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Bacteriological and Mycological Profile of Chronic Obstructive Pulmonary Disease (COPD) In Tertiary Care Center

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Abstract:

Background: Chronic obstructive pulmonary disease (COPD) is a common disease characterized by persistent airflow obstruction with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. The aim of the present study was to obtain comprehensive insight into the bacteriological and fungal profile and their antibiotic sensitivity pattern in sputum culture of AECOPD patients.

Methods: The study was conducted in Saveetha Medical College and Hospital for the period of Six months from April 2017 to September 2017.110 sputum samples were analysed for bacterial and fungal culture and antimicrobial and antifungal susceptibility pattern.

Results: 110 sputum samples diagnosed for AECOPD included with age group comprising from 40 > 70 years of age in which 21 was positive for Bacterial and 9 was positive for Fungi.

Conclusion: Among gram negative bacteria predominantly *Pseudomonas aeruginosa* is the commonest bacteria. The other important organisms are *Klebsiella pneumoniae, E. coli, Acinetobacter species*. Antibiotics showing high activity against Gram negative organisms are Piperacillin tazobactam, carbapenemase, and quinolones. Candida is most common fungi to be isolated followed by *Aspergillus flavus*. Amphotericin B, Itraconazole were most effective antifungals. *Aspergillus flavus* in our study was found to be resistant to both Fluconazole and Itraconazole by broth micro dilution method

Keywords: AECOPD, Bacteria, Fungi, Antimicrobial susceptibility pattern

INTRODUCTION

Chronic lung diseases are with the mankind for centuries together. It is an ancient disease and one of the major public health problems in the developing countries of the world today. It has made its impact felt throughout the ages, no other diseases have so much sociological, economic and health significance as Chronic lung disease [(1)]. Even nowadays it has become the killer number one in the developing countries like India. Chronic obstructive pulmonary disease (COPD) is the progressive chronic disease which is characterized by persistent airflow obstruction with an enhanced chronic inflammatory response in the airways and the lung to noxious particles and gases [(2)].

COPD also known as Chronic Obstructive Airway disease (COAD), chronic airflow limitation (CAL) and chronic obstructive respiratory disease (CORD) [(1)], Global Initiative for chronic obstructive disease (GOLD) [(3)].

COPD is the major cause of morbidity and one of the principle causes of the death worldwide(2).It is the fourth leading cause of the death in the word [(2)].Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is defined as sustained worsening of patient's condition, from the stable state and beyond normal day today variations that is acute in onset and necessitates a change in regular medications in a patient with underlying COPD(2).It is the important factor of mortality in COPD patients[(3)].

Bacterial infections are most common cause of AECOPD. It is estimated that bacterial infections are responsible for more than 40% of all exacerbations in India[(2)].It has been found that use of antibiotics as well as type of antibiotics used to treat AECOPD has an impact on the failure[(4)].Bacterial flora of AECOPD is changing from usual pathogen[(3)].More than 90% of patients with AECOPD are treated with antibiotics, due to emergence of resistant strains of most common respiratory pathogens effectiveness of many is uncertain in past 15 years[(6)].Now a day's investigators needs culture studies for proper selection of antibiotic but it is time consuming process and the facility is not available in most of the institutions[(2)]. The choice of antibiotic should be based on the local resistance pattern [(1)].

Pathogenic bacteria organisms are found in 50-80% of patients with COPD during exacerbations [(8).] The predominant organisms believed were *Streptococcus pneumoniae, H.influenzae*, Moraxella *catarrhalis*, are been recognized as causes of purulent exacerbations of COPD[(4).] *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been found in more severe cases [(4).]

Fungal infections have been recently emerged as a worldwide health care problem [(5).] *Aspergillus* infection is the commonest invasive fungal infection which involves respiratory tract. Man acquires the infection through inhalation of fungal spores though infection has also been documented by exposure to water aerosols contaminated with *Aspergillus* spores [(5).]Chronic Obstructive Pulmonary Disease (COPD) has been recognized as a risk factor for invasive aspergillosis [(6).]According to the National Nosocomial Infection Surveillance System, Aspergillus species were responsible for 1.3% of the nosocomial fungal infections [(7)].

The potential role of fungal colonization and infection in the pathogenesis of COPD is poorly understood as bacteria and viruses were usually considered as the major cause of COPD exacerbations [(3).]

Antibiotic therapy almost requires empiric [6], which is frequently unavoidable with the background of little information available from Indian literature [5]. On this basis of study was undertaken to know the bacteriological and mycological profile of COPD patients with exacerbations and design a proper antibiotic susceptibility pattern, which will have a beneficial effect on the morbidity and mortality of the disease [7].

MATERIALS AND METHODS

This prospective study was undertaken over a period of six months (April 2017-September 2017) at the Department of Microbiology, Saveetha Medical College and Hospital Thandalam after obtaining approval from Institutional ethical committee. Sputum samples were collected from COPD patients with exacerbation and further processed for isolation of pathogenic bacteria and fungi. Patients diagnosed with COPD on the basis of history of exposure to risk factors, clinical history and examination supported by spirometry and chest x ray and Broncho alveolar lavage (BAL) and Endotracheal (ET) aspiration were excluded. Sputum culture and sensitivity reports were analysed for the bacteriological and fungal profile and each patient's data were categorised under age, sex.

BACTERIAL AND FUNGAL CULTURE

The samples were examined microscopically after staining with Gram's stain and grading was done by Bartlett's grading system [12] plated directly on Blood agar, Chocolate agar, MacConkey's agar and Sabouraud's Dextrose agar. Plates were incubated aerobically at 37°c and Sabouraud's Dextrose agar 24°c and 37°c for 24 hours. On one blood agar streaking with Staphylococci was done to facilitate growth of *Haemophilus influenza*. Bacterial isolates were identified on the basis of colony morphology; Gram staining and standard biochemical reactions followed by Antibiotic susceptibility test [12] using antibiotics from HI media. Fungal identification were done by Lactophenol Cotton Blue Staining (LPCB) [13] and followed by appropriate identification tests for species identification and antifungal susceptibility test. For Candida identification isolates was inoculated into HI Chrome agar and followed by Sugar Assimilation test [14].

ANTIFUNGAL SUSCEPTIBILITY TEST USING DISC DIFFUSION METHODFO*R Candida* [14]

- Muller Hinton Agar with 0.5 g/ml methylene blue and 2%glucose
- The inoculums were made by lawn culture
- Then the antifungal disc is placed in the plate
- Incubated for 24 hours at 37 C and zone size is measured and compared with CLSI guidelines

MICRO BROTH DILUTION METHOD FOR *Aspergillus* [15]

The RPMI 1640 medium with glutamine but without sodium bicarbonate are buffered with 0.165M morpolinepropanesulfonic acid (MOPS) at Ph 7.0. The medium is sterilized by filtering the solution using 0.22 micro litre filters.

PROCEDURE OF THE INOCULUM

- 100 micro litres of conidial suspension to 5ml of RPMI 1640 medium
- Column 1 was filled with 200 micro litres of RPMI 1640 medium without drug or inoculums suspension, to serve as sterility control
- 100microlitre of the inoculums and 100 micro liter of drug free RPMI 1640 medium to serve as growth controls.

RESULTS & DISCUSSION

The present study consisted of 110 patients admitted in Saveetha Medical College and Hospital during the period of six months. Out of 110 Sputum culture 21 were found to be bacterial isolates and 9 were found to be fungal isolates. Comparatively similar study was done by Iyer et al analysed that bacterial pathogens can be isolated from sputum in 45% of patients with AECOPD during exacerbations [16].

Among 110 patients 89 (81%) are male patients, and 21 (19%) are female patients. Similar findings were observed in other studies which conclude that AECOPD is more common among male [17]. This male predominance can be explained by the fact that male patients included in our study were found to be smokers. Moreover, smoking habits are more common in males that constitute one of the factors for development of COPD.

It was observed that AECOPD was more prevalent in 45-80-year age group. High prevalence of AECOPD among males may be contributed to the fact that, were they were more involved in smoking & start it in younger age groups Madhavi et al [12] reported that maximum numbers of AECOPD were ≥ 65 years of age.

In our study, bacterial culture was positive for 21(19%) out of 110 sputum samples. Iyer et al [16] analysed that bacterial pathogens can be isolated from sputum in 45% of patients with COPD during exacerbations. Comparatively lower culture positivity in our study can be explained by the overuse of antibiotics by patients which are easily available even without prescription. This overuse of antibiotics may lead to drug resistance in the community in future.

Pseudomonas aeruginosa 10(48%) was commonest pathogen followed by *Klebsiella sps* 7(33%), *E. coli* 3(14%), *Acinetobacter* 1(5%). In studies done elsewhere in India by Madhavi et al [12] on AECOPD also reported *Klebsiella pneumoniae* as the predominant organism isolated.

In our study all gram-negative bacilli were found to be resistant to Ampicillin. However, for *Pseudomonas spp* resistance to 3rd generation cephalosporin's were comparatively less Ceftazidime (30%), Cefepime (50%). 40% isolates of *Pseudomonas* were also found to be resistant to Imipenem and piperacillin tazobactam. So, piperacillin tazobactam, carbapenemase and third generation cephalosporin's are still drugs of choice against *Pseudomonas* in our set up. Resistance to quinolones was also found to be high (60%) for *Pseudomonas*. This can be explained by the fact of overuse abuse of quinolones and

penicillin's as over the counter drugs by patients in the community.

For *Klebsiella* sps resistance to 3rd generation cephalosporin was high Cefepime (100%), Ceftriaxone (57%). But resistance to amino glycosides like Amikacin and Gentamicin was least (14%) & (28%) respectively.28% isolates were found to be resistant for Imipenem, piperacillin tazobactam and Ciprofloxacin. So, piperacillin tazobactam, carbapenemase, quinolones, amino glycosides are drug of choice against *Klebsiella* sps in our study. Third generation cephalosporin have less efficacy against *Klebsiella* as shown in our study.

For *E. coli* resistance to 3rd generation cephalosporin was high Cefepime (100%), Ceftriaxone (67%) which is almost similar to *Klebsiella*. No resistance was observed in amino glycosides, carbapenemase and isolates were found to be resistant for ciprofloxacin (66%). Piperacillin tazobactam was less resistant (33%). So, Piperacillin tazobactam, amino glycosides, carbapenemase are drug of choice for *E. coli*

Single isolate of *Acinetobacter spp* in our study was found to be resistant to all drugs tested i.e. Multi Drug Resistance (MDR) which was sensitive to only higher drug like Colistin and polymyxin B

Figure 1 Chrome Agar plate

Figure 2 sugar assimilation test

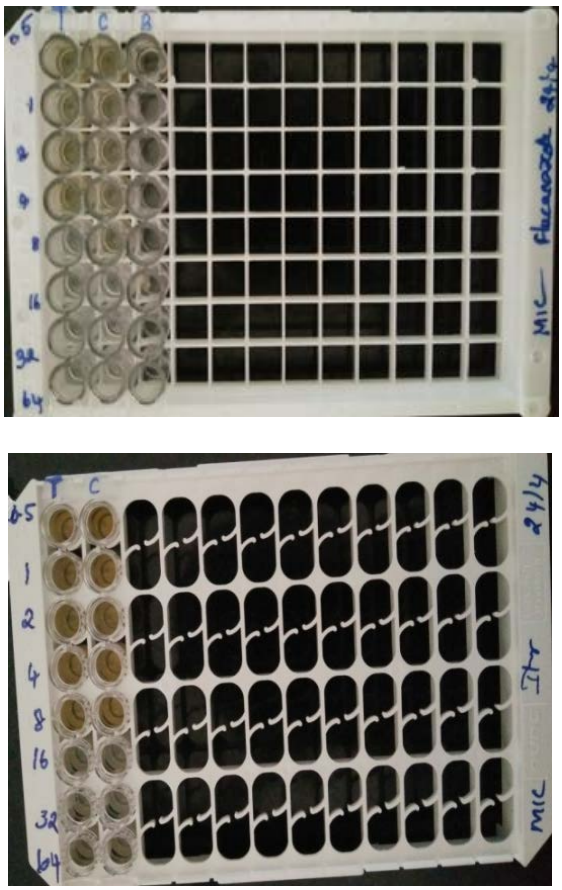

Figure 3 MIC for *Aspergillus flavus*

In Indian study by Sharon et al [20] it was found that piperacilline+tazobactum was the most effective antibiotic resistant to all gram-negative organisms along with Amikacin. Chawla et al [21] observed that piperacillin – tazobactam was effective against gram-negative organism *Pseudomonas aeruginosa.* Ceftriaxone was also most effective against gram-negative organism like *Klebsiella* and *E. coli.* These findings are similar to our finding which concludes that piperacillin tazobactam and amino glycosides are the most effective antibiotics against *Enterobacteriaceae and Pseudomonas.* Though 3rd generation cephalosporin's have efficacy against *Pseudomonas* as found in our study the same cannot be applicable for *Enterobacteriaceae*.

In our study, 9(11%) sputum samples were positive for fungus by both KOH and fungal culture. *Candida* (89%) was most common fungus isolated and followed by one isolate of *Aspergillus flavus* (11%). So, in our study bacteria was isolated from majority of patients of COPD compared to fungus (9/110). Ronald et al [23] reported 13% positivity for candida from sputum samples of COPD patients which are similar to our study. Patients with previous severe exacerbations are more likely to receive a higher number of antibiotic therapies as well as steroids for symptomatic relief. These patients are at high risk of developing fungal infections. One limitations of our study we have used only sputum samples for fungal culture. Use of Bronchoalveolar lavage (BAL) may increase the

positivity rate for fungus in such patients which were negative for bacterial culture. Guinea et al [23] reported a high isolation rate of 20% for *Aspergillus fumigatus* using both sputum and BAL samples of COPD patients.

Jayalakshmi et.al [24] reported resistance to Fluconazole as 11.7% and 12.1% among *candida* spp isolated from AECOPD cases. Patients on Fluconazole suppressive therapy are more likely to have infection caused by non-albicans species

In our study among *candida* isolates, *Candida albicans and tropicalis* were the commonest species. However, among albicans, one isolate was resistant to all the azoles and Amphotericin B. Resistance to Azoles was 66% among *Candida albicans.* All the 3 isolates of *Candida tropicalis* in our study were sensitive to Itraconazole and Amphotericin B. Two isolates of *Candida glabrata* were sensitive to only Amphotericin B and resistant to Azoles which can be explained by the fact that glabrata is intrinsically resistant to azoles.

In this study, candida isolates showed 66% resistance to Fluconazole, Itraconazole, Amphotericin B and similar to the study which is done by in gevandenbossche et.al [20]

The single isolate of *Aspergillus flavus* in our study was found to be resistant to both Fluconazole and Itraconazole by broth micro dilution method. *A.flavus* contributes to the cause of airflow obstruction or is a consequence of a damaged and remodelled airway and thus more likely in subjects with severe COPD.

CONCLUSION

COPD is one of the common lung disorders encountered in clinical practice and sputum culture and sensitivity is one of the good and less expensive method to study the aetiology and associated conditions with COPD. Antibiotic sensitivity also helps in determining antibiotic protocol treatments in patients with COPD for better prognosis and reducing morbidity and mortality. It is concluded from the present study that bacterial infection is more common than fungal infection. Gram negative bacteria are seen predominantly *Pseudomonas aeruginosa* is the commonest bacteria, precipitating COPD.The other important organisms are *Klebsiella pneumoniae, E. coli, Acinetobacter species*.

Antibiotics showing high activity against Gram negative organisms are Piperacillin tazobactam, carbapenemase, and quinolones.

Fungi candida is most common fungi to be isolated followed by *Aspergillus flavus*. Antibiotics showing high activity against Amphotericin B, Itraconazole

Aspergillus flavus in our study was found to be resistant to both Fluconazole and Itraconazole by broth micro dilution method**.**

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