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Epidemiological Studies on Relation between *Streptococcus pneumoniae* with Respiratory Diseases

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Abstract

Streptococcus pneumoniae is the leading cause of bacterial infections worldwide and causes a range of illness from otitis media to pneumonia and meningitis.
The study was carried out in Mansoura university hospitals, Egypt over period of 12 month. On a number of 71 patients, all of them are suffering from respiratory tract diseases (Acute bronchitis, chronic obstructive pulmonary disease, Pneumonia, Chronic suppurative lung disease, bronchial asthma) their ages ranged from 15 -75 years. Results showed that *Streptococcus pneumoniae* bacteria that were isolated from sputum samples were (34%), *S. pneumoniae* isolated from patients in Age ≤35 was (17%), and in age ≥3 5 *Streptococcus pneumoniae* was (37.3%). *Streptococcus pneumoniae* isolated from patients with chronic bronchitis was 25%, while pneumonia was (50%), while chronic obstructive pulmonary disease was 14.3%, and chronic suppurative lung disease was (33.8%). The sensitivity patterns proved that *Streptococcus pneumoniae* in antibiotic prepared disc is highly sensitive to Levofloxacin.

Keywords: Respiratory tract diseases, *Streptococcus pneumoniae*, Antibiotics.

Introduction

Respiratory tract infections (RTIs) are one of the major public health problems and a leading cause of morbidity and mortality in many developing countries. Respiratory tract is the most common site for infection by pathogens because it comes into direct contact with the physical environment and is exposed to microorganisms in the air. *Streptococcus pneumoniae* causes an acute bacterial infection. Bacteria isolated from respiratory tract infection were Staphylococcus aureus, Streptococcus pneumonia, Haemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Moraxella subgenus Branhamella catarrhalis Goto H; et al (2005). The common bacteria causing respiratory tract infections were Streptococcus pneumoniae has the highest percentage of occurrence, followed by Klebsiella pneumoniae, Escherichia coli (20.9%) and Staphylococcus aureus, respectively. D. W. Taura, et al. (2013).
Streptococcus pneumoniae (pneumococcus) remains an important cause of pneumonia, meningitis,

bacteremias, and acute otitis media worldwide. Antimicrobial resistance among pneumococci has escalated dramatically over the past three decades, and is influenced by patterns of antibiotic use, population density, and spread of a few international clones. ***Lynch JP 3rd, Zhanel GG.(2010).***Each year, approximately half of the 2.6million deaths due to acute respiratory infections in children under five are due to pneumococcal pneumonia . Invasive pneumococcal disease (IPD, pneumonia, meningitis, and sepsis) in children is particularly associated with mortality and is a major cause of hospitalizations and long-term neurologic sequelae. ***Carla A. Talarico (2009)***

Antibiotics are the mainstay of acterial treatment, the goal of these drugs is to kill the invading bacteria without harming the host ***Archer and Ronald, (2001)***. Antibiotic effectiveness depends on mechanism of action, drug distribution, site of infection, immune status of the host, and resistance factors of bacteria .Antibiotics work through several mechanisms. Some (such as vancomycin and penicillin) inhibit formation of bacterial cell walls. Erythromycin, tetracycline, and chloramphenicol interrupt protein synthesis. Still others inhibit bacterial metabolism (sulfa drugs) or interfere with DNA synthesis (ciprofloxacin, rifampin) and/or cell membrane permeability (polymyxin b) ***Conte JE, (2002).*** When antibiotics were discovered in the 1940s, they were incredibly effective in bacterial infection treatment. Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance ***Perez et al., 1990; Barie, 1998; Domin, (1998).*** Bacteria may be naturally resistant to different classes of antibiotics or may acquire resistance from other bacteria through exchange of resistant genes. Indiscriminate, inappropriate, and prolonged use of antibiotics have selected out the most antibiotic-resistant bacteria ***Petrosillo and Pantosti, (2002)***. Antibiotic-resistant strains have emerged in hospitals, long-term care facilities, and communities worldwide ***Levin and Levy, (2003).***

The study was carried out in Mansoura university hospitals, Egypt over period of 12 month. on a number of 71patients (40males and 31females) all of them are suffering from respiratory tract diseases (Acute bronchitis, chronic obstructive pulmonary disease, Pneumonia, Chronic suppurative lung disease, bronchial asthma) their ages ranged from 15years to 75 years, , 38 patients nonsmoking and 33 patients smoking .Data entry was completed using Microsoft excel. SPSS Program and medical programs for statistical analysis (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Material and methods:

The present study was conducted on a number of 71patients (males and females) were subjected to clinical examination in hospitals of Mansoura university, Egypt their ages ranged from 15years to 75 years, This study done over period of 12 months. In this study there are 40 male and 31females all of them are suffering from respiratory tract diseases (Chronic bronchitis, chronic obstructive pulmonary disease, Pneumonia, Chronic suppurative lung disease). All patients were subjected to the history (Sex, Age and smoking habit), clinical examination and a complete physical examination including symptoms and signs indicating the development of bacterial infection.

Sample collection:

Sputum samples were collected in sterile plastic container under complete aseptic conditions, the best sample is the early morning sputum. The patient should expectorate from deep down in the lung. If patient failed to expectorate, sputum may be induced by expectorant or by inhalation of heated saline hypertonic first morning sputum sample was collected directly into a sterile wide mouthed container and transported to laboratory according to standard protocol **(Cheesbrough. M. 2000).**

Preparation of sputum samples:

Add to the sputum about 5 ml of sterile physiological saline to obtain as pure a culture for isolation of *streptococcus pneumoniae* as possible of a respiratory

pathogen it is necessary to reduce the number of commensal inoculated. **(Cowan and Steel, 1974) and (Cheesbrough, 2000)**.

Isolation and identification of bacterial strains:

Isolation was performed using horse blood agar media (Oxoid), each Plate was inoculated by spreading aloobful of prepared sputum sample. Incubation of culture plate's media was placed in an inverted position for 24 to 48 hours at 37°C. Isolates were identified according to colonial morphology and appearance, growth characteristics, hemolytic pattern, microscopically by Gram's stain and biochemically (Catalase test, Coagulase test, alpha hemolysis on blood agar media, Bile solubility test, Optochin susceptibility test) according to Bergey's Manual of Determinative Bacteriology **(Holt etal., 1994)**

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed using the disc diffusion method. Fourteen different antibiotics (Levofloxacin, Azithromycin, Meropenem, Ciprofloxacin, Gentamycin, Imipenem, Cefotaxime, Cloxacillin, and Amoxicillin\clavulanic acid, Ceftazidime, Spiramycin, Piperacillin, Ampicillin\sulbactam, and Ampicillin) were tested for determination of their antibiotic effects on the isolated strains.

Statistical analysis

All data were run on IBM compatible personal computer using the software Statical Package for Social Scientist (SPSS) Program and medical programs for statical analysis (SPSS Inc. Chicago, IL, USA).The trend x^2 test for statistical comparisons between the groups and a p P < 0.05 was considered as statistically significant.

Results:

Seventy one patients their ages ranged from 15 years to 75 years, 40 male and 31females all of them are suffering from respiratory tract diseases (12 chronic bronchitis, 22 Pneumonia, 21 chronic obstructive pulmonary disease, 16 chronic suppurative lung disease), 38 patients non Smoking and 33 patients smoking.

The respiratory tract infections in Man were chronic bronchitis (9.8%), Pneumonia (31.71%), chronic obstructive lung disease (COPD) (26.83%), chronic suppurative lung disease (CSLD) (31.71%) While in woman were chronic bronchitis (13.33%), Pneumonia (33.33%), Chronic obstructive lung disease (COPD) (36.67%), Chronic suppurative lung disease CSLD (16.67%).

There was a significant relationship between the sex and the diagnosis of patients (P* =0.01).

The respiratory tract infections in Age ≤ 35 were chronic bronchitis (25%), Pneumonia (50%), chronic obstructive lung disease (COPD) (17%) and chronic suppurative lung disease (CSLD) (8%). While Age ≥ 35 are chronic bronchitis (8%), pneumonia (29%), chronic obstructive lung disease (COPD) (34%), chronic suppurative lung disease CSLD (29%).

There was a significant relationship between the Age group and the diagnosis of patients (P* =0.02).

The respiratory tract infections in Nonsmoking patients were chronic bronchitis (15.8%), pneumonia (39.5%), chronic obstructive lung disease (COPD) (31.5%), and chronic suppurative lung disease (CSLD) (13.2%), while Smoking patients are chronic bronchitis (6.1%), Pneumonia (24.8%), COPD 30.3 %, and Chronic suppurative lung disease (CSLD) (39.4%).

There was a significant relationship between the smoking habit and the diagnosis of patients (P* =0.0116).

Colonies of twenty four plates appear as small, grey, moist (sometimes mucoidal), and characteristically produce a zone of alpha-hemolysis (green),isolates gave positive Gram stain , negative Catalase test, negative Coagulase test, alpha hemolysis on blood agar media, Positive Bile solubility test, positive Optochin susceptibility test that show they were ***Streptococcus pneumoniae*****.**

According to sex *Streptococcus pneumoniae* isolated from infected male was (31.7%), while in females was (36%). There was a significant relationship between the occurrence of the isolates and the gender of patients (P* =0.02).

According to age *Streptococcus pneumoniae* isolated in Age ≤ 35 was (17%), in age ≥ 35 *Streptococcus pneumoniae* was (37.3%). There was a significant relationship between the occurrence of the isolates and the age of patients (P* =0.01).

Table (1): Relation between diagnosis of cases with Gender, Age and Smoking habit:

Diagnosis		Gender		Age		Smoking habit		Total of cases
		Male	female	Age≤35	Age≥35	Non Smoking	Smoking	
Chronic Bronchitis	Count	4	4	3	5	6	2	8
	%	9.8	13.33	25	8	15.8	6.1	11.27
pneumonia	Count	13	10	6	17	15	8	23
	%	31.71	33.33	50	29	39.5	24.2	32.39
COPD	Count	11	11	2	20	12	10	22
	%	26.83	36.67	17	34	31.5	30.3	30.98
CSLD	Count	13	5	1	17	5	13	18
	%	31.71	16.67	8	29	13.2	39.4	25.35
Total	Count	41	30	12	59	38	33	71
	%	57.7	42.7	17	83	53.52	46.48	100

Chronic obstructive pulmonary disease: COPD
Chronic superlative lung disease: CSLD ((Lung abscess, Bronchiectasis))

Table (2): Relation between *Streptococcus pneumonia*e of cases with Gender, Age and Smoking habit of cases:

Diagnosis		Gender		Age		Smoking habit	
		Male	Female	Age ≤ 35	Age ≥ 35	Non Smoking	Smoking
Chronic Bronchitis	Count	1	2	0	3	0	3
	%	2.44	6.66	0	5.1	0	7.9
pneumonia	Count	5	6	2	9	8	3
	%	12.2	20	16.66	15.3	24.24	7.9
COPD	Count	2	1	0	3	2	1
	%	4.9	3.33	0	5.1	6.1	2.6
CSLD	Count	5	2	0	7	2	5
	%	12.2	6.66	0	11.9	6.1	13.2
Total	Count	13	11	2	22	12	12
	%	57.7	42.7	17	83	53.52	46.48

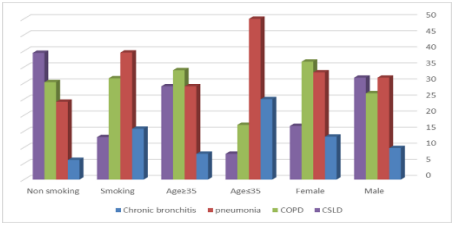

Figure (1): Relation between diagnosis of cases with Gender, Age and Smoking habit:

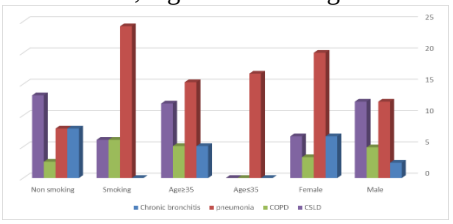

Figure (2): Relation between *Streptococcus pneumonia*e of cases with Gender, Age and Smoking habit of cases

Streptococcus pneumoniae isolated from patients with chronic bronchitis was 25%, while pneumonia was (50%), while chronic obstructive pulmonary disease was 14.3%, and chronic suppurative lung disease was (33.8%). (**Tab.3).**

Streptococcus pneumoniae isolated from nonsmoking patients was *(31.58%)*, while smoking patients was *Streptococcus pneumoniae* (36.4%). There was no significant relationship between the occurrence of the isolates and the smoking habit of patients (P* =0.2743).

Table (3): Relation between *Streptococcus pneumonia*e bacteria and diagnosis of all cases:

Diagnosis	*Streptococcus pneumoniae*		No of cases	% of cases
	No	%		
Chronic bronchitis	3	25	12	16.9
pneumonia	11	50	22	30.99
COPD	3	14.3	21	29.58
CSLD	7	43.75	16	22.54
Total	24	33.8	71	100

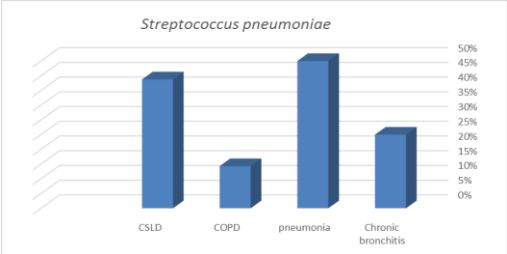

Figure (3): Relation between *Streptococcus pneumonia*e bacteria and diagnosis of all cases

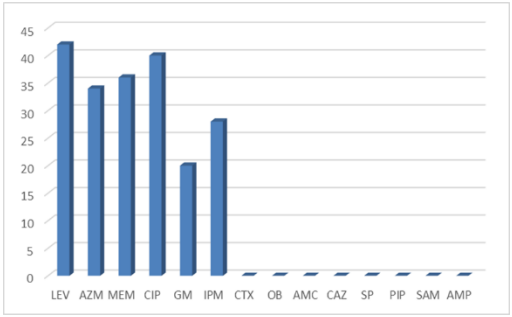

Figure (4): Sensitivity test of *Streptococcus pneumoniae*

Screening of antibiotic sensitivity of tested bacteria:

Streptococcus pneumoniae isolated was tested for their antibiotic sensitivity to fourteen different antibiotics (Levofloxacin, Azithromycin, Meropenem, Ciprofloxacin, Gentamycin, Imipenem, Cefotaxime, Cloxacillin, Ceftazidime, and Amoxicillin\clavulanic acid, Spiramycin, Piperacillin, Ampicillin\sulbactam, and Ampicillin).

Data in table show that *Streptococcus pneumonia*e were highly susceptible for Levofloxacin (42mm) followed by Ciprofloxacin (40mm), Meropenem (36mm), Azithromycin (34mm), Imipenem (28mm), and Gentamycin (20mm) respectively *S.pneumonia*e resistant to Cefotaxime, Cloxacillin , Amoxicillin \ clavulanic Acid, Ceftazidime , Spiramycin, Piperacillin, Ampecillin\sulbactam, Ampicillin (**Tab. 4).**

Table (4): Antibiotic Sensitivity test of *Streptococcus pneumoniae:*

Antibiotic	Abbreviations	Clear zone	Sensitivity
Levofloxacin	LEV	42	S
Azithromycin	AZM	34	S
Meropenem	MEM	36	S
Ciprofloxacin	CIP	40	S
Gentamycin	GM	20	S
Imipenem	IPM	28	S
Cefotaxime	CTX	0	R
Cloxacillin	OB	0	R
Amoxicillin\clavulanic Acid	AMC	0	R
Ceftazidime	CAZ	0	R
Spiramycin	SP	0	R
Piperacillin	PIP	0	R
Ampecillin\sulbactam	SAM	0	R
Ampicillin	AMP	0	R

S: Sensitive I: Intermediate R: Resistant

Discussion:

Respiratory tract infections are the most frequently reported of all human infections. According to recent WHO report on the epidemiology of top ten infectious diseases, Lower respiratory tract infection tops the list in the developing countries and it becomes fourth in developed countries. ***Ndip et al., (2008)***. In this study the occurrence of bacterial pathogens varies with age, in that, age group more than 35 year 83% more infected than below 35 years 17%. Nearly similar result were reported by ***Goto H, Iwasaki M. (2007) ,Goto H, Kumagai S. (2008) , Goto H, Kumagai S.(2009) , Goto H, Iwasaki M. (2010), Goto H, Iwasaki M. (2011)***Found that the majority number (57.7%) of the patients with respiratory infection were aged 70 years or older, while ***Salman khan, et al (2013)*** find out that Age group of 1-10 years old was at a higher risk .

This study found that among smoking habit Nonsmoking people 53.52% are more infected than smoking people 46.48%. ***Marcy TW etal, (1989)*** investigated that Cigarette smoking alters the respiratory tract ability to defend itself from 110 infection. Some subjects with chronic bronchitis have colonization of the lower respiratory tract with bacteria. Both patients with chronic respiratory disease and healthy smokers appear to have a higher frequency of respiratory infections and an increased severity of symptoms when infected. Children exposed passively to cigarette smoke have higher rates of respiratory illnesses, ***Lidia Arcavi, etal. (2004)*** found that cigarette smoking appears to be a major risk factor for respiratory tract and other systemic

infections. Both active and passive cigarette smoke exposure increase the risk of infections, and ***Kozielski J (2008)*** show that Tobacco smoke exposure leads to development of structural and immunological changes in the lungs, these changes favor development on inflammation in the respiratory tract. Cigarette smoking is a substantial risk factor for important bacterial and viral infections.

This study found that commonest respiratory tract infection was Bacterial pneumonia (32.39 %) followed by Chronic obstructive pulmonary disease (COPD) (30.39%), Chronic suppurative lung disease (CSLD) (25.35%) and chronic bronchitis (11.27%). The commonest respiratory tract infections in Man were Pneumonia (31.71%), Chronic suppurative lung disease (CSLD) (31.71%) followed by Chronic obstructive lung disease (COPD) (26.83%) and chronic bronchitis (9.8%) .While in woman are Chronic obstructive lung disease (COPD) (36.67%), followed by Pneumonia (33.33%), Chronic suppurative lung disease CSLD (16.67%), and chronic bronchitis (13.33%) **.** The commonest respiratory tract infections in Age ≤35 are Pneumonia (50%), followed by chronic bronchitis (25%), Chronic obstructive lung disease (COPD) (17%) and Chronic suppurative lung disease (CSLD) (8%). While Age ≥35 are chronic obstructive lung disease (COPD) (34%), followed by Pneumonia (29%), chronic suppurative lung disease CSLD (29%) and chronic bronchitis (8%) .

According to data in **[Tab. 1]** The commonest respiratory tract infections in Nonsmoking people was pneumonia (39.5%) followed by Chronic obstructive lung disease (COPD) (31.5%), chronic bronchitis (15.8%) and Chronic suppurative lung disease (CSLD) (13.2%),While Smoking people was Chronic suppurative lung disease (CSLD) (39.4%), followed by COPD 30.3 %, Pneumonia (24.8%) and chronic bronchitis (6.1%).

In this study common Bacteria causing respiratory tract infections were *Streptococcus pneumoniae (34%),* nearly similar results investigated by ***Taura, et al .(2013)*** who reported that common Bacteria causing respiratory tract infections are *S.pneumoniae* 25.6% , A number of investigators, ***Goto et al (2005)*** reported that common Bacteria causing respiratory tract infections were *Streptococcus pneumoniae* , followed by *Staphylococcus aureus ,Haemophilus influenzae , Pseudomonas aeruginosa, Klebsiella pneumoniae , and Moraxella subgenus Branhamella catarrhalis ,**Goto H, Iwasaki (2007,2010,2011)*** find out that common Bacteria causing respiratory tract infections were *Staphylococcus aureus , Streptococcus pneumoniae, Haemophilus influenzae ,Pseudomonas aeruginosa , Klebsiella pneumoniae* , and *Moraxella catarrhalis.* ***Goto , Kumagai (2008, 2009)*** find out that common Bacteria causing respiratory tract infections were: *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Moraxella catarrhalis*. ***Ndip et al.2008*** find out the common Bacteria causing lower respiratory tract infections are *Pseudomonas aeruginosa, followed* by *Haemophilus influenzae, Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus* and *Escherichia coli.*

Salman khan, et al (2013) demonstrated that Gram-negative bacteria were more than gram-positive bacteria. Respectively *P.aeruginosa* and *S.pneumoniae* were the most common Gram negative and Gram positive bacterial isolates recovered from lower respiratory tract infections. ***Asati Rakesh Kumar (2013)*** found out that *Staphylococcus aureus* (36.7%) accounted most common organism *and Klebsiella pneumonia* (21.1 %) accounted second most common organism. Other organisms were *Pseudomonas spp*. (18.3 %), *Escherichia coli (12.7 %), Staphylococcus aureus (9.8%) and Proteus spp. (1.4 %).*

This study found that Men 58% reported respiratory tract infections more frequently than women 42%. This result agree with other studies conducted by ***Panda et al., (2012)*** whose reported that, out of the 101 isolated organisms, 64 (63.4%) were from males while 37 (36.6%) were from females and ***Matthew (2007)*** reported that most Respiratory Tract Infections (RTIs) is more severe in males than in females, However, these results contradicts the data obtained by ***El-***

Mahmood et al., (2010), in which in a similar study, out of 232 total isolates,114 (49.1%) were from males while 118 (50.9%) from females. In this study the commonest Bacteria in infected male is *Streptococcus pneumoniae* (31.7%) and in females are *S.pneumoniae*, *(36*%)

The commonest Bacteria isolated in age ≥ 35 is *Streptococcus pneumoniae*, ***Panda et al., (2012)***, recorded higher occurrence of *K. pneumoniae* among patients ranging from 51-60 and 60-70 years. and ***D. W. Taura,et al .(2013)*** find out that Age ranges 20 – 29 and 30 – 39 have the highest percentage of pathogens isolated.

Streptococcus pneumoniae is the commonest Bacteria isolated from non smoking and smoking patients (31.58%), (36.4%) *respectively*.

In this study the commonest bacteria isolated from patients with pneumonia was *Streptococcus pneumonia*e (50%) This result agree with *Goto H; et al (2005)*who investigated that the commonest bacteria frequently isolated from the patients with bacterial pneumonia were *Streptococcus pneumoniae* (23.4%), on the other hand , ***Goto H, Iwasaki M. (2007)*** find out that the commonest bacteria frequently isolated from the patients with bacterial pneumonia were *Staphylococcus aureus* (21.9%) .In this study *Streptococcus pneumonia*e isolated from 25% of patients infected with chronic bronchitis, **3** % of patients infected with COPD, 7% of patients infected with CSLD.

In this study *Streptococcus pneumoniae* highly susceptible for Levofloxacin followed by Ciprofloxacin, Meropenem, Azithromycin, Imipenem, and Gentamycin respectively *S.pneumonia*e resistant to Cefotaxime, Cloxacillin, Amoxicillin\clavulanic Acid , Ceftazidime, Spiramycin, Piperacillin, Ampecillin\ sulbactam, Ampicillin. **[Tab. 4]**. Number of investigators**, *Rosa del Campo, etal (2005)*** investigated that Forty-eight *Streptococcus pneumoniae* isolates recovered from sputum samples High over all resistance rates were observed: to penicillin, 73%; to Cefotaxime, 33%; to erythromycin, 42%; to tetracycline, 58%; to chloramphenicol, 48%; and to trimethoprim-sulfamethoxazole, 67%. Resistance to fluoroquinolones was not detected. Multi resistance was a common feature (60%), ***Iroha Ifeanyichukwu Romanus, etal (2013)*** found out that *Streptococcus* spp. were susceptible to ciprofloxacin, Ceftazidime and amikacin but resistant to amoxicillin/clavulanic acid, and ampicillin.

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الملخص العربي

عنوان البحث: الدراسات الوبائية على العلاقة بين بكتريا Streptococcus pneumoniae وأمراض الجهاز التنفسي

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تعتبر بكتريا *Streptococcus pneumoniae* السبب الرئيسي للعدوى البكتيرية في جميع أنحاء العالم وتسبب مجموعة من الأمراض من التهاب الأذن الوسطى والتهاب رئوي والتهاب السحايا.
وقد أجريت الدراسة في مستشفيات جامعة المنصورة، مصر خلال فترة 12 شهر. على عدد من 71 مريضا، جميعهم يعانون من أمراض الجهاز التنفسي (التهاب القصبات الحاد، مرض الانسداد الرئوي المزمن، الالتهاب الرئوي وأمراض الرئة القيحي المزمن، الربو القصبي) تراوحت أعمارهم من 15 -75 عاما. وأظهرت النتائج أن بكتيريا *Streptococcus pneumoniae* التي تم عزلها من عينات البلغم كانت (34٪)، *Streptococcus pneumoniae* المعزوله من المرضى في سن ≥35 كانت (17٪)، وفي سن ≤35 كانت النسبه (37.3٪)وكانت *Streptococcus pneumoniae* المعزولة من المرضى الذين يعانون من التهاب الشعب الهوائية المزمن (25٪)، في حين كان الالتهاب الرئوي (50٪)، في حين كان مرض الانسداد الرئوي المزمن (14.3٪)، وكان المزمن مرض الرئة قيحية (33.8٪). أثبتت أنماط الحساسية للمضادات الحيوية ان البكتريا حساسة جدا لليفوفلوكساسين.