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Microorganisms resistant to conventional antibiotics therapy in hospitalized patients with severe acute exacerbation of chronic obstructive pulmonary disease

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Abstract:

BACKGROUND AND AIM: The major objective of management of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is the identification of pathogen. In the early treatment of patients with AECOPD, empirical antibiotic selection is very important for patient recovery. The aim of this study is to recognize the contribution of microorganisms resistant to conventional antibiotics therapy (MRCT) in hospitalized patients with severe AECOPD and to identify the risk factors and clinical characteristics associated with infection by these microorganisms.

MATERIALS AND METHODS: This cross-sectional study was conducted on 100 patients with AECOPD. The total and differential leukocyte count, spirometric-indices, sputum gram stain, semi-quantitative sputum culture using the colony-forming unit, and assessment of the susceptibility of the isolated bacterial species to 25 antibiotics by disc-diffusion methods were done for all patients.

RESULTS: MRCT was isolated in 57% of the studied patients. The most common isolated MRCT species were *Klebsiella* (50.8%), *Pseudomonas* (15.8%), *Escherichia coli* (10.5%), methicillin-resistant *Staphylococcus aureus (MRSA)* (8.8%), *Acinetobacter* (8.8%), *Citrobacter* (3.5%), and *Enterobacter* (1.8%) which were significantly resistant to amoxicillin/clavulanate, piperacillin/tazobactam, azithromycin, erythromycin, tetracycline, doxycycline, clindamycin, and penicillin/sulbactam ($P < 0.05$). AECOPD patients with MRCT have significantly lower spirometric indices and eosinophil than those with microorganisms sensitive to conventional antibiotics therapy (MSCT). Increased chronic obstructive pulmonary disease (COPD) severity, presence of comorbidities, male sex, age/year, higher smoking package/year, and total leukocyte count cm^3 were the predictive risk factors of infections with MRCT.

CONCLUSIONS: MRCT was predominant among AECOPD patients, among them *Klebsiella, Pseudomonas,* and *MRSA* were the most common isolated species. Higher COPD severity and presence of comorbidities were the most significant risk factors for infections with MRCT in patients with AECOPD. Increased COPD severity had 2.571 folds increased risk for MRCT infection among AECOPD by odd ratio.

Keywords:

Anti-bacterial agents, chronic obstructive, cross-sectional studies, exacerbation, pulmonary disease, resistance to antimicrobials

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Introduction

Bacterial flora of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) keeps changing from time to time and choice of antibiotics for empirical therapy should depend upon the local bacterial prevalence and resistance pattern. This is essential as to permit for effective and cost saving management policy and decreasing the emergence of drug resistance.[1]

However, antimicrobial treatment for AECOPD remains controversial**.** The most common reasons of AECOPD are infections caused by bacteria (40%–60%), viruses (about 30%), and atypical bacteria (5%–10%).[2] The common causative bacterial pathogens include *Streptococcus pneumoniae*, *Haemophilus influenzae, and Moraxella catarrhalis*.[3] Thus, currently approved for antimicrobial treatment is amino-penicillin with or without clavulanic acid, a macrolide or tetracycline.[4]

The AECOPD is uncommonly caused by microorganisms such as *Pseudomonas aeruginosa,* methicillin-resistant *Staphylococcus aureus* (MRSA), *Stenotrophomonas maltophilia, or Enterobacteria* that are not responding to these treatments. Guidelines and previous studies of severe AECOPD suggest that these patients have increased frequencies of exacerbations, previous antibiotic use, previous hospital admissions, and more severe airflow limitations.[5] Therefore, both the existence and type of bacteria in the airways in chronic obstructive pulmonary disease (COPD) are not static phenomena what means that bacterial infection occurrence and type in COPD are variables and liable for change and may be affected by the factors such as disease progression, exacerbations, and treatments including antibiotics and inhaled corticosteroids. Conversely, the presence of bacteria can influence disease progression and exacerbations in COPD patients.[6]

It has recently been accepted that antibiotic resistance is a main public-health problem worldwide, and international efforts are needed to counteract its emergence. Repeated and improper use of antibiotics is increasingly being recognized as the main cause of this emerging resistance. Granulocytic pattern, smoking index, and presence of certain comorbidities may be defined as contributing factors for this resistance. Therefore, the detection of clinical characteristics that identify patients with EACOPD that can be resistant to conventional antibiotics therapy is extremely important.[7]

Accordingly, this study was carried out to recognize the contribution of microorganisms resistant to conventional antibiotics therapy (MRCT) in admitted patients with severe AECOPD and to identify the hazard factors and clinical characteristics accompanying with infection by these microorganisms.

Materials and Methods

Study design and patients selection

Study was approved by the institutional review board of faculty of medicine (IRB 2019010171). An informed written consent was gotten from every patient before participations into the study regarding adherence to the guidelines of the declaration of Helsinki.

This cross-sectional study was conducted on 100 patients presented by the clinical picture of AECOPD. The sample size was calculated by Epi info, Atlanta, Georgia (US) according to the annual flow of COPD cases and its prevalence in Egypt 6.6[8] with margin of error 5% and confidence level 95%. All patients were hospitalized at chest diseases department during the period from November 2019 to August 2020. Of the 217 patients hospitalized with severe AECOPD throughout the study period, 117 (53.9%) were excepted due to study noneligibility, history of antibiotics use in the last month to avoid impacts of recent antibiotic use in normal respiratory flora,[9] chest radiography showing evidence of bronchiectasis or pneumonia, inability to perform acceptable spirometric maneuvers, and inability to collect good quality sputum specimens. The inclusion criteria were patients who had been diagnosed with AECOPD. The diagnosis and COPD severity were established according to the modified criteria defined in GOLD (2019)[10] (had irreversible/partially reversible airflow obstruction (postbronchodilator forced expiratory volume in 1 s/forced vital capacity [FEV_1/FVC%] <0.7, FEV_1 <80% of percent predicted and an increase in FEV_1 <200 mL, or <12% of baseline measurements 20 min after inhalation Salbutamol [400 μg] given via a metered-dose inhaler). Clinically, an exacerbation was defined as a worsening of respiratory symptoms that led the patient to contact health-care facilities and assessed using the Anthonisen criteria.[11] Furthermore, we searched for the presence of associated comorbidities, for example, hypertension, diabetes, ischemic heart disease, and autoimmune diseases.

Study tools

Pulmonary function tests

Spirometry was performed during stable status that preceded the studied exacerbation using FUKUDa DENSHI Spirosift SP-5000, Japan, before and after the inhalation of short-acting β_2-agonist, the following indices were recorded FEV_1% predicted, FVC% predicted, FEV_1/FVC ratio, and forced expiratory flow rate 25%–75% predicted (FEF25%–75%) according to the European Respiratory Society recommendations.[12]

Total leukocytic and differential leukocytic count

Blood samples were collected before antibiotic starting and measured using a hematological analyzer (Sysmex

XE-21N, Kobe, Japan), the following indices were recorded; total leukocyte count (TLC)/cm^3, neutrophils %, lymphocytes %, and eosinophils %. The total and differential leukocytic count was categorized into normal, decreased, or increased according to the following cutoff; 4.5–10/cm^3 for TLC, 45%–75% for neutrophils, and 20%–40% for lymphocytes,[13] while eosinophilia is generally defined as blood eosinophils greater or equal to 2%.[14]

Microbiological examination of sputum

Gram's Stain and microscopically examination

Sputum samples were collected before starting antibiotics therapy. It was collected according to Shepherd[15] and transported fresh for immediate processing. Sputum specimens appropriate quality was assessed as specimen should contain ≥10 leukocytes with mucus, and <25 squamous epithelial cells per low-power field × 10.[16] The isolated organism was identified after staining as Gram-positive or Gram-negative bacteria. Gram-positive bacteria detected by the test include *(S. aureus spp and S. pneumoniae spp);* Gram-negative bacteria detected by the test include *(Klebsiella pneumonia spp, P. aeruginosa spp, Escherichia coli (spp, Acinetobacter spp, Citrobacter spp and Enterobacter spp).*

Sputum culture and colony-forming unit count

Sputum cultures were done on routine media used for the isolation and identification of respiratory pathogens including (blood agar, chocolate agar, and MacConkey agar). Quantitative cultures were done using the calibrated loop method. 0.1 ml of specimen was plated onto solid media and colonies forming unit (CFU) were counted after 24 h incubation,[17] Specimens with CFU count <10^4/ml were considered colonization (negative microbiology), whereas specimens with CFU count ≥10^4/ml were considered infection and further processed for the identification of bacteria using biochemical reactions.[18] The studied AECOPD patients were classified into three groups based on the isolated microorganisms: (1) Patients with the isolation of microorganism sensitive to conventional antibiotic therapy (MSCT) according to the GOLD guidelines (i.e., aminopenicillin with clavulanic acid, a tetracycline, or a macrolide); (2) patients with microorganism resistant to conventional treatment (MRCT) isolation, (i.e., *P. aeruginosa*, MRSA, *S. maltophilia*, *Enterobacteriaceae* producer of extended spectrum of beta lactamase, and *Acinetobacter baumannii*); and (3) patients with negative microbiology results who had either growth of normal respiratory flora or growth of others organisms with CFU <10^4/ml).[19]

Antibiotics susceptibility (disk diffusion methods)

Commercially prepared disks for the most commonly prescribed antibiotics were used to assess antimicrobial susceptibility of the isolated microorganisms. The zone diameters of each antibiotic were interpreted using the criteria set by the Clinical and Laboratory Standards Institute (NCCLS, 2009).[20]

Statistical analysis of data

Recorded data were analyzed using the Statistical Package for the Social Sciences, software version 23.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard deviation. Qualitative data were expressed as frequency and percentage. Data were explored for normality using the Kolmogorov–Smirnov and Shapiro–Wilk test.

The following tests were done:

- A one-way analysis of variance when comparing between more than two means and *post hoc* test: Tukey's test was used for the multiple comparisons between different variables
- Chi-square test of significance was used to compare the proportions between qualitative parameters
- Multivariate logistic regression analysis: Odds ratios (ORs) with 95% confidence intervals were calculated for the evaluation of the overall association between each possible risk factor and the occurrence predictive for the isolation of MRCT in patients with AECOPD. Variable selection strategy when performing logistic regression depended on demographic data and significant parameters of studied patients
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. Hence, the *P* value was considered significant as the following:
- Probability (*P* value)
  - $P < 0.05$ was considered significant
  - $P < 0.001$ was considered highly significant
  - $P > 0.05$ was considered insignificant.

Results

Table 1 shows that there was male predominance among AECOPD patients (91% male versus 9% female) with mean age 62.2 years, mean smoking inde × 61.4 package/year, and means body mass index (BMI) 31.6 kg/m^2. Seventy-one percent of studied AECOPD patients had comorbidities. Mean total leukocytic count, mean neutrophil, mean lymphocyte, and mean eosinophil were (9.5/cm^3, 71.6%, 27.6%, and 2.07%), respectively. According to spirometric indices among AECOPD patients, mean postbronchodilator FEV1/FVC, mean FEV1, mean FVC and mean FEF25–75 were (63.5%, 53.2%, 74.9%, and 51.4%), respectively. As regard GOLD stage classification, 58% of studied patients were GOLD II stage, 31% GOLD III stage, and 11% GOLD IV stage.

There were 28% of the studied AECOPD patients have negative microbiology, 15% have growth of MSCT,

and 57% have growth of MRCT based on isolated bacterial species in sputum culture [Figure 1]. Among those with MRCT, the isolated bacterial species in the descending order were *Klebsiella spp* (50.8%), *P. aeruginosa spp* (15.8%), *E. coli* (10.5%), *MRSA spp* (8.8%), *Acinetobacter spp* (8.8%), *Citrobacter spp* (3.5%), and *Enterobacter spp* (1.8%) [Figure 2].

Table 2 demonstrates that the FEV_1/FVC ratio was significantly decreased in both MRCT subgroup compared to both negative microbiology subgroup and MSCT subgroup. The $FEV_{1\%}$, FVC%, and FEF25%–75% were significantly decreased in either MSCT or MRCT subgroups as compared to negative microbiology subgroup and in MRCT subgroup compared to MSCT subgroup. On the other hand, the COPD severity was significantly increased in either MSCT or MRCT subgroups compared to negative microbiology subgroup and in MRCT subgroup compared to MSCT subgroup. Regarding the leukocytes indices, the TLC, and neutrophils % were significantly increased in AECOPD subgroups with growth of either MSCT or MRCT compared to negative microbiology subgroup. On the other hand, the eosinophils percentage was significantly decreased in AECOPD subgroups with growth of either MSCT or MRCT compared to negative microbiology subgroup. All leukocytes indices were not significantly differed between AECOPD subgroups with either MSCT or MRCT.

Table 1: Characteristics of the patients (*n*=100)

Demographic data	AECOPD patients (mean±SD)
Sex, *n* (%)	
Male	91 (91)
Female	9 (9)
Comorbidities	72 (72.0)
Age (years)	62.2±7.8
Smoking (package/years)	61.4±18.9
BMI (kg/m^2)	31.6±4.5
TLC (cm^3)	9.5±3.6
Neutrophils (%)	71.6±12
Lymphocytes (%)	27.6±8.2
Eosinophils (%)	2.07±0.9
Post-BD FEV_1/FVC ratio	63.5±4.5
FEV_1%	53.2±14.3
FVC%	74.9±14.8
FEF 25%-75%	51.4±8.8
GOLD stage, *n* (%)	
GOLD II	58 (58)
GOLD III	31 (31)
GOLD IV	11 (11)

AECOPD: Acute exacerbation of chronic obstructive pulmonary disease, BMI: Body mass index, TLC: Total leukocytic count, BD: Bronchodilator, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1st s, FEF25%-75%: Forced expiratory flow rate 25%-75%, GOLD: Global initiative for chronic obstructive lung disease, SD: Standard deviation

Table 3 demonstrates that the predictive risk factors of infections with MRCT in patients with AECOPD by odd ratios in the descending orders were increased COPD severity (P = 0.002, OR = 2.571), presence of comorbidities (P = 0.818, OR = 1.776), male sex (P = 0.024, OR = 1.463), age/year (P = 0.835, OR = 1.233), smoking package/year. (P = 0.029, OR = 1.226), TLC cm^3 (P = 0.331, OR = 1.145), increased neutrophils % (P = 0.025, OR = 1.142), BMI (P = 0.767, OR = 1.132), lower FVC% (P = 0.042, OR = 1.131), lower FEF 25%–75% (P = 0.011, OR = 1.110), lower FEV_1 (P = 0.010, OR = 1.065), lower FEV_1/FVC ratio (P = 0.026, OR = 0.896), and finally eosinophil% (P = 0.111, OR = 0.515).

Table 4 shows that the MRCT was highly significantly resistant to Amoxicillin\Clavulanate, Piperacillin\Tazobactam, azithromycin, erythromycin, tetracycline, and doxycycline (P < 0.001). MRCT was significantly resistant to clindamycin and penicillin/

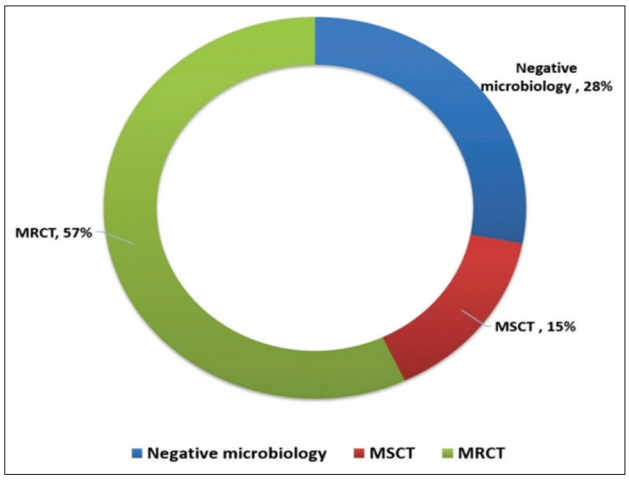

Figure 1: Classification of AECOPD patients based on isolated bacterial species in sputum culture. AECOPD: Acute exacerbation of chronic obstructive pulmonary disease, MSCT: Microorganism sensitive to conventional therapy, MRCT: Microorganism resistant to conventional therapy

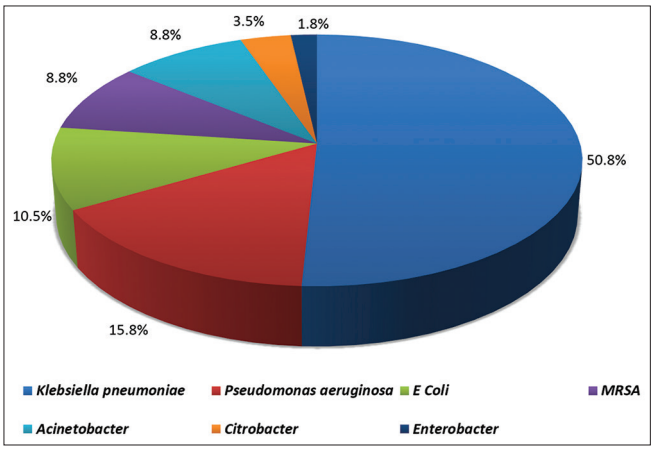

Figure 2: Distribution of MRCT among the patients with AECOPD. AECOPD: Acute exacerbation of chronic obstructive pulmonary disease, MRCT: Microorganism resistant to conventional therapy

Table 2: Comparison of spirometric indices, chronic obstructive pulmonary disease severity, and leukocytic indices between the three studied acute exacerbation of chronic obstructive pulmonary disease subgroups

Items	AECOPD subgroups (mean±SD)			*P*
	MRCT (*n*=57)	MSCT (*n*=15)	Negative microbiology (*n*=28)	
FEV_1/FVC ratio	61.5±5.2#,§	65.4±2.6	66.0±2.0	0.001*
FEV_1%	46.8±12.7#,§	54.6±14.6#	64.2±9.7	0.001*
FVC%	67.7±13.8#,§	77.0±13.2#	86.7±8.5	0.001*
FEF25%-75%	47.1±9.2#,§	53.2±7.0#	58.1±2.5	0.001*
TLC (cm^3)	10.6±3.5#	9.8±3.7#	7.3±2.4	0.001*
Neutrophils (%)	77.1±9.2#	75.5±7.6#	58.7±9.5	0.001*
Lymphocytes (%)	26.4±9.2	27.6±6.3	30.0±7.1	0.161
Eosinophils (%)	1.8±0.7§	2.2±0.9	2.5±1.1	0.001*
GOLD stage‡, *n* (%)				
GOLD II	24 (42.1)	9 (60.0)	25 (89.3)	0.001*
GOLD III	24 (42.1)	4 (26.7)	3 (10.7)	
GOLD IV	9 (15.7)	2 (13.3)		

*Significant *P* value, ‡Chi-square test, *post hoc* test; Tukey's test (#Significant difference with negative microbiology group, §Significant difference with MSCT group). Using: One-way ANOVA. ANOVA: Analysis of variance, AECOPD: Acute exacerbation of chronic obstructive pulmonary disease, MSCT: Microorganism sensitive to conventional therapy, MRCT: Microorganism resistant to conventional therapy, TLC: Total leukocytic count, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1st s, FEF25%-75%: Forced expiratory flow rate 25%-75%, GOLD: Global initiative for chronic obstructive lung disease, SD: Standard deviation

Table 3: Multivariate logistic regression analysis for predictive risk factors for the isolation of microorganism resistant to conventional therapy in patients with acute exacerbation of chronic obstructive pulmonary disease (*n*=57)

Item	95% CI for OR	OR	*P*
Constant	1.287-2.733	2.340	0.001*
Male sex	0.804-1.708	1.463	0.024*
Age (years)	0.740-1.621	1.233	0.835
Smoking (package/years)	0.797-1.802	1.226	0.029*
BMI	0.792-1.845	1.132	0.767
Comorbidities	1.332-3.194	1.776	0.818
Post-PD FEV_1/FVC ratio	0.493-1.046	0.896	0.026*
FEV_1%	0.639-1.400	1.065	0.010*
FVC%	0.735-1.661	1.131	0.042*
FEF 25%-75%	0.777-1.810	1.110	0.011*
COPD severity	1.928-4.622	2.571	0.002*
TLC (cm^3)	0.607-1.272	1.145	0.331
Neutrophils (%)	0.708-1.572	1.142	0.025*
Eosinophils (%)	0.335-0.757	0.515	0.111

*Significant *P* value. OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, TLC: Total leukocytic count, BD: Bronchodilator, FVC: Forced vital capacity, FEV_1: Forced expiratory volume in 1st s, FEF25%-75%: Forced expiratory flow rate 25%-75%, COPD: Chronic obstructive pulmonary disease

sulbactam ($P < 0.006$, $P < 0.020$), respectively. On the other hand, the MSCT was highly significantly resistant to imipenem, Meropenem ($P < 0.001$) and significantly resistant to Gentamycin ($P < 0.008$) compared to MRCT.

Discussion

AECOPD is sometimes triggered by microorganisms that are not responding to therapies suggested by the guidelines.[21] Therefore, in our study, we compare AECOPD patients with MRCT, patients with MSCTs, and patients with negative microbiology aiming to recognize the contribution of bacterial species MRCT in AECOPD and to detect the risk factors and clinical characteristics associated with exacerbation by these microorganisms.

The main finding of the current study is that in patients with AECOPD, the sputum cultures were positive in 72% and negative in 28% (negative microbiology). There was predominance of MRCT (57%) (*Klebsiella* [50.8%], *Pseudomonas* [15.8%], *E. coli* [10.5%], *MRSA* [8.8%], *Acinetobacter* [8.8%], *Citrobacter* [3.5%], and *Enterobacter* [1.8%]), whereas MSCT was detected in 15% of the AECOPD patients. These findings highlight that there is a major shift of bacterial etiology in AECOPD from Gram-positive species previously known to be isolated in such case (e.g. *H. influenzae, M. catarrhalis, and S. pneumoniae)* to a virulent and difficult to treat bacterial species. Welte and Miravitlles[22] assumed that a complex host–pathogen reaction in the airways regulates the outcome of each new bacterial strain presence in COPD, and the balance between host defense mechanism and pathogen virulence directs the proliferation level of the pathogen, which, in turn, regulates airway inflammation surge. Moreover, marked increases in airway inflammation lead to greater physiological alterations, which cause sufficient symptoms to be recognized as an AECOPD. Lung microbiome dysbiosis is a main cause of chronic respiratory problems that can interrupt homeostasis in the lung causing lung inflammation and infection.[21] However, we did not detect *H. influenza* and *M. catarrhalis* in our study, which could be explained by the fact that our patients had severe AECOPD necessitating hospital admission in which these bacterial classes are less prevalent than mild exacerbation. This change could also be clarified by dissimilar environment circumstances and unintended use of antibiotics in our locality. Lower prevalence of positive sputum culture

Table 4: Comparison of antibiotics resistance between acute exacerbation of chronic obstructive pulmonary disease patients with microorganism sensitive to conventional therapy and those with microorganism resistant to conventional therapy

Antibiotics	MSCT (*n*=15), *n* (%)	MRCT (*n*=57), *n* (%)	*P*
Imipenem‡	8 (53.3)	5 (8.7)	<0.001*
Meropenem#	13 (86.8)	9 (15.8)	<0.001*
Piperacillin\Tazobactam#	15 (100)	31 (54.4)	<0.001*
Penicillin\Sulbactam‡	9 (60)	51 (89.5)	0.020*
Cefoperazone\Sulbactam‡	7 (46.7)	43 (75.4)	0.066
Amoxicillin\Clavulanate#	0	57 (100.0)	<0.001*
Ceftriaxone‡	7 (46.7)	31 (54.4)	0.809
Cefotaxime#	14 (93.3)	52 (91.2)	1.000
Cefepime‡	9 (60.0)	31 (52.6)	0.923
Ceftazidime#	11 (73.3)	42 (73.7)	1.000
Ciprofloxacin#	2 (13.3)	6 (10.5)	0.669
Levofloxacin#	2 (13.3)	4 (7.0)	0.598
Ofloxacin‡	5 (33.3)	11 (19.2)	0.415
Gentamycin#	5 (33.3)	3 (5.3)	0.008*
Amikacin#	1 (6.7)	7 (12.3)	1.000
Tobramycin‡	10 (66.7)	35 (61.4)	0.940
Doxycycline#	9 (60)	57 (100)	<0.001*
Trimethoprim sulfamethoxazole#	12 (80)	42 (77.2)	0.746
Linezolid#	14 (93.3)	56 (98.2)	0.376
Vancomycin#	13 (86.7)	56 (98.2)	0.108
Colistin sulfate#	15 (100)	49 (85.9)	0.191
Erythromycin#	0	57 (100)	<0.001*
Azithromycin#	0	57 (100)	<0.001*
Clindamycin#	11 (73.3)	56 (98.2)	0.006*
Tetracycline#	0	57 (100)	<0.001*

‡Chi-square test, #Fisher's exact, *Significant *P* value. MSCT: Microorganism sensitive to conventional therapy, MRCT: Microorganism resistant to conventional therapy

and MRCT was reported by Estirado *et al*.[19] as 44% had positive sputum cultures and 56% had negative cultures. AECOPD was concomitant with MRCT isolation in 40% and MSCT isolation in 60% of cases. In the patients group with MRCT, the most frequent microorganism was *P. aeruginosa* (74%), methicillin resistant *S. aureus* (6%), *S. maltophilia* (3%), *A. baumannii* (3%) and 15% had polymicrobial etiology. Our results were in concomitant with Sheng-Hsiang *et al*.[23] who investigated the microbiology of AECOPD in patients admitted in Taiwan hospital and found that the predominant bacteria were *K. pneumoniae* (19.6%), *P. aeruginosa* (16.8%) *and H. influenzae* (7.5%), followed by *A. baumannii* (6.9%) and *Enterobacter* species (6.1%). Furthermore, our results were concomitant with Messous *et al*.[24] who as sputum cultures were considered significant in (73%), 16.5% cultures were positive, and 31 microorganisms were isolated which the most frequent were *P. aeruginosa* (25.8%) and *K. pneumoniae* (16.2%). Almost half (40.9%) of the isolates were resistant to conventional first-line antibiotics (43.7% amoxicillin-clavulanic acid). In fact, nonfermenting Gram-negative bacilli such as *P. aeruginosa* are reported for their multidrug resistance (multiresistance bacteria).[10] Furthermore, another study in Egypt[25] reported predominance of Gram-negative bacilli with *K. pneumonia, P. aeruginosa, and Acinetobacter* as the most common isolates followed by *S. aureus*. These findings may suggest the significant issue of an appropriate anti-pseudomonal antibiotic empirical treatment in COPD patients with these risk factors. Furthermore our results are nearly close to Makled *et al*.[26] who reported that *Klebsiella spp.* represented 35.4% of all isolates from the studied groups.

Our study revealed that there was significant decrease of all spirometric-indices and more advanced GOLD stage in our studied AECOPD patients with MRCT isolates compared to those with either negative microbiology or MSCT isolates. Moreover, the increased COPD severity and lower FEV_1/FVC ratio lower FEF 25%–75%, lower FEV_1 are the predictive risk factors for infections with MRCT. Higher COPD severity had 2.571 folds increased risk for MRCT infection among AECOPD by odd ratio. These findings means that the functional impairment severity in COPD determines the bacterial cause of exacerbations, it also determines the type of bacterial classes that cause such exacerbations. Moreover, patients with severe COPD have obvious structure changes, recurrent hospital admission, use of high dose inhaled corticosteroids, and may be treated with frequent courses of systemic steroids or antibiotics that favor infection with these potentially pathogenic microorganisms (changes in

airways microbiota). On the other hand, infections with Gram-negative bacterial species induce intense airways inflammatory response with more functional impairment and worsening of spirometric indices than Gram-positive species. Thairu *et al*.[27] mentioned that the Gram-negative bacteria are more pathogenic due to their outer membrane structure. In addition, Gram-negative bacteria have lipopolysaccharide in their outer membrane, an endotoxin that augments the severity of inflammation. Gram-positive infections are generally less severe because the human body does not contain peptidoglycan; in fact, humans produce an enzyme (lysozyme) that attacks the open peptidoglycan layer of Gram-positive bacteria.

The present study revealed that there was significant increase of TLC and neutrophils % in patients with growth of either MSCT or MRCT compared to those with negative microbiology, with no difference between MRCT and MSCT. In addition, the increased neutrophils % was predictive risk factors and had (1.02) folds for infection risk with MRCT. These findings might be explained by the fact that bacterial exacerbations induce an influx of neutrophils into the airways. Consequent neutrophils activation and degranulation in the lumen releases large amounts of proteolytic enzymes in the airways. Similarly, Kang *et al*.[28] reported that the AECOPD has been associated with neutrophilic airway inflammation. Neutrophilic exacerbations COPD patients experienced poorer clinical outcomes than those with eosinophilic exacerbation did. Furthermore, Sharma *et al*.[1] found that although both increased TLC and predominant neutrophilia were detected in patients whose sputum had bacterial growth as compared to no growth ($P = 0.62$).

Our study revealed that AECOPD patients with MRCT revealed significant lower eosinophils % in patients with either MRCT or MSCT compared to those with negative microbiology, with no difference between MRCT or MSCT subgroups. These findings may be attributed to the reported inverse relationship between eosinophil's and bacterial infections, as blood eosinophil counts are known to be reduced during severe bacterial infection as postulated by Kolsum *et al*.[29] On the other hand, bacterial infections are known to cause eosinopenia and the patients with eosinophils ≤2% may have greater bacterial colonization.[30] Similar results were reported by Kang *et al*.[28] as eosinophilic airway inflammation accounted for a considerable proportion of AECOPD (30%).

Our study showed although smoking status and smoking package/year are not significantly differed between the three studied groups MRCT, the higher smoking package/year was a risk factors for MRCT isolation with OR = 1.005. It is known that smoke rises airways colonization.[31] This is possibly related to the reduced phagocytic ability of alveolar macrophages and the reduced cytokine response which is smoking associated.[19] These results were agreed with Estirado *et al*.[19] who reported that only 11.8% of patients MRCT patients subgroup were smokers.

MRCT were significantly resistant to amoxicillin\clavulanate, piperacillin\tazobactam, azithromycin, erythromycin, tetracycline, doxycycline, clindamycin, and penicillin\sulbactam compared to MSCT. This higher resistance rate could be due to ill-advised use of antimicrobial during prior AECOPD, with subsequent development of antibiotics resistance. Soler-Cataluña *et al*.[32] documented that the unnecessary use of antibiotics plays a significant role in increasing bacterial resistance, greater medical costs, and more drug-related adverse actions.

As contamination by upper airway secretions which may frequently harbor potential pathogens is a main concern in sputum culture; therefore, the main strength of the current study is that we did quantitative culture with CFU and culturing only good quality sputum sample.

However, our study has limits that should be mentioned.

Our study was done at only one center in Egypt; sample size restricted the analysis of certain factors per organism. The noninfectious causes of exacerbation were not excluded. Finally, viral and atypical bacteria were not evaluated in the current study, we would prefer to evaluate them, but the technical and financial obstacles prevented us from studying these agents.

Conclusions

MRCT was predominant among the studied patients with AECOPD as well as they were significantly resistant to amoxicillin/clavulanate, azithromycin, erythromycin, tetracycline, penicillin/sulbactam, doxycycline, clindamycin, and piperacillin/tazobactam. On the other hand, the MSCT was significantly resistant to Piperacillin/Tazobactam, imipenem, Meropenem, and Gentamycin compared to MRCT.

Among isolated MRCT *Klebsiella, Pseudomonas and MRSA* were the most common isolated MRCT species. Higher COPD severity, presence of comorbidities, male sex, age/year, higher smoking package/year, and TLC cm^3 were the most predictive risk factors for infections with MRCT in patients with AECOPD. Higher COPD severity had 2.571 folds increased risk for MRCT infection among AECOPD by odd ratio.

To improve and adjust antibiotics therapy, we recommend evaluating the bacterial profile of AECOPD from time to time alongside with the antibiotic resistance pattern of the bacterium. Judicious use of antibiotics based on sputum

culture and antibiogram seems to be the safest approach to prevent antibiotics resistance. The identification of the predictive factors of bacterial etiology in AECOPD in the current study could represent achievement step in the progress of a prediction model for bacterial exacerbation in COPD. However, this model will require to be confirmed with larger AECOPD cohort studies from several centers.

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Conflicts of interest

There are no conflicts of interest.

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