

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.504.012>

Bacteriological Profile of Patients with AECOPD- Hospital Based Study

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ABSTRACT

Keywords

Haemophilus influenza,
Chronic obstructive pulmonary disease,
Tuberculosis.

Article Info

Accepted:
09 March 2016
Available Online:
10 April 2016

COPD is defined as a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. Exacerbations, mostly of bacterial, are a frequent cause of morbidity in COPD patients. A total of 148 sputum samples were collected from AECOPD patients. All collected sputum samples were subjected to standard microbiological procedures. Out of 148 sputum samples, growth of pathogenic organisms was obtained in 109 (73.65%) sputum samples. Gram negative organisms were isolated predominantly 68(62.39%). While Gram positive organisms accounted for 41(37.61%). *Haemophilus influenzae* 38(34.86) was the predominant bacteria isolated followed by *Streptococcus pneumoniae* 25(22.94). Eight patients had positive mycobacterial sputum culture on Lowenstein Jensen medium. Majority of isolates were sensitive to piperacillin/ tazobactam followed by amikacin. *H. influenzae* was the commonest bacterium isolated predominantly. Piperacillin/ tazobactam and amikacin were the most active antibacterial agents in our study. It is essential to screen for sputum AFB in endemic areas of tuberculosis.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a usually progressive decline in lung function and worsening of breathlessness, exercise capacity and impairment of quality of life with time.¹ Exacerbations are episodes of acute worsening of clinical condition in patients with COPD. An episode of acute exacerbation of COPD (AECOPD) was defined as, “an acute event characterized by worsening of the patient’s respiratory

symptoms that is beyond normal day-to-day variations and leads to a change in medication”. (GOLD, 2014) One of the earliest studies to know the prevalence of COPD in India was carried out by Wig *et al* in rural Delhi (Wig *et al.*, 1964). COPD is the fourth leading cause of death in the world and will become the third leading cause of death worldwide by 2020. Four to ten percent adult male population of India and 3.5 to 6.5% population in Asia pacific countries suffer from this disease (Patel *et al.*, 2015).

Exacerbations can be of bacterial, viral or non-infectious etiology, and a significant proportion can be multi-factorial (Papi *et al.*, 2006). Cigarette smoking or inhalation of dust or fumes are important contributing factors (Jindal *et al.*, 1983) The prevalence of AECOPD varying from 1% in urban non-smoker to 21% in rural smokers and mortality rate of 24% if the patient required ICU admission. This mortality rate increased to 30% if the patient was above 65 years (Sharan, 2015) It is estimated that bacterial infections are responsible for more than 40% of all exacerbations in India (Chawla, 2008).

In previous studies, the predominant organisms during acute infective exacerbations were believed to be *Streptococcus pneumoniae*, nontypable *Haemophilus influenzae*, and to some extent *Moraxella catarrhalis* (Murphy, 1987). However, these studies were based on heterogeneous patient groups and the results are difficult to interpret because of methodologic differences related to the use of either expectorated sputum or specimens obtained by bronchoscopy (Monso, 1995). Bacterial flora of AECOPD is changing from usual pathogen. The choice of the antibiotic should be based on the local bacterial resistance pattern. Therefore the knowledge of bacterial flora of patients of AECOPD of that geographical area is required (Patel, 2015).

Hence we aimed at determining the bacteria which is predominantly responsible for AECOPD. The study was also done to assess their antimicrobial susceptibility pattern of these isolates so that we can design a proper antibiotic regimen which will have a beneficial effect on the morbidity and mortality of the disease.

Materials and Methods

This is a prospective, observational study in

which a total of 148 sputum samples were collected from AECOPD patients who attended the Inpatient and Outpatient Departments of Chennai Meenakshi Multispeciality Hospital, Luz Churuch Road, Chennai-600 004, India over a period of one year from January 2014 until December 2014.

Inclusion Criteria

Patients were diagnosed by the clinician concerned depending upon the presence of two of the following symptoms:

Increased cough

Increased purulence and/or volume of expectorations

Increased severity of dyspnoea.

Exclusion Criteria

Previous diagnosis of bronchial asthma, cystic fibrosis, or bronchiectasis

Diagnosis of neoplasia

Clinical-radiologic evidence of pneumonia

Any type of antibiotic treatment received over the 5 days prior to sampling the sputum for culture.

Specimens

Early morning expectorated sputum samples were collected in wide mouth sterile containers after giving appropriate instructions for all patients. The quality of the sputum was assessed by both macroscopic and microscopic examination. All sputum samples were subjected to Gram staining and reported based on Bartlett's grading system (Koneman *et al.*, 2006). All unsuitable specimens were discarded and a

repeat specimen was collected. The suitable sputum samples were inoculated onto MacConkey's agar, chocolate agar and two blood agar plates. On one blood agar streaking with Staphylococci was done to facilitate growth of *Haemophilus influenzae*. All the plates were incubated at 37°C for 24 hours in 7-10 % CO₂ concentration. Acid fast staining and culture for Mycobacterium species onto Lowenstein-Jensen medium after decontamination of sputum specimens were performed according to standard procedures (Murray *et al.*, 2006)

Antibiotic Sensitivity Testing

Antimicrobial susceptibility of the isolates was determined against the following antibacterial agents by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2011).

Amikacin (AK-30µg), Ceftazidime (Ca- 30 µg), Cefotaxime (Ce-30µg), Ciprofloxacin (Cf- 5µg), Cotrimoxazole (Co-25µg), Gentamycin (G-10µg), Piperacillin-Tazobactam (Pt- 100/10µg), Imipenem [I 10µg], Cephoxitin (Cn-30µg), Clindamycin (Cd-2µg), Erythromycin (E-15µg), chloramphenicol (30 µg), Penicillin (P-10 units), Linezolid (Le-30µg), Vancomycin (Va-30 µg) (Hi Media, Mumbai).

All the analysis was performed using simple percentage method.

Results and Discussion

A total of 148 cases were included in the present study 124 (83.79%) males and 24 (16.21%) females having mean age 54.7 years. All males were smokers while all females though non-smokers, but exposed to biomass fuel. Growth of pathogenic organisms was obtained in 109 (73.65%) sputum samples. It was observed that

purulent sputum gave better isolation of pathogens than mucoid sputum. (Table 1) Gram negative organisms were isolated predominantly 68 (62.39%). While Gram positive organisms accounted for 41 (37.61%).

Haemophilus influenzae 38 (34.86) was the predominant bacteria isolated followed by Streptococcus pneumonia 25 (22.94), Klebsiella pneumonia 11 (10.09%), Pseudomonas aeruginosa 19 (17.43%) and Staphylococcus aureus 8 (7.34%). A total of eight patients had positive mycobacterial sputum culture. Out eight patients with positive mycobacterial growth, *M. tuberculosis* (MTB) was identified in five (4.59%) patients whereas atypical mycobacterium was identified in three (2.75%) patients. All three atypical mycobacteria were identified as *M. chelonae*.

Mixed infections were identified in 17 (15.60%) sputum samples. Only one patient had positive growth on both routine culture media (Pseudomonas aeruginosa) and Lowenstein Jensen medium (*Mycobacterium tuberculosis*).

Antibiotic sensitivity pattern to all organisms is shown in Table 2. Majority of isolates were sensitive to piperacillin/tazobactam followed by amikacin irrespective of gram positive or negative organisms. All gram negative bacteria were susceptible to Imipenem except Pseudomonas aeruginosa, where four isolates were resistant. All gram positive bacteria were susceptible to Vancomycin and Linezolid uniformly. Chronic obstructive pulmonary disease (COPD) is a leading cause of mortality worldwide and is associated with an important morbidity related in large part to acute COPD exacerbations (AECOPD) (Ai-Ping, 2005).

Table.1 Demographic Data of Patients with COPD and Physical Characteristics of Sputum Samples

Age	Mean 54.7
Sex	
Male	124(83.79%)
Female	24(16.21%)
Smoking status	
Smokers	42 (28.38%)
Ex-smokers	77 (52.02%)
Non-smokers	29 (19.59%)
Biomass fuel	17(11.49%)
Physical characteristics of sputum	
Purulent samples	112/148
Mucoid samples	36/148
Significant growth of pathogen	
Purulent samples	94/112(83.93%)
Mucoid samples	15/36(41.67%)

Table.2 Antibiotic Sensitivity Pattern of Bacteria

Isolate	AK	G	I	PIT	E	CiP	mox	Cefataxi	Cefuroxi me	VA	LZ
<i>H.influenzae</i> (38)	35 (92.11%)	15 (59.47%)	38 (100%)	36 (94.74%)	19 (50%)	17 (44.74%)	31 (81.58%)	19 (50%)	11 (28.95%)	NT	NT
<i>Streptococcus Pneumoniae</i> (25)	21 (84%)	19 (76%)	NT	22 (88%)	15(60%)	13 (52%)	15 (60%)	11 (44%)	15 (60%)	25 (100%)	25 (100%)
<i>Pseudomonas aeruginosa</i> (19)	15 (78.95%)	11 (57.89%)	16 (84.21%)	16 (84.21%)	NT	9 (47.37%)	10 (52.63%)	8 (42.11%)	9 (47.37%)	NT	NT
<i>Klebsiella Pneumoniae</i> (11)	9(81.82%)	5 (45.45%)	11 (100%)	11 (100%)	NT	4 (36.36%)	4 (36.56%)	6 (54.55%)	2 (18.18%)	NT	NT
<i>Staphylococcus aureus</i> (8)	6 (75%)	2 (25%)	NT	7 (87.5%)	2 (25%)	4 (50%)	6 (75%)	2 (25%)	1 (12.5%)	8 (100%)	8 (100%)

Sputum culture was positive in 73.65% of patients with AECOPD which was higher as compare to other studies. According to the study conducted by Patel *et al.* (2015) Sputum culture was positive in 82% of

patients with AECOPD which was higher as compare to other studies. (Ko *et al.*, 2008)

This difference may be because of the fact that culture positivity depends on nature of

sputum, time of collection sputum and previous antibiotic use. Males were affected more than females because they were more involved in smoking and therefore more chance of inhalation and increased environmental exposure or temperature variation. (Raza *et al.*, 2013)

All females though non-smokers, but had constant exposure to biomass fuel. Majority of females encountered in our study are from sub urban areas. Worldwide, about 50% of all households and 90% of rural households use biomass fuel (wood, charcoal, other vegetable matter, and animal dung) and coal as their main source of domestic energy. About 50% of deaths from COPD in developing countries are attributable to biomass smoke, of which about 75% are of women. (Lopez *et al.*, 2006). More than 80% of homes in China, India, and sub-Saharan Africa use biomass fuel for cooking. (Reddy *et al.*, 1996)

Gram negative organisms were isolated predominantly 68 (62.39%). While Gram positive organisms accounted for 41 (37.61%). According to the study conducted by Dalia *et al* (2016), Gram positive bacteria represented 80% of isolates, while Gram negative bacteria represent the remaining 20%. However other studies reported the predominance of Gram negative bacteria. (Viswambhar *et al.*, 2013 and Madhavi *et al.*, 2012) In our study *Haemophilus influenzae* 38(34.86%) was the most common pathogen followed by *Streptococcus pneumonia* 25(22.94%). This finding is contrary to other studies reported from by Chawla *et al.* (2008) who had found *Pseudomonas aeruginosa* while Madhavi *et al.* (2012) had found *Klebsiella pneumonia* as the most common organism. However, Groenewegan *et al.* (2003) reported *Hemophilus influenza* (45%) as most frequent organism followed by *streptococcus pneumonia* (27%).

Screening for TB in patients with AECOPD is essential in this locality with a high background prevalence of TB infection as systemic steroid is often prescribed for AECOPD in order to speed up recovery of airway function and reduce hospital length of stay. (Niewoehner *et al.*, 1999) In our study *M. tuberculosis* (MTB) was identified in five (4.59%) patients whereas atypical mycobacterium was identified in three (2.75%) patients. Atypical mycobacterial growth in sputum of these patients might be due to colonization rather than genuine infection. Nevertheless, careful follow-up with repeated sputum cultures and radiological examination is needed in these patients.

In the present study, amikacin, piperacillin-tazobactam and imipenem showed good response among Gram negative bacilli. Ciprofloxacin and cephalosporins showed decreased sensitivity. All strains of Gram positive cocci were sensitive to vancomycin and linezolid. However, vancomycin-intermediate and vancomycin resistant *S. aureus* (VISA and VRSA) strains have been reported recently from various parts of the country. (Menezes *et al.*, 2008) According to the study conducted by Patel *et al.* (2015) piperacilline/tazobactam was the most effective antibiotic against all organisms (gram positive and gram negative) which is in agreement with the present study.

In conclusion, *H. influenzae* was the commonest bacterium isolated predominantly. Piperacillin/ tazobactam should be the first choice empirical antibiotic. It is essential to screen for sputum Acid Fast Staining in endemic areas of tuberculosis. Further studies are required to formulate an antibiotic policy at regular intervals.

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How to cite this article:

M.F. Aleemullah, V. Krishnamurthy, M.Harish and C. Arshad Akeel. 2016. Bacteriological Profile of Patients with AECOPD- Hospital Based Study. *Int.J.Curr.Microbiol.App.Sci*. 5(4): 84-90. doi: <http://dx.doi.org/10.20546/ijcmas.2016.504.012>