Burden of bacterial exacerbation in bronchial asthma in Assiut University Hospitals, Egypt

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Background
Asthma is one of the most common chronic respiratory diseases. Despite advances in asthma management, acute exacerbations continue to occur and impose considerable morbidity and mortality on patients and constitute a major burden on healthcare resources.
Objective
This study aimed to determine the associations between bacterial infections and adult asthma exacerbations, together with detection of antibiotic resistance patterns in clinical practice.
Patients and methods
Sputa were collected from 60 adult asthmatic patients recruited from both Internal Medicine Department and Chest Disease Department and their critical care units during exacerbation attacks. Patients underwent thorough clinical examination, laboratory investigations, and pulmonary function tests. Bacterial isolates were identified using the standard diagnostic methods. Susceptibilities of the isolated bacterial strains were determined using disk diffusion method.
Results
Significant bacterial growth was detected in 47 (78%) patients. Single etiological agent was detected among 44 (73%) patients, whereas mixed infection was found in three (5%) patients. A total of 52 bacterial strains were isolated from our asthmatic patients. The predominant bacterial strains were as follows in decreasing order: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Gram-negative bacilli constituted 52% (27 isolates) of the total bacterial isolates during the exacerbation attacks. Non-multidrug-resistant bacteria were 15 (30%) in number, 22 (44%) bacterial isolates were multidrug resistant, six (12%) bacterial isolates were extensively drug resistant, and seven (14%) isolates were pandrug resistant.
Conclusion
Acute exacerbation of asthma was associated with infection in most patients. Gram-negative bacteria and *S. pneumoniae* form a relevant part of the microbial pattern of exacerbation of asthma. Antibiotic resistance among bacterial strains remains a challenge for the management of asthma exacerbations in clinical settings.

Keywords:
acute exacerbation, bacteria, bronchial asthma

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Introduction

Asthma is one of the most common chronic respiratory diseases. Despite advances in asthma management, acute exacerbations continue to occur and impose considerable morbidity and mortality on patients and constitute a major burden on healthcare resources [1]. Infections precipitate asthma exacerbations [2]. Bacteria are well-known causative organisms in the exacerbation of asthma, and pose significant problems for patients and their clinicians. Bacterial organisms can increase airway hyper-responsiveness and inflammation in the patient with known asthma [3]. On in-vitro examination, it was found that many bacteria are capable of activating several allergic inflammatory cells, including mast cells, eosinophils, bronchial epithelial cells, and smooth muscle cells. Mast cells express toll-like receptor 4 (TLR 4) on their surface, which is a receptor for bacterial lipopolysaccharide. After stimulation of TLR4 ligands, mast cells induce a subset of genes that include a Th2 cytokine and chemokines that recruit Th2 cells and eosinophils [4]. Eosinophils express TLR7 and TLR8 that inhibit bacterial replication in the lung and prevent bacterial-induced airway hyper-reactivity [5].

Objective

In the present study, the associations between bacterial infections and adult asthma exacerbations, together with detection of antibiotic resistance patterns, were evaluated in clinical practice.

Patients and methods

Ethical considerations

This study was approved by the Ethical Committee of our university and oral consent was obtained from each participant. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Study design and population

Adult asthmatic patients who were admitted in both the internal medicine department and the chest disease department and their critical care units due to asthma exacerbations from March 2015 to February 2016 were recruited in this prospective study. Respiratory specimens were collected from eligible patients who were over 18 years of age and had a clinical diagnosis of asthma supported by one or more other characteristics: variability in peak expiratory flow of more than 20%; airway reversibility by inhaled β2 agonist; hyper-responsiveness to methacholine challenge; and recurrent dyspnea episodes with wheezing [6]. Assessment of asthma severity was performed according to previously published criteria [7]. Asthmatic inpatients who were admitted for other diseases or who did not require systemic steroid treatment were excluded. Questionnaires were administered and included demographic and clinical data, and associated risk factors (e.g. smoking, immunosuppressives, and other comorbid conditions). Smoking history was calculated as number of packs/year=number of cigarettes smoked per day×number of years smoked/20 (one pack has 20 cigarettes) [8]. Patients underwent thorough clinical examination, laboratory investigations, and pulmonary function tests, which included spirometry, peripheral oxygen saturation, and chest radiography. Forced expiratory volume in the first second and forced vital capacity were obtained from the flow-volume curve using a spirometer (Zan 300 Sensor Medics MGA USB; Germany) (ZAN 300 MGA USB, nSpire Health GmbH, Oberthulba, Germany). Static lung volumes were measured using the closed-circuit helium dilution method. The reference values used were those of the American Thoracic Society standards before and 20 min 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. Echocardiography was performed for almost all patients, especially for those with severe attacks during admission, and the remaining underwent echocardiography on discharge.

Resting transthoracic echocardiography was performed using Philips Envisor 2002 (Andover, Massachusetts, USA). The procedure was performed with a 2.5 MHz multiphase array probe in standard parasternal and apical views according to the recommendations of the American Society of Echocardiography Segmental wall motion abnormalities. Ejection fraction and valvular function were assessed in the apical two-chamber and four-chamber view. The mean pulmonary artery pressure (mPAP) was calculated from systolic artery pressure (sPAP) using the formula: mPAP=0.61×sPAP+2 mmHg [9].

Bacterial strains and susceptibility testing

Sputa from asthmatic patients were collected in the early morning. Bacterial isolates were identified using the standard diagnostic methods. Susceptibilities of the isolated bacterial strains were determined for ampicillin, piperacillin/tazobactam, cephalosporins (ceftriaxone and ceftazidime), tetracycline, aminoglycosides (gentamicin and amikacin), ciprofloxacin, vancomycin, averozolid, oxacillin, and aztreonam. All antibiotics were purchased from Bioanalyse (Ankara, Turkey). The test was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines [10]. Bacterial isolates were classified as pandrug resistant (PDR), extensively drug-resistant, multidrug resistant (MDR), and nonmultiresistant, if resistance is found to all, to all except one or two, to greater than or equal to three, and to less than three antibiotic classes, respectively [11]. Isolation of atypical bacteria and anaerobes was not considered.

Statistical analysis

Data were analyzed using SPSS: IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp.), statistical package and were presented as number and percentage, or mean±SD as appropriate. Statistical significance was assessed using χ^2 or Fisher's exact test for categorical variables and analysis of variance (ANOVA) for continuous variables. A *P*-value less than 0.05 was considered statistically significant.

Results

Clinical characteristics of patients

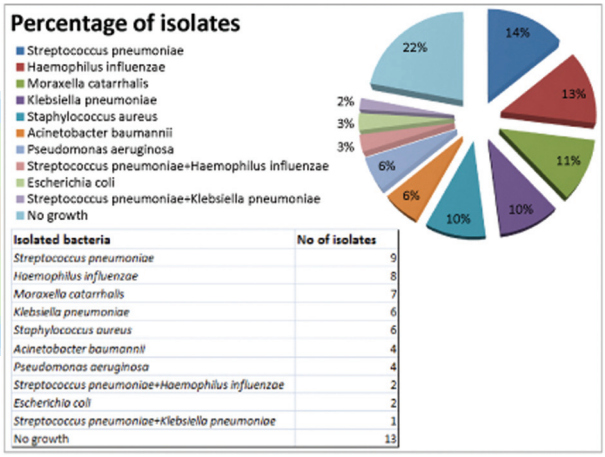
The study included 60 asthmatic patients with a mean age of 46.8±12.87 years (range: 21–72 years). There were 43 (72%) male patients and 17 (28%) female patients. Patients were mainly admitted in the chest

department (30; 50%), followed by the Internal Medicine Department (16; 26.6%), and then equal numbers were admitted in both the chest ICU and internal medicine ICU (both seven patients; 11.7%). Most patients (46; 77%) experienced moderate exacerbation attack, whereas 12 (20%) patients experienced acute severe attack and two (3%) patients had life-threatening attacks. The mean duration of hospital stay was 5.6±2.84 days (range: 1–14 days). It was significantly longer in patients with life-threatening asthma exacerbation and acute severe attacks versus those with moderate attacks (ANOVA; P=0.001 and 0.018, respectively). Fifty-eight (97%) patients experienced one exacerbation attack during the study period, whereas two (3%) patients had two attacks. Most of the patients with moderate asthma exacerbation had normal O_2 and CO_2 tensions, whereas patients with acute, severe, or life-threatening asthma exacerbations had hypoxia and hypercapnia (ANOVA; P=0.017, 0.006, and <0.001, 0.034 of moderate asthma vs. acute severe or life-threatening asthma exacerbations for O_2 and CO_2 pressures, respectively) (Table 1). Using Fisher's exact test, a statistically significant difference was found between the severity of asthma exacerbation with the incidence of respiratory failure (P=0.002). Most of the patients were either nonsmokers (30%) or exsmokers (52%). All female participants included in the study were nonsmokers. Male patients were either exsmokers or current smokers. Some of the patients had associated cardiopulmonary conditions. All patients received corticosteroid therapy as a standard treatment during the exacerbations. Most patients had elevated white blood cell count (49; 92%) and considerable number had elevated erythrocyte sedimentation rate (32; 53%).

Isolated bacteria and antibiotic sensitivity

Significant bacterial growth was found in 47 (78%) of the 60 participating patients during 49 (79%) of the 62 exacerbation attacks. No growth was found in 13 (22%) patients during 13 (21%) attacks (Table 1). Single etiological agent was detected among 44 (73%) patients during 46 (74%) attacks, whereas mixed infection was found in three (5%) patients during three (4.8%) attacks. The distribution of bacterial isolates in asthma exacerbations is shown in Fig. 1. A total of 52 bacterial strains were isolated from our asthmatic patients. The predominant bacterial strains were as follows in decreasing order: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Thus, Gram-negative bacilli constituted 52% (27 isolates) of the total bacterial isolates during the exacerbation attacks. *S. pneumoniae* and *M. catarrhalis* were mainly sensitive to ampicillin, piperacillin/tazobactam, tetracycline, cephalosporins, and aztreonam. *H. influenza* was mostly sensitive to piperacillin/tazobactam, cephalosporins, gentamicin, amikacin, and aztreonam. *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were mainly resistant to most tested antibiotics. *S. aureus* strains were mainly sensitive to piperacillin/tazobactam, tetracycline, vancomycin, and aztreonam. One *S. aureus* strain had intermediate sensitivity to vancomycin, whereas three *S. aureus* isolates showed resistance to oxacillin. Sensitivity of bacterial strains to different antibiotics is shown in Fig. 2. Non-MDR bacteria were 15 (30%) in number, 22 (44%) bacterial isolates were MDR, six (12%) bacterial isolates were extensively drug-resistant, whereas seven (14%) isolates were PDR. There was a statistically significant

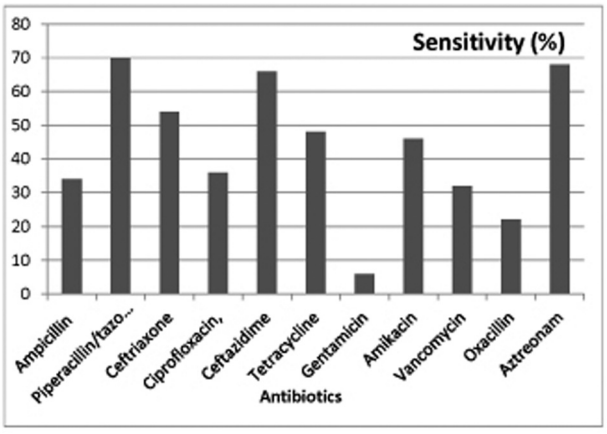
Figure 1



Isolated bacteria	No of isolates
Streptococcus pneumoniae	9
Haemophilus influenzae	8
Moraxella catarrhalis	7
Klebsiella pneumoniae	6
Staphylococcus aureus	6
Acinetobacter baumannii	4
Pseudomonas aeruginosa	4
Streptococcus pneumoniae+Haemophilus influenzae	2
Escherichia coli	2
Streptococcus pneumoniae+Klebsiella pneumoniae	1
No growth	13

Number and percentage of isolated bacterial strains during asthma exacerbation

Figure 2



Antibiogram (sensitivity) of 52 bacterial isolates in the sputum of asthmatic patients

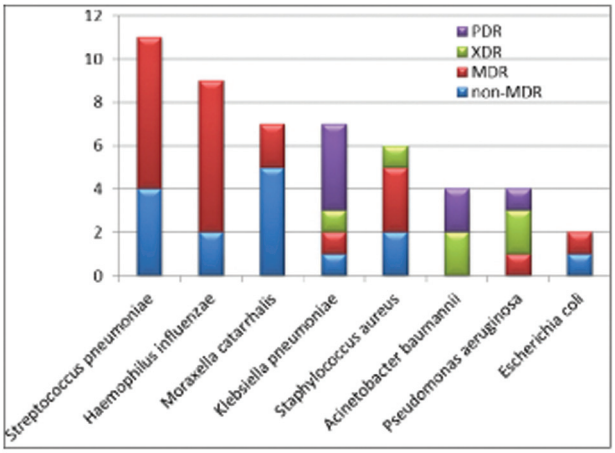
Table 1 Clinical and radiological characteristics of asthmatic patients (*n*=60)

	N (%)
O_2 tension	
Moderate asthma exacerbation (46 patients)	
Normal	27 (59)
Mild hypoxia	13 (28)
Moderate hypoxia	6 (13)
Acute severe asthma (12 patients)	
Normal	1 (8)
Mild hypoxia	8 (67)
Moderate hypoxi	3 (25)
Life-threatening asthma (2 patients)	
Severe hypoxia	2 (100)
CO_2 tension	
Moderate asthma exacerbation (46 patients)	
Normal CO_2 tension	45 (98)
Hypercapnia	1 (2)
Acute severe asthma (12 patients)	
Normal CO_2 tension	4 (33)
Hypercapnia	8 (67)
Life-threatening asthma (2 patients)	
Normal CO_2 tension	0 (0)
Hypercapnia	2 (100)
Chest radiography	
Normal chest radiography	39 (65)
Bronchial wall thickening	9 (15)
Lobar consolidation	6 (10)
Pneumonic infiltrates	6 (10)
Smoking	
Nonsmokers	18 (30)
Exsmokers	31 (52)
Smoker	11 (18)
Mild smokers	2 (3)
Moderate smokers	6 (10)
Heavy smokers	3 (5)
Associated cardiopulmonary condition	
RF	3 (5)
DCP	2 (3)
MV	2 (3)
IHD	1 (2)
Bacteriological diagnosis	
Significant bacterial growth during exacerbations attacks	62 attacks/60 patients
Single etiological agent	46 (74) attacks/44 (73) patients
Mixed infection	3 (4.8) attacks/3 (5) patients
No bacterial growth	13 (21) attacks/13 (22) patients

DCP, decompensated cor-pulmonale; IHD, ischemic heart disease; MV, mechanical ventilation; RF, respiratory failure.

difference between bacterial isolates as regards antibiotic sensitivity patterns (Fisher's exact test; *P*=0.001). Resistance rates were higher for Gram-negative bacilli compared with other bacterial isolates (χ^2; $P<0.001$), especially for *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* where considerable number of those strains showed PDR pattern (Fig. 3).

Figure 3



Drug resistance pattern of isolated bacterial strains. MDR, multidrug resistance; PDR, pandrug resistance; XDR, extensively drug resistance

Both *S. pneumoniae* and *M. catarrhalis* had the highest sensitivities to most tested antibiotics (χ^2; $P<0.001$).

Discussion

Asthma is a common emergency department presentation in many parts of the world. In both the pediatric and adult populations, asthma is responsible for ~10 to 15/1000 emergency department visits. Indeed, there is a well-known cardiopulmonary comorbidity association, and acute asthma exacerbation may precipitate acute cardiac events if not well treated. Acute care for exacerbations may be received in the emergency department or clinic setting where patients require assessment and additional therapeutic interventions due to an exacerbation of their airway disease [12]. Exacerbations are responsible for much of the mortality, morbidity, and expense of asthma [13]. Our data showed that acute exacerbation of asthma was associated with infection in 78% of our patients. Association of bacterial infection with asthma exacerbation was proved in many previous studies [13–16]. The mechanistic hallmark of asthma is colonization of the lower airways. Investigations of the microbiome of the lower airways, including bronchoalveolar lavage [17], brushings taken at bronchoscopy [2,18], and induced sputum [19], have revealed that the microbiome in asthmatic airways has an altered bacterial composition as compared with the microbiome in healthy airways. Although there are some inconsistencies between studies [17], a greater microbial richness and diversity may exist in asthmatic samples [18,19]. If

bacterial diversity is increased in asthma, certain bacterial species may prevail. In several studies, an increased abundance of the *Haemophilus* spp. was more frequently detected in the lower airways of adult asthmatic patients [18]. There are also hints for an association of the presence of *M. catarrhalis* or members of the genera *Streptococcus* in lower airways with a certain phenotype of asthma, characterized by neutrophilic inflammation and resistance to treatment with steroids [16]. As evidenced in our work, most of our patients had elevated white blood cell count and elevated erythrocyte sedimentation rate, which indicated the inflammatory process in those patients. The strongest evidence for a causal relationship between the local airway microbiome and asthma exacerbation comes from prospective studies in which microbial colonization before the onset of disease has been investigated. In the prospective study by Bisgaard *et al.* [15], the occurrence of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in the hypopharyngeal region of neonates was associated with an increased risk for asthma later in life. This detection in neonatal samples before disease onset as well as in asthmatic patients with established disease suggests that those potential pathogens may persist and possibly impact disease exacerbation and progression. *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* were detected during the exacerbation attacks of our asthmatic patients with considerable rates, which is consistent with previous findings [2]. The response of asthmatic patients to antibiotics also suggests the importance of acute and chronic bacterial infections in the pathogenesis of disease [2]. Epidemiological research suggested the need to understand the extent and nature of normal airway flora in understanding asthma exacerbation [20]. In our study, *S. pneumoniae* was the most commonly isolated bacteria. This is in accordance with previous studies [6]. More than 50% of bacterial isolates in our patients were Gram-negative bacilli. Previous reports demonstrated airway expansion of specific Gram-negative bacteria, which trigger inflammatory process and induce corticosteroid resistance [17]. Our data suggested that *S. aureus* are also present in excess in the airways of asthmatic patients during exacerbations. Our patients, especially male, were mostly exsmokers or current smokers, which reflected the effect of smoking as a risk factor for severe asthma exacerbations [21,22]. Smoking perpetuates an ongoing inflammatory response that leads to airway narrowing and hyperactivity, and hence patients become more prone to infection exacerbation attacks [23]. Moreover, cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma [24]. Smoking cessation is an effective therapy for asthma exacerbation and is associated with a decrease in symptoms, and improved health status. Although half of our patients were ex-smokers, they still experienced exacerbation attacks, which imply that smoking cessation was too late and the disease progression continued even after smoking cessation. All our asthmatic patients received corticosteroids as a standard treatment of asthma. Systemic corticosteroids markedly reduce the need for hospital admission and relapses in patients with severe asthma. The benefits are greatest in patients with life-threatening asthma and those not currently receiving steroids. Significant benefit with systemic steroid therapy is observed within 4 h of administration [12]. Nevertheless, in a considerable number of our patients, no significant bacterial growth was detected. Atypical bacteria and/or viral infections are not to be excluded in those patients. It is likely that wider exploration of adults with asthma will identify a range of organisms associated with the disease, including *Mycoplasma* spp. and *Chlamydia* spp.

Conclusion

Our data showed that acute exacerbation of asthma was associated with infection in most patients. Exacerbations are responsible for much of the mortality, morbidity, and expense of asthma. Gram-negative bacteria and *S. pneumoniae* form a relevant part of the microbial pattern of exacerbation of bronchial asthma that must be taken into account in patients who require hospitalization, particularly those with acute severe and life-threatening attacks. Antibiotic resistance among bacterial strains remains a challenge for the management of asthma exacerbations in clinical settings.

Study limitations

Our work had small sample size. More research advances with isolation of atypical bacteria and anaerobes is recommended to fulfill the best and good management of asthmatic patients.

Acknowledgements

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Nil.

Conflicts of interest

There are no conflicts of interest.

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