Bacteriological Study on Acute Exacerbation of Chronic Obstructive Pulmonary Disease (COPD) Patients

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Abstract:

Background and Aims: COPD characterized by sputum production, bacterial colonization, neutrophilic bronchial airway inflammation and poor health status, and this lead to cause morbidity and mortality worldwide. This study was designed to find out the most common bacteria that responsible for acute exacerbation of COPD, determine the antimicrobial sensitivity against the bacteria isolated.

Patients and Methods: This study is done on patients admitted with acute exacerbation of COPD to hospital. The selection process of Patients was depending on clinical examination, the radiological test and pulmonary function test. The ages of patients were from 40 years and up. 80 patients, 48 males and 32 females. 80 patients with stable COPD serves as controls. A morning specimen of sputum was collected after appropriate preparation and physical character of the sputum were noted. Sputum was immediately sent to microbiology lab for culture. Sensitivity patterns in potentially pathogenic microorganisms (PPMs) were also investigated.

Results: Fifty-two patients (65%) had positive sputum cultures, Indicating the presence of bacterial infection. Streptococcus pneumoniae (26.25%), Escherichia coli (7.5%) , Klebsiella pneumoniae (7.5%), Pseudomonas aeruginosa (5%) ,Acinetobacter baumannii (3.75%),Staphylococcus aureus(3.75%) , Stenotrophomonas maltophilia (2.5%) , Enterobacter cloacae (1.25%), Leclercia adecarboxylata (1.25%), Moraxella catarrhalis (1.25%) and Raoultella ornithinolytica (1.25%).The majority of bacterial pathogens isolated in this study were susceptible to Imipenem, Meropenem, Doxycycline and Chloramphenicol.

Conclusions: Our results show Incidence of bacterial infection during acute exacerbations of COPD is about 65%. Streptococcus pneumoniae has been the most Pathogen frequently isolated; Bacterial airway infections play a great role in many, but not in all, of the cases of AECOPD. So there is the need to do a sputum bacterial culture examination on each patient with AECOPD, And with appropriate antibiotics to contribute to curing of them.

Keywords: COPD; acute exacerbation and bacterial infection.

Introduction:

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease characterized by persistent airflow limitation that is usually progressive, and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients [1]. Related diagnoses include chronic bronchitis (cough and sputum on most days for at least 3 months, in each of 2 consecutive years) and emphysema (abnormal permanent enlargement of the airspaces distal to the terminal bronchioles, accompanied by destruction of their walls and without obvious fibrosis); Extra – pulmonary effects include weight loss and skeletal muscle dysfunction [2].The prevalence of COPD is directly related to the prevalence of tobacco smoking and, in low and middle –income countries, the use of biomass fuels. Current estimates suggest that 80 million people worldwide suffer from moderate to severe disease [3] .In 2005, COPD contributed to more than 3 million deaths (5% of deaths globally but, by 2020, it is forecast to represent the third most important cause of death worldwide. The anticipated rise in morbidity and mortality from COPD will be greatest in Asian and African countries as a result of their increasing tobacco consumption[4].Cigarette smoking represents the most significant risk factor, and the risk of developing COPD relates to both the amount and the duration of smoking. It is unusual to develop COPD with less than 10 pack years (1 pack year = 20 cigarette / day/ year) and not all smokers develop the condition [1].Bacterial colonization is one of a serious factor that play the important role in COPD development and causing of acute exacerbations (AECOPD)[5].The most common of isolated bacteria is *Streptococcus pneumoniae* [6][7]. Other organisms have the pathogenic effect in the stage of acute exacerbations of COPD include *Acinetobacter, Klebsiella, Pseudomonas, Escherchia coli, Stenotrophomonas, Enterobacter , Leclercia* [8] and *Staphylococcus aureus* [9] .

Patients & Methods: Eighty samples of sputum were collected from COPD patients at acute exacerbation stage from Al-Yarmouk teaching hospital, Medical city hospital and Medical Al khademeain emamaain city, during the six month period from January, 2015 to June, 2015.The process of collection the sputum was doing in a sterile laboratory container, the specimens collected in the early morning and before the breakfast, initially asked the patient to breathe deeply and try to cough, and sometimes we use warm water in the event of an inability to cough. Nasal secretions and saliva were rejected.

At first the sputum underwent to Gram's stain to investigate that matching sample standard or not

before any other measures. By using the examined microscopically, Sputum sample of good quality is considered when the numbers of squamous cells lower than 10 and leukocyte more than 25. [10] Also, each sample subjected to dye Ziehl-Neelson for the detection of Mycobacterium tuberculosis, according to the recommendations of the WHO. [11] After checking stage, the sputum was culture directly on the plates (Blood agar, MacConky agar, and Chocolate agar). And the plates incubated for 24 hours at 37ºC after that exanimated .[12] Identification of the isolates was depending on morphological and biochemical characteristics by using API test and confirm the results with Vitek system. During this study we obtained growth of *Candida* colony, for identification this organism used the Gram stain utilized in the laboratory is beneficial in the discovery of yeasts like *Candida spp.*.

Candida albicans were gram positive and able to keep the purple color of Crystal violet, although there is a decolorized stage. The reason is due to the structural cell walls for *Candida* spp. Whereas others fungi not agree this stain. [13] And for more confirmed we used Germ Tube Procedure. This is included making suspension from the yeast colony in the serum and incubated for (2-4) hours at 37C°. After that, bring one drop from the suspension and putted on slide with cover, examine under the microscope for searching on the germ formation.

RESULTS

Results of 80 sputum samples of acute exacerbation COPD were collected from three hospitals, the number of males was 48 (60%) while the females was 32 (40%) as shown in figure (1),

probability sampling technique by pooling all the patients attending the dermatology outpatient clinic in both hospitals on daily basis during the four month period of the study. After taking the verbal consent of the patients; a full history was taken from

each (after the diagnosis has been settled by the senior dermatologist), then a thorough clinical

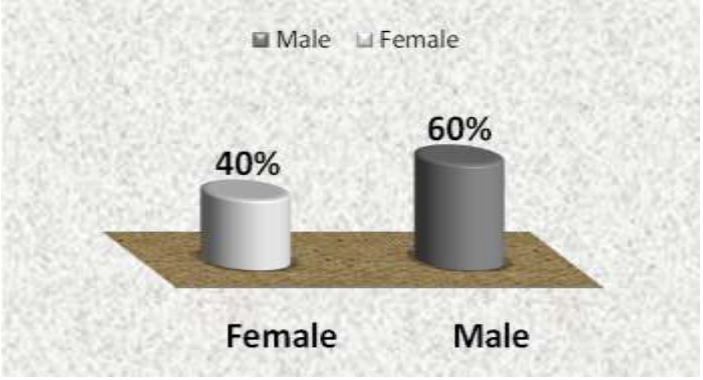

Figure 1. The percentage distribution according the sex of 80 patients with COPD.

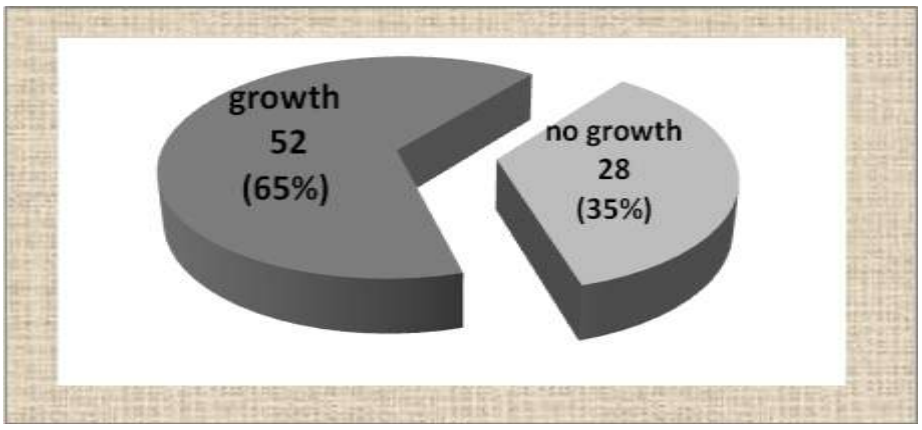

Figure 2. The number of patients that have growth in the culture and no growth with their percentages.

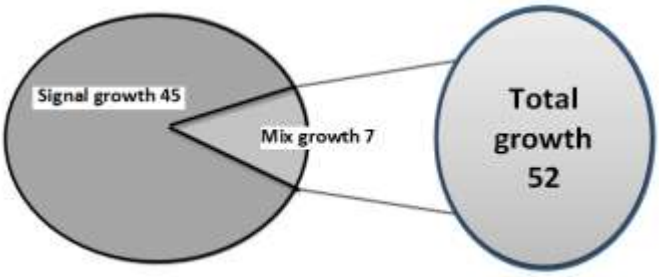

Figure 3. Distribution of patients with single or mixed infections.

Figure 1. The percentage distribution according the sex of 80 patients with COPD.

The growth was observed in 52 samples (65%) , as it is shown in Figure (2) and reminding 28 (35%) were no growth .

Patients with single infection was 46 patients(88.4%) and number of patients with mix infection was 6(12%) as shown in figure (3).

The total number of bacteria isolated in 52 patients, were 49 isolates and three cases were positive for AFB. **Figure 3.** Distribution of patients with single or mixed infections.

The isolation, including 21 (26.25%) *Streptococcus pneumoniae* ,6 (7.5 %) *Klebsiella pneumoniae* ,6 (7.5 %) *Escherichia coli* , 4 (5%) *Pseudomonas spp.*, 3 (3.75%) *Acinetobacter baumannii* , 3 (3.75%) *Staphylococcus aureus* , 2 (2.5 %) *Stenotrophomonas maltophilia* , 1(1.25%) *Enterobacter cloacae* , 1 (1.25%) *Leclercia adecarboxylata,*1(1.25%)*Moraxella catarrhalis* and 1(1.25%) *Raoultella ornithinolytica* And 3 (3.75%) *Mycobacterium tuberculosis*, also there were 6 (7.5%) from *Candida* isolates as shown in figure (4) and figure (5).

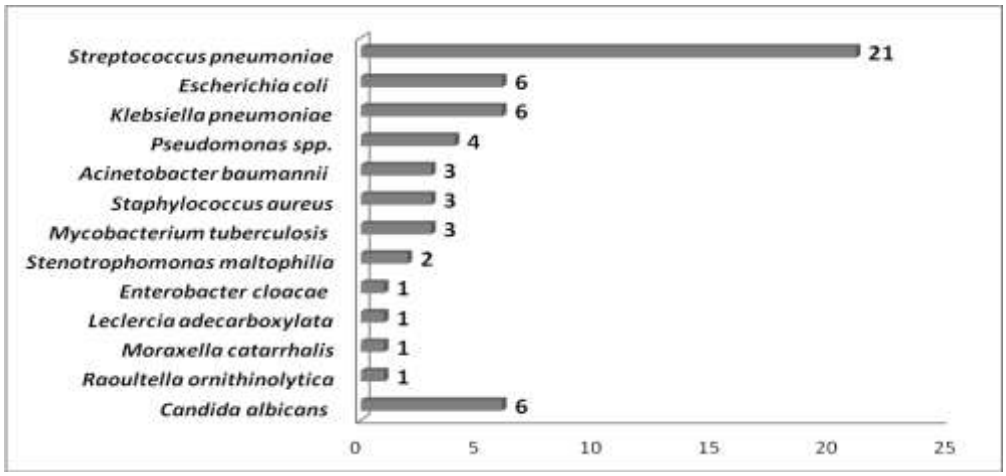

Figure 4. The distribution of pathogens detected in 80 adult patients with COPD.

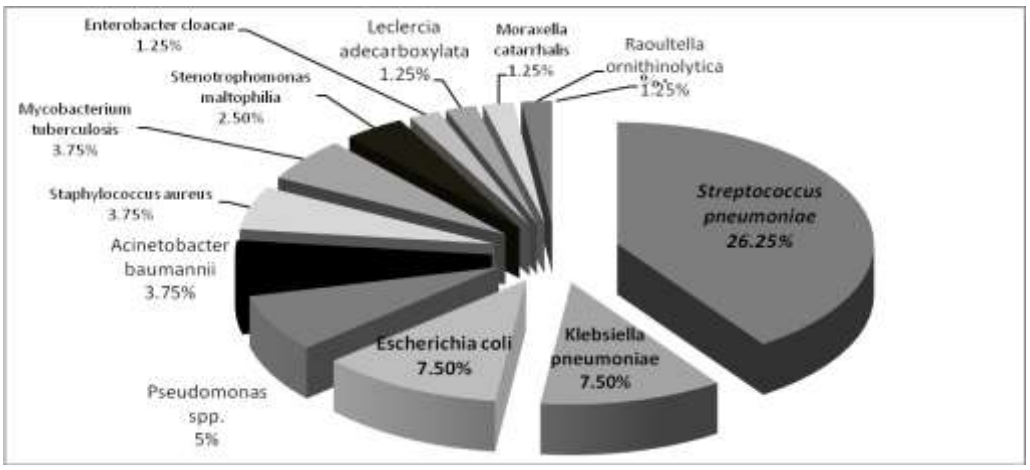

Figure 5. The percentage of bacteria isolation**.**

Table (1) illustrated the bacterial growth ,distribution between single and mix growth , with their percentages .According to experimental results from the 10 antibiotics,the sensitivity of bacterial isolates to Meropenem was (94%) while (92%) was from share Imipenem, (88%) Doxycycline, (82%) Chloramphenicol ,(63.2%) for each one Azithromycin &Ceftriaxone , (55.1%) Tetracycline ,(45%) Gentamicin ,(39%) Amoxicillin and (27%) Erythromycin as shown in figure (6).

Table 1. Distribute total numbers and percentages the bacteria in 52 patients with COPD.

Name of Bacteria	Signal infection No (%)	Mix Infection No (%)	Total No (%)
	42 (80.7 %)	10 (19.2 %)	52 (65 %)
Streptococcus pneumoniae	18 (85.7)	3 (14.28)	21 (26.25)
Klebsiella pneumoniae	4(66.6)	2 (33.3)	6 (7.5)
Escherichia coli	5 (83.3)	1 (16.6)	6 (7.5)
Pseudomonas spp.	4 (100)	_____	4 (5)
Acinetobacter baumannii	2 (66.6)	1 (33.3)	3 (3.75)
Staphylococcus aureus	3 (100)	_____	3 (3.75)
Mycobacterium tuberculosis	3 (100)	_____	3 (3.75)
Stenotrophomonas maltophilia	1 (50)	1 (50)	2 (2.5)
Enterobacter cloacae	1 (100)	_____	1 (1.25)
Leclercia adecarboxylata	1 (100)	_____	1 (1.25)
Moraxella catarrhalis	_____	1 (100)	1 (1.25)
Raoultella ornithinolytica	_____	1 (100)	1 (1.25)

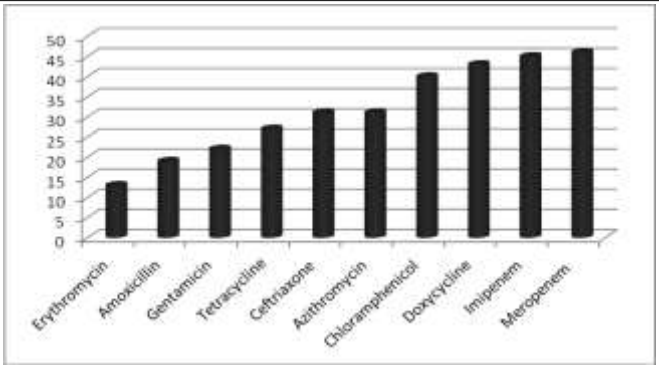

Figure 6.the antimicrobial susceptibility of 49 bacterial isolates**.**

Discussion:

COPD exacerbation are high, mortality and morbidity that caused by infections of lower-respiratory-tract. [14] Infections represent main role to investigation of acute exacerbations. [15]The infections constitute about 80% of the etiology of the disease. [16, 17] Bacteria occupy the bulk of this percentage where it constitutes 50-60% of the agents that caused exacerbation in COPD. [17, 18, 19, 20, 21, 22] This study corroborates the previous results documented that a higher occurrence of bacterial infection in the patients whom suffering from deterioration the function of the lung through its admission to the hospital in the acute exacerbations. So in this study, we using modern device in the field of bacterial diagnosis were identified 52 of patients having a positive culture from bacteria growth in sputum and exceed about 50% of the patients, despite the use of different samples. For example the proportion of bacteria was 51.6% that obtained by use protected specimen brush .[23] another study utilized the bronchoalveolar lavage fluid for patients used the mechanically ventilated in acute exacerbation of COPD and the bacteria percentage existed in 50% of patients. [24] Higher percentage of identifying pathogenic bacteria were reported by Sanjay Sethi and Mette V, *et al*., they found that microbial agents were identified in (60%) of patients with COPD. [17,25] Lower percentage was reported by R.I.M. ElKorashy, *et al*., Who observed the microbial agents were identified in (37%) of

patients.[29] These differences in the final results may be due to several factors, including utilized different laboratory tests and different samples, or there is impediment to make an accurate diagnosis of the microbial agents that causing COPD**.**

The difficulties that we faced in the process of collection of samples for a study that about 40% of patients had taken antimicrobial treatment before admission to the hospital and this lead to decrease the identification process the causative agents.

Some patients were tired and no ability to cough especial who the age over 60 years, And therefore no ability to extract phlegm so we have re-sample more than once to get to the required sample. Bacterial species were isolated from 52 (65%) patients with COPD at acute exacerbation in this study, similar the results M.R. Bari, *et al*.,[27] and their percentage also was (65%). This agrees with Mette V, *et al* ; S. Madhavi, *et al*., and N. Arora, *et al.,* they reported that bacterial pathogens were isolated from (60% , 55%and 52% patients respectively).[17,22,27]

The highest percentage of bacterial isolates was 72% reported by Soler N, *et al*., and S .Ewig ,*et al* .[27,32] While this study was in contrast with Vesna cukic; D. Stolz, *et al*., and R.I.M. ElKorashy, *et al*., , they reported that typical bacterial pathogens were isolated from (41.3% , 38.29% and 37% of patients respectively).[26,30,31]

Other studies have similar results of the bacteria isolates percentage was 50% and these studies include Larsen MV *et al* ; L. Erkan ,*et al* ; Veeramachaneni SB *et al* ; Murphy TF *et al* ; Karin H. Groenewegen *et al* ; Sanjay Sethi, *et al* ; Soler N *et al* ; Monso E *et al* . [19,20,21,23,24,32,33,34] Mix infection has been formed in this research proportion was 7.5% and this agree with results of Xue-Jun Li *et al* [35] and their percentage 7% ,while other studies , like Karin H. *et al* ; Vesna cukic and R.I.M. ElKorashy *et al* ; were formed (26% ,4% and 33.3% respectively).[21,26,30] And explains the difference in results between researches in the use of different tests for diagnosis and take the patient's antibiotic treatment during sample collection. The results of the bacteria isolation in table (1), shows that *Streptococcus pneumoniae* was the most frequent bacteria isolated from patients suffering from COPD, with the percentage of isolates (25.26%). The present study compatible with that of Sanjay Sethi *et al* ; N.Arora, *et al* ; and Karin H, *et al* , showed that *S. pneumoniae* was isolated from (25%, 25.8%,and 28.0% of patients respectively).[21,22,34]

Higher percentage was observed by Pela R, *et al* ; and IS Patel *et al* ,they reported that the organism was isolated from (48%, 33.3%, respectively) of patients with COPD.[36,37]

Lower percentage was reported by L. Erkan, *et al*; Vesna Cukic; H.Cabello et *al*; YE Fenge, *et al*; Soler N *et al* and S. Ewig *et al.* , they reported that *S. pneumoniae* was isolated from (5.3% , 9.33% , 10.3%, 11.7% , 12% and 13.8% respectively) of patients with COPD.[24,29,30,33,39] The higher rate of previous antibiotics uses, difficulty to take an adequate specimen from the sputum and different in diagnostic technique all these factors may reduce the rate of detection the bacteria. The present study reveals that percentage of Gram Negative Bacilli (30%) among patients with COPD. The present study agree with the researchers that gram negative bacilli was responsible for (25.9%, 30% and 34%) of COPD cases (R.I.M. ElKorashy *et al* ; S. Ewig *et al* and S.Madhavi *et al*).[26,28,29]

While a higher proportion of gram negative bacilli isolates was 53.3% , according the results of M.R.Bari *et al* .[27] These results come in consistency with study of Koulenti D. and Rello, J ,they mention that the recurrence of Gram-negative bacteria in COPD were increasing.[39]

Candida infection considers a common danger factor related to COPD patients .[40] Usually observed *Candida spp.* in tracheobronchial secretion from intubated patients and who use mechanical ventilation especially when the duration range from 5-30 days ,the proportion of isolated increase in the 35% .[41]

Sobieraj shown in his study ,that rate of oral Candidiasis increase in the COPD patients who depended on the treatment with inhaled corticosteroids.[42]

The present study has shown the percentage of *Candida* was (7.5%) from the total isolation.

While Nunes FP *et al* reported that found 10-15% of cases study suffering from laryngeal candidiasis.[43] Kaushik Saha *et al* was detected strain from candida in one patient with obstructive pulmonary disease.[44]

In this study, used antibiotic groups which are existing in all hospitals often.

Some studies have suggested that these drugs have become resistant by bacteria while other studies have confirmed the contrary, So the goal from screen sensitivity was to know how much the extent of compliance with these researches.

In the present study, Carbapenems were the most effective antibiotics against of most isolated bacteria.And this result was identical with Egyptian study, that bacteria sensitivity was 100% .[26]A recent study was taken 1142 subjects who treatment by utilizing Azithromycin about 250mg daily for one year that found a reduction in the rate of exacerbations in COPD patients.[45]

Also Seemungal *et al*, was shown the reduction in rate of exacerbation in his study on 109 patients were used erythromycin in treatment [46], the present study was shown sensitivity to Azithromycin (63%). Several studies were confirmed that used macrolides observed decrease the rate of exacerbations in COPD like Yamaya M, *et al* , Blasi F, *et al* and Crosbie P.A.J.& Woodhead M.A. [47,48,49] Wenzel *et al* was recommended that used macrolides (like azithromycin) for patients whom suffering at least from 2 exacerbations in the past year because considered the optimal therapy .[50] The

results of this study were shown that sensitivity to Ceftriaxone 63.2%,Whereas ElKorashy reported that sensitivity to Ceftriaxone was 73.4% and the resistance to the Gentamicin was 14.3%. [26]

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