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Research Article

INCIDENCE AND SENSITIVITY PATTERN OF *PSEUDOMONAS AERUGINOSA* IN PATIENTS OF ACUTE EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

This study was taken up to find out incidence and sensitivity pattern of *Pseudomonas aeruginosa* in patients hospitalized in J. N. Medical College, A.M.U., Aligarh for AECOPD (acute exacerbation of chronic obstructive pulmonary disease) as knowledge of possible sensitivity patterns facilitates the orientation of antibacterial treatment. The most common cause of AECOPD is an upper respiratory infection caused by an increase in airway bacterial load or the emergence of a new bacterial strain. In addition, viral infections, air pollution and some unidentified pathogens can also cause AECOPD. 200 clinically diagnosed cases of AECOPD of age ≥40 years were included in the study. Sputum sample was obtained from the patients and processed according to standard lab procedures. The prevalence of AECOPD was more common in the age group of 50-60 years (49.5%) with ratio between male and female of 2.8:1. Among 200 patients hospitalized for AECOPD *Pseudomonas aeruginosa* incidence was found to be 20.7%, isolated organism were sensitive to Amikacin, Piperacillin tazobactum, Levofloxacin and Meropenem. However a high level of resistance was seen with Ceftazidime, Aztreonam and Cefixime.

Keywords: AECOPD, *Pseudomonas aeruginosa*, Sensitivity pattern

1. INTRODUCTION

COPD is a common, preventable, and treatable disease that is 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. Chronic inflammation causes structural changes, small airways narrowing, and destruction of lung parenchyma. A loss of small airways may contribute to airflow limitation and mucociliary dysfunction, a characteristic feature of the disease."(GOLD 2019)

Estimated numbers of COPD cases were 384 million in 2010 with a global prevalence of 11.7%. Globally there are annually around three million deaths [1]. The prevalence of COPD is expected to rise over next 30 years and by 2030 there may be over 4.5 million deaths annually from COPD and related conditions [2].In 2005 COPD was the eighth leading cause of DALYs lost across world but by 2013 COPD was ranked as fifth leading cause of DALYs lost [3].

Acute exacerbation of COPD (AECOPD) is defined as a sustained worsening of the patient's condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD. 4]. Bacterial infection is one of the important cause of AECOPD.

Pseudomonas aeruginosa is isolated from the sputum of 4-15% of adults with COPD in many cross-sectional studies [5, 6]. The role of *P. aeruginosa* in the course of COPD is less well characterized but has been the subject of increasing recent interest. *P. aeruginosa* is more likely to be isolated from patients with severe disease, particularly among patients who require mechanical ventilation for severe exacerbations [7, 8].

2. MATERIAL AND METHODS

A total of 200 patients admitted with the diagnosis of AECOPD were selected as study group. All the patients in the study group were more than 40 years of age. ***Inclusion criteria:*** Patients (previously and currently diagnosed COPD) were selected as per GOLD guidelines and Anthosien criteria for exacerbation of COPD. ***Exclusion criteria:*** Patients of COPD having bronchiectasis, sputum positive tuberculosis, lung malignancy and other evident disease on chest X ray

(pneumothorax, hydro pneumothorax, pleural effusion, lung mass, lung abscess) were excluded.

All the samples were collected under strict aseptic precautions in sterile containers, properly labeled and were transported to the laboratory in appropriate conditions and processed according to standard guidelines. Bartlett grading system was used. A few glass beads (2.5-3.5 mm) and an equal volume of 2% (w/v) N-acetyl-l cysteine (NAC) were added to each specimen. The NAC solution was freshly prepared each day by dissolving 2 g NAC in 13 ml 1N NaOH and diluting to a final volume of 100 ml with PBS. The pH of the solution was adjusted to 7.3. Homogenized sputa were processed within 30 minutes Sputum samples were mechanically homogenized with sterile glass beads using vortex machine. Tenfold serial dilutions of the homogenized sample were made in brain heart infusion broth and with 0.01 ml loop were plated out onto the surface of a range of different media including blood agar, chocolate agar, MacConkey agar [9-12].

- MacConkey agar plate, at 37°C in ambient air for 24 hrs
- 5% sheep Blood agar plate, with 5-10% CO_2, 37°C for 24 hrs
- Chocholate agar plate, with 5-10% Co2, 37°C for 24 hrs

The isolated colonies were identified by means of Gram's stain, motility, catalase test, oxidase test, coagulase test and by of various other biochemical reactions like Indole test, Methyl red test, Vogesproskauer test, Citrate utilization test, Urease test, Triple sugar iron agar, Nitrate reduction test, Hugh-Leifsons oxidation fermentation test, coagulase production (for *Staphylococcus*), Optochin Sensitivity (for *Streptococcus pneumoniae*) were performed. Sugar fermentation tests with sugars viz: Glucose, Lactose, Sucrose, Maltose, Mannitol, Xylose, Arabinose and Dulcitol, inositols etc were done to identify the isolate according to standard laboratory procedures.

Antimicrobial susceptibility testing for Non fastidious organisms was done by disc diffusion method using Kirby bauer technique on Mueller Hinton agar (HiMedia, Mumbai), using appropriate antimicrobial drugs as directed by CLSI (clinical and lab standards institute) guidelines.

Antimicrobial susceptibility testing for Fastidious organism was done by disc diffusion method using Kirby bauer technique on Mueller Hinton agar supplemented with 5% sheep blood, using antimicrobial drugs, as directed by CLSI guidelines. Phenotypic screening and confirmatory tests were done to detect ESBL (extended spectrum beta lactamases) production among isolates. Screening tests for MBL (metallobetalactamases) detection was also done.

3. RESULTS

A total of 200 patients were included in the study with the diagnosis of AECOPD admitted at our centre J.N. Medical College, A.M.U., Aligarh. Statistical analysis was done by using statistical package for social sciences (SPSS) version 20.The test applied in the current study was modified chi square comparison of proportion to analyse statistical significance for antibiotic sensitivity. P value <0.05 was taken as statistically significant.

Out of 200 patients 78.5% were culture positive while 21.5% were culture negative (table 1).

Table 1: Sputum culture Results

Culture	No. of patients	%
Gram negative	115	57.5
Gram positive	42	21.0
Culture negative	43	21.5

Gram negative organisms (57.5%) predominated gram positive organisms (21%) among which monobacterial isolates were more common than polybacterial isolates (93% vs 7%) (Table 2).

Table 2: Monobacterial and Polybacterial culture results

	No. of patients	%
Monobacterial	146	93
Polybacterial	11	7

Table 3: Polybacterial isolates

Isolates	No. of patients
Pseudomonas aeruiginosa + E . coli	2
Pseudomonas aeruginosa + Proteus sp.	2
Pseudomonas aeruginosa + Staph. aureus	1
Pseudomonas aeruginosa + Citrobacter sp.	1
Staph. aureus + Streptococcus pneumoniae	2
Klebsiella sp. + Proteus sp.	1
Klebsiellasp +E. coli	1
Citrobactersp + E. coli	1

Table 4: Different organisms isolated from the study group

Isolates	No. of patients	Percentage
Klebsiella sp.	34	21.6
Pseudomonas aeruginosa	32	20.7
Moraxella catarrhalis	26	16.5
Staph. aureus	21	13.3
Streptococcus pneumoniae	15	9.5
E . coli	12	7.6
Citrobacter sp.	11	7.0
CONS	3	1.9
Enterococcus sp	3	1.9
Total	157	100

Pseudomonas aeruginosa was isolated in 32 patients (20.7%) (Table 4).Out of 32 patients 25 were male and 7 were female.10 patients were in the age group 41-50 years, 13 patients in 51-60 years, 8 patients in 61-70 years and one patient more than 70 years. Out of 25 male patients 23 were smokers and 2 were non smokers and 1 female patient had history of bidi smoking while the other 6 female patients were non smokers (Table 5).

Table 5: Association of smoking as the risk factors of COPD among study group

	MALE	%	FEMALE	%
Smoker	23	92%	1	14.3%
Non smoker	2	8%	6	85.7%

All the patients presented with symptoms of increased dyspnea, wheezing and increase in sputum production. On the basis of arterial blood gas analysis done at the time of admission type two respiratory failure was diagnosed in 15 out of 32 patients. Endotracheal intubation was done in 7 out of 15 patients with respiratory failure, rest 8 patients were managed by non invasive ventilation. On 2D echo screening corpulmonale was found in 9 of 32 patients with *Pseudomonas infection*. 6 patients were diagnosed with type 2 diabetes mellitus along with COPD.

Out of 32 isolates sensitivity pattern was seen as 27 isolates were sensitive to amikacin (84.3%)($p < 0.01$), 27 isolates were sensitive to piperacillin tazobactum (84.3%)($p<0.01$), 26 isolates were sensitive to levofloxacin (81.2%) ($p<0.01$), 23 isolates were sensitive to meropenem (71.8%)($p<0.01$). No resistance was seen with polymyxin B, tigecycline and colistin. However a high level of resistance was seen with Ceftazidime (93.8%), Aztreonam (93.8%) and Cefixime (75%)(table 6). These results were found to be statistically significant.

Table 6: Antimicrobial susceptibility of *Pseudomonas aeruginosa*

Antibiotics	*Pseudomonas aeruginosa* (N=32)	
	S(%)	R(%)
Amikacin	27(84.3)	5(15.7)
Cefixime	8(25)	24(75)
Levofloxacin	26(81.2)	6(18.8)
Piptaz	27(84.3)	5(15.7)
Meropenem	23(71.8)	9(28.2)
Polymyxins (n=6)	6(100)	0(0)
Tigecycline (n=6)	6(100)	0(0)
Ceftazidime	2(6.2)	30(93.8)
Aztreonam	2(6.2)	30(93.8)
Colistin (n=6)	6(100)	0(0)

Table 7: Determination of ESBL isolates

Isolates	Screening (Ceftazidime)		Confirmatory (Piperacillin tazobactum)	
	R	%	R	%
Pseudomonas aeruginosa (n=32)	30	93.8%	9	28.1%

Table 8: Determination of MBL production

Isolates	Screening-Disc diffusion method	
	R	%
Pseudomonas aeruginosa (n=32)	**9**	**28.1%**

In this study ESBL production by screening disc diffusion method using ceftazidime was positive in 30 (93.8%) isolates but by phenotypic confirmatory method using *Piperacillin tazobactum* ESBL production was seen in 9 (28.1%) isolates out of 32 isolates. (table7). MBL production among isolated *Pseudomonas aeruginosa* by screening disc diffusion method using meropenem was found in 9 (28.1%) isolates out of 32 isolates (table 8).

4. DISCUSSION

In the present study incidence and sensitivity pattern of *Pseudomonas aeruginosa* was analysed in 200 patients

admitted with AECOPD in J. N. Medical College A.M.U., Aligarh. *Pseudomonas aeruginosa* was isolated in 32 out of 200 patients included in the study and most of the patients were in the age group of 51-60 years of age (13 out of 32 patients) with a predominance of males (78.2%) over females (21.8%). This can be explained by the fact that COPD has the highest prevalence in fifth and sixth decade of life. As age advances, the physiological decrease in lung function is accentuated by the cumulative damage done by smoking and other co morbid conditions. Other studies also showed a similar results as done by Soniya Saxena et al (43% in 55-65 years age; 68% male, 32% female), Gerard Rakesh et al [13] (the most common age group was fifty five years constituting 43% ;70% male, 30% female).

Men have pronounced smoking habits and are exposed more to outside environment as compared to females. Smoking leads to decreased mucociliary clearance and innate immunity thereby leading to increased bacterial colonization that can give rise to increased airway inflammation and thus exacerbation. Out of 32 patients smoking was associated with 92 % were male and only 1 female was smoker. Among the 85.7 % of non smoker females majority of them had the history of chulha smoking. There is growing evidence that indoor biomass exposure to modern and traditional fuels used during cooking may predispose women to develop COPD in many developing countries [14, 15]. Occupational exposure, organic and inorganic dusts, chemical agents and fumes are an underappreciated risk factor for COPD among non smokers [16]. Other studies also showed a predominance of smokers in AECOPD like by Sharan H et al (62.5% smokers) [17], Gerard Rakesh et al (70% smokers) [13].

Culture results (78.5% culture positive vs 21.5% culture negative) of current study were similar as found in other studies like Madhavi et al,(55% culture positive) [18], Alamoudi OS et al. (69.8% culture positive), Arora et al. (72% culture positive). Gram negative organisms (73.5%) predominated gram positive organisms (26.5%) which were in accordance with results of studies done by Soniya et al(gram negative 65.95% ; 34.04% of gram positive) [19], Sharan H et al [17] (gram negative 61.54%; gram positive 38.46%). Gerard et al, (gram negative 51.3%; 48.64% gram positive isolates.) [13], Madhavi et al (75% gram negative; 25% gram positive) [18]. In this study it was found that monobacterial isolates were more common than polybacterial isolates (93% vs 7%). Similar results were observed by Gerard Rakesh et al. (51.35% of monobacterial isolates and 5% of polybacterial isolates) [13], Chawla K et al. (monobacterial growth 92.85% and growth of two organisms was isolated in 7.14% cases), Soniya Saxena et al, (37% single bacterial isolates and 5% double bacterial isolates).

In this study *Pseudomonas aeruginosa* was isolated from sputum sample of 32 patients (20.7%) of AECOPD, studies done by other workers showed isolation of *Pseudomonas aeruginosa* as Pradhan KC et al found Pseudomonas aeruginosa in 13% patients, Madhavi et al. [18] found Pseudomonas aeruginosa in 15% patients, Deephti babu et al, found *Pseudomonas aeruginosa* in 24.68% patients, Soniya Saxena et al, found *P. aeruginosa* in 14.89% patients, Erkan L et al, *Pseudomonas aeruginosa* in 8% patients and, Sharan H et al isolated *Pseudomonas aeruginosa* in 10.26 % patients.

Pseudomonas aeruginosa in sputum at hospital admission is more frequent in patients with poorer scoring on the BODE index, previous hospital admissions, oral corticosteroids and prior isolation of PA. *Pseudomonas aeruginosa* is uncommon and is usually associated with the greatest degree of functional impairment [20, 21]. *P. aeruginosa* is more likely to be isolated from patients with severe disease, particularly among patients who require mechanical ventilation for severe exacerbations [7, 8]. In this study also it was seen that on the basis of arterial blood gas analysis done at the time of admission type two respiratory failure was diagnosed in 15 out of 32 patients requiring admission to respiratory ICU. Endotracheal intubation was done in 7 out of 15 patients with respiratory failure, rest 8 patients were managed by non invasive ventilation.

The isolated organisms in the current study were found sensitive to Amikacin (84.3%)($p < 0.01$), Piperacillin Tazobactum (84.3%) ($p<0.01$), Levofloxacin (81.2%) ($p<0.01$), Meropenem (71.8%) ($p<0.01$). No resistance was seen with Polymyxin B, Tigecycline and Colistin. However a high level of resistance was seen with Ceftazidime (93.8%), Aztreonam (93.8%) and Cefixime (75%).

Similar results were observed by Somya Saxena et al, sensitivity of *Pseudomonas aeruginosa* in their study was Meropenem (92.8%), Piperacillin tazobactum (85.7%), Amikacin (78.5%) and Levofloxacin (78.5%), Sharan H et al, [17] in their study did not found any resistance to Amikacin and Meropenem. However they observed a resistance of 75% *Pseudomonas aeruginosa* to Ceftazidimie. Gauri Kulkarni et al, also found a resistance of 66%

Pseudomonas aeruginosa to Ceftazidime. Isolates were sensitive to Piperacillin tazobactum (80%) and Levofloxacin (63%).

In this study ESBL production by screening disc diffusion method using ceftazidime was positive in 30 (93.8%) isolates (table 7) and MBL production among isolated *Pseudomonas aeruginosa* by screening disc diffusion method using meropenem was found in 9 (28.1%) isolates out of 32 isolates (table 8). However, ESBL production was seen in only 9 (28.1%) isolates out of 32 isolates by phenotypic confirmatory method using *piperacillin-tazobactum* (table 7). Confirmatory test for MBL production was not done in current study.

5. CONCLUSION

Exacerbations punctuate the clinical course of COPD in many patients. Exacerbations, mostly of an infectious etiology, are a frequent cause of morbidity in COPD patients. Purulent sputum sample is a good and easy to obtain, non invasive sample that provides preliminary idea about the pathogens, thereby helping in selecting antibiotics for empirical antibiotic therapy as the culture positivity is high in these samples. Antibiotics are important in treatment of AECOPD. This study was done with the purpose of knowing the incidence and sensitivity pattern of *Pseudomonas aeruginosa* in patients hospitalised for AECOPD and formulating antibiogram for the same. Resistance is emerging even in the community acquired infections. A high level of resistance to commonly used antibiotics is emerging due to incorrect diagnosis and inappropriate use of antibiotics. The choice of antibiotics should be based on the local antibiotic policy and the pattern of local pathogens. Hence Periodic isolation and identification of resistant status of pathogens responsible for AECOPD will help us to formulate appropriate treatment protocol which will be of immense use in reducing mortality and morbidity besides reducing the volume of antibiotics and development of resistance to antibiotics.

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