

# **Antibiotic Susceptibility Pattern among *Streptococcus pneumoniae* Isolates**

## **Thesis**

Submitted for Partial Fulfillment of Master Degree  
in Clinical Pathology

**By**

***Mennat Allah Samir Mohamed***

MB Bch

Faculty of Medicine - Ain Shams University

**Under Supervision of**

**Professor / Amira Mohammed Mokhtar**

Professor of Clinical Pathology

Faculty of Medicine - Ain Shams University

**Doctor/ Sally Mohammed Saber**

Assistant Professor of Clinical Pathology

Faculty of Medicine - Ain Shams University

**Faculty of Medicine**

**Ain Shams University**

**2018**

## List of Contents

Title	Page
▪ List of Abbreviations.....	I
▪ List of Tables.....	III
▪ List of Figures .....	V
▪ Introduction.....	1
▪ Aim of the Work .....	4
▪ Review of Literature	
I. Streptococcus Pneumoniae .....	5
II. Antibiotic-Resistant Streptococcus Pneumoniae .....	21
III. Diagnosis of Pneumococcal Infection.....	29
IV. Treatment of Pneumococcal Infection .....	49
V. Prevention and Control of Pneumococcal Infection .....	53
▪ Materials and Methods .....	58
▪ Results .....	75
▪ Discussion .....	88
▪ Conclusion and Recommendations .....	100
▪ Summary.....	102
▪ References.....	104
▪ Arabic Summary.....	--

## List of Abbreviations

<b>ARMed</b>	Antibiotic Resistance Surveillance & Control in the Mediterranean Region
<b>AST</b>	Antimicrobial susceptibility test
<b>CAP</b>	Community acquired pneumonia
<b>CHD</b>	Coronary heart disease
<b>CLSI</b>	Clinical and Laboratory Standards Institute
<b>COPD</b>	Chronic Obstructive Pulmonary Disease
<b>CPS</b>	Capsular Polysaccharide
<b>CWPS</b>	Cell Wall Polysaccharide
<b>DD</b>	Disk Diffusion
<b>DM</b>	Diabetes Mellitus
<b>DNA</b>	Deoxyribonucleic Acid
<b>DRSP</b>	Drug Resistant S. Pneumoniae
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>FDA</b>	Food and Drug Administration
<b>HAP</b>	Hospital Acquired Pneumonia
<b>HCB</b>	Hospital Central da Beira
<b>HIV</b>	Human Immunodeficiency Virus
<b>HUS</b>	Hemolytic Uremic Syndrome
<b>ICT</b>	Immunochromatographic Membrane Test
<b>IDSA</b>	Infectious Disease Society of America
<b>IPD</b>	Invasive Pneumococcal Disease

**LA** .....Latex Agglutination  
**LRT** .....Lower Respiratory Tract  
**MALDI-TOF MS** ....Matrix-Assisted Laser Desorption-  
Ionization Time-Of-Flight Mass  
Spectrometry

## List of Abbreviations

**MDR** .....Multidrug Resistant  
**MHA** .....Muller Hinton Agar  
**MIC**.....Minimal Inhibitory Concentration  
**MLSb** .....Macrolide, Lincosamide, Streptogramins  
**PBP**.....Penicillin Binding Protein  
**PCR** .....Polymerase Chain Reaction  
**PCV** .....Pneumococcal Polysaccharide  
Conjugate Vaccine  
**PERCH** .....Pneumonia Etiology Research for  
Child Health  
**PPSV**.....Purified Pneumococcal Polysaccharide  
Vaccine  
**PRP**.....Penicillin Resistant S. Pneumoniae  
**S. Pneumoniae** .....Streptococcus Pneumoniae  
**S. viridans**.....Streptococcus Viridans  
**SMG**.....Streptococcus Mitis Group  
**SXT** .....Trimethoprim-Sulphamethoxazole  
**UTI** .....Urinary Tract Infection  
**VGS** .....Viridans Group Streptococc

## List of Tables

Table No.	Title	Page
<b>Table (1):</b>	Taxonomic classification of S. pneumonia.....	7
<b>Table (2):</b>	The main pneumococcal virulence factors and their main role in invasive pneumococcal disease.....	11
<b>Table (3):</b>	Resistance to other antibiotics.....	28
<b>Table (4):</b>	Molecular detection of genes responsible for penicillin and macrolide resistance.....	48
<b>Table (5):</b>	Summary of pneumococcal vaccine serotype content.....	54
<b>Table (6):</b>	CLSI List of used Antibiotic discs and their concentration .....	66
<b>Table (7):</b>	Resistant Phenotype detection of S. pneumoniae by vitek .....	71
<b>Table (8):</b>	Demographic data of clinical samples .....	75

<b>Table (9):</b>	Antibiogram of studied <i>S. pneumoniae</i> isolates by disk diffusion .....	77
<b>Table (10):</b>	Antibiogram of studied <i>S. pneumoniae</i> isolates by vitek.....	79
<b>Table (11):</b>	Correlation between disk diffusion and vitek results of penicillin.....	80

## List of Tables

Table No.	Title	Page
<b>Table (12):</b>	Correlation between disk diffusion and vitek results of Erythromycin.....	81
<b>Table (13):</b>	Correlation between disk diffusion and vitek results of levofloxacin.....	82
<b>Table (14):</b>	Correlation between disk diffusion and vitek results of Tetracycline .....	83
<b>Table (15):</b>	Correlation between disk diffusion and vitek results of SXT .....	84
<b>Table (16):</b>	Correlation between disk diffusion and vitek results of vancomycin .....	85
<b>Table (17):</b>	Correlation between disk diffusion and vitek results of linezolid .....	86
<b>Table (18):</b>	Number of isolates of S.pneumoniae at various MIC ranges to Benzylpenicillin.....	86
<b>Table (19):</b>	Number of isolates of S.pneumoniae at various MIC ranges to Erythromycin .....	86
<b>Table (20):</b>	MIC50, MIC90 for Benzylpenicillin, and Erythromycin .....	87
<b>Table (21):</b>	Detected resistant S.pneumoniae phenotypes .....	87

## List of Figures

Figure No.	Title	Page
<b>Fig. (1):</b>	Invasive and Non-invasive pneumococcal disease .....	17
<b>Fig. (2):</b>	Pneumococcal serogroups which are most responsible for Invasive disease ..	19
<b>Fig. (3):</b>	<i>S. pneumoniae</i> lancet-shaped diplococci in Gram stain; note the encapsulated organisms as evident by the clear “halo” .....	32
<b>Fig. (4):</b>	Principle of MALDI-TOF MS .....	36
<b>Fig. (5):</b>	A) Negative quellung reaction, B) positive quellung reaction, the capsule appears as an enlarged clear halo surrounding the dark blue stained cell.....	40
<b>Fig. (6):</b>	Flow chart for identification and characterization of a <i>S. pneumoniae</i> .....	41
<b>Fig. (7):</b>	Flow chart for standard diagnosis of Pneumonia in immuno-competent adults .....	42
<b>Fig. (8):</b>	Vitek® 2 compact system .....	61
<b>Fig. (9):</b>	Vitek® 2 antibiotic susceptibility cards AST-ST01 .....	61
<b>Fig. (10):</b>	Colonies of <i>S. pneumoniae</i> on blood agar mucoid with draughtsman appearance .....	62



**Fig. (11):** Gram stain of *S. pneumoniae* ..... 63

## List of Figures

Figure No.	Title	Page
<b>Fig. (12):</b>	Optochin test. A) <i>S. viridians</i> , resistance to optochin B) <i>S. Pneumoniae</i> , susceptible to optochin...	64
<b>Fig. (13):</b>	Antibiotic susceptibility test by disk diffusion.....	67
<b>Fig. (14):</b>	Inducible clindamycin resistance in <i>S. pneumoniae</i> .....	68
<b>Fig. (15):</b>	Vitek® 2: AST of <i>S. pneumoniae</i> .....	70
<b>Fig. (16):</b>	Frequency of studied clinical samples .....	76
<b>Fig. (17):</b>	Percentage of antibiotic sensitivity by disk diffusion method.....	77
<b>Fig. (18):</b>	Percentage of antibiotic sensitivity by Vitek .....	79

## Abstract

*Streptococcus pneumoniae* is the leading cause of community-acquired pneumonia around the world and in Severe pneumococcal disease is associated with a high mortality in adults. Antimicrobial resistance of *S. pneumoniae* against the most commonly used antimicrobial drugs is increasing worldwide, principally affecting  $\beta$ -lactam, macrolide and sulfonamide sensitivity.  $\beta$ -lactam antibiotics, and penicillin in particular, are amongst the most widely used antimicrobial drugs for empirical treatment of pneumonia 1. Evaluation of antimicrobial resistance of *S. pneumoniae* is fundamental to guide the empirical treatment of PID, as well as to encourage reflections to support immunization policies2 .

This study was done on 50 *Streptococcus pneumoniae* isolates recovered from samples referred to the Central Microbiology Laboratory, Ain Shams University Hospitals, for routine culture and sensitivity. the mean value of their ages was  $45.8 \pm 16.3$ . they were 37 males and 13 females. antibiotic susceptibility test was done by disk diffusion method and vitek method. there was a significant association between the disk diffusion method and vitek method ( $p=0.022$ ) except for penicillin .

In this study the resistance rate of MDR isolates by vitek method was (16/50) 32% .the percentage of PRP was (19/50) 38%. The percentage of MLSb is (8/50) 16%. the percentage of vancomycin resistant *S.pneumonia* was (6/50) 12%.

**Keyword:** Antibiotic, *Streptococcus pneumonia*, Clinical Pathology

## INTRODUCTION

*Streptococcus pneumoniae* (*S.pneumoniae*) is a leading cause of invasive and noninvasive bacterial pneumonia, meningitis, and sepsis in infants, children, and adult worldwide. However, many countries lack national estimates of disease burden. To support local and global policy decisions on pneumococcal disease prevention and treatment, country-specific incidence of serious cases and deaths was estimated in children younger than 5 years. *S. pneumoniae* causes around 11% of all deaths in children younger than 5 years. Child mortality reduction can be accelerated by prevention and treatment of pneumococcal disease, especially in regions of the world with the greatest burden (*Kumar, 2015*).

Although there are varieties of antibiotics for treatment of (*S. pneumoniae*), but the pneumococcal multidrug resistance (MDR) has become a serious concern in the treatment of invasive pneumococcal diseases globally (*Song and Wayne, 2013*). The percentage of multidrug-resistant strains have reached up to 95.6% (*Kim et al., 2012; Song and Wayne, 2013*).

Globally the percentage of *S. pneumoniae* resistant strains is 100% for erythromycin and cotrimoxazole, 86.9% for clindamycin, 82% for cefuroxime, 42,6% for penicillin, 36.1% for meropenem, 18% for ceftriaxone, and 13% for cefotaxime (*Liu et al. (2013)*).

A retrospective multicenter study conducted in 5 hospitals in Egypt revealed an increase in penicillin resistance (37%), and little resistance to ceftriaxone (84% susceptible) and ciprofloxacin (82% susceptible) among CSF isolates (*El Kholly et al., 2003*).

Another sentinel meningitis surveillance program showed a marked increase in penicillin resistance (50%) among CSF isolates in Egypt (*Wasfy et al., 2005*).

Also, *Afifi et al. (2007)*, reported high rates of multidrug resistance (MDR) in *S. pneumoniae*, reached 8 out of the 206 isolates tested (4%).

Additionally, a surveillance report of the Antibiotic Resistance Surveillance & Control in the Mediterranean Region (ARMed) project which started in 2003 and continued for 2 years in the southeastern Mediterranean, reported 30% penicillin resistance and 25% erythromycin resistance among the *S. pneumoniae* Egyptian isolates (*Borg et al., 2009*).

Generally, serotype 19A seemed to have a higher resistance rate than other serotypes. Serotype 19A is not a serotype that is covered by the pneumococcal conjugate vaccine (PCV-7), which should be of concern. Studies in many countries have shown that use of PCV-13 decreased the overall incidence of invasive pneumococcal disease (IPD) among children (*Weil-Olivier et al., 2012*).

Extensive studies are required to continually update antimicrobial susceptibility patterns. The unregulated use of antibiotics in developing countries is common, stressing the importance of surveillance for antibiotic resistant pathogen to guide empirical treatment (***Borg et al., 2009***).

For *S. pneumoniae* isolated from CSF, penicillin, cefotaxime, ceftriaxone and meropenem should be tested by a reliable Minimal inhibitory concentration (MIC) method. Such isolates can also be tested against vancomycin using the MIC or disk diffusion. For non-meningitis isolates oxacillin zone or penicillin (MIC) can predict susceptibility to  $\beta$ -lactams. Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution using the single-well test (containing both erythromycin and clindamycin) (***Clinical and Laboratory standards Institute “CLSI”, 2017***).

## **AIM OF THE WORK**

The aim of this work is to detect the antibiotic susceptibility pattern among *S. pneumoniae* isolates which recovered from clinical samples referred to the Central Microbiology Laboratory, Ain Shams University Hospitals.