Research Article

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GRAM NEGATIVE ORGANISMS AS A CAUSE OF ACUTE EXACERBATION OF 'COPD' AND ASSESS THE MULTI DRUG RESISTANT IN THE GRAM-NEGATIVE ISOLATES

S. Vasuki Srinivasan [1]* and S. Ahmed John [2]

[1]Research and Development Center, Bharathiar University, Coimbatore, Tamilnadu, India

[2]Post Graduate and Research Department of Botany, Jamal Mohamed College (Autonomous), Tiruchirapalli, India

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*Corresponding author
E-mail: vasukimicrolab@gmail.com

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ABSTRACT

The cause of an acute exacerbation of COPD is most often infectious and related to a viral and bacterial infection. Gram negative bacteria stated to be a considerable etiology of acute exacerbation of COPD. The current study to determine the prevalence of Gram negative bacterial etiology of acute exacerbation COPD in hospitalized patient and asses the incidence of multidrug resistance gram negative bacteria. 289 tracheal specimen was collected from the laboratory of hospitals using sterile containers and subjected to Gram's stain, culture on blood agar, MacConkey agar, Hi-chrome agar and Chocolate agar. The isolated organisms subjected to biochemical testing. The ESBL producer's screened by double disc synergy test. Tracheal culture for pathogenic bacteria showed 232 isolates including 182 Gram negative organism, 27 Gram positive organisms, 23 fungus and 57 mixed flora. The culture test reveal the commonest isolate was *Klebsiella sps* 68 (24%), followed by *Pseudomonas sps* 57 (20%). *Enterobacter* sps 29(10%), *Acinetobacter* 10 (3%). The drug sensitivity reveals that the gram-negative isolates were sensitive to Colistin (100%), Imipenem and Meropenem (50-60%), Cefaperazone/Sulbactam (10-15%) and Piperacilling/Tazobactam (5-40%). Multidrug resistant strains were 31%. This study conclude Gram negative bacteria were frequently isolated in the study group and antimicrobial sensitivity results very efficiently used to start the antimicrobial treatment, in the emerging of an increasing rate of isolation of resistant organisms.

Keywords: Chronic Obstructive Pulmonary Disease, Multidrug resistant, Bacterial infection.

INTRODUCTION

Chronic Obstructive Pulmonary Disease or COPD, chronic obstructive respiratory disease (CORD), refers to a group of diseases that cause airflow blockage and breathing-related problem[1]. Chronic obstructive pulmonary disease (COPD) is one of the most common lung diseases. It makes it difficult to breathe. There are two main forms of COPD. Chronic bronchitis, which involves a long-term cough with mucus. Emphysema, which involves destruction of the lungs over time. Most people with COPD have a combination of both conditions[2].

The cause of an acute exacerbation of COPD (AECOPD) is most often infectious and related to a viral and bacterial infection [3]. The predictor of an exacerbation in any given patient appears to be a history of previous exacerbation[4].

Bacterial infection is only one of numerous stimuli causing inflammation in COPD and evidence is lacking that increased inflammation due to bacterial infection alone leads to progression of airflow obstruction. However, when infective exacerbation occurs, they have a major impact on the patient overall health status, which may take 6 weeks or more to recover and infection can lead to serious complications requiring hospitalization[5,6]. Severe airflow obstruction, hypoxemia and the presence of hypercapnia are all risk factors leading to poor outcome[7].

Sputum and tracheal cultures are still useful in researching the pathogenesis of exacerbations of COPD, as they provide the pathogens to be studied further. It is important to note that all studies to date of bacterial causation of exacerbations have used a single culture of sputum at the time of presentation and standard or semi quantitative culture techniques[8,9].

Bacteria are isolated from sputum in 40 to 60 % of acute exacerbation of COPD. Three predominant bacterial species isolated from patients experiencing exacerbation of COPD are non typable *Haemophilus inflenza, Moraxella catarrhalis and Streptococcus pneumoniae* [10]. Several studies have reported that the other organism commonly play pathogenic role in acute exacerbation of COPD are *Klebsiella, Pseudomonas, Enterobacter and Acinetobacter* [11].

Acinetobacter baumannii is an important opportunistic pathogen in hospital, and the multidrug resistant isolates of *A. baumannii* have been increasingly reported in recent years[12].

Several recent studies have reported the presence of multidrug –resistant bacteria at hospital admission in patient with severe COPD exacerbation[13,14]. Non fermenting Gram-negative bacilli including *Pseudomonas, Acinetobacter baumannii* and lactose fermenting *Klebsiella, Enterobacter* are the most frequently isolated multidrug-resistant bacteria were defined as Ceftazidime or Imipenem resistant *Pseudomonas* aeruginosa, *Acinetobacter baumannii*, S. maltophilia and extended-spectrum Beta-lactamase producing Gram negative bacilli[15].

MATERIALS AND METHODS

Collection of samples

The aim of the study is to assess the incidence of gram negative bacterial organisms as a cause of acute exacerbation of COPD and multidrug resistant in the gram-negative isolates. This

cohort prospective study samples collected from the private hospitals laboratory in the South Tamilnadu, India.

The following criteria included in the study was the physical properties of the specimen. Saliva or purulent, sputum yellow with blood stained. 289 Tracheal samples collected in a sterile container as in the first 48 h of admission. The processing of the specimen was done as per (CLSI) guideline.

Isolation and Identification of Pathogens

All the samples were inoculated on different selective and non-selective culture media as the per the standard techniques. The following medium used for the isolation of organism, EMB agar, Blood agar, MacConkey agar. The media were incubated aerobically overnight at 37^0 C. The organisms were identified on the basis of colony morphology, Gram staining, motility and biochemical reaction according to Bergey's Manual of Determinative Bacteriology.

Antibiotic Susceptibility Testing

All isolated bacteria were subjected to antimicrobial susceptibility test by Kirby-Bauer disc diffusion method. The antimicrobial used were Amikacin (30 µg/disc), Amoxicillin/ clavulanic acid (30 µg/disc), Cefaperazone/ Sulbactam (75/30 µg/disc), Cefotaxime (30 µg/disc), Piperacillin/Tazobactam (100 µg/10 µg) Imipenem (10 µg), Meropenem (10µg), Colistin (10µg).

ESBL- Detection

Double disc synergy test was performed using discs of Ceftazidime (30 µg/disc), Ceftriaxone (30 µg/disc), Cefotaxime (30 µg/disc), Amoxicillin/clavulanic acid (30 µg/disc) and combined drugs of Cefotaxime /clavulanic acid (30/10 µg/disc), ceftazidime /clavulanic acid (30/10 µg/disc). The discs were placed at a distance of 30 and 16 mm from each other and incubated for 37^0 C for 24 hrs. Increased zone of inhibition in the combined disks (cefotaxime /clavulanic acid (30/10 µg/disc), ceftazidime /clavulanic acid (30/10 µg/disc) than the Cefotaxime (30 µg/disc), Amoxicillin/clavulanic acid (30 µg/disc). This type of sensitivity pattern producing bacteria considered harboring ESBL.

Quality Control

According to the CLSI guidelines, quality of used media, reagents, antibiotics checked with (ATCC) strains of *Pseudomonas aeruginosa* 27853, ATCC *Escherichia coli* 25922, ATCC *Staphylococcus aureus* 25923.

RESULT AND DISCUSSION

Endo tracheal samples (n=289) were from the private hospitals laboratory of hospitalized patient and were processed for the isolation of pathogenic bacteria. Bacteria were isolated according to Bergey's manual. 289 Tracheal culture for pathogenic bacteria showed 232 isolates including 182 Gram negative, 27 Gram positive, 23 fungus, and 57 mixed flora that details listed in Table 1, Figure 1.In culture positive, commonest isolate was *Klebsiella* sp 68 (24%), followed by *Pseudomonas* sp 57(20%). *Enterobacter* 29(10%), *Acinetobacter* 10(3%). The results were reported in Table 2, Figure 2, 3.

The drug sensitivity result reveals that the gram-negative isolates were sensitive to Colistin (100%), Imipenem and Meropenem (50-60%), Cefaperazone/Sulbactam (10-15%) and Piperacillin/Tazobactam (5-40%). The results were reported in Table 3. Multidrug resistant strains were *Klebsiella*, *Entrobacter* sp, *Acinetobacter* 107 (37.2%) The gram-negative isolates *Klebsiella* resistant to Amikacin (97.1%), Amocyclav (100%), Ceftazidime (100%), Imipenem (19.1%), Meropenem (26.4%). E*nterobacter*, *Acinetobacter* were resistant to Amikacin (100%), Amocyclav (100%), Cefotaxime, Ceftazidime (100%). *Acinetobacter* resistant to Imipenem (50%), Meropenem (80%). *Enterobacter* resistant to Imipenem (48.2%), Meropenem (65.5%). *Pseudomonas* sp resistant to Cefotaxime and Ceftazidime (91.2%) Table 4. According to the definition of multidrug resistant organisms, Cefatazidime resistant and / or Imipenem resistance:

107/182 with a percent (58.7% of the Gram-negative organism)
107/289 with a percent (37.2% of the total sample from COPD patient with infective exacerbation.

Table 1: Culture positive and Negative details from the Clinical specimen

Total number of Sample Tested	289
Culture positive	232
Culture negative	57
Number of Gram negative organisms	182
Number of Gram positive organisms	27
Fungus	23

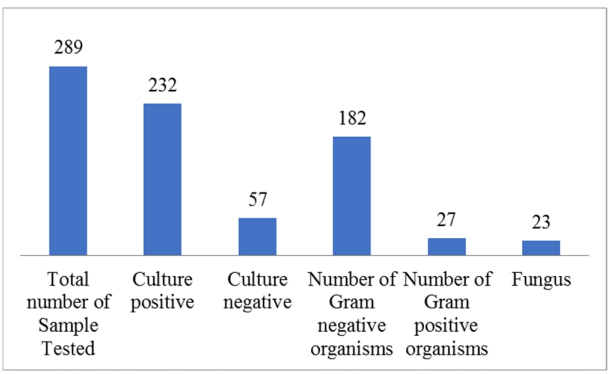

Figure 1: Isolation details from the study sample

Table 2: Percentage of the cultures o f gram positive and negative isolates

Name of the organisms	Number of isolates	Percentage
Klebsiella	68	24%
Pseudomonas aeruginosa	57	20%
Enterobacter	29	10%
Acinetobacter	10	3%
Escherichia coli	18	6%
Haemophilus influenza	Nil	Nil
MRSA	4	1%
Staphylococcus aureus	20	7%
Strptococcus pneumoniae	3	1%
Candida	23	8%
Filamentous fungi	Nil	0%
TB+ve	Nil	0%
Normal flora	57	20%

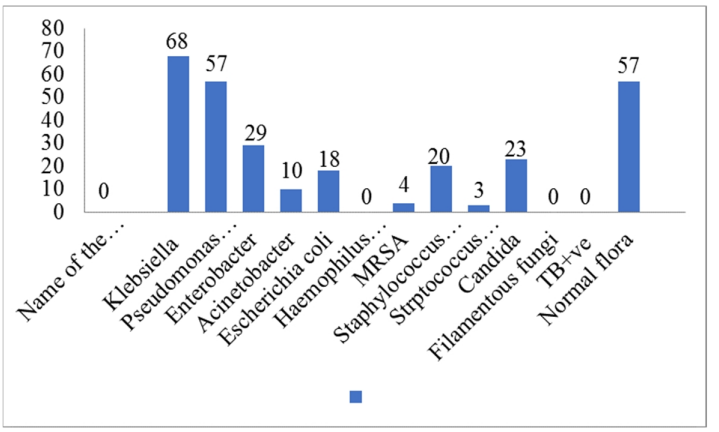

Figure 2: Graphical representation of microbial details isolated from the ICU COPD patient
MRSA: Methicillin resistant *Staphylococcus aureus*

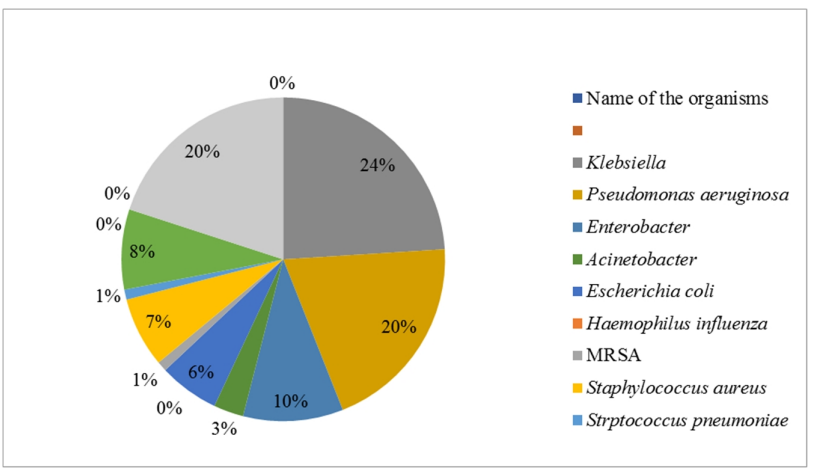

Figure 3: Diagrammatic representation of microbial details isolated from the hospitalized COPD patient in percentage
MRSA: Methicillin resistant *Staphylococcus aureus*

Table 3: Number of sensitivity of the isolated Gram negative bacteria to different antibiotics

Name of Antibiotics	*Klebsiella*	*Pseudomonas*	*Enterobacter*	*Acinetobacter*
Amickacin	2/68	15/57	0/29	0/10
Amoxyclav	0/68	10/57	0/29	0/10
Ceotaxime, Ceftazidime	0/68	5/57	0/29	0/10
Cefaperazone/sulbactam	15/68	12/57	7/29	4/10
Piperacillin/tazobactam	15/68	37/57	3/29	2/10
Imipenem	55/68	57/57	15/29	5/10
Meropenem	50/68	57/57	10/29	2/10
Colistin	68/68	57/57	29/29	10/10

Table 4: Number of resistance of the isolated Gram negative bacteria to different antibiotics

Organisms	Ak	Ac	CF, CZ	CFS	PT	IM	MR	CL
Klebsiella	66/68	68/68	68/68	53/68	53/68	13/68	18/68	0/68
	97.10%	100%	100%	77.90%	77.90%	19.10%	26.40%	0%
Pseudomonas	42/57	47/57	52/57	45/57	20/57	0/57	0/57	0/57
	73.60%	82.40%	91.20%	78.90%	35.10%	0%	0%	0%
Enterobacter	29/29	29/29	29/29	22/29	26/29	14/29	19/29	0/29
	100%	100%	100%	75.80%	89.60%	48.20%	65.50%	0%
*Acinetobacte***r**	100%	100%	100%	60%	80%	50%	80%	0%

Ak: Amikacin; Ac: Amoxyclav; CF: Cefotaxime; Cz: Ceftazidime; PT: Piperacillin/Tazobactam; IM: Imipenem; MR: Meropenem; CL: Colistin.

CONCLUSION AND RECOMMENDATIONS

Klebsiella sp and *Pseudomonas* sp is most predominant bacterial pathogens in hospitalized patient with acute exacerbation COPD. Colistin displayed significant susceptibility against the ESBL and MDR. Imipenem, Meropenem and Piperacillin/Tazobactam, Cefaperazone/Sulbactam were the most active antibacterial agents and, therefore, the drug of choice in treating COPD. Infection with Acinetobacter in COPD patient are one of the major concerns to treat because of their intrinsic and acquired resistance, so antimicrobial sensitivity pattern must be checked for the causative organism and for the effective treatment of patient.

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