

**DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASES PATIENTS**

SHANGAMITHRA R\*, SARASWATHI R, ANUPRIYA A, CHITRA RAJALAKSHMI P, LALITHAMBIGAI J

Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre,  
Trichy, Tamil Nadu, India.

\*Corresponding author: Shangamithra R; Email: shangamithra18@gmail.com

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**ABSTRACT**

**Objective:** Chronic obstructive pulmonary disease (COPD) is the leading cause of morbidity and mortality worldwide which includes chronic bronchitis and emphysema. 80% of acute exacerbation of COPD (AECOPD) are triggered by respiratory viruses, atypical bacteria, and aerobic Gram-positive and Gram-negative bacteria. The aim of our study was to analyze the prevalence and antimicrobial susceptibility testing of *Klebsiella pneumoniae* and detection of extended spectrum beta lactamases (ESBL)-producing *K. pneumoniae* isolates from COPD patients.

**Methods:** Sputum samples received from seventy-five diagnosed cases of COPD from the department of General Medicine and Chest Medicine, TSRM MCH and RC, Trichy, from March 2022 to August 2022. Direct Gram stain was done for all sputum samples. The suitable sputum samples were cultured. The identification of organism and antimicrobial susceptibility testing was done by standard microbiological techniques.

**Results:** Out of seventy-five cases, 64% were males and 36% were females. Forty-seven were sputum positive, and the prevalence of Gram-negative bacteria was 64%. *K. pneumoniae* was the most common bacteria isolated (42.2%), followed by *Pseudomonas aeruginosa* (15.5%). Antimicrobial susceptibility testing of *K. pneumoniae* was sensitive to imipenem (78.94%), aminoglycosides (78.94%), amoxicillin-clavulanic acid (63.15%), ciprofloxacin (57.89%), Piperacillin-Taxobactam (57.89%), and the phenotypic tests showed 13 (68%) isolates were ESBL producers.

**Conclusion:** This study showed that *K. pneumoniae* and *P. aeruginosa* are the most common organisms associated with AECOPD and increased rate of ESBL producers were observed.

**Keywords:** *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, Chronic obstructive pulmonary disease, Antibiotic sensitivity testing, Extended spectrum beta lactamases.

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**INTRODUCTION**

Chronic obstructive pulmonary disease (COPD) is a spectrum of airway diseases that includes chronic bronchitis and emphysema [1]. The World Health Organization says it is the third leading cause of death worldwide [2]. COPD is defined as a preventable and treatable disease characterized by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases, exacerbation is the acute worsening of clinical condition of COPD patients [3].

Acute exacerbation of COPD (AECOPD) is defined as a sustained worsening of the patient's condition, from normal day-to-day activities. The prevalence of AECOPD varying from 1% in urban non-smoker to 21% in rural smokers and mortality rate of 24% of the patients required intensive care unit admission [4]. This mortality rate increased to 30%, if the patient was above 65 years [5]. The clinical guidelines have included "The Winnipeg criteria," which are based on increased breathlessness, sputum purulence, and sputum volume, to diagnose the patients and grade the severity of acute exacerbations of COPD [6].

AECOPD is triggered by respiratory viruses (Influenza, Para influenza, Rhinovirus, Coronavirus, Adenovirus, and RSV), atypical bacteria (*Mycoplasma pneumoniae* and *Chlamydia pneumoniae*), and aerobic Gram-positive and Gram-negative organisms. Early diagnosis and knowledge of local bacteriological profile and antibiogram help us to reduce the number of failure cases recorded with empirical treatment during AECOPD. The present work was

done to find out the aerobic bacteria and their antibiotic sensitivity pattern in AECOPD. The aim and objective of the present study were to analyze the prevalence and antimicrobial susceptibility testing of *Klebsiella pneumoniae* and also to detect the extended spectrum beta lactamases (ESBL) producers of *K. pneumoniae* isolates from AECOPD patients.

**METHODS**

Seventy-five sputum samples of AECOPD patients from General Medicine and Chest Medicine were collected and processed over a period of 6 months (March 2022 to August 2022) after getting the approval from the ethical committee of our institute.

Variables included for the study were age, sex, smoking, comorbidities, signs, and symptoms of the patient. The information regarding these variables was collected using a pretested questionnaire.

**Inclusion criteria**

- All clinically diagnosed severe AECOPD cases
- Patient requiring inpatient ward admission
- Adequate sputum sample based on Bartlett's grading: <10 squamous epithelial cells and >25 pus cells/LPF [7].

**Exclusion criteria**

- Bronchial asthma/lung abscesses/Interstitial lung disease/lung cancer
- Known case of pulmonary tuberculosis
- Ischemic heart disease
- Subjects who were recently started on antibiotic therapy.

### Ethical considerations

Before the commencement of the study, ethical approval was obtained from the institutional review board. Written consent was obtained from the study participants or their guardians. Confidentiality of individual patient's information was maintained during data collection, analysis, and interpretation.

### Specimen collection

Early morning samples were obtained from clinically diagnosed cases of AECOPD. Patients were instructed to collect deep coughed sputum into a sterile wide mouth container with a screw cap after rinsing the mouth twice with plain water. Material is transported and processed immediately within 30 min from the time of collection [8].

### Culture methods

Direct Gram stain was done from sputum sample and reported according to Bartlett's grading system. A score of one and above was considered suitable sample. The suitable sputum samples were inoculated onto Nutrient agar, Mac-Conkey'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 h in 7–10% CO<sub>2</sub> concentration. The isolated organisms were identified by standard microbiological techniques [8]. All the confirmed isolates of *K. pneumoniae* were tested for antimicrobial susceptibility by Kirby–Bauer disk diffusion method on Muller–Hinton Agar plates. The suspension of the isolated organism's broth was adjusted to 0.5 MacFarlands standard, and lawn culture was done and incubated at 37°C overnight. The sensitivity and resistant patterns were reported according to the latest CLSI guidelines [9]. The detection of ESBL producers of *K. pneumoniae* was done by combined disc test method [9].

### RESULTS

A total of seventy-five sputum samples of AECOPD were included in the present study, 51 were males and 24 were females. Among them, 49 (65.3%) belong to the age group of 51–70 years and 17 (22.6%) were above 70 years (Table 1).

Out of fifty males, 32 (64%) were chronic smoker and 18 (36%) were non-smokers and 54 (72%) were diabetes and the remaining 21 (28%) were non-diabetic (Fig. 1).

Table 1: Age-wise distribution

Age	Frequency (n)	Percentage
30–50 years	9	12.0
51–70 years	49	65.3
Above 70 years	17	22.6
Total	75	100

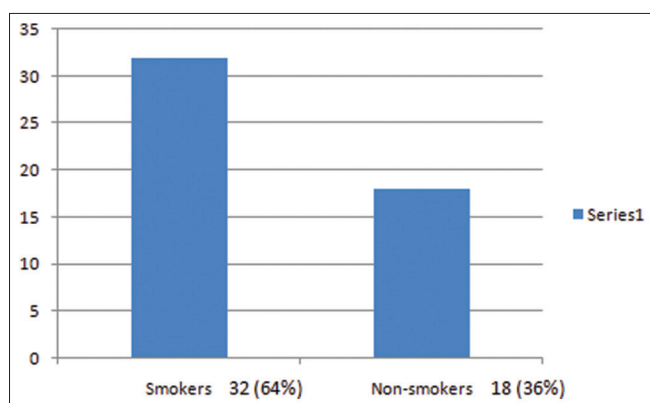


Fig. 1: Distribution of smokers and non-smokers

Gram staining findings were noted in 89.4% (67/75) cases and culture positive for pathogenic organism was obtained in 62.7% (47/75) cases. Among these, 36 (76.5%) isolates were Gram-negative organisms and 11 (23.4%) isolates were Gram-positive organisms.

Among forty-seven culture-positive patients, *K. pneumoniae* was the most common bacteria 42.2% (n=19), followed by *Pseudomonas aeruginosa* 15.5%, Methicillin-sensitive *Staphylococcus aureus* 13.3%, *E. coli* 11.1%, *Streptococcus pneumoniae* 6.6%, *Citrobacter* species 4.4%, methicillin-resistant *S. aureus* 4.4%, and *Acinetobacter* species 2.3% cases (Table 2).

Antimicrobial susceptibility testing was done for nineteen isolates of *K. pneumoniae* and found to be highly sensitive to Imipenem (78.94%), Aminoglycosides (78.94%), Amoxicillin clavulanic acid (63.15%), Ciprofloxacin (57.89%), Piperacillin Taxobactam (57.89%), and least sensitive to Cephalosporins and Ampicillin (Table 3a).

These isolates were further subjected to phenotypic tests, which showed, thirteen (68%) isolates were ESBL producers, four (21%) were carbapenemase producers, and two (10%) were non-ESBL producers (Table 3b).

### DISCUSSION

It was observed that AECOPD was prevalent in 51–70-year age group. Thus, AECOPD was common in advanced age group as respiratory tract is more susceptible due to the impairment of immunological defense mechanism, associated comorbid illness, increased duration of seasonal variation, and tobacco smoking. This is concordance with the study conducted in Bhubaneswar by Rao et al., [10] which states 70% belongs to the age group of 61–75 years. Males were affected more than females because they were more involved in smoking and start it in younger age group; therefore, more chance of inhalation and increased

Table 2: Distribution of bacterial isolates

Organisms isolated	Number	Percentage
<i>Klebsiella pneumoniae</i>	19	42.2
<i>Pseudomonas aeruginosa</i>	7	15.5
MSSA	6	13.3
<i>E. coli</i>	5	11.1
<i>Streptococcus pneumoniae</i>	3	6.6
<i>Citrobacter</i> spp.	2	4.4
MRSA	2	4.4
<i>Acinetobacter</i> spp.	1	2.3

Table 3a: Antimicrobial susceptibility of *Klebsiella pneumoniae*

Antibiotics	Sensitive	Resistance
Ampicillin	10.52	63.15
Amoxy+clav	63.15	36.84
Ciprofloxacin	57.89	42.10
Co-trimoxazole	57.89	42.10
Gentamicin	78.94	21.0
Amikacin	78.94	21.0
Ceftriaxone	42.10	57.89
Cefotaxime	36.84	63.15
Cefipime	59.36	40.64
Piparacillin+tazobactam	57.89	42.10
Aztreonam	47.36	52.63
Imipenem	78.94	21.0

Table 3b: Phenotypic distribution of *Klebsiella pneumoniae*

	Number	Percentage
Non-ESBL	2	10
ESBL producers	13	68
Carbapenemase producers	4	21

environmental exposure or temperature variation. In non-smokers, especially among women, exposure to indoor air pollution was an important factor. This is concordance with a study done by Mythri *et al.* [11] and Sharan [12], respectively, and both states 60–70% were smokers. Out of seventy-five patients, 72% were diabetic and the remaining 28% were non-diabetic. This is concurrent with the study done at Thanjavur by Mary *et al.*, stating 60% of diabetic patients are involved acute exacerbation.

In our study, Gram staining findings were in correlation with the culture findings in 89.4% of cases; aerobic culture positivity was 62.7% which is similar to other studies. The prevalence of Gram-negative isolates was 76.5%, as compared to 23.4% of Gram-positive isolates corresponding to other studies. This corresponds to the study done at Karimnagar by Bannaravuri *et al.* [13] and Mussema *et al.*, in Southwest Ethiopia [14], in which >70% of culture positive were Gram-negative bacilli.

*K. pneumoniae* and *P. aeruginosa* were the common bacteria in our study. The bacterial isolates depend on the prevalence of bacteria in hospital environment; in the community, antibiotic prophylaxis and severity of exacerbation. *H. influenzae* was not isolated in our study which can be explained by the fact of temperature variations and the use of antibiotics either self or by prescription. This corresponds to the study done at Bangalore by Narayanagowda *et al.*, [15] and also in concordance with the study done by Bannaravuri *et al.*, [13] showed >40% prevalence of *K. pneumoniae* in COPD patients sample. A study done at Egypt by Ghanem *et al.*, [16] which states the prevalence of *K. pneumoniae* is 58% followed by *P. aeruginosa* 14% which is concurrent with our study. Although few study done by Sharma *et al.*, [17] it is not concurrent which states the prevalence of *S. pneumoniae* is 13%.

AST pattern of *K. pneumoniae* was found to be sensitive to Imipenem, Aminoglycosides, Amoxicillin-clavulanic acid, Ciprofloxacin, and Piperacillin-Taxobactam. This is similar to the study conducted in Indore by Sharan [12], which showed 40% resistant to amoxicillin and clavulanic acid, 30% resistant to aminoglycosides, and 5% resistant to Carbapenams.

Among these, 68% isolates were ESBL producers and 21% were carbapenemase producers. This corresponds to the study done at Nagpur by Hansotia *et al.*, [18] which showed 57% were ESBL producers.

## CONCLUSION

The optimal usage of antibiotics and effective antimicrobial therapy can significantly diminish health-care costs and maintain the quality of life, especially in the elderly patients. In our study, Imipenem, Aminoglycosides, Amoxicillin-clavulanic acid, and Ciprofloxacin were the most active antibacterial agents and the emergence of ESBL and Carbapenemase-resistant cases are highly increasing nowadays. More studies are required to be conducted on antibiotic susceptibility pattern at regular interval, to formulate the antibiotic policy for improvement in patients' conditions mainly in acute exacerbations of COPD, which in turn reduces the morbidity and mortality.

## Recommendation

- Proper hand hygiene measures, health education of the patients regarding strict compliance to the prescribed antibiotics regimen
- Mandating judicious usage of antibiotics by the clinical practitioners to overcome ESBL and carbapenemase-resistant strains
- Formulation of antibiotic policies for health institutions and antibiotic stewardship programs can aid to a great extent in proper antibiotic usage and the prevention of further spread of resistance, thereby paving a way for a healthy community.

## Limitations of the study

- Test sample size is low
- It is a single-center-based study
- Phenotypic test has to be confirmed by genotyping.

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## AUTHORS CONTRIBUTIONS

R. Saraswathi and R. Shangamithra conceptualized the study. R. Shangamithra, A. Anupriya performed the experiments and analyzed the data and R. Saraswathi and R. Shangamithra wrote the manuscript.

## CONFLICTS OF INTEREST

All the authors declare that they have no conflicts of interest.

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