing ceftazidime 30 µg & cefotaxime 30 µg are placed 20-30 mm away from the central disc on the same inoculated plate and incubated at 37°C for 18-24 hours.

Interpretation

After incubation, an extension in the zone of inhibition around the peripheral discs towards the centrally placed amoxicillin/Clavulanic acid 30 mcg/10 mcg disc indicated ESBL production.

Control- *K. pneumoniae*- ATCC-700603 (CLSI-2016-17).

RESULTS

Gram Negative Bacterial isolates showing resistance to 2nd and 3rd Generation Cephalosporins were subjected to ESBL phenotypic confirmation by Double Disk Synergy Test. 22 (78.5%) out of total 28 K.pneumoniae isolates, 10 (83.3,6%) out of total 12 A.baumanii, 6 (75%) out of total 8 P.aeruginosa, 1(50%) out of total 2 E.coli and 1 (100%) out of total 1 Enterobacter species' isolates were positive for ESBL production.

The overall prevalence of ESBL production was 78.4 %. 56% isolates were obtained from BAL fluid of which 51% were Gram negative and 5% ware Gram positive as the Gram positive isolates were less in number Gram negative isolates were processed for ESBL detection. Out of the100 samples collected 56% were collected by bronchoscopic technique and 44% by Metra`s technique (mini BAL). This blinded invasive procedure has been advocated because of the potential risk of invasive bronchoscopic technique in critically ill patients too unstable to undergo bronchoscopy.[18-19] According to a study conducted by Richards Metal and stevenetal, BAL fluid sample obtained by either brobchoscopic and Metras technique showed similar diagnostic accuracy.[19-20]

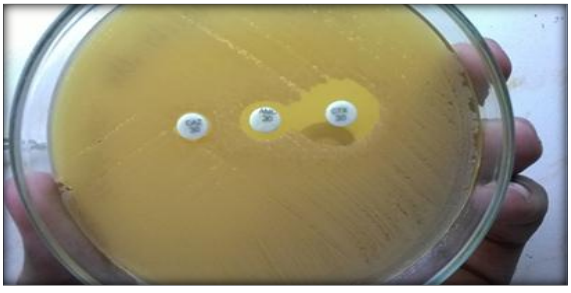

Figure 1: Showing E.S.B.L Confirmatory test by Double Disk Synergy Test

Table 1: Phenotypic prevalence of potential ESBL producers of gram negative micro organisms

S. No	Isolates	Total No. of Gram negative Isolates	Screening positive	No. of Positives for ESBL (confirmative)	Percentage
1.	Klebsiella pneumoniae	28	24	22	78.5%
2.	Acinetobacter baumannii	12	10	10	83.33%
3.	Pseudomonas aeruginosa	8	7	6	75%
4.	Escherichia coli	2	1	1	50%
5.	Enterobacter species	1	1	1	100%
TOTAL		51	43	40	78.43%

DISCUSSION

ESBLs have become a widespread serious problem. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromises the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBLs presents diagnostic challenges to the clinical microbiology laboratories.

The overall prevalence of ESBL production was 78.43%. In the present study, Acinetobacter baumannii showed 83.3% of ESBL production, these studies correlated with the previous studies conducted by Perumal P.G.et al. and they revealed that in their study ESBL production is 84%.[16]

The present study found that Acientobacter baumannii 83.3% is major gram negative organism. Klebsiella pneumoniae showed 78.5%& E.coli 50% & Enterobacter species' (n=1) 100% ESBL production. These results agreement with that of previous finding conducted by Shashidhar et al at KMC revealed klebsiella pneumoniae 65.9%,E.coli 70.6% and Enterobacter 100%.[17] klebsiella pneumoniae 65.9%,E.coli 70.6% and Enterobacter 100%.[16]

In the present study, another major gram negative Pseudomonas aeruginosa isolates showed 75% ESBL production. These results accordance with that of previous results reported by Manojkumar et al and revealed that ESBL production is 41.2%.[18]

According to a study by Giantsou E, et al reported a significantly higher de-escalation rates of antibiotics when guided by results of quantitative BAL fluid cultures.[15] Hence this invasive approach & quantitative culture susceptibility analysis of BAL fluid can lead to significantly more antibiotic modification there by enabling better patient management with decreased morbidity & mortality.

ESBL strains are usually multi-drug resistant. Because these strains become resistant to available antibiotics and they can pass the gene to other clinical strains, the quick detection of these strains in microbiology laboratories is very important. Antimicrobial therapy has played an important role in the treatment of human bacterial infections, but the drug resistance that has emerged in the treatment of bacterial infections due to ESBL enzymes degrades all beta lactam antibiotics and thus bacteria become multidrug resistant.[8]

New technologies such as molecular techniques and modified mass spectrometry technique (matrix assisted light desorption ionization time-of-flight) are being suggested as quicker alternatives for routine laboratory diagnosis. However these are available only in research facilities and tertiary care center's,routine detection of ESBLs by conventional methods should be done in every laboratory where molecular methods cannot be performed.[7]

CONCLUSION

The present study highlights the prevalence of ESBL-producing bacteria in the, tertiary care center, India. All the strains isolated were multi drug resistant and retained their sensitivity against imipenem. Further this study extended for molecular detection and identification of beta lactamases would be essential for a reliable epidemiological investigation of antimicrobial resistance. These enzymes can be chromosomal or plasmid mediated, which may help in the dissemination of antimicrobial drug resistance in health care settings. Therefore, ESBL producing organisms should be identified quickly so that appropriate antibiotic usage and infection control measures can be implemented.

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