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

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].

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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, averyzolid, 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 χ2 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
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 (\(\chi^2\); \(P<0.001\)), especially for \(K.\ pneumoniae\), \(A.\ baumannii\), and \(P.\ aeruginosa\) where considerable number of those strains showed PDR pattern (Fig. 3).

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

| characteristic                      | N (%)     |
|-------------------------------------|-----------|
| **O2 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 hypoxia                    | 3 (25)    |
| Life-threatening asthma (2 patients) |           |
| Severe hypoxia                      | 2 (100)   |
| **CO2 tension**                     |           |
| Moderate asthma exacerbation (46 patients) |         |
| Normal CO2 tension                  | 45 (98)   |
| Hypercapnia                         | 1 (2)     |
| Acute severe asthma (12 patients)   |           |
| Normal CO2 tension                  | 4 (33)    |
| Hypercapnia                         | 8 (67)    |
| Life-threatening asthma (2 patients) |           |
| Normal CO2 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.

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

### 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 4h 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.

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Conflicts of interest
There are no conflicts of interest.

References
1. Jackson DJ, Sykes A, Mallia P, Johnston SL. Asthma exacerbations: origin, effect, and prevention. J Allergy Clin Immunol 2011; 128:1165–1174.
2. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, et al. Disordered microbial communities in asthmatic airways. PLoS One 2010; 5:e8578.
3. Kraft M. The role of bacterial infections in asthma. Clin Chest Med 2000; 21:301–313.
4. Okumura S, Kashiwakura J, Tomita H, Matsumoto K, Nakajima T, Saito H, et al. Identification of specific gene expression profiles in human mast cells mediated by Toll-like receptor 4 and FcepsilonRI. Blood 2003; 102:2547–2554.
5. Drake MG, Kaufman EH, Fryer AD, Jacoby DB. The therapeutic potential of toll-like receptor 7 stimulation in asthma. Inflamm Allergy Drug Targets 2012; 11:484–491.
6. Iikura M, Hojo M, Koketsu R, Watanabe S, Sato A, Chino H, et al. The importance of bacterial and viral infections associated with adult asthma exacerbations in clinical practice. PLoS One 2015; 10:e0123584.
7. Aldington S, Beasley R. Asthma exacerbations 5: assessment and management of severe asthma in adults in hospital. Thorax 2007; 62:447–458.
8. Indrayan AD, Kumar RM, Dwivedi SD. A simple index of smoking. COBRA Preprint Series 2008: -40.
9. Carroll JD, Hess OM. Assessment of normal and abnormal cardiac function. In: Zipes DP, Libby P, Borow RO, Braunwald E, editors. Braunwald’s heart disease: a text book of cardiovascular medicine. Vol. 2. 7th ed. Philadelphia, PA: Elsevier Saunders; 2005: p. 498.
10. Institute CaLS, M100-S22: performance standards for antimicrobial susceptibility testing; twenty-two informational supplement. Wayne, PA: Institute CaLS; 2012.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012; 18:268–281.
12. Rowe BH, Spooner CH, Ducharme FM, Bretzlaif JA, Bota GW. Corticosteroids for preventing relapse following acute exacerbations of asthma. Cochrane Database Syst Rev 2007; 18:CD000195.
13. Dickson RP, Martinez FJ, Huffnagle GB. The role of the microbiome in exacerbations of chronic lung diseases. Lancet 2014; 384:691–702.