British Microbiology Research Journal
7(6): 288-305, 2015, Article no.BMRJ.2015.121
ISSN: 2231-0886

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Bacterial Profile and Antibiotic Susceptibility Patterns of Acute Exacerbation of Chronic Obstructive Pulmonary Disease in Assiut University Hospitals, Upper Egypt; a One-year Prospective Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MSEM and MAEM designed the study, performed the bacteriological and statistical analysis, wrote the protocol, and wrote the manuscript and managed literature searches. Author ATH performed the clinical assessment of patients, arranged the clinical data and participated in writing the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/16317
Editor(s):
(1) Hung-Jen Liu, Institute of Molecular Biology, National Chung Hsing University, Taiwan.
Reviewers:
(1) Xiuhui Zhong, Institute of Traditional Chinese Veterinary Medicine, China.
(2) Gulsen Meral, Kagithane State Hospital, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=993&id=8&aid=8767

Original Research Article

Received 25th January 2015
Accepted 17th March 2015
Published 10th April 2015

ABSTRACT

The majority of chronic obstructive pulmonary disease exacerbations are caused by infections of the tracheobronchial tree. Previous data on bacterial exacerbations of COPD in Upper Egypt are limited. Hence, this study was conducted for the identification of the causative bacteria in exacerbations of COPD, and to illustrate their antimicrobial susceptibility patterns at Assiut University Hospitals, Upper Egypt. A total of 116 COPD patients who underwent 167 infection exacerbation attacks participated in this prospective study during 2013. Significant bacterial growth was found in 143 (86%) out of the 167 exacerbation attacks. The most common detected bacteria

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were *Haemophilus influenzae* (19.4%), *Escherichia coli* (18%), *Streptococcus pneumoniae* (16.7%), *Klebsiella pneumoniae* (14%), *Streptococcus pyogenes* (10%), *Pseudomonas aeruginosa* (5.6%), methicillin resistant *Staphylococcus aureus* (5.6%), *Acinetobacter baumannii* (4.2%), and *Moraxella catarrhalis* (2.8%). The majority of the isolated strains showed high resistance rates to most groups of antibiotics where 91 (63%) of the isolated strains were multidrug resistant, 37 (26%) strains were extreme drug resistant and 16 (11%) bacterial strains were pandrug resistant. High resistance rates were observed against penicillins and cephalosporins. Moderate resistance rates were detected against the fluoroquinolones. High susceptibilities were detected to the carbapenem group. All the isolated Gram-positive bacteria were sensitive to linezolid.

Keywords: Chronic obstructive pulmonary disease; infection exacerbation; antimicrobial resistance; Upper Egypt.

ABBREVIATIONS

Acin. baumannii	*Acinetobacter baumannii*
AECOPD	*acute exacerbation of Chronic obstructive pulmonary disease*
ANOVA	*Analysis of variance*
CLSI	*Clinical and Laboratory Standards Institute*
COPD	*Chronic obstructive pulmonary disease*
DCP	*decompensated Core-pulmonale*
DVT	*deep venous thrombosis*
E coli	*Escherichia coli*
EMB	*Eosin Methylene Blue*
ESR	*erythrocyte sedimentation rate*
FEV_1	*Forced expiratory volume in the first second*
FVC	*forced vital capacity*
GOLD	*Global Initiative for Obstructive Lung Disease*
H. influenzae	*Haemophilus influenzae*
I	*intermediate*
ICU	*Intensive Care Unit*
IHD	*ischemic heart disease*
Kl. pneumoniae	*Klebsiella pneumoniae*
LTOT	*long term oxygen therapy*
M. catarrahlis	*Moraxella catarrahlis*
MDR	*Multidrug-resistant*
MRSA	*methicillin resistant Staphylococcus aureus*
MV	*mechanical ventilation*
PDR	*pandrug-resistant*
Ps. aeruginosa	*Pseudomonas aeruginosa*
R	*resistant*
RF	*Respiratory failure*
S	*susceptible*
SD	*Standard deviation*
SPSS	*Statistical package for social sciences*
Staph. epidermidis	*Staphylococcus epidermidis*
Strep. pneumoniae	*Streptococcus pneumoniae*
VTE	*Venous thromboembolism*
XDR	*extensively drug-resistant*

1. INTRODUCTION

Exacerbation of chronic obstructive pulmonary disease (COPD) 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 may warrant additional treatment in a patient with underlying COPD [1]. COPD exacerbations increase the rate of hospitalization and mortality and decrease the quality of life. The economic and social

burden of AECOPD is extremely high. It is estimated that almost 35-45% of the total per capita health-care costs for COPD attributed to exacerbations alone [2]. Especially that more than half of the patients often require re-admission in the subsequent period [3]. The majority of COPD exacerbations are caused by infections of the tracheobronchial tree [4]. A key characteristic of airway inflammation in COPD is the persistent presence of bacteria in the lower airways. The most commonly isolated bacteria in the lower respiratory tract of COPD patients were *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*, with growing evidence of the significance of *Pseudomonas aeruginosa* infections in severe COPD disease [5]. Congestive heart failure, systemic infections, pulmonary embolism, pneumonia, air pollution, cold air, allergies, and smoking associated with 20-40% of COPD exacerbations [6]. People with moderate COPD have one exacerbation per year on average; those with severe COPD have two. However, these averages mask wide heterogeneity: many patients with COPD have exacerbations never or very infrequently; a few experience them almost every month [7]. Patients who experience frequent exacerbations may present an accelerating rate of lung function decline. Thus, the management of exacerbations by prompt diagnosis and effective treatment should be a major goal in COPD [8]. Previous data on infection exacerbations of COPD in Upper Egypt are limited. Hence, this study was conducted for the identification of the causative bacteria in acute exacerbation of COPD (AECOPD), and to illustrate their antimicrobial susceptibility patterns at Assiut University Hospitals, Upper Egypt.

2. MATERIALS AND METHODS

2.1 Study Design and Population

COPD patients admitted at the Chest Department, Assiut University Hospitals, Upper Egypt who met the Global Initiative for Obstructive Lung Disease (GOLD) guidelines [9] and experienced one or more exacerbation attacks during the period between January 2013 and December 2013 were invited to participate in this prospective study. Tuberculous patients were excluded from the study. All participants signed the informed consent form that approved by the Institutional Ethics Committee. Questionnaires with demographic and clinical data were fulfilled. Smoking index was calculated as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (1 pack has 20 cigarettes) [10].

2.2 Clinical Assessment

Patients underwent thorough clinical examination and pulmonary function tests that included spirometry, peripheral oxygen saturation, and chest X-ray. Forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were obtained from the flow-volume curve using a spirometer (Zan 300, Sensor Medics MGA USB, Germany). Static lung volumes were measured by closed-circuit helium dilution method. The reference values used were those of the American Thoracic Society standards before and 20 minutes after β-agonist (fenoterol 400 mcg) inhalation. The highest value of at least three measurements was selected and expressed as a percentage of reference values [9].

2.3 Laboratory Tests

Venous blood samples were obtained from patients for performing relevant chemical investigations; blood glucose level, liver function tests, kidney function tests, complete blood count, erythrocyte sedimentation rate (ESR). Arterial blood samples were obtained for measurement of blood gases.

2.4 Bacteriological Diagnosis

Valid early-morning sputum samples were collected into sterile cups from patients through effective coughing sometimes assisted by physiotherapy to obtain lung secretions as described previously [11]. Samples were transported directly to the Microbiology and Immunology Department, Faculty of Medicine, Assiut University where the bacteriological analyses were performed.

2.5 Identification of the Causative Bacterial Strains

Samples were examined microscopically after staining with Gram´s stain and cultured directly on nutrient, blood, chocolate, mannitol salt, bile

esculin, CHROMagar, MacConkey´s, and Eosin Methylene Blue (EMB) agar plates. The cultured plates were incubated aerobically at 37ºC for 24-48 hours. Blood and chocolate agar plates were incubated at 35–36ºC with 5% CO2 for 48 hours for isolation of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* strains. Bacterial isolates were identified based on colonial morphology, Gram staining, and standard biochemical reactions according to the Bergey's Manual of Systematic Bacteriology [12].

2.6 Antibiotic Susceptibility Testing

Susceptibilities of the isolated bacterial strains were determined to penicillins (amoxicillin and amoxicillin / clavulanic acid), phenicols (chloramphenicol), cephalosporins (ceftriaxone, cefepime, ciprofloxacin, cefaclor, ceprodoxime, and cefotaxime), fluoroquinolones (levofloxacin, ofloxacin, and lomefloxacin), and tetracyclines (doxycycline) (Bioanalyse, Turkey). In addition, susceptibilities of Gram-positive bacterial strains were tested against other penicillins (penicillin, oxacillin, methicillin, and carbencillin), polypeptides (bacitracin), macrolides (erythromycin), glycopeptides (vancomycin and teicoplanin), and oxazolidinones (linezolid). Susceptibilities of Gram-negative stains were tested also against aminoglycosides (gentamicin, amikacin, tobramycin, and neomycin), carbapenems (imipenem and meropenem), and monobactams (aztreonam). The test was performed using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The results were interpreted as susceptible (S), intermediate (I), or resistant (R). Multidrug-resistant (MDR) bacteria was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) bacteria was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and pandrug-resistant (PDR) bacteria was defined as non-susceptibility to all agents in all antimicrobial categories [14].

2.7 Statistical Analysis

The SPSS program version 19.0 was used for the statistical analysis of data. Data were presented as mean and standard deviation or number and percentage as appropriate. A *P* value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Characteristics of COPD Patients

The study included 116 COPD patients consisted of 108 (93%) males and 8 (7%) females with age range 42-72 years (mean±SD, 57.6±8 years). Most (78; 67%) patients aged >55 years while, 38 (33%) patients aged 42-55 years. Eighty four (72%) patients were admitted to the Intensive Care Unit (ICU) while 32 (28%) patients were admitted to the Chest Department (Table 1). All patients were residents of Upper Egypt, primarily residents of Assiut (70%), followed by residents of the Governorates Qena (15%), Aswan (7%), Luxor (5%) and Sohag (3%). Of the 116 patients, 88 (76%) patients had very severe (stage IV) COPD, 18 (15%) patients had severe (stage III) COPD, 8 (7%) patients had moderate (stage II) COPD and 2 (1.7%) patients had mild (stage I) COPD. The duration of hospital stay ranged from 3-49 days (mean+/-SD, 14±9 days). It was significantly longer in patients with very severe versus those with severe, moderate, and mild COPD (ANOVA, *P*=0.024, *P*=0.001, and *P*=0.000, respectively) and in patients with severe COPD versus moderate and mild COPD (ANOVA, *P*=0.003 and *P*=0.000, respectively). The patients' group (n=116) suffered from 167 infection exacerbations attacks during the study period from January to December 2013 with 79 (68%) patients experienced one attack/year, 24 (21%) patients experienced two attacks/year, 12 (10%) patients experienced three attacks/year, and one patient (~1%) experienced infection exacerbations four times / year (Table 1). The duration between attacks ranged from 3-225 days (mean±SD, 64.4±69.9 days). No significant difference was found regarding the frequency of exacerbation attacks / year or the duration between attacks with COPD stage. Eighty seven (75%) patients were smokers. Heavy-smokers were 52 (45%) patients, moderate-smokers were 33 (28%) patients, mild-smokers were 2 (2%) patients, ex-smokers were 17 (15%) patients, while 12 (10%) patients were non-smokers (Table 1). A significant positive correlation was observed between the COPD stage and the smoking index (r=0.438, *P*=0.000). A total of 104 (~90%) COPD patients had hypoxia. Mild, moderate, and severe hypoxia was detected in 18 (16%), 70 (60%), and 16 (14%) patients, respectively, while 12 (10%) patients had normal O2 tension. Hypercapnea was detected in 80

(69%) COPD patients while, 36 (31%) patients had normal CO2 tension. Chest x ray was normal in stage I COPD patients and one patient in stage II, while characteristic COPD changes had been detected in all COPD stages III and IV cases. Chest x ray showed pneumonic infiltrates in 18 (15%) cases (Table 1). Cardiopulmonary co-morbidities were found in many COPD patients. Respiratory failure (RF) was found in 86 (74%) patients, decompensated Core-pulmonale (DCP) was detected in 58 (50%) of patients, 18 (16%) patients were managed with long term oxygen therapy (LTOT), 8 (7%) patients were under mechanical ventilation (MV). Other cardiopulmonary co-morbidities were detected in few patients; pleural effusion in 4 (3%) patients, alpha1-antitrypsin deficiency in 3 (~3%) patients, ischemic heart disease (IHD) and lung carcinoma were detected in 2 (~2%) patients each (Table 1). Laboratory tests showed that 78 (67%) COPD patients had high erythrocyte sedimentation rate (ESR), 44 (38%) patients had anemia, 30 (26%) patients were diabetic, 28 (24%) patients had leucocytosis, 12 (10%) patients had hypoalbuminemia, and 8 (7%) patients were hypertensive. Venous thromboembolism (VTE) was found in 13 (11%) patients with 6 (5%) patients suffered from pulmonary embolism and 7 (6%) patients had deep venous thrombosis (DVT). Impaired liver and renal functions were detected in 8 (7%) and 6 (5%) patients, respectively. Stage IV (very severe) COPD was significantly associated with the presence of DCP, RF, and high ESR (Fisher´s exact test, *P*=0.003, 0.003, and 0.001, respectively). Other co-morbidities had no significant association with COPD stage. Most (84%) of COPD patients were treated regularly with corticosteroids (Table 1).

Thirty four (20%) exacerbation attacks detected in January, 28 (17%) of exacerbations detected in May, 25 (15%) in April, 22 (13%) during November, the least exacerbations were detected in September (4 attaks; 2%), August (3 attacks; 1.8%), and June (2 attacks; 1.2%) (Fig. 1).

3.2 Bacteriological Analysis

Significant bacterial growth was found in 88 (76%) out of the 116 COPD patients during 143 (86%) out of the 167 exacerbation attacks either single (127 attacks; 89%) or mixed infections (16 attacks; 11%). In 18 (24%) patients (24 attacks; 14%), no significant bacterial growth was found (Table 1). A total of 144 bacterial strains were isolated in exacerbations of COPD either solely (113 strains) or mixed (31 strains). The distribution of bacterial isolates in different COPD stages is shown in Fig. 2. The predominant bacterial strains were in decreasing order; *Haemophilus influenzae* (*H. influenzae*) (19.4%) that isolated in 22 attacks as a single pathogen and in 6 attacks combined with other pathogens. *Escherichia coli* (*E. coli*) were isolated in 18% of the attacks (singly in 18 attacks and mixed in 8 attacks), *Streptococcus pneumoniae* (*Strep. pneumoniae*) were found in 16.7% of attacks (singly in 20 attacks and mixed in 4 attacks). Other bacterial isolates were: *Klebsiella pneumoniae* (*Kl. pneumoniae*) (14%; singly in 14 attacks and mixed in 6 attacks), *Streptococcus pyogenes* (*Strep. pyogenes*) (10%; singly in 13 attacks and mixed in one attack), Each of *Pseudomonas aeruginosa* (*Ps. aeruginosa*) and, methicillin resistant *Staphylococcus aureus* (MRSA) were detected in 5.6% of the attacks (singly in 6 attacks and mixed in 2 attacks), *Acinetobacter baumannii* (*Acin. baumannii*) (4.2%; singly in 5 attacks and mixed in one attack), *Moraxella catarrahlis* (*M. catarrahlis*) (2.8%; singly in 4 attacks). Each of *Staphylococcus epidermidis (Staph. epidermidis)*, Enterobacter, and Enterococci were detected in two (1.4%) attacks (Fig. 2). Thus, Gram-negative bacilli were detected in 82(49%) attacks. COPD patients infected with either *M. catarrahlis* or *Staph. epidermidis* were ≥ 60 years old with associated cardiopulmonary and/or systemic co-morbidities. Optochin-sensitive *Strep. pneumoniae* strains were 17 (71%) in number, while seven (29%) strains were optochin-resistant. There was no significant association between the types of bacterial isolates or the optochin-sensitivity patterns of *Strep. pneumoniae* strains and the severity of COPD.

3.3 Antibiotic Susceptibility Patterns

High resistance rates were observed among the isolated bacterial strains against most groups of antibiotics where, 91 (63%) of the isolated strains were MDR, 47 (33%) strains were XDR and 6 (4%) bacterial strains were PDR (Table 2). Most isolates were resistant to amoxicillin, amoxicillin / clavulanic acid, cephalosporins (with exception to ciprofloxacin), ofloxacin, and lomefloxacin. About half the isolates were resistant to chloramphenicol, ciprofloxacin, levofloxacin, and doxycycline (Table 2). Among the Gram-positive

bacteria, resistance rates were highest against the penicillin group and erythromycin. Resistance to bacitracin, vancomycin, and teicoplanin ranged from 58-64%. All the isolated Gram-positive bacteria were sensitive to linezolid (Table 2). For Gram-negative isolates, the resistance rates to the aminoglycosides group ranged from high level to tobramycin and gentamicin to a slightly lower level (44%) to amikacin. Resistance rate was also high (81%) to aztreonam. Only few isolates (8.5%) showed resistance to the carbapenem group that belonged to *H. influenzae*, *E. coli*, *Ps. aeruginosa*, and *M. catarrahlis* strains (Table 2). Most (84%) of *H. influenza*e strains were MDR, 2 (7%) strains were XDR, and another 2 (7%) strains were PDR. About 54% of detected *E. coli* strains were MDR, 31% of the isolates were XDR, and 15% of the isolates were PDR. About 40% of the isolated *Kl. pneumonia*e strains were MDR, while 60% were XDR and no isolates were PDR. XDR was found in all (100%) *Enterobacter* strains, 43% of *Strep. pyogenes*, 42% of *Strep. pneumoniae*, 37% of MRSA, 33% of *Acin. baumannii*, 25% of *Ps. aeruginosa*, 17% of *Strep. pneumoniae,* and 14% of *Strep. pyogenes* strains. All (100%) *M. Catarrahlis*, *Staph. epidermidis*, and enterococcal strains were MDR. While 75%, 67%, 63%, 58%, and 57% of *Ps. aeruginosa*, *Acin. baumannii*, *MRSA*, *Strep. pneumoniae*, and *Strep. pyogenes* strains were MDR, respectively (Table 2). All *Staph. aureus* strains were methicillin and oxacillin-resistant. About 63% of the isolated *Strep. pneumonia*e strains were penicillin-resistant (Table 2). No statistically significant difference was found between different antibiotic resistance patterns in the duration of hospital stay. The patterns of antibiotic resistance in different COPD stages are shown in Fig. 3. Death rates among MDR, XDR, and PDR infected patients were 2%, 6%, and 67%, respectively. The death rate was significantly higher for patients infected by PDR bacteria than those infected by XDR or MDR bacteria (Fisher's exact test; *P*=.000). On the other hand, there was no significant difference between different COPD stages regarding the antibiotic resistance patterns. For COPD cases (18 patients; 24%) with no significant bacterial growth, 17 patients had recovered and discharged while one patient died that had underlying pulmonary embolism.

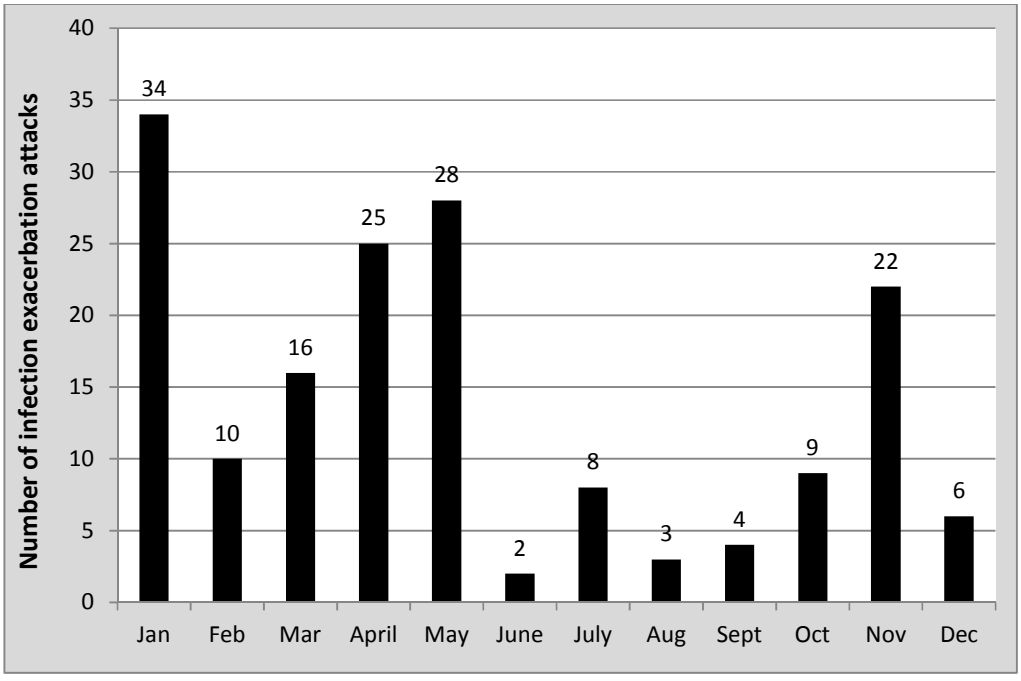

Fig. 1. Monthly distribution of COPD infection exacerbations during January-December, 2013 (n=167)

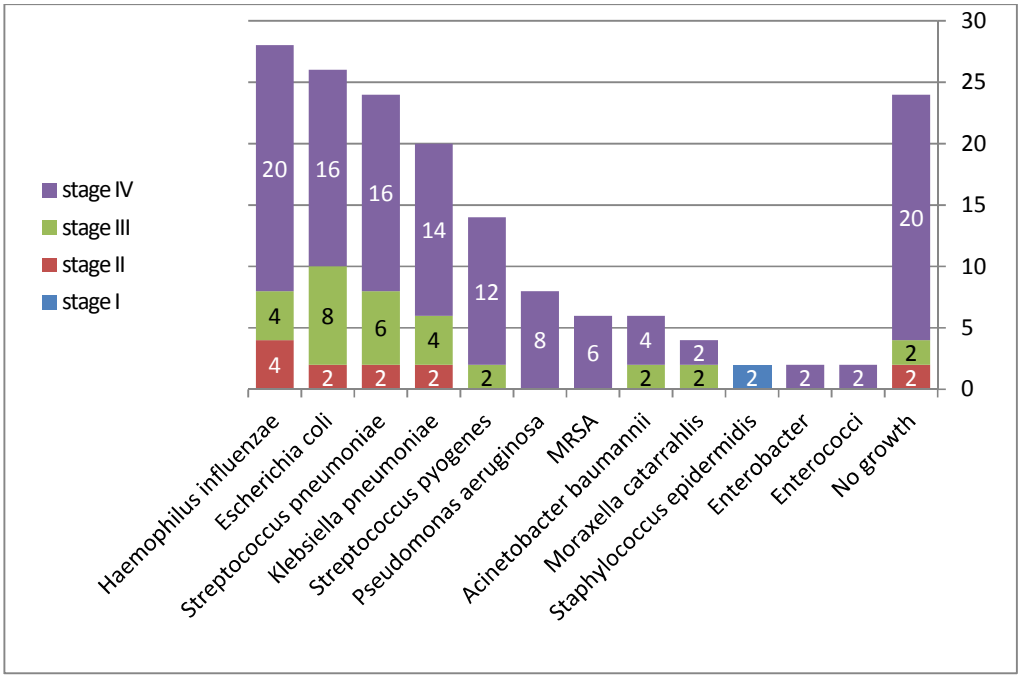

Fig. 2. Bacterial strains detected during infection exacerbation of different COPD stages. Each number of bacterial-positive samples is represented both with a bar and absolute values in the abscissa. No bacterial isolates were detected in 24 attacks. Abbreviations: MRSA=methicillin resistant *Staphylococcus aureus*

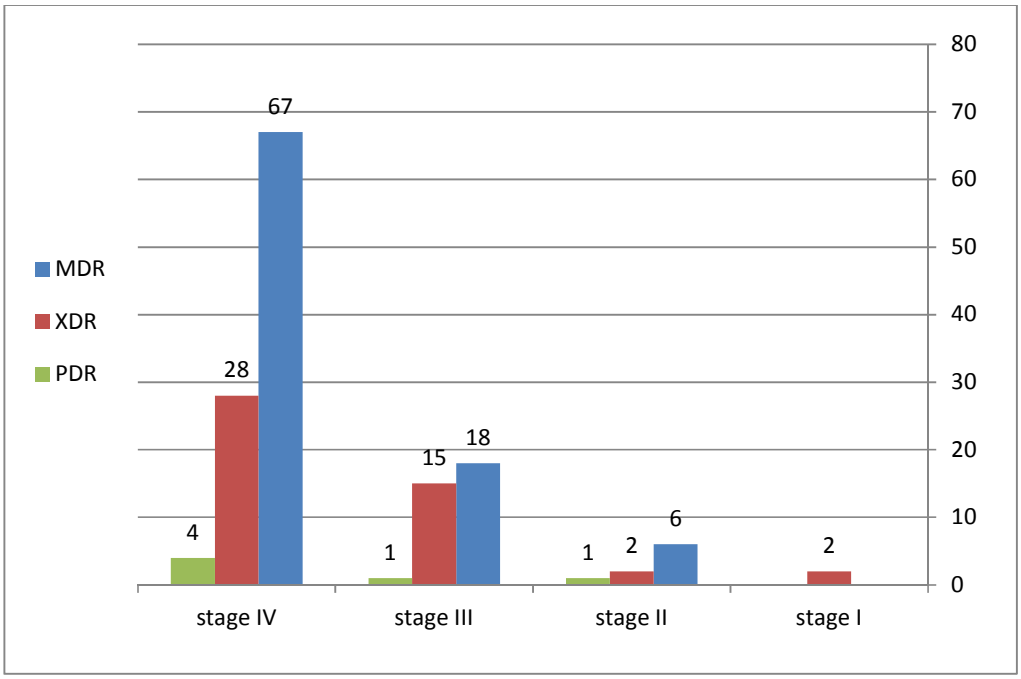

Fig. 3. Antibiotic resistance patterns in different COPD stages (n=144 attacks)

Table 1. Demographic and clinical characteristics of COPD patients (n=116)

Patients' characteristics		N (%)	
Sex			
	Female	8 (7)	
	Male	108 (93)	
Geographical area			
	Assiut	80 (70)	
	Sohag	4 (3)	
	Qena	18 (15)	
	Luxor	6 (5)	
	Aswan	8 (7)	
Admission			
	ICU	84 (72)	
	Chest Department	32 (28)	
COPD stage			
	I (mild)	2 (1.7)	
	II (moderate)	8 (7)	
	III (severe)	18 (15)	
	IV (very severe)	88 (76)	
Number of attacks (no=167)/year			
	One attack	79 (68)	
	Two attacks	24 (21)	
	Three attacks	12 (10)	
	Four attacks	1 (1)	
Smoking index			
	0 (non-smokers)	12 (10)	
	Ex-smokers	17 (15)	
	Mild-smokers	2 (2)	87 (75)
	Moderate-smokers	33 (28)	
	Heavy-smokers	52 (45)	
Arterial blood gases			
O2 tension	Normal	12 (10)	
	Mild hypoxia	18 (16)	104 (90)
	Moderate hypoxia	70 (60)	
	Severe hypoxia	16 (14)	
CO2 tension	Normal	36 (31)	
	Hypercapnea	80 (69)	

Patients' characteristics		N (%)
Chest X ray		
Normal chest x ray	stage I: 2(2%); stage II: 1(0.9%)	3 (2.9)
COPD changes *	stage II:7(6%); stage III:18(15%); stage IV:88(76%)	113 (97)
Pneumonic infiltrates (co-morbid pneumonia)	stage III: 2(2%) stage IV: 16(14%)	18 (15)
Associated cardiopulmonary condition		
	DCP	58 (50)
	RF	86 (74)
	MV	8 (7)
	LTOT	18 (16)
	Pulmonary embolism	6 (5)
	Pleural effusion	4 (3)
	IHD	2 (2)
	Lung carcinoma	2 (2)
	Alpha1-antitrypsin deficiency	3 (3)
Other systematic condition		
	Anaemia	44 (38)
	DM	30 (26)
	Hypoalbuminemia	12 (10)
	Leucocytosis	28 (24)
	Hypertension	8 (7)
	DVT	7 (6)
	Impaired liver function	8 (7)
	Impaired renal function	6 (5)
	Increased ESR	78 (67)
	Previous corticosteroid therapy	98 (84)
Bacteriological diagnosis		
Significant bacterial growth during exacerbations (no of patients=116/ no of attacks=167)		**88 patients (76%)/143 attacks (86%)**
	Single etiological agent	72 patients (82%)/127 attacks (89%)
	Mixed infection	16 patients (18%)/ 16 attacks (11%)
No bacterial growth		**18 patients (24%)/ 24 attacks (14%)**

*Abbreviations: ICU=Intensive Care Unit; DCP=decompensated Core-pulmonale; RF=respiratory failure; MV=mechanical ventilation; LTOT=long term oxygen therapy; IHD=ischemic heart disease; DM=diabetes mellitus; DVT=deep venous thrombosis; ESR=erythrocyte sedimentation rate. * COPD changes include: hyperinflation of the lung, increase bronchovascular marking, low flattened diaphragm, ribbon-shaped heart, and cardiomegaly*

Table 2. Resistance patterns of isolated bacterial strains to antimicrobial agents

5	1				6	7	8	9	10			11		12	MDR	XDR	PDR
doxycycline	penicillin	oxacillin	methicillin	carbencillin	bacitracin	erythromycin	vancomycin	linezolid	neomycin	tobramycin	amikacin	gentamicin	meropenem	imipenem	aztreonam		
4(14)	ND**	ND	ND	ND	ND	ND	ND	ND	14(50)	22(79)	5(18)	24(86)	2(7)	2(7)	18(64)	24(86)	2(7)
18(69)	ND	ND	ND	ND	ND	ND	ND	ND	20(77)	22(85)	13(50)	18(69)	4(15)	4(15)	24(92)	14(54)	8(31)
12(60)	ND	ND	ND	ND	ND	ND	ND	ND	14(70)	16(80)	10(50)	14(70)	0(0)	0(0)	14(70)	8(40)	12(60)
4(50)	ND	ND	ND	ND	ND	ND	ND	ND	6(75)	6(75)	5(63)	6(75)	1(12.5)	0(0)	8(100)	6(75)	2(25)
6(100)	ND	ND	ND	ND	ND	ND	ND	ND	0(0)	6(100)	2(33)	2(33)	0(0)	0(0)	6(100)	4(67)	2(33)
0(0)	ND	ND	ND	ND	ND	ND	ND	ND	4(100)	4(100)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)
2(100)	ND	ND	ND	ND	ND	ND	ND	ND	2(100)	2(100)	2(100)	2(100)	1(50)	2(100)	2(100)	0(0)	2(100)
6(25)	15(63)	22(92)	22(92)	17(71)	12(50)	16(67)	14(58)	0(0)	ND	ND	ND	ND	ND	ND	ND	14(58)	10(42)
6(43)	10(71)	12(86)	12(86)	10(71)	10(71)	12(86)	10(71)	0(0)	ND	ND	ND	ND	ND	ND	ND	8(57)	6(43)
0(0)	0(0)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)	0(0)	ND	ND	ND	ND	ND	ND	ND	2(100)	0(0)
6(75)	8(100)	8(100)	8(100)	8(100)	8(100)	8(100)	4(50)	0(0)	ND	ND	ND	ND	ND	ND	ND	5(63)	3(37)
0(0)	2(100)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)	0(0)	ND	ND	ND	ND	ND	ND	ND	2(100)	0(0)
64(44)	35(70)	44(88)	44(88)	39(78)	32(64)	38(76)	30(60)	0(0)	60(64)	78(83)	41(44)	70(75)	8(8.5)	8(8.5)	76(81)	91(63)	47(33)

Bacterial isolates	Total	1*		2	3							4		
	No (%)	amoxicillin	amoxicillin/ clavulanic acid	chloramphenicol	ceftriaxone	cefepime	ciprofloxacin	ceprodoxime	cefaclor	cefotaxime	levofloxacin	ofloxacin	lomefloxacin
H. influenzae	28 (19.4)	28(100)	16(57)	4(14)	12(43)	24(86)	10(36)	24(86)	18(64)	26(93)	8(29)	10(36)	14(50)
E. coli	26 (18)	26(100)	22(85)	12(46)	22(85)	22(85)	20(77)	22(85)	26(100)	26(100)	16(62)	16(62)	22(85)
Kl. pneumoniae	20 (14)	20(100)	16(80)	10(50)	14(70)	16(80)	12(60)	14(70)	16(80)	16(80)	13(65)	12(60)	12(60)
Ps. aeruginosa	8 (5.6)	8(100)	6(75)	4(50)	8(100)	8(100)	0(0)	8(100)	6(75)	6(75)	0(0)	2(25)	6(75)
Acin. baumannii	6(4.2)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)
Mor.catarrahlis	4 (2.8)	4(100)	4(100)	0(0)	4(100)	4(100)	2(50)	4(100)	2(50)	2(50)	0(0)	3(75)	2(50)
Enterobacter	2 (1.4)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
Str. pneumoniae	24 (16.7)	22(92)	14(58)	12(50)	20(83)	20(83)	14(58)	22(92)	18(75)	16(67)	14(58)	18(75)	20(83)
Str.pyogenes	14 (10)	14(100)	8(57)	4(29)	12(86)	6(43)	6(43)	14(100)	12(86)	14(100)	4(29)	12(86)	14(100)
Staph.epidermidis	2 (1.4)	2(100)	2(100)	0(0)	2(100)	2(100)	0(0)	2(100)	2(100)	2(100)	0(0)	0(0)	0(0)
MRSA	8 (5.6)	8(100)	4(50)	4(50)	8(100)	8(100)	8(100)	6(75)	8(100)	8(100)	6(75)	8(100)	8(100)
Enterococci	2 (1.4)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	2(100)	2(100)
Total	144(100)	140(97)	100(69)	58(48)	110(76)	118(82)	80(56)	124(86)	116(81)	124(86)	71(49)	91(63)	108(75)

*1-*penicillins and penicillin combinations; 2-phenicols; 3-cephalosporines; 4-fluoroquinolones; 5-tetracyclines; 6-polypeptides; 7-macrolides; 8-glycopeptides; 9-oxazolidinones; 10-aminoglycosides; 11-carbapenems; 12-monobactams.*** ND=not determined*

4. DISCUSSION

This study aimed to diagnose the spectrum of bacterial pathogens associated with AECOPD and their antimicrobial susceptibility patterns during the year 2013. The study included 116 AECOPD patients that admitted during the study period at Assiut University Hospitals, Upper Egypt. Most of the participated patients were old aged, suffered severe or very severe COPD, had associated cardiopulmonary or systemic co-morbidities, and were critically ill that required admission at the ICU. Older age is a predictive factor for increased hospitalizations in COPD due to the higher degree of disability and co-morbidity in the older population [15]. The co-morbid conditions can trigger AECOPD and their presence is a predictor of poor clinical outcome [16]. The close association between COPD and cardiovascular diseases had been established during the last 15 years. It is estimated that the diagnosis of COPD increases the risk of cardiovascular disease by an OR of 2.7 [17]. Anemia and hypoalbuminemia that have been detected in our patients reflected the underlying nutritional status, and increased ESR and leucocytosis highlighted the underlying inflammatory process in those patients that are often observed during COPD exacerbations [18] and affect the clinical outcome of the disease [19]. Our patients were mostly smokers which reflected the effect of current smoking as a risk factor for severe exacerbations. Smoking perpetuates an ongoing inflammatory response that leads to airway narrowing and hyperactivity so patients become more prone to infection exacerbation attacks [20]. From a public health perspective, smoking cessation is the single most effective therapy for COPD and is associated with a decrease in symptoms, and improved health status [21]. Also we found that some ex-smokers had experienced exacerbation attacks which imply that smoking cessation was too late and the disease progression continued even after smoking cessation.

Almost all patients in this study had hypoxia and mostly had hypercapnea. Chronic hypoxia and hypercapnia were responsible for the pathogenesis of COPD [22] and hypercapnea is an independent risk factor for AECOPD and represents a marker of disease severity [23]. In our study, the duration of hospital stay was significantly longer in patients with severe and very severe COPD versus those with mild or moderate disease which corresponds to previous reports [24,25]. Shorter durations of exacerbations were a predictor of success of treatment while longer durations were a predictor of need for ventilatory support and poor outcome of the disease [3]. Most of our patients were under steroid therapy. Corticosteroids were routinely described in AECOPD patients as they reduce the airway inflammation [26]. However, they may be associated with adverse effects like fluid retention, hypertension, diabetes mellitus, and osteoporosis. Therefore, their use in AECOPD must be balanced against adverse effects [27]. In this work, the peak of exacerbation attacks occurred during January and a large proportion occurred during November. AECOPD attacks occurred mainly during winter months compared with summer [28]. A previous global study of exacerbation seasonability demonstrated that in the Northern Hemisphere, about 9% of patients had exacerbations between December and February compared to 5% in June to August with 80% winter excess in exacerbations, whereas in the southern hemisphere, 12% of patients had exacerbations in their winter compared to 7% in summer with 71% winter excess. A higher proportion of patients in the southern region reported an exacerbation in any seasonally adjusted month compared with the northern region [29]. In another study, it was found that the mean monthly exacerbation rates during winter were 2.16 fold higher than during summer [30]. In our work, a large proportion of AECOPD also occurred during April and May. This can be explained by the relatively constant temperatures in Egypt with a mean of ≥18ºC all year round.

Bacterial infections are generally considered to be the most common cause of AECOPD [31]. Previous studies have shown that approximately one third of COPD patients are colonized at any time [32]. In this work, Gram-negative bacilli were detected in about half of AECOPD. Gram-negative bacilli were also the predominant organisms in the study done by Siripataravanit et al. [33] in Thailand. Gram-negative bacilli and *Enterobacteriaceae* were the most common isolated bacteria in cases of AECOPD also in another study in China [34]. A change in the microbial pathogens seen during AECOPD from the usual pathogens to Gram-negative bacteria is in parallel with the deterioration of the patient´s lung function [31]. Our study population included admitted cases of AECOPD where there is deterioration of their lung function, hence, Gram-negative bacteria were most commonly isolated. *H. influenzae* was the most common bacteria detected in our study. This is in correspondence

with previous works in Egypt [35] and other countries [36,37,38,39,40]. Strains of *H. influenza*e stimulate mucus hypersecretion and inhibit ciliary beat frequency. Furthermore, they can cause direct epithelial damage and their endotoxin increase epithelial expression of the pro-inflammatory cytokines thus providing potential mechanisms to upregulate the process of inflammation in COPD [41]. *E. coli* strains were the second common organism isolated in AECOPD in this study. In a previous study in Germany [42], E coli were the most common organism isolated in cases of AECOPD. *Strep. pneumoniae* strains were detected in 16.7% of AECOPD in this work. A previous documentation has shown that airway colonization with *S. pneumoniae* increases the risk of a first COPD exacerbation [43]. Also Sethi et al. [44], showed a significant increase in exacerbations when *S. pneumoniae* was isolated. About 29% of the isolated *S. pneumoniae* strains in this study were optochin-resistant. Optochin-resistant *S. pneumoniae* strains were first described in 1987 [45] and since then, their incidence in clinical sources increased steadily during the last decade [46]. Similar to our findings, *H. influenzae* and *S. pneumoniae* were the most prevalent organisms isolated in AECOPD previously in Pakistan [47], Germany [48], and the Netherlands [49]. *Kl. pneumoniae* were detected in 14% in AECOPD attacks in this study. This detection rate is higher than that detected previously [50,51]. A substantial number of our patients had *P. aeruginosa* with a percentage (5.6%) similar to previous report that was 6.3% [36]. On the other hand, our percentage was lower than other reports with a prevalence rate of 15% [49,52]. MRSA were detected in 5.6% of the participated patients. COPD was considered as an independent factor in the isolation of MRSA in ICU [53]. *Acin. baumannii* strains were found in 4.2% of AECOPD in our work. This isolation rate was comparable to previous studies in Bosnia and Herzegovina [50] and in Taiwan [54]. While *Acin. baumannii* is a major pathogen in nosocomial infections, community-acquired acinetobacter infections are of an increasingly concern because they mainly affect patients with certain co-morbidities such as COPD [55]. In our findings, the percent of detection of *M. catarrhalis* was 2.8%. Both of *Acin. baumannii* and *M. catarrhalis* were previously detected in cases of AECOPD in Bangladesh [56]. Our COPD patients that were infected by *M. catarrhalis* were old aged with associated co-morbidities. Wright et al. [57] found that the majority of respiratory isolates containing *M. catarrhalis* are from elderly patients with underlying cardiopulmonary diseases. Adherence of *M. catarrhalis* to epithelial cells increases in elderly patients [58]. *M. catarrhalis* has emerged as a main pathogen over the last two to three decades in patients with chronic obstructive pulmonary disease (COPD) [59]. In contrast to a previously reported data [60] that found a relationship between the severity of COPD and the type of isolated bacterial strains, our results found no significant association between the type of bacterial isolates and severity of COPD. This difference may be due to different demographic data and the small sample size of our study. A larger sample size is required to prove these findings.

To obtain high susceptibilities to antimicrobial agents, we tested the susceptibilities of the isolated bacterial strains to major groups of antibiotics that have effect against both Gram-negative and -positive bacteria. Our findings demonstrated high resistance rates among the isolated bacterial strains to different groups of antibiotics. Resistance was at the highest level to amoxicillin followed by the cephalosporins group (with exception to ciprofloxacin). This was similar to a previous study in Egypt [35]. Our data proved that the fluoroquinolones cannot be considered as the first option for treatment of AECOPD as recommended in previous reports [61,62] as their frequent usage can lead to the emergence of resistant strains that have been demonstrated in our results. About half of the bacterial isolates in our study were sensitive to chloramphenicol, ciprofloxacin, levofloxacin, and doxycycline. A similar rate of sensitivity to ciprofloxacin was also observed in a previous data from India [31]. Previous studies evidenced the high bacteriological eradication rate in AECOPD patients when treated by levofloxacin [63]. Levofloxacin appears to be only marginally affected in term of resistance rate [64]. In comparison to other members of the aminoglycoside group used in this study, resistance rate to amikacin was slightly lower among the Gram-negative isolates. This is similar to previous reports from Egypt [35,51]. Sensitivity of our Gram-negative bacteria was at the highest level to the carbapenem group which was similar to previous studies from Egypt [51] and China [65]. Nevertheless, some isolates (8.5%) showed resistance to that group. Resistance to imipenem was reported also previously in cases of AECOPD in coal workers [66]. In our study, as with previous findings [67,68,69], *E. coli* demonstrated a very high microbial resistance to antibiotics where 15% of

the isolated *E. coli* strains were PDR. The majority of Gram-positive isolates in this study were resistant to penicillin which was similar to other findings [38]. A high resistance rate was also detected in *Strep. pneumoniae* strains in this study to penicillin. In recent years, resistance to penicillin increased rapidly among *Strep. pneumonia*e strains [60]. All *Staph. aureus* strains in our COPD patients were oxacillin-resistant which was similar to a previous report in India [70], while methicillin-resistance was higher than the rates in previous reports [60,71,72]. All the isolated Gram-positive bacteria in this work were sensitive to linezolid, the first commercially available oxazolidinone antibiotic. Similarly, linezolid was active against Gram-positive isolates in previous studies in United Kingdom [73,74]. In this study, MDR bacteria were isolated in a rate of 63% which is higher than previous reports [51,75]. Isolation of MDR bacteria in cases of respiratory infections was recorded in previous studies from Vietnam [76], China [77,78], and Thailand [79]. Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and -negative bacteria are both affected by the emergence and rise of antimicrobial resistance [14]. The situation is compounded by cross-resistance within and between classes of antibacterial agents, which further limits treatment options [80].

5. CONCLUSION

From our results we concluded that, Gram-negative bacilli are the leading pathogens in patients with AECOPD in Upper Egypt with predominance of *H. influenzae*. Our bacteriological profiles highlighted the distribution of other pathogens, including *E. coli*, *Strep. pneumoniae*, and *Kl. pneumoniae in* AECOPD. The isolated bacterial strains characterized by high resistance rates to most groups of antimicrobials. Sensitivity was relatively high to the carbapenem group.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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