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RESEARCH ARTICLE

A STUDY ON DETECTION OF BACTERIAL AND FUNGAL ISOLATES IN BRONCHO ALVEOLAR LAVAGE FLUID SPECIMEN WITH A SPECIAL MENTION ON ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL ISOLATES

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Key words:-
Broncho-Alveolar Lavage Fluid, Bacterial Isolates, Antimicrobial Sensitivity and Fungal Isolates

Abstract

Introduction: Chronic respiratory diseases are a group of chronic disease affecting the airways and other structures of lungs. Common causes of infections in these patients are viruses, bacteria and fungi[4]. Respiratory Tract Infection (RTI) is by large one of the leading causes of the morbidity and mortality in the world[3]. Early diagnosis and proper choice of antimicrobials is crucial for management of these patients**.**

Aims and objectives: The Aims and Objectives of this study, is to identify the bacteriological and mycological isolates in Broncho-alveolar lavage fluid and detect antimicrobial susceptibility pattern of bacterial isolates.

Materials and Methods:A total of 203 Broncho-alveolar lavage fluid in paired samples obtained from patients were analyzed.The pathogens were identified and speciated by using the set of biochemical reactions as per standard reference.

Results: This prospective study was done for a period of twelve months (September 2017 to August 2018) with 203 Broncho-alveolar lavage fluid samples. Growth was observed in 115 samples (56.65%). Of the culture positive samples, 77(67%) were bacterial, 21(18%) samples were mycobacterium tuberculosis and 17 (16%) were fungal isolates. The most common bacterial isolate was Klebsiellapneumoniae (28.57%), followed by Staphylococcus species (24.64%), Escherichia coli (20.77%), Pseudomonas species (14.28%), and Acinetobacter species (11.68%). Among the Staphylococcus aureus isolates,40% were Methicillin resistant and 60% were Methicillin sensitive.The fungal isolates were Candida(35.29%),Aspergillus(29.41%),(17.64%),Penicillium(11.76%)a nd Paecilomyces (5.88%).

Conclusion: Systematicculture of BAL fluidgives a definite diagnosis of chronic pulmonary infections which would guide in selection of appropriate antimicrobials for management of pneumonia..A study like this would help and guide the physicians in prescribing the right combinations of anti-microbial to limit and prevent the emergence of multi-drug resistant strains of Bacteria.

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Introduction:-

Chronic respiratory diseases are a group of chronic disease affecting the airways and other structures of lungs[1]. They present with symptoms of cough with sputum production,breathlessness and chest pain [1]. Common causes of infections in these patients are viruses, bacteria and fungi[4].Respiratory Tract Infection is by large one of the leading causes of the morbidity and mortality in the world[3].Early diagnosis andtreatment with the properchoice of antimicrobials is crucial for management of these patients.

Routine non-invasive investigations for bacterial pneumonia include sputum sampling, blood cultures, and urine antigen detection. The sputum Gram stain is rarely informative, and the yield of sputum bacterial cultures is low, although it improves with optimal sampling and absence of prior antibiotic therapy.Quantitativeculture approach tries to discriminate between bacterial colonization and true bacterial infection by determining the bacterial burden[4].

Bronchoalveolar lavage (BAL) is a medical procedure in which a bronchoscope is passed through the mouth or nose into the lungs[2]. Fluid is then squirted into the small parts of the lung and then recollected for analysis[2]. The main constituents of BAL fluid originate from secretions of the alveoli[2]. When sputum induction is not possible, such as in ventilator-associated pneumonia (VAP), the value of Broncho-alveolar lavage (BAL) fluid culture is significant, as the survival rate is dependent on the empirical therapy[4]. The advent of bronchoscopy and quantitative invasive techniques like Broncho-alveolar lavage has improved sensitivity and specificity of diagnostic techniques in diagnosis of pulmonary infections[2].

It has been reported that clinical microbiologists in diagnostic laboratories have a critical role to play in the diagnosis and management of Lower Respiratory Tract Infection, as overtreatment of acute uncomplicated pulmonary infections led to unparalleled levels of multi drug resistance among pathogens.[3]

Aims and objectives:-

The Aims and Objectives of this study,is to identifythe bacteriological and mycological isolates in Broncho - alveolar lavage fluid and detect antimicrobial susceptibility pattern.

Materials and Methods:-

This study was done in the Department of Microbiology, Stanley Medical College-Chennai. Bronchoscopy was done as an outpatient procedure and the lavage fluid samples received in the Microbiology Department were analyzed. This is a prospective study done from September 2017to August 2018. A total of 203 Broncho-alveolar lavage fluids in paired samplesobtained from patients were analyzed.

Themicrobiological analysis included Gram's staining,AFB staining,bacterial culture, fungal culture and antimicrobial susceptibility testing.

Bacteriology:

Broncho-alveolar lavage fluid (BAL) specimens were mixed andspread on 2 cm diameter area on microscopy slideswith 4mm loop. The smears allowed to dry fixed and stained with Gram's staining .The number of bacteria per oil immersion field reported. Semi-quantitative loop method was used for culture. Two mm diameter loop was usedto spreadsample on MacConkey agar, Chocolate agar(plate kept in candle jar) and blood agar plates incubated at 37^0 C for 24 hr.

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

The Gram Positive and Gram Negative Bacterial isolates were further identified and species identifications were done by using the set of biochemical reactions as per standard reference. The following biochemical tests were put

up for identification of Gram Positive isolates.-Catalase Test, Coagulase test (Slide and Tube method), O.F Sugars (Oxidative Fermentative Sugars) and 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.

Antibiotic sensitivity testing of all isolates was done by Kirby Bauer's disc diffusion method on Muller Hinton agar and results were interpreted as per CLSI guidelines[14]. Standard antibiotic discs like Amikacin (30mcg), Amoxy-clav (20/10mcg), Ampicillin (30mcg), Penicillin Cefipime (30mcg), Cefoxitin(30mcg), Cefotaxime (30mcg), Ceftazidime (30mcg), Ciprofloxacin (5mcg), Levofloxacin Clindamycin (2mcg), Cotrimoxazole (25mcg), Erythromycin (15mcg), Gentamicin (10mcg), Imipenem (10mcg), Meropenem Linezolid (30mcg), Piperacillin/tazobactam (100/10mcg), ChoramphenicolTeicoplanin (30mcg), Tetracycline (30mcg), Tigecycline and Vancomycin (30mcg) were used. E-strips were used to determine MIC values for Colistin&Tigecycline for Gram negative bacilli and Vancomycin for Staphylococcus aureus.

Fungus and AFB study:

Ten ml of each sample were centrifuged at 3000 rpm for 20 min and the deposits were wet mounted with 10%potassium hydroxide for fungus examination and acid fast staining smear examination by Kinyoun's technique. The samples from the deposits were inoculated onto Sabourauds agar slants for fungus culture and Lowenstein Jensen slants for mycobacterial culture.Fungus culture was incubated at 30^{o}C aerobic conditions for 4 weeks.All the species found in the culture isolates were identified by standard methods.

Statistical Analysis:-

Data were collected and analyzed using SPSS software by descriptive statistical methods by computing means and proportion with95% confident interval .A 'p' value than less 0.05 was considered as statistically significant.

Results:-

A total of 203 samples were analyzed using the standard protocols. Among these 126 were male and 77 were female.

Table-1:- Age and sex wise distribution of the cases.

S. No	Age group (in years)	Male	Female	Total	Percentage
1	18-30	19	10	29	14%
2	31-40	21	17	38	18%
3	41-50	28	21	49	24%
4	51-60	34	18	52	25%
5	61 and above	24	11	35	17%
	Total	126(62%)	77(38%)	203	

Maximum samples received were males with 62%,with predominant age group of 51 to 60 years

Pathogens Isolated:-

The analysis revealed pyogenic infections of 48% (77), fungal infections 14% (17) and 17% Tubercular infections (21) as shown in fig 1.

Fig 1:- Pie chart of Pathogens Isolates.

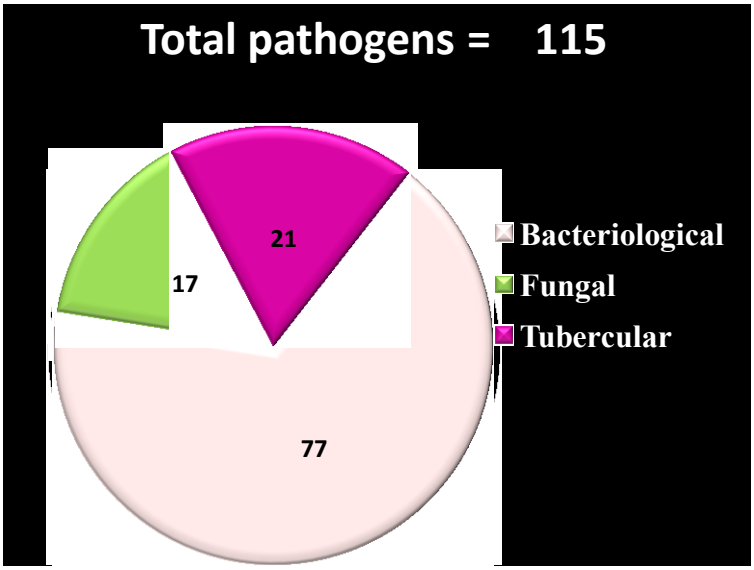


Fig 2:- Bar chart of bacterial Isolates.

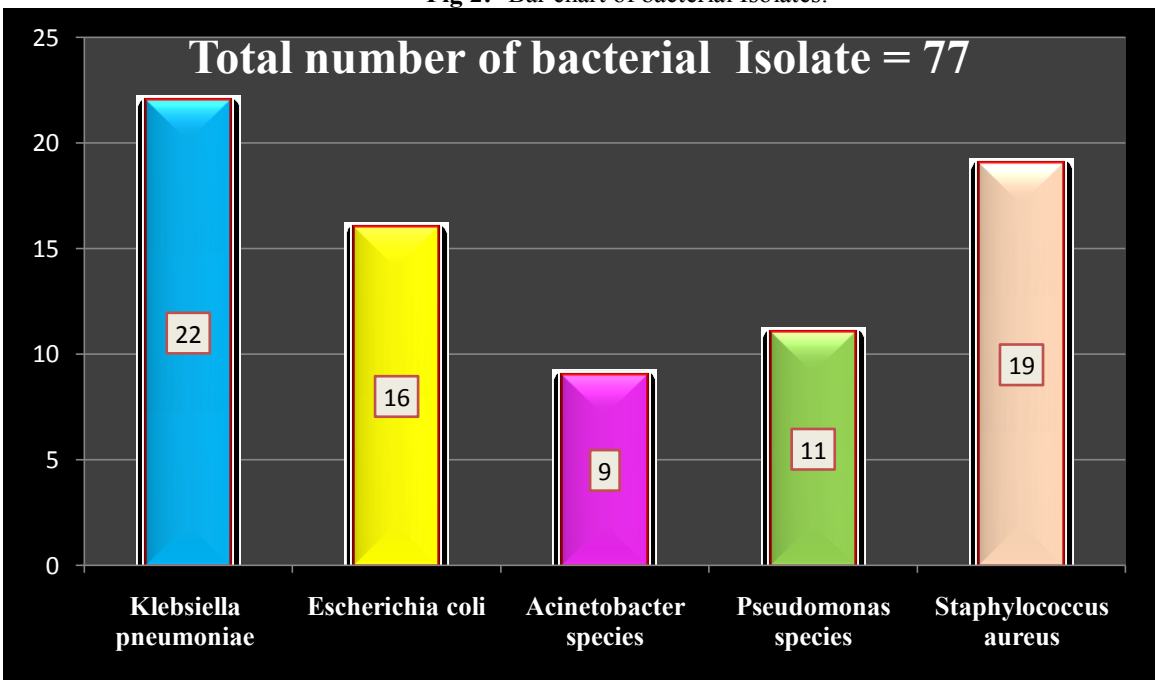


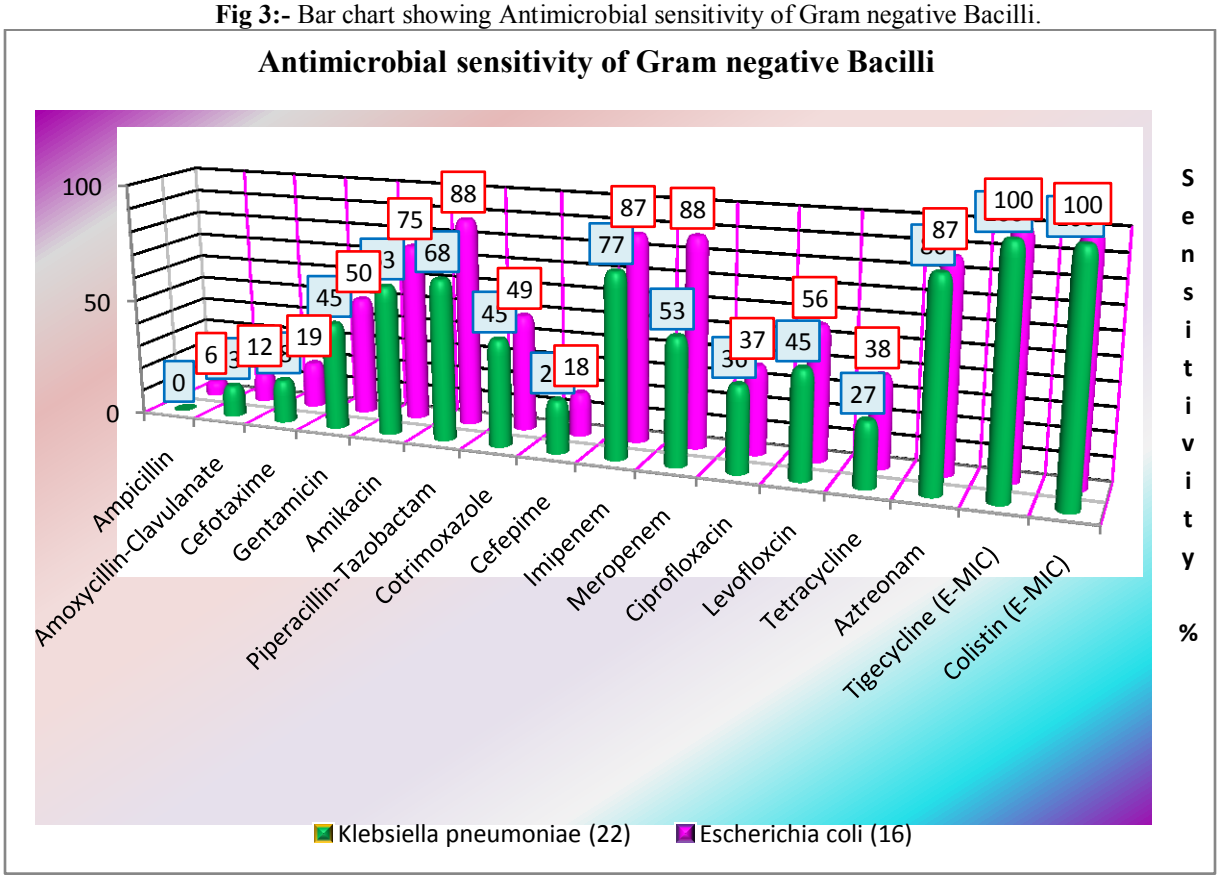
Fig 2 shows that of the total 77 organisms isolated,22 were Klebsiellapneumoniae, 16 were Escherichia coli,9 were Acinetobacter,11 were Pseudomonasand 19 wereStaylococusaureus.

Table 2:- Antimicrobial sensitivity of Gram negative Bacilli.

Drugs	Klebsiellapneumoniae (22)	Escherichia coli (16)
Ampicillin	0	6
Amoxycillin-Clavulanate	13	12
Cefotaxime	18	19
Gentamicin	45	50

Amikacin	63	75
Piperacillin-Tazobactam	68	88
Cotrimoxazole	45	49
Cefepime	22	18
Imipenem	77	87
Meropenem	53	88
Ciprofloxacin	36	37
Levofloxcin	45	56
Tetracycline	27	38
Aztreonam	86	87
Tigecycline (E-MIC)	100	100
Colistin (E-MIC)	100	100

Fig 3:- Bar chart showing Antimicrobial sensitivity of Gram negative Bacilli.



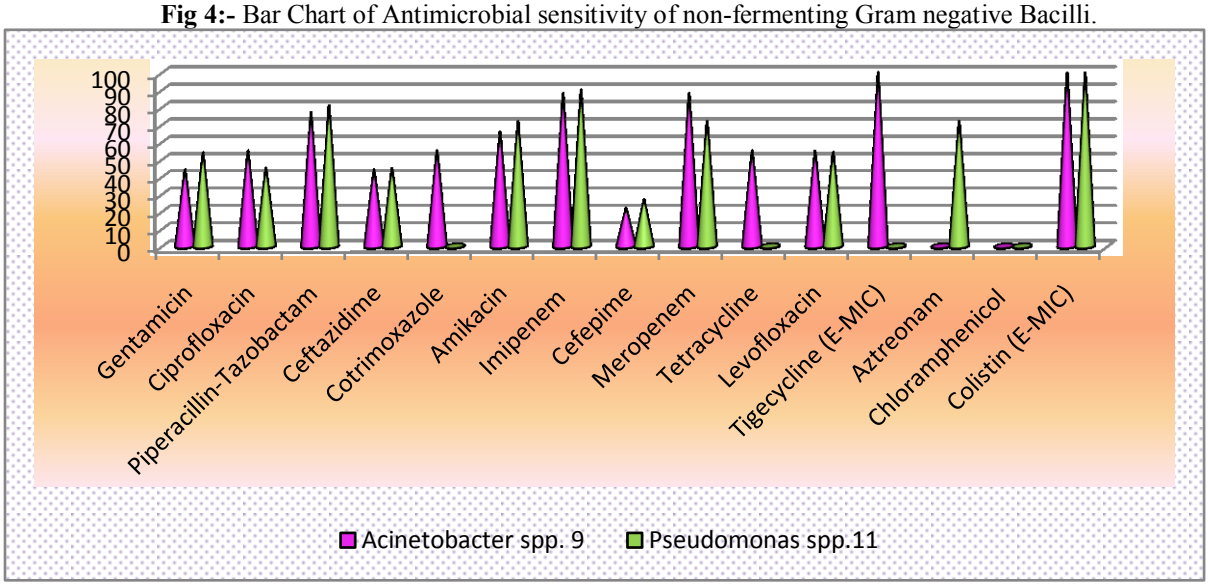
Klebsiellapneumonia and E-coliwere highly sensitive to Tigecycline,Colistin,Imipenem,Aztreonam .Moderately sensitive to Piperacillin-Tazobactam,Amikacin,Levofloxacin,Ciprofloxacin ,Meropenem and least sensitive to Amoxycillin-Clavulanate and Cefotaxime as shown in Fig3.

Table 3:- Antimicrobial sensitivity of non-fermenting Gram negative Bacilli.

Drugs	**Acinetobacter spp. 9**	**Pseudomonas spp.11**
Gentamicin	44	54
Ciprofloxacin	55	45
Piperacillin-Tazobactam	77	81
Ceftazidime	44	45
Cotrimoxazole	55	IR
Amikacin	66	72

Imipenem	88	90
Cefepime	22	27
Meropenem	88	72
Tetracycline	55	IR
Levofloxacin	55	54
Tigecycline (E-MIC)	100	IR
Aztreonam	IR	72
Chloramphenicol	IR	IR
Colistin (E-MIC)	100	100

Fig 4:- Bar Chart of Antimicrobial sensitivity of non-fermenting Gram negative Bacilli.

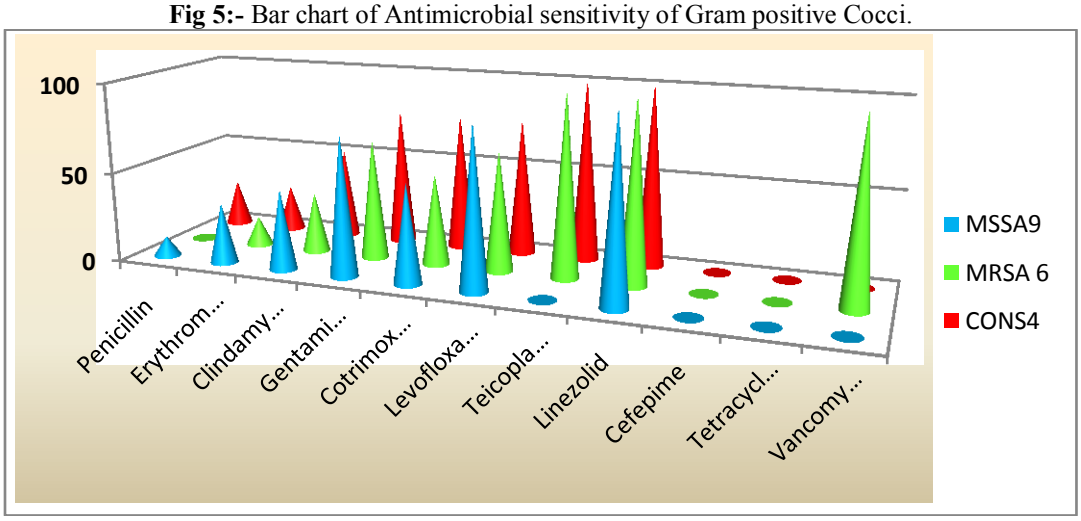


Pseudomonassps were highly sensitive to Colistin,Imipenem,Piperacillin-Tazobactam, Meropenem and Aztreonam. Acinetobactersps were highly sensitive Colistin, Tigecycline,Meropenem and Imipenem as shown in fig 4.

Table 4:- Antimicrobial sensitivity of Gram positive Cocci.

Antibiotics	**MSSA9**	**MRSA 6**	**CONS4**
Penicillin	11	0	25
Erythromycin	33	16	25
Clindamycin	44	33	50
Gentamicin	77	66	75
Cotrimoxazole	55	50	75
Levofloxacin	88	66	75
Teicoplanin(E-MIC)	-	100	100
Linezolid	100	100	100
Cefepime	-	-	-
Tetracycline	-	-	-
Vancomycin(E-MIC)	-	100	-

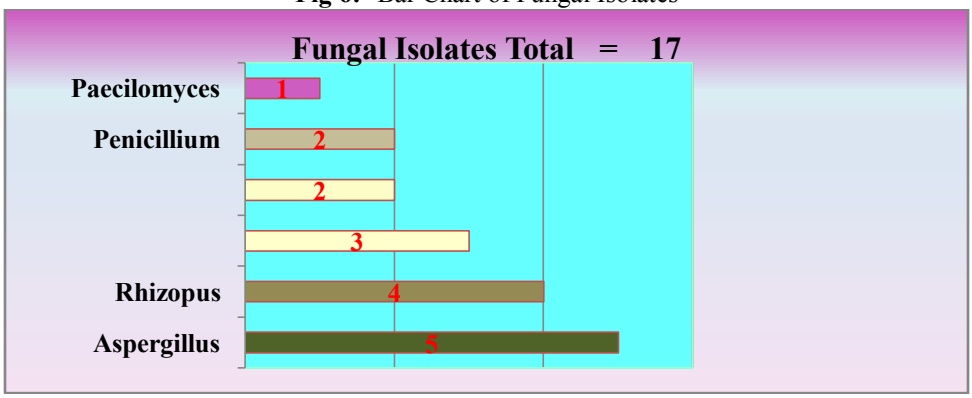
Fig 5:- Bar chart of Antimicrobial sensitivity of Gram positive Cocci.



Staylococusspecies were the only gram positive cocci pathogens isolated. Methicillin sensitive Staylococusaureuswere 9 and resistant were 6.Cogulase Negative Staylococusaureus were 4. All were highly sensitive to Linezolid as shown in fig 5.

Fungal Isolates:

Fig 6:- Bar Chart of Fungal Isolates



Fungal pathogens detected were17 consisting of Aspergillus, Paecilomyces, Penicillium, Rhizopus and Candida albicans and non albicans as shown in fig 6. Gram's staining was 87% sensitive correlating with culture positive.

Discussion:-

A total of 203 BAL samples were evaluated.In this study the most of the chronic respiratory diseases belong to51-60 years with 25% closely followed by 41-50 years with 24% comparable to the results in a study done by Syed Abdul Bari and Maimoona Mustafa. Predominance of male (62%) over female patients (38%) can be explained by the fact that males are exposed because of their mobility as compared to females.

Of the culture positive samples, 77 (67%) were bacterial infection, 21 (18%) samples were mycobacterium tuberculosis and 17 (16%) were fungal isolates.

The most common gram negative bacterial isolate was Klebsiellapneumoniae (28.57%) which correlates with studies conducted byVivek KU, Nutan Kumar DM,Butin contrast to the study conducted by Rahul Magazine, BhartiChogtuetal which showed d Pseudomonas aeruginosa. The other bacteria were Escherichia coli (20.77%), Pseudomonas species (14.28%) and Acinetobacterspp(11.68%).

Staphylococcus aureus species(12.86%) were the common gram positive cocci in this study, similar to the study bySyed Abdul Bari and Maimoona Mustafa.Among Staphylococcus aureus 40% were Methicillin resistant and 60% were Methicillin sensitive.

Klebsiellapneumoniae and E-coli were highly sensitive to Tigecycline,Colistin,Imipenem, and Aztreonam. They were moderately sensitive to Piperacillin-Tazobactam,Amikacin,Levofloxacin,Ciprofloxacin,and Meropenem. But they were leastsensitive to Amoxycillin-Clavulanate and Cefotaxime. These findings correlate with the study done by Rahul Magazine, BhartiChogtuetal.

Non-fermenting Gram negative Bacilli:Pseudomonasspecies were highly sensitive to Colistin,Imipenem, Piperacillin-TazobactamMeropenem and Aztreonam. Acinetobacterspecies were highly sensitive Colistin, Tigecycline,Meropenem and Imipenem.

The fungal isolates were Candida species (35.29%),Aspergillus species (29.41%), Rhizopus(17.64%), Penicillium(11.76%)and Paecilomyces (5.88%).

Conclusion:-

Systematicculture of BAL fluidgives a definite diagnosis of chronic pulmonary infections which would guide in selection of appropriate antimicrobials for management of pneumonia.The microbiologic profile of the BAL fluid isolated from the chronic respiratory infections shows predominance of gram negative pathogen. With increasing incidence of antibiotic resistance in gram negative isolates mainly because of indiscriminate use of empirical antibiotics.A study like this would help and guide the physicians in prescribing the right combinations of anti-microbial to limit and prevent the emergence of multi-drug resistant strains of Bacteria.

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