Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

The Most Common Aerobic Bacteria Species Isolated from Some Asthmatic Inhabitants of Thi-Qar Province / Iraq

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Abstact

Background: Asthma is a common and chronic respiratory disease that affects over 300 million people worldwide and causes significant sickness and mortality.

Objective: This study aimed to determine the prevalence of most common Aerobic bacteria among asthma patients and its relation to demographic data in Iraqi population

Materials and Methods: 130 people participated in the current study, which was carried out at the Al-Nasiriya Teaching Hospital from October 2023 to June 2024. Of them, 100 patients had asthma, 70 had bacterial infections, 30 did not, and 30 served as a control group. The ages span from 3 to 80. The conventional methods that rely on culture to identify common pathogenic bacterial species

Result: This study was involved 70 patients with asthma for identification of common bacteria and showed that the most common isolated bacteria was S. aureus 24 (34.29%, then S. pneumonia 20 (28.57%), then P. aeruginosa 14(20.00%), while the lowest isolated bacteria was K. pneumonia 12 (17.14%), the present study also recorded a non-significant decrease in the frequency of bacteria in asthmatic patients.

Kew Words: Asthma, S. aureus, S. pneumonia, P. aeruginosa, K. pneumonia, Bacterial Infection

Introduction: Asthma is a chronic inflammatory illness that affects the airways, causing symptoms like wheezing, coughing, breathing difficulties, and a chest that feels heavy. The symptoms of asthma are caused by inflammation of the airways, which results in the formation of mucus, changes to the wall of the airway, and bronchial hyper-responsiveness (BHR), the smooth muscle cells' propensity to react to stimuli that are not specific, such as cold air. [1]. Although people might develop asthma later in life (late-onset asthma), it usually first appears in childhood

(childhood-onset asthma). There are differences between asthma that develops in childhood and asthma that develops later in life. Adult-onset asthma is more severe and has a weaker correlation with allergies than asthma that develops in infancy. Atopy, or the genetic predisposition to develop allergy disorders, decreased lung function, and respiratory tract infections—especially rhinovirus-related ones—are important risk factors for children's asthma to continue[2]. The pathogenicity of respiratory viruses and the underlying inflammation in children with asthma are not fully understood, nor is the significance of several early-life viral infections in the development of asthma. [3]. Since inflammation plays a major part in the development of asthma, it seems sense that the primary goals of asthma treatment should be to effectively control symptoms and address the underlying inflammation in order to stop the condition from recurring episodes [4]. The study employed an unsupervised clustering algorithm to investigate the asthma disease progression and clinical features. The findings demonstrated the diversity of asthma and the wide range of patient differences in asthma severity, age of onset, related risk factors, presence of co-occurring disorders, and responsiveness to treatment [5]. The respiratory system is a refuge for respiratory channel and the external environment. and there is a large number of them, there is a normal flora and confined to the upper respiratory tract and prepare gram positive bacteria, is the most common such as streptococci and staphylococci , also there are types of Gram negative bacteria present in this part of the respiratory system, such as Pseudomonas. aeruginosa and klebsiella. pneumonia although ,these organisms can becomes opportunistic and causes many diseases in the upper respiratory tract if a person's immunity is weak for any reason [6].

Materials and Methods

Patients Group :One hundred thirty people were involved in this study: 30 patients were not infected with bacteria. and 70 patients, 30 of whom were female and 40 of whom were male, were diagnosed with asthma by a respiratory specialist. Their ages ranged from 3-80.

Control Group : Thirty healthy individuals (15 males and 15 females) ranging in age from 3 to 73 made up the control group.

Exclusion Criteria : People with autoimmune disorders, diabetes, high blood pressure, and other chronic illnesses who also have asthma.

Samples Collection

Sputum Samples :were collected from controls and asthmatic patients using sterile containers, and within an hour of collection, they were sent straight to the lab for analysis.

Bacteria Culture : The following media were used to diagnose the gram-positive and gram-negative bacteria: blood agar, MacConkey agar, Mannitol salt agar, brain heart infusion broth, and urease activity test media.

Isolation of organisms : The obtained specimens were cultured one at a time by adding a loop's worth or a swab's worth of undiluted sputum samples to media such as blood agar, MacConkey agar, and chocolate agar. Plates were kept in an incubator with 5% CO2 for the entire night. Sub-

culturing was carried out multiple times in order to purify the bacterial isolates prior to taking any additional diagnostic measures.

Identification of the isolated bacteria

1-Primary diagnosis by light microscopic examination (ordinary)

It is used Gram stain to investigate the bacterial morphological characteristics and to discriminate between two broad communities of bacteria - Gram-positive and Gram-negative based on their components in the wall. Gram-positive bacteria are purple-spotted, as they have a dense layer of peptidoglycan in their walls. A thick layer of peptidoglycan layer can maintain the violet crystal which is used to dye. Conversely, owing to their thinner peptidoglycan wall but Gram-negative bacteria are stained red that does not maintain crystal violet in the decoloring phase. A bacterial isolate colony had been brushed with a burnt loop and located in a sterile glass slide. A droplet of saline was taken via the loop, and then the bacterial colony was coated on the glass slide. The glass slide was heated and the frost was allowed to dry. A droplet of crystal violet, the primary stain was located on smear. After 1 min., the crystal violet was carefully washed off the glass slide. A drop of the mordant, iodine was next added and then after one minute, the iodine was carefully washed off the slide. A droplet of 70% ethanol was located as the decolorizing agent and washed off after 15 seconds. Safranin, the counterstain was added and after 60 seconds it was washed off the glass slide. The slide was allowed to dry off completely, after which it was observed under the microscope.

2- Biochemical tests for gram-positive bacteria:

1-Catalase test

One colony is combined with a few drops of hydrogen peroxide on a slide. This test is used to distinguish between Staphylococci spp. (which give positive test results) and Streptococci spp. (which provide negative test results). The positive result is displayed as bubbles.

2- Coagulase test

This test is done by adding drops of plasma on a glass slide to single colony a positive result is indicate by clotting of plasma. This test used for recognition of the coagulase positive Staphylococci. aureus (coagulase positive), while coagulase negative to the other species of Staphylococci, is appeared as negative test.

3- Oxidase test

A few colonies were added to filter paper that had been soaked in a particular reagent for this test in order to identify whether the results were positive (purple) or negative (no change).

4- Indole test

The pure bacterial colony is added to the peptone water medium and incubated for 24 hours at 37 °C. Following this, drops of Kovacs reagent are added, and the appearance of a red ring on the media's surface indicates a successful test.

5- Kliglers Iron agar test

After making the agar slant, the isolated bacteria were taken by loop, injected into the bulgt agar, and streaked over the surface of the slant before being incubated for twenty-four hours at 37 °C by MacFaddin, 2000).

6- Voges-proskauer test

The ability of bacteria to create acetyl methyl carbonyl is determined by preparing MR-VP media, inoculating it with bacterial culture inoculation, and then heating it to 37 °C for 24 hours. After 15 minutes, a few drops of potassium hydroxide and the alpha-naphthol reagent are added, and the affirmative result turns red.

7- Methyle red test

To prepare the test, inoculate the tube with MR-VP media, inoculate a new bacterial culture, and then incubate it at 37 °C for the entire night.5 drops of methyl red reagent are then added. The outcome is dependent on whether it is red or yellow, indicating a favorable or negative outcome.

8- Citrate utilization test

Fresh culture of bacteria are inoculated and then incubated at 37 °C for 24 h. The positive result is indicated by change the color of the media from green to blue.

9- Culturing on Mannitol Salt agar (MSA)

Positive for coagulase When Staphylococci are grown on this medium, the medium turns yellow and begins to ferment mannitol sugar. Coagulase-negative Staphylococci do not ferment mannitol, and the PH indicator's color does not change.

10- API20 System

This system is used to confirm the diagnosis of bacterial isolates and to identify the bacterial species that recovered in this study. This system is manufactured by the French company BIOMERIEUX (2018).

Approval of the ethical committee: The presented revision has been permitted by the Directorate of Health in the Thi-Qar Committee (No. 210 ⁄ 2023 in 26/10/2023). The patient's consent was taken verbally in hospitals and clinics, when visiting them in their homes, or by mobile phone conversation for an invitation for blood and sputum samples**.**

Statistical Analysis: The data of the current study was statistically analysis by using SPSS version 26, based on using Kruskal-Wallis H for mean and range and Chi-square for independent at p. value < 0.05.

Results

Identification of Gram-positive Bacteria in Asthmatic Patients. This study was involved 70 patients with asthma for identification of common gram-positive bacteria and showed that the most common isolated bacteria was S. aureus 24 (34.29%, then S. pneumonia 20 (28.57%), while the lowest isolated bacteria was K. pneumonia 12 (17.14%), the present study also recorded a non-significant decrease in the frequency of bacteria in asthmatic patients.

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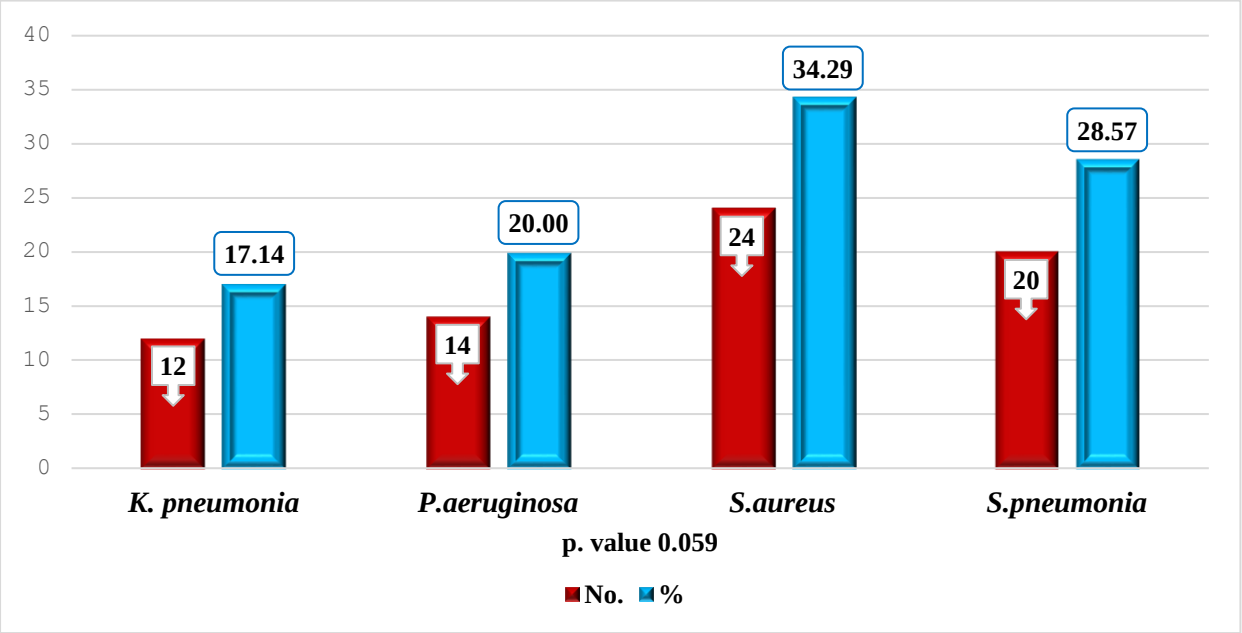
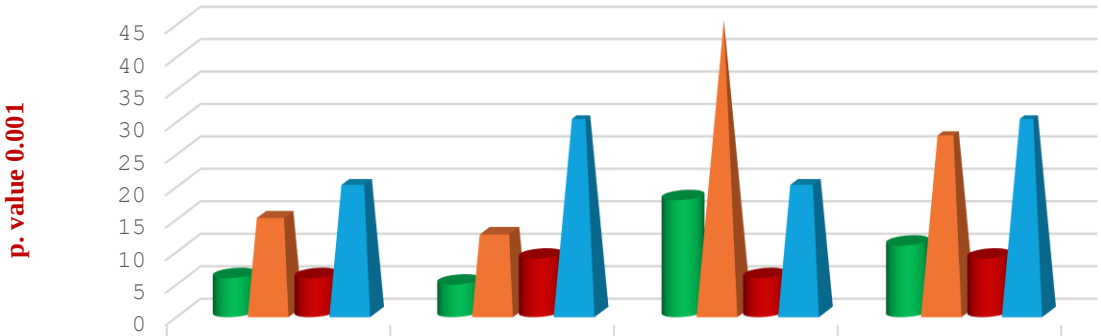


Figure (1): Identification of gram-positive bacteria and gram negative bacteria in asthmatic patients

Prevalence of Gram-positive and Gram-negative Bacteria in Asthmatic Patients According to the Sex

The present study was conducted a significant difference in the frequency of bacteria in asthmatic patients according to the sex at p. value < 0.05,. the prevalence of bacteria was in 40 (57.15%) of male group, while 30 (42.85%) in female group. in the other hand the most isolated bacteria in male group was S. aureus 18 (45%), while in female group P. aeruginosa 9 (30%), as in the figure below.



	K. pneumonia	P. aeruginosa	S. aureus	S. pneumonia
Male No.	6	5	18	11
Male %	15	12.5	45	27.5
Female No.	6	9	6	9
Female %	20.00	30.00	20.00	30.00

Figure (2): Identification of gram-positive and Gram-negative bacteria in asthmatic patients according to the sex

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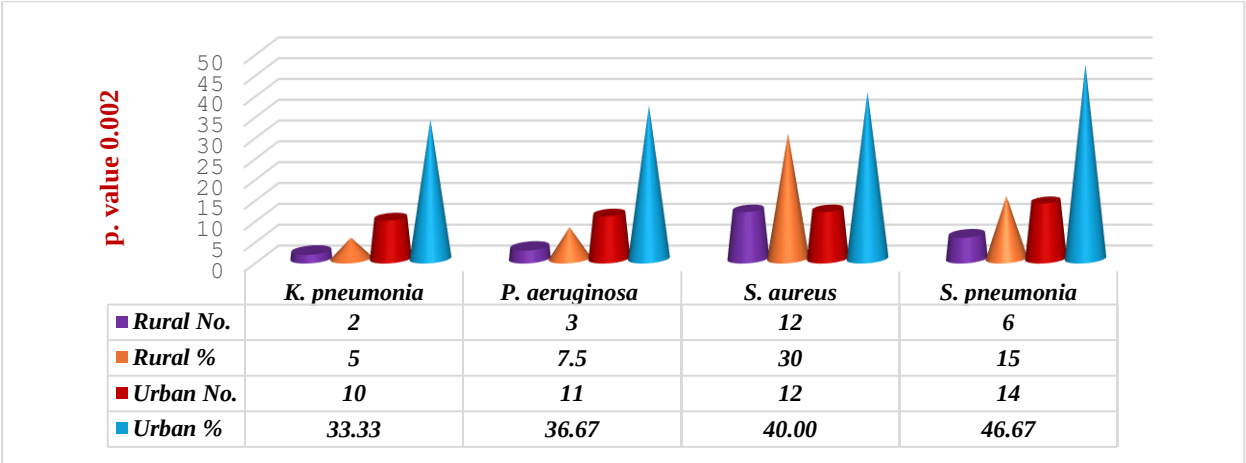
Prevalence of Gram-positive Bacteria in Asthmatic Patients According to the Residency

The current study was found a significant difference in the frequency of bacteria in asthmatic patients according to the residency at p. value < 0.05. our result showed that 23 (32.85%) of the patient sample came from rural residence, while 47 (67.14%) was from urban residence, in the other hand the most isolated bacteria in rural residence was S. aureus 12 (30%), while in urban residence was S. pneumonia 14 (46.67%), as shown in the figure below.



Figure(3): Identification of gram-positive and Gram-negativ bacteria in asthmatic patients according to the residency

Distribution of Asthmatic Patients (with and without Bacterial Infection) and Control Group According to the Sex

Our findings conducted a non-significant difference between patients' groups, and within asthmatic patients groups as related to sex at p. value < 0.05. it was noted 47 (56.95%) of patients groups were males compared with 56 (43.08%) were females, in addition the males were higher percentage in both asthmatic patients, while equal to females in the control group, as in table below.

Table (1): Distribution of asthmatic patients (with and without bacterial infection) according to the sex

Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
With Infection	40	57.14	30	42.86	70	53.84
Without Infection	19	63.33	11	36.67	30	23.08
Control	15	50.00	15	50.00	30	23.08
Total	74	56.92	56	43.08	130	100
All Groups $Calx^2$= 3.44 $Tabx^2$= 5.99 DF= 2 P. Value $0.178^{Non-Sig}$ Patient Groups $Calx^2$= 0.75 $Tabx^2$= 3.84 DF= 1 P. Value $0.386^{Non-Sig}$						

Average of Age of Asthmatic Patients (with and without Bacterial infection) and Control Group

This study was find a non-significant difference between asthmatic and control groups according to average of age at p. value < 0.05. the average of age in control (40.6 years), increased non-significantly, when compared with asthmatic patients without bacterial infection 33.0 years, as in table below.

Table (2): Average of age of asthmatic patients (with and without bacterial infection) and control group

Study Groups	Age Mean ± S. D	Rang Of Age
With Infection	36.3 ± 20.8	3 – 82
Without Infection	33.0 ± 21.6	3 – 80
Control	40.6 ± 18.3	3 - 73
Kruskal-Wallis H P. Value	0.231 non-Sig	

Distribution of Asthmatic Patients (with and without Bacterial Infection) and Control Group, According to the Age Groups

The current study was recorded a significant difference between asthmatic groups, and within asthmatic patients groups according to the age groups at p. value < 0.05. the highest age group in bacterial infected asthmatic patients was second age group 20 (28.57%), while in the non infected asthmatic patients, were both first and third age groups 9 (30.0%) was the highest. the highest age group in the control group was fourth age group 12 (40%), as in table below.

Table (3): Distribution of asthmatic patients (with and without bacterial infection) according to the age groups

Age Groups In Years	With Infection		Without Infectio		Control		Total	
	No.	%	No.	%	No.	%	No.	%
3 – 15	13	18.57	9	30.00	4	13.33	26	20.00
16 – 30	20	28.57	5	16.67	5	16.67	30	23.08
31 - 45	14	20.00	9	30.00	6	20.00	29	22.31
46 – 60	13	18.57	3	10.00	12	40.00	28	21.54
> 60	10	14.29	4	13.33	3	10.00	17	13.07
Total	70	53.84	30	23.08	30	23.08	130	100
All Groups Calx^2= 36.0 Tabx^2= 15.51 Df= 8 P. Value <0.001 sig Patients Groups Calx^2= 36.0 Tabx^2= 9.49 Df= 4 P. Value 0.034 sig								

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Distribution of Asthmatic Patients (with and without Bacterial Infection) and Control Group According to Residency

This study was conducted that a significant difference between studies groups, while a non-significant difference within asthmatic patients groups according to residency at p. value < 0.05. there were 83 (63.85%) of asthematic groups were urban residents compared with 47 (36.15%) were rural residents. in addition the urban residents were higher in both infected and non infected asthmatic patients, while the rural and urban residence in the control group, showed as in table (4).

Table (4): Distribution of asthmatic patients with and without bacterial infection according to residency

Residency	Rural		Urban		Total	
	No.	%	No.	%	No.	%
With Infection	23	32.86	47	67.14	70	53.84
Without Infection	8	26.67	22	73.33	30	23.08
Control	16	53.33	14	46.67	30	23.08
Total	47	36.15	83	63.85	130	100
All Groups Calx^2= 15.7 Tabx^2= 5.99 DF= 2 P. Value <0.001^{Sig} Patients Groups Calx^2= 0.857 Tabx^2= 3.84 DF= 1 P. Value 0.355$^{Non-Sig}$						

Distribution of Asthmatic Patients (with and without Bacterial Infection) According History of Disease

This study was conducted that a non-significant difference between asthmatic patients groups according to history of disease at p. value < 0.05 .of asthmatic patients with bacterial infection there were 37 (52.86%) had history for disease compared with 17 (56.67%) of asthmatic patients without bacterial infection had not history for disease, as in table below.

Table (5): Distribution of asthmatic patients (with and without bacterial infection) according to history of disease

History Of Disease	Hereditary		Non-Hereditary		Total	
	No.	%	No.	%	No.	%
With Infection	37	52.86	33	47.14	70	70.0
Without Infection	17	56.67	13	43.33	30	30.0
Total	54	54.0	46	46.0	100	100
Calx^2= 0.32 Tabx^2= 3.84 DF= 1 P. Value 0.570$^{Non-Sig}$ OR Infected/Noninfected 0.85 (0.48 – 1.48)						

Discussion

Prevalence of Gram-positive and Gram negative Bacteria in Asthmatic Patients

The current study was noted among 70 asthmatic patients infected with bacteria, the most common isolated bacteria was S. aureus 24 (34.29%, then S. epidermidis 20 (28.57%), P. aeruginosa 14 (20.0%), while the lowest isolated bacteria was K. pneumonia 12 (17.14%). The present study was agreed with study of Kim et al. [7], which noted the most abundance bacteria in asthmatic patients was S. aureus 38.7%, furthermore, their study also investigated the patients with S. aureus that colonize nasal cavity suffering from sever asthma. Also, previous studies that done by both Pesek and Lockey [8], and Brealey et al. [9], also recorded that the young asthmatic patients had abundant gram-positive bacteria than gram-negative, and the most bacteria was H. influenza then S. pneumonia, furthermore, it was determined that pneumococcal infection was linked to a higher likelihood of asthma exacerbation in patients. Additionally, younger patients who had nasopharyngeal pneumococcal colonies were found to have an elevated risk of developing severe respiratory syncytial virus infection.

The administration of the pneumococcal vaccine resulted in changes in the colonization of the nasopharynx, specifically an increase in the population of H. influenzae and a decrease in was linked to enhanced management of asthma. However, it did not influence the occurrence of upper respiratory infections (URI) and lower respiratory infections (LRI), nor did it alter the rate of antibiotic usage. Research conducted on mice with asthma shown that infection with pneumococcus or immunization with a peptide 27 deletion mutant effectively controlled allergic asthma by stimulating the production of Treg cells, Thus, inactivation of the pep27 gene (Δpep27) renders pneumococci nonlytic and unable to invade the lungs, blood, and brain. Curiously, those with asthma exhibited an improved immune response to 23-valent pneumococcal immunizations in comparison to those without asthma, because this vaccine composed from 23 capsular polysaccharide types of S. pneumoniae, representing at least 85% to 90% of pneumococcal disease [10, 11].

The study of Caruso et al. [12] recorded that the asthmatic patients suffering from nasal colonization with S. aureus, and that patients with bacterial infection had high level of both IL-5 and IgE. Also, a study of Bachert et al. [13] demonstrated that S. aureus exerts control over the immune response in the airway mucosa through its proteins, such as superantigens, serine-protease-like proteins, and protein A. The respiratory epithelium releases IL-33, which activates innate lymphoid cells through its receptor ST2. This leads to the release of type 2 cytokines from both innate lymphoid cells and T helper 2 cells. Additionally, it triggers mast cell degranulation, significant local activation of B-cells, and the creation of IgE. All these processes contribute to the severity of the condition.

The study of Eklöf et al. [14], showed among 21408 asthmatic patients the prevalence of P. aeruginosa was 3.6%, also showed the most bacterium isolated from patients)that use inhaled corticosteroids was showed a positive correlation between uses of inhalation and nasal bacterial colonization.

It is well known that primary risk factors contributing to the prevalence of this condition include genetic susceptibility, environmental pollution, viral infection, a family history of allergies or asthma, and cigarette smoking. To investigate the impact of environmental pollution on the occurrence of asthma, it is necessary to compare the incidence of the condition in areas with and without various types of pollution. Therefore, the results of the current analysis indicate a higher prevalence of asthma in urban areas compared to rural areas [15].

Age, Sex, and Residency of Studies Groups

The current study included 59 male and 41 female. It is showed the average of age the patients in asthmatic patients with and without bacterial infection was 36.3 years, and 33.0 years respectively,

A local study conducted in Thi-Qar province by Salih and Salman, [16], showed a significant difference according to sex of patients was observed among 100 patients when the sample included 68 female and 32 males. The study of Zhang et al. [17], showed that the age average in sever asthmatic patients was 47.9 years, the older age compares with this study, and in non-sever asthma 45.2 years, while noted a non-significant difference with regard sex of patients, but the sever asthma increased in female than male. A recent study conducted by Caruso et al. [12], showed the average of age was older (51 years) compared with current study, but their result agreed with regard sex that was a non-significant difference noted. Eklöf et al. [14], indicated a non-significant difference between male and female in occurrence of disease, it was noted the 54.6% of patients were female and 45.4% male. The study of Hailemaryam et al. [18], was showed among 240 patients with asthma there is not significant difference according to sex, age groups and residency, while there was a significant difference according to family size and family education status. Based on their residential address, asthma patients are categorized into two geographic groups: 32.85% live in rural areas, and 67.14% reside in urban areas. It is caused by environmental or genetic factors, air pollution from factories and automobile exhaust, and pollution of the surrounding environment. These findings are consistent with those of Larche et al. (2003) [19], who mention that a person's genetic background may increase their body's production of antibodies against important environmental allergens, which in turn increases their susceptibility to asthma.

This result is consistent with Kelly and Fussell's [20], suggestion that a variety of environmental variables, such as fungal allergens, air pollutants, and other environmental chemicals, have been linked to various elements of asthma development and exacerbation.

Conclusion :This study noted the most abundance bacteria in asthmatic patients was S. aureus 38.7%, furthermore, their study also investigated the patients with S. aureus that colonize nasal cavity suffering from sever asthma.

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