

Bacteriological Profile in Acute Exacerbation of Chronic Obstructive Lung Disease (AECOPD).

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ABSTRACT

Background: In COPD, acute exacerbation is the common problem during natural course. Studies of sputum samples using standard culture and molecular techniques have demonstrated that it is associated with increased prevalence of bacteria. **Methods:** 200 clinically diagnosed cases of AECOPD of age ≥ 45 years were recruited. Two sputum samples each were processed by conventional methods. Preparation of media, reagents, Gram staining, identification of culture isolates, different tests, including antibiotic sensitivity tests were carried out following standard laboratory. **Results:** The prevalence of AECOPD was more common in the age group of fifty six to sixty five years (43%) with ratio between male and female of 2.12:1. *Klebsiella pneumonia* was the predominant organism isolated in 42.55%, followed by *Staphylococcus aureus* in 28.73%, *P. aeruginosa* in 14.89%, *E coli* in 8.51%, CONS in 4.26% and *S. pneumoniae* in 1.06%. Gram negative bacteria were most sensitive to meropenem, imipenem, amikacin, followed by cefotaxime ceftriaxone, levofloxacin, Cefepime and aztreonam. Gram-positive bacterial isolates were most sensitive to linezolid (34.04%) followed by vancomycin (32.98%), ceftazidime (31.91%). **Conclusion:** Sputum culture is a good and simple diagnostic tool to study the etiology due to bacteria in AECOPD. Antibiogram helps in the formation of the correct treatment protocol, screening resistant pathogens and better drug for treatment, thereby helping to decrease the mortality and morbidity.

Keywords: AECOPD, bacteria, antibiogram.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a spectrum of disorders that results in airflow obstruction. COPD constitutes 30% of cases seen in chest clinics and accounts for 1-2.5% admissions in hospitals all over India. Males are more often affected than females.^[1] According to Anto J. M. *et al*^[2] COPD is a leading cause of mortality and disability worldwide.

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Acute exacerbation of COPD showed a hospital mortality rate of 24% if the patient required ICU admission. Globally, COPD by 2020 expected to rise to be at the third position as a cause of death and at fifth position as the cause of loss of disability adjusted life years. In COPD, acute exacerbation is the common problem during natural course. Both the

prevalence and mortality from this disease has been increasing worldwide.^[4]

Pathogens that have been implicated as causing acute exacerbation of COPD by infecting the lower respiratory tract are: aerobic Gram-positive and Gram-negative bacteria and respiratory viruses. The relative contributions of these different classes of pathogens may change depending on the severity of the underlying obstructive airway diseases.^[5]

Now it has also become apparent that patients with chronic airways disease (asthma and COPD) have a different spectrum of bacteria in the lower respiratory tract than those with normal lungs.^[7,8-12] Approximately 50% of exacerbations of COPD are associated with the isolation of bacteria from the lower respiratory tract.^[13,14]

Studies of sputum and bronchoscopy samples using standard culture and molecular techniques have clearly demonstrated that COPD exacerbations are associated with a markedly increased prevalence of bacteria. Sputum samples are the first-line investigation used. Overall sputum cultures are still useful in researching the pathogenesis of

exacerbations of COPD, as they provide the pathogens to be studied further.^[15]

It is also essential to know effective and cost saving antibiotic strategy to reduce the emergence of drug resistance. Therefore, this study was undertaken to isolate and identify the bacterial etiological agents causing acute exacerbations in COPD patients and to study the antibiotic sensitivity patterns of the isolates.

MATERIALS AND METHODS

Clearance and permission of institutional ethical committee was taken for this cross-sectional analytical study. 200 clinically diagnosed cases of AECOPD of age ≥ 45 years were recruited from clinics and wards of department of medicine and department of TB Chest of L N Medical College and research centre, Bhopal. The exclusion criteria were patients on maintenance treatment of oral steroids, Subjects with history of recent antibiotic therapy within 7 days, Patient having bronchial Asthma, lung abscesses or lung cancer, Known case of Pulmonary Koch's, diabetes mellitus or HIV and AECOPD Patient on ventilatory support.

Deeply expectorated two sputum samples after an oral gargle with water was collected directly into a sterile and wide mouthed disposable universal container. After proper collection Sputum, samples were transported immediately to Microbiology laboratory for further processing by conventional methods. Murray and Washington's^[21] grading system was followed for assessing the quality of sputum sample of stained smear.

Appropriate sputum sample was inoculated on 5% Sheep Blood agar, Chocolate agar and MacConkey's agar. These inoculated plates were then incubated for a period of 18-24 hours after which they were examined for evidence of bacterial growth. A single well separated colony was identified. Preliminary tests like Grams staining of the colony, Hanging-drop preparation, Catalase test and Cytochrome oxidase test were done. Biochemical tests like Indole test, Methyl red test, Voges proskauer test, Citrate utilization test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifson's oxidation fermentation test, coagulase production (for Staphylococci), Optochin Sensitivity (for *S. pneumoniae*) were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols were done to identify the isolate. These tests were performed according to standard methods.^[22]

Antibiotic sensitivity test of the isolates were performed by Kirby Bauer Disc Diffusion method^[23] using Mueller Hinton agar and antibiotic discs, as described by Clinical Laboratory Standard Institute (CLSI) guidelines^[24] was followed to perform antibiotic sensitivity test.

Antibiogram was read, that is zones of inhibition were measured and sensitivities to various antibiotics were determined using CLSI guidelines^[24] for each antibiotic regarding the zone of inhibition and sensitivity.

RESULTS

The individual bacterial isolates and their sensitive pattern to various antibiotics were also recorded in all two hundred (200) patients.

Age Distribution

Table 1: Age Wise Distribution of AECOPD Cases.

Age (in years)	Number	Percentage (%)
45 – 55	40	20
56 – 65	86	43
66 – 75	64	32
76 – 85	10	5
>85	0	0
Total	200	100

In this study the prevalence of AECOPD in patients aged between 56 and 65 were 86 (43 %), between 45 and 55 were 40 (20%) between 66 and 75 were 64 (32%), and lastly between 75 and 85 were 10 (5%). [Table 1].

Sex Distribution

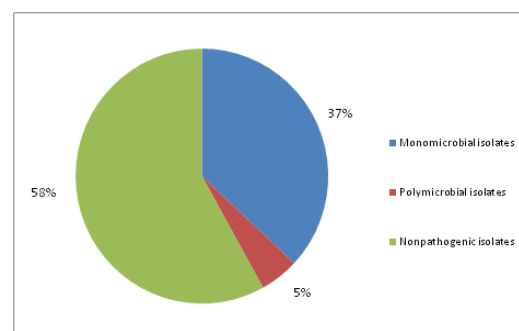
Table 2: Sex Wise Distribution of AECOPD Cases.

Sex	Number	Percentage (%)
Males	136	68
Females	64	32
Total	200	100

It is evident from that out of 200 patients admitted 136 (68%) were males and 64 (32%) were females. The ratio between male and female is 2.12:1. [Table 2].

Bacteriological Profile

Out of a total of 200 cases 84 yielded positive sputum cultures giving a success rate of 42%. Out of 200, monomicrobial isolates were 74 (37%), polymicrobial isolates were 10 (5%) and non pathogenic growth was 116 (58%) [Graph 1].



Graph 1: Distribution of Isolates in AECOPD.

Table 3: Bacterial Strains in Sputum Samples.

S. No	Gram negative bacilli (GNB)	Number	Percentage (%)
1	<i>K. pneumoniae</i>	40	42.55
2	<i>P. aeruginosa</i>	14	14.89
3	<i>E. coli</i>	8	8.51
	Total GNB	62	65.95
	Gram positive cocci(GPC)		
5	<i>S. aureus</i>	27	28.73
6	CONS	4	4.26
7	<i>S. pneumoniae</i>	1	1.06
	Total GPC	32	34.05
	Total no. of isolates	94	100

Out of ninety four (94) pathogenic bacteria, *Klebsiella pneumoniae* were the commonest bacteria isolated, in forty (40) cases, followed by *Staphylococcus aureus* isolated in twenty seven (27) cases, *Pseudomonas aeruginosa* in fourteen (14) cases, *Escherichia coli* in eight (8) cases. *Coagulase*

negative staphylococcus was isolated in four (4) cases. *Streptococcus pneumoniae* was isolated in one (1) case [Table 3].

Table 4: Bacterial Strains in Sputum Samples (poly microbial).

Isolates	No. of cases
<i>Klebsiella pneumoniae</i> + <i>Pseudomonas aeruginosa</i>	4
<i>Klebsiella pneumoniae</i> + <i>Staphylococcus aureus</i>	2
<i>E. coli</i> + coagulase negative <i>Staphylococcus</i>	2
<i>Klebsiella pneumoniae</i> + <i>E. coli</i>	2
Total	10

Out of eighty four (84) positive sputum cultures, ten (10) samples showed more than one isolates [Table 4].

Antibiotic Sensitivity Patterns of the Isolates

Table 5: Antimicrobial Susceptibility Pattern of Isolated Gram Negative Bacilli.

Antibiotics	<i>K. pneumoniae</i> n = 40	<i>P. aeruginosa</i> n = 14	<i>E. coli</i> n = 8	Total n = 62	Percentage(%) 65.96
Ampicillin	18	5	1	24	25.53
Ampicillin sulbactam	33	5	3	41	43.61
Aztreonam	34	11	6	51	54.25
Gentamicin	33	11	6	50	53.19
Amikacin	37	11	7	55	58.51
Amoxycylave	28	6	1	35	37.23
Cefuroxime	31	NR	6	37	39.36
Ceftriaxone	36	NR	7	43	45.74
Cefotaxime	37	NR	7	44	46.8
Cefoxitin	34	NR	6	40	42.55
Ciprofloxacin	30	11	5	46	48.93
Imipenem	40	12	8	60	63.83
Cotrimoxazole	14	10	4	28	29.79
Piperacillin tazobactam	30	12	4	46	48.94
Ceftazidim	33	12	5	50	53.19
Meropenem	40	13	8	61	64.89
Cefepime	34	12	6	52	55.31
Piperacillin	25	11	2	38	40.42
Levofloxacin	36	11	7	54	57.44
Tobramycin	34	12	6	52	55.32
Ticarcillin	NR	12	NR	12	12.77
Colistin	NR	13	NR	13	13.83
Polymyxin-B	NR	13	NR	13	13.83
Gatifloxacin	NR	9	NR	9	9.57
Netilmicin	NR	11	NR	11	11.7

NR: Not Recommended by CLSI; hence not tested

Klebsiella pneumoniae was the most common isolate and it was sensitive to meropenem, Imepenem, cefotaxime, amikacin, ceftriaxone, levofloxacin, cefepime and aztronam [Table 5]. It was resistant to cotrimaxazole and ampicillin. *Pseudomonas aeruginosa* was mainly sensitive to meropenem, colistine, polymyxin b, piperacillin- tazobactam, tobramycin, ceftazidime, cefepime. It was resistant to ampicillin, ampicillin sulbactam, cefuroxime and amoxycylave. *Escherichia coli* were sensitive to meropenem, imipenem, amikacin, ceftriaxone, cefotaxime, levofloxacin. They were resistant to ampicillin and amoxycylave.

Staphylococcus aureus, which was the next common isolate, was sensitive to amoxycylavulinate, penicillin, erythromycin, gentamicin amikacin, netilmicin, and co-trimoxazole. 7.40% of *Staphylococcus aureus* were methicillin resistant (MRSA). *Streptococcus pneumoniae* was sensitive to penicillin, ampicillin, erythromycin, amoxycylave, co-trimoxazole and penicillin and resistant to tetracycline, gentamycin, amikacin and ofloxacin. *Coagulase negative Staphylococcus aureus*, was sensitive to cefoxitin, linezolid, vancomycin, amikacin, ceftazolin and quinpristin-dalfopristine. It was resistant to penicillin, erythromycin, clindamycin and chloramphenicol [Table-6].

Table 6: Antimicrobial Susceptibility Pattern of Isolated Gram Positive Cocci.

Antibiotics	<i>S. aureus</i> n =27	<i>S. pneumoniae</i> n = 1	CONS n = 4	Total 32	Percentage (%) 34.04
Penicillin	6	0	0	6	6.38
Cefoxitin	25	1	4	30	31.91
Erythromycin	17	1	1	19	20.21
Clindamycin	19	1	2	22	23.4
Linezolid	27	1	4	32	34.04
Cotrimoxazole	16	1	3	20	21.28
Vancomycin	26	1	4	31	32.98
Ciprofloxacin	21	NR	3	24	25.53
Gentamycin	21	NR	3	24	25.53
Amikacin	23	NR	4	27	28.72
Novobiocin	27	NR	4	31	32.98
Ceftaroline	25	1	4	30	31.91
Oxacillin	25	1	4	30	31.91
Levofloxacin	18	0	3	21	22.34
Tetracycline	NR	0	NR	0	0
Chloramphenicol	20	0	2	22	23.4
Quinpristine- dalfopristine	18	1	4	23	24.47

NR: Not Recommended by CLSI; hence not tested

Resistance Pattern among Bacterial Isolates

Table 7: Distribution of ESBL And MRSA Among Bacterial Isolates

	Number	Percentage (%)
ESBL	1	1.06
MRSA	2	2.13
Total	3	3.19

Out of ninety four (94), one (1) isolate expressed extended spectrum β lactamase type of resistance and two (2) were found to show methicillin resistant *Staphylococcus aureus* [Table 7].

DISCUSSION

In the present study, bacteriological spectrum was analyzed in 200 AECOPD cases. It was observed that AECOPD was prevalent in 45-85 year age group. The majority of cases that is 43% belongs to 55-65 year age. As reported by Madhavi *et al* [25] maximum numbers of AECOPD were ≥ 65 years of age, which can be explained by the fact that chronic bronchitis has the highest prevalence in fifth and sixth decades. 67% patients aged >55 years while, 38 (33%) patients aged 42-55 years in the study done by Mohamed *et al*. [26] In one study done by Hari Saran *et al* [27] 40.19% patient belonged to age 51-60 years and 3 (2.80%) were aged more than 80 years. It was observed that AECOPD was prevalent in 45-80 year age group. However, among them, 45-65 year age group constituted 75%.

We have concluded here that AECOPD was higher in males 136 (68%) than females 64 (32%). One study observed a total of 107 cases, out of which 72 (67.29%) were males and 35 (32.71%) females (M:F ratio 2.06:1). [27] Our study has a similar distribution of males and females with nearly same ratio. A similar observation was made by Gerard Rakesh *et al* [28] who had 68 males and 32 females in his study, with a sex ratio of 2.1:1. 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.

Iyer *et al* [29] analysed that bacterial pathogens can be isolated from sputum in 45% of patients with COPD during exacerbations. Erkan Let *al* [30] observed bacterial pathogens in 55% AECOPD cases. We have isolated bacterial pathogens from sputum in 42% of patients with AECOPD. This could be due to declining lung function.

In this study, the prevalence of gram negative isolates was 65.95% as compared to 34.04% of gram positive. This is in accordance with other studies, like Hari Saran *et al* [27] where the prevalence of gram negative isolates was 61.54% and the prevalence of gram positive isolates was 38.46%. Gerard *et al* [28] observed gram negative isolates as predominant pathogens that is 51.3% as compared to 48.64% of gram positive isolates.

Among pathogenic microorganisms, *Klebsiella pneumoniae* was the predominant organism isolated 42.55 %, followed by *Staphylococcus aureus* 28.73 %, *P. aeruginosa* 14.89%, *E coli* 8.51 %, CONS 4.26%, *S pneumoniae* 1.06 % . In a study done by Erkan Let *al* [30] on 100 cases of AECOPD, had similar results. It was observed by them that *Klebsiella* species as most common isolate in 40% cases. It was followed by *Pseudomonas aeruginosa* in 8%, *Escherichia coli* in 2% and *Proteus* species in 1%. Bari MR *et al* also has reported *Klebsiella pneumoniae* 59% as commonest isolate, followed by *Pseudomonas aeruginosa* 15%, *Staphylococcus aureus* 13.6% , *Streptococcus pneumoniae* 6.8% and *Streptococcus pyogenes* 4.5%. In another study by Sethi Sanjay *et al* [5] examined 50 patients and obtained lower airway secretions for culture. Predominant bacteria isolated in their study was *Pseudomonas aeruginosa* 42.85. *Staphylococcus aureus* is being reported as second most common isolate overall and most common isolate among gram positives. This is in accordance with many

other studies like that of Kamat S.R. et al, Pradhan K.C. et al, and Arora usha et al.

H. Influenzae is reported to be the most common isolate by many investigators like Tager Ira et al^[17], Niroumand Mitra. et al^[20] and others. *H. Influenzae* was not isolated in the present study. It is well known that the frequency of infection resulting in AECOPD by various microorganisms varies from one geographical area to another. Our country has a wide climatic variation and COPD is more common in northern India because of long, cold winters, small houses and high levels of indoor pollution^[3,6].

In the present study, it was observed that out of 58% pathogenic isolates 37% were single bacterial isolates and 5% were double bacterial isolates. Double bacterial growth pattern in the present study was as follows: *Klebsiella pneumoniae* with *Pseudomonas aeruginosa* in 4 cases, *Klebsiella pneumoniae* with *Staphylococcus aureus* in 2 cases, *E. coli* with coagulase negative *Staphylococcus* in 2 cases and *Klebsiella pneumoniae* with *E. coli* in 2 cases. Shimizu et al^[32] observed in their study as *S. pneumoniae* in 8% AECOPD cases, *H. influenzae* 2% cases, double bacterial infection with *Klebsiella pneumoniae* and *H. influenzae* in 4% cases, and with *P. aeruginosa* and *S. aureus* in 2% cases. In a study done by Bari MR et al^[31] out of the 90 sputum samples, double organisms like, *Pseudomonas* with *Klebsiella* in 2 cases and *Pseudomonas* with *Acinetobacter* in the 1 case were isolated.

In the present study it was analyzed that gram negative isolates were most sensitive to meropenem (64.89%) followed by imipenem (63.83%), amikacin (54.25%). Resistance was noted towards ampicillin (25.55%), cotrimoxazole (29.79%). MDR, XDR, PDR were not found in the present study. Only 1 ESBL among *klebsiella* was revealed. Korashy et al^[33] revealed that the gram negative isolates were mostly sensitive to carbapenems (100%) followed by aminoglycosides like amikacin. Multidrug resistant strains were only found in 28.6% of the gram negative organisms and 7.4% of the total cases of AECOPD. Hariom Sharan et al^[27] concluded that meropenem & piperacillin-tazobactam, amikacin & levofloxacin were most effective for gram negative bacilli. This study clearly showed that about 60% of isolated *Pseudomonas aeruginosa* and 50% of *Acinetobacter* spp. were resistant to the commonly used first and second generation Cephalosporins. In the present study no MDR, XDR, PDR were found, this in accordance with previous data reported from India by Madhavi et al^[25]. Relatively low resistance pattern in the present study can be explained by lesser use of higher antibiotics in most of the patients from central India as they are not affordable to them.

In the present study, Gram positives were most sensitive linezolid (34.04%) followed by vancomycin (32.98%), cefoxitin (31.91%). These

were mostly resistant towards erythromycin (20.21%), co-trimoxazole (21.28%) followed by levofloxacin (22.34%). In present study out of 27 *Staphylococcus aureus* 2 were MRSA. Our study had lower incidence of MRSA that is 2.13%. They were mostly sensitive to levofloxacin, linezolid, and vancomycin [Table 5 & 6]. Hariom Sharan et al^[27] concluded that vancomycin, linezolid, azithromycin and clarithromycin were the most effective drugs for gram positive cocci and amikacin & levofloxacin for both gram positive cocci & gram negative bacilli. Anand k et al^[35] analysed that piperacillin-tazobactam was the most effective antibiotic against all organisms. Quinolones were less effective. A high resistance rate was also detected in *Strep. pneumoniae* strain in this study to penicillin. All the isolated Gram-positive bacteria in this study were found sensitive to linezolid, the first commercially available oxazolidinone antibiotic.

CONCLUSION

AECOPD have a major impact on the quality of life of patients with the condition. They are a major cause of hospital admission and health care utilization. Bacterial infection in AECOPD was seen more in the age group of 55-65 years. *Klebsiella pneumoniae* (42.55%) was the commonest isolate followed by *Staphylococcus aureus* (28.73%), and *Pseudomonas aeruginosa* (14.89%). Antimicrobials effective against gram-negative bacteria were meropenem, imipenem, amikacin and antimicrobials effective against gram-positive bacteria were linezolid, cefoxitin and vancomycin.

Sputum culture is a good and simple diagnostic tool to study the etiology due to bacteria in AECOPD. Antibiogram helps in the correct treatment protocol during the management of AECOPD. It also helps in screening resistant pathogens and better drug for treatment, thereby helping to decrease the mortality and morbidity. In future, more elaborated similar studies are required, incorporating additional laboratory interpretations with personal, local socio-economical and epidemiological markers.

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