STUDY ON SEROTYPES AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF STREPTOCOCCUS PNEUMONIAE AMONG HEALTHY AND PNEUMONIC CHILDREN IN ADEN CITY, REPUBLIC OF YEMEN

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in
Clinical Microbiology

By

Yasser Mansour Mohammed Matran

Registration Number: 12004343

Supervised By

Dr. Sandeep Sharma (23995)
Department of Medical Laboratory Sciences
College of Applied Medical Sciences
Lovely Professional University.

Co-Supervised by

Dr. Ahmed Mohammed Al-Haddad
Department of Medical Laboratory Sciences
College of Medicine and Health Sciences,
Hadhramout University, Yemen.

LOVELY PROFESSIONAL UNIVERSITY
2024

DECLARATION

I hereby declare that the presented work in the thesis entitled "Study on Nasopharyngeal Carriage and Antimicrobial Susceptibility Patterns of Streptococcus Pneumoniae Among Children in Aden City, Yemen" in fulfilment of the degree of Doctor of Philosophy (Ph.D.), is the outcome of research work carried out by me under the supervision of Dr. Sandeep Sharma, who works as Professor of Clinical Microbiology in the Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Lovely Professional University, and Dr. Ahmed Mohammed Al-Haddad, who works as Professor of Medical Microbiology and Molecular Biology at the Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Hadhramout University, Yemen. In keeping with the general practice of reporting scientific observations, due acknowledgments have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or in full to any other University or Institute for the award of any degree.

Yasser Mansour Mohammed Matran

Registration No.: 12004343

School of Allied Medical Science

Lovely Professional University, Punjab, India.

SUPERVISOR CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Study on Nasopharyngeal Carriage And Antimicrobial Susceptibility Patterns of *Streptococcus Pneumoniae* Amone Children In Aden City, Yemen" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Clinical Microbiology at School of Allied Medical Science, college of Medical science is a research work carried out by Yasser Mansour Mohammed Matran bearing Registration No. 12004343, is bonafide record of his original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

Supervisor

Dr. Sandeep Sharma (23995)

Professor of Clinical Microbiology

Department of Medical Laboratory Sciences

College of Applied Medical Sciences

Lovely Professional University, Punjab, India.

CO-SUPERVISOR CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "Study on Nasopharyngeal Carriage And Antimicrobial Susceptibility Patterns of *Streptococcus Pneumoniae* Amone Children In Aden City, Yemen" submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Clinical Microbiology at School of Allied Medical Science, college of Medical science is a research work carried out by Yasser Mansour Mohammed Matran bearing Registration No. 12004343, is bonafide record of his original work carried out under my Co-supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course

A. Al-Haddad

Co-Supervisor

Dr. Ahmed Mohammed Al-Haddad

Department of Medical Laboratory Sciences

College of Medicine and Health Sciences,

Hadhramout University, Yemen.

Acknowledgments

Firstly, I would like to convey my sincere appreciation to my supervisor, Professor Dr. Sandeep Sharma, and my co-supervisor, Professor Dr. Ahmed M. Al Haddad, for their steadfast support, supervision, and invaluable insights throughout my research journey.

My thanks are extended to Dr. Iman Ba Sedik, Professor of Pediatrics at the Faculty of Medicine and Health Sciences, University of Aden, for her valuable advice, especially in refining the study questionnaire, which played an essential role in improving the quality of the research.

Additionally, I wish to offer my sincere appreciation to Mr. Salem Madram, General Director of the Centre of Public Health Laboratories, for graciously providing the necessary space for conducting the practical aspects of this study. Without their continuous encouragement and support, this research would not have been possible. As well, heartfelt thanks go to the Head Department of Medical Microbiology at the Center of Public Health Laboratories, Aden, Mr. Abdullah Omer Ba Omer, for his unwavering support during the practical work, and to his team, especially Mr. Gamal Al Hasani and Mr. Samih Laaor, for their invaluable assistance during the investigation process. Furthermore, I am indebted to the Head of the Department of Molecular Biology, Miss Nahed M. Salem, and her team, especially Miss Mida Jafer and Miss Shima Ahmed, for their steadfast support during the molecular identification phase of the research. My appreciation also extends to the Head of the Department of Virology for providing facilities to preserve our specimens. I would also like to acknowledge the Director of Al Sadkah Teaching Hospital and Al Basaten Health Care Center and their teams for their cooperation during the crucial phase of sample collection.

Finally, I express my gratitude to all those who contributed effort and support, playing a pivotal role in the successful completion of this research, even if I may have missed mentioning their names and titles specifically.

Dedication

To the soul of my father, whose wisdom and guidance remain my compass in this scholarly journey, may Allah grant him mercy.

To the river of affection, My Mother, whose unwavering love has been the bedrock of my aspirations.

To my support and strength in this life, my wife, who has been a source of inspiration, encouragement, and understanding. Her unwavering support has fueled my pursuit of knowledge, even in the face of the bitterness of distance and separation.

To my brothers and sisters, for their constant support throughout the challenges I have faced.

To my sons and daughters, who inspire me to strive for excellence and leave a legacy of learning.

Table of Contents

List of Terminology.

Abbreviation	Full Name
AMR	Antimicrobial resistance
AOM	Acute Otitis Media
BAL	Bronchoalveolar Lavage
CBPs	Choline-Binding Proteins
CLSI	Clinical and Laboratory Standards Institute
cps	capsular polysaccharide synthesis locus
CSF	cerebrospinal fluid
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthase
E-test	Epsilometer Test
EVs	Extracellular Vehicles
GBA	Gentamycin Blood Agar
H_2O_2	Hydrogen Peroxide
IPD	Invasive Pneumococcal Disease
LTA	Lipoteichoic Acid
lytA	Pneumococcal Autolysin
MIC	Minimum Inhibitory Concentration
Nan	Neuraminidase
NESp	nonencapsulated *S. pneumoniae* strains
NVS	Non-Vaccine Serotypes
PBPs	Penicillin Binding Protein
PCV	Pneumococcal Conjugate vaccine
pGSN	Plasma Gelsolin
PsaA	Pneumococcal Surface Adhesin A
PspC	Pneumococcal Surface Protein C
PVSR	pneumococcal vaccine serotype replacement

ROS	Reactive Oxygen Species
rTF	Recombinant Trigger Factor Protein
RT-PCR	Real Time Polymerase Chain Reaction
SGG	Skim milk Glycerol Glucose broth
SIgA	Secretory Immunoglobulin A
STGGB	Skim milk Tryptone Glucose Glycerin Broth
TAF	Thrombocyte Activating Factor
TF	The Trigger Factor Protein
TH-HYC	Hewitt/Hemoglobin/Yeast/Charcoal/Agar
TI-2	T-Cell-Independent Type 2
TLRs	Toll-Like Receptors

List of Tables

List of Figures

List of Appendices

Abstract

Background: *Streptococcus pneumoniae* (*S. pneumoniae*, also known as Pneumococci) remains primarily a challenge to children's health worldwide. Pneumococcal diseases like pneumonia, meningitis, and sepsis are leading causes of child mortality internationally. High rates of antibiotic resistance aggravate the danger of pneumococcal infections in pediatric populations globally. Moreover, the shifting of vaccine serovars with non-vaccine serotype that has highly sophisticated pathogenic activity and antibiotic resistance worsen the burden of pneumococci. This investigation intended to determine the carriage rate of pneumococci among Yemeni kids, identify the most common distributing pneumococcal serotypes in Yemeni children after twelve years of application of pneumococcal conjugate vaccine, assess the patterns of penicillin alternative antibiotics by measuring their breakpoint concentrations and along with that create a warning alarm for the public health service and health care regarding multi drug-resistant strains of pneumococci to enhance the national surveillance and public awareness program.

Material & Methods: A sample of 385 nasopharyngeal swabs were gathered from kids aged 2-17 years in Aden City, Yemen, including 185 pneumonic children from pediatric clinics and 200 asymptomatic children from health care centers and primary schools. Swabs were inoculated on Gentamicin Blood Agar, the isolates were confirmed as *S. pneumoniae* depending on morphology characteristics, optochin susceptibility, and solubility of isolates in bile salt. Antimicrobial susceptibility was screened by Kirby-Bauer technique followed by E-test to determine MIC for resistant isolates as per Clinical Laboratory Standard Institute guidelines 2021. Serotyping was studied by utilizing Pneumotest latex agglutination kit. The Real-time PCR directing at the *lytA* gene provided molecular detection of *S. pneumoniae*. The sample size was calculated using Thompson's equation to provide representative data.

Data analysis was made by Statistical Package for the Social Sciences version 22 and EZ SPSS Tutorials. The analysis involved determining carriage rates and resistance

percentages for age groups and serotypes. Chi-square and odds ratios were applied to evaluate the associations between pneumococcal carriage, associated risk factors, and antibiotic resistance. Ethical clearance had been gained from the Research and Ethics Committee of the University of Aden, Yemen under the Research Regulation REC-98-2021, and parents provided informed consent prior to enrollment.

Results. The pneumococcal carriage was 44.4% and 57.1% respectively by culture and RT-PCR. In fact, the RT-PCR detected *S. pneumoniae* in 220 of 385 nasopharyngeal swabs, including 170 culture-positive samples. In contrast, culture only identified 171 positive cases. RT-PCR showed greater sensitivity (99.5%) and infinite specificity versus culture. The positive and negative predictive values of RT-PCR were 100%, and 99.4% respectively, and giving a high accuracy (99.7%) for pneumococcal detection. There was a considerable statistical association between carriage rate and having apartment of a single room (OR = 7.9; p = 0.00001), sharing a bedroom (OR = 15.1; p = 0.00001), and low monthly incomes (OR = 2.02; p = 0.007).

The total vaccination rate was found to be 76.1%. The pneumonic group had 73.5% vaccination rate, whereas it was 78.5% in healthy children. The observed coverage rate of PCV 13 was 76%. The common isolated PCV 13 serotypes were 19, 1, 4, 5, 23, 3, 6, 22, 7, 9, 14, and 18 in the two cohorts. While 2, 8, and 15 were the common non-PCV 13 isolates in the two study groups.

The study found a considerably higher antimicrobial resistance rate among children of Aden city, particularly for Penicillin at the CLSI meningitis breakpoint, the resistance rate was high in isolates of both the healthy and pneumonic groups, with rates of 95.3% and 97.6%, respectively. Furthermore, it was demonstrated that among the resistant strains, 75.6% in healthy children and 87.1% in pneumonic children had resistance rates greater than two times the meningitis CLSI breakpoint for resistance. In contrast, the non-meningitis Penicillin resistance was 13.3% in the total cohort.

The resistance rate to Cefepime (meningitis breakpoint) was found in 15.8% of isolates from the total cohort, there was no resistance for the non-meningitis breakpoint. Ceftriaxone resistance (meningitis breakpoint) was observed in 16.4% of isolates from the total cohort. However, there was no resistance at the non-meningitis breakpoint among healthy children and only 1.2% among pneumonic children. Additionally, among the percentage of resistant strains, 9.3% in healthy children and 1.2% in pneumonic children had resistance greater than two times the resistance cut-off. In addition, the amoxicillin/clavulanate gave 100% sensitivity against *S. pneumoniae*.

In connection with Erythromycin resistance, 44.2% of isolates from healthy children and 51.8% from pneumonic children showed resistance. Among these resistant strains, 40.7% in healthy children and 36.5% in pneumonic children had resistance rates greater than two times the CLSI cut-off.

In favor of Azithromycin resistance, 26.7% of strains from healthy children and 35.3% from pneumonic children exhibited resistance. Among these resistant strains, 2.3% in healthy children and 3.5% in pneumonic children had resistance greater than two times the specified threshold.

Doxycycline resistance was observed in 50% of strains from healthy children and 56.5% from pneumonic children. The current survey confirmed that 22.1% of resistant isolates in healthy children and 34.1% in pneumonic children had resistance rates greater than two times CLSI cutoff. There was no difference in terms of antimicrobial resistance between the two study cohorts. Nevertheless, a notable discrepancy in Penicillin resistance between the two groups was established when applied the CLSI non-meningitis breakpoints ($\chi^2 = 3.9$; $p = 0.04$).

The most common Penicillin resistant serotypes based on the CLSI meningitis cut-off were 19, 1, 2, 5, 4, 22, 23, and 3 among strains from healthy children. Additionally, 19, 2, 5, and 23 were the prominent pneumococcal serotypes resistance to Ceftriaxone, while serotypes 19, 3, and 5 were the most resistant to Cefepime. Multiple resistance to

Penicillin, Ceftriaxone, and Cefepime, as tested according to the CLSI meningitis breakpoint, was found in serotypes 19, 1, 5, 4, 3, 6, 7, and 8 among isolates from healthy children. While the common serotypes which isolated from children with pneumonia displaying high Penicillin resistance according to the CLSI meningitis cut-off were 4, 1, 2, 3, 5, 6, 19, 22, and 23. The serotypes with multiple resistance for the tree β lactam antibiotics among children with pneumonia were 4,1, 2, 3. 5and 19.

The prevalent pneumococcal serotypes resistant to Erythromycin in the healthy group were 1, 2, 4, 5, 19, 15, and 23. Similarly, for Azithromycin, the resistant serotypes were 1, 19, 5, 2, 4, and 23. Additionally, resistance to Doxycycline was observed in serotypes 1, 2, 4, 5, 15, 19, and 23. Notably, serotypes 1, 2, 4, 5, 6, 7, 8, 15, 18, 19, and 23 were the most prevalent resistant serotypes for Erythromycin, Azithromycin, and Doxycycline in this group.

In the pneumonic population, resistance to Erythromycin was observed in various serotypes of *S. pneumoniae*, including 1, 3, 4, 5, 6, and 19. Similarly, the most resistant serotypes to Azithromycin were 1, 4, and 19. Resistance to Doxycycline was also commonly found in serotypes 1, 3, 5, 6, 12, 19, and 23. Notably, serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 19, 22, and 23 were the most commonly observed serotypes exhibiting resistance to Erythromycin, Azithromycin, and Doxycycline in this cohort.

The most common serotypes with resistance to six antibiotics out of seven were 1, 2, 4, 5, 19, 7, and 22. Multiple drug resistance was observed in 85.7% of cases, with serotypes 1, 2, 4, and 19 being prominent in this phenomenon.

Conclusion. Our investigation established a high pneumococcal carriage rate in kids of Aden city. During the investigation process, The RT-PCR achieved a high level of accuracy in detection of *S. pneumoniae* from the nasopharyngeal swabs. In contrast, culture identified fewer cases. RT-PCR exhibited high sensitivity and perfect specificity. Its positive predictive value indicated a positive test reflecting the target condition, while the negative predictive value correctly ruled out the condition. This efficacy underscoring

its impact on *S. pneumoniae* detection outcomes. Moreover, the pneumococcal carriage was notably elevated in children who slept in a single room with family members, lived in households with only one bedroom, or had a low monthly income. These findings may reflect the consequences of humanitarian crisis for this community class.

In the current investigation, three-quarters of isolated serotypes among a total cohort were included in the PCV13. The most dominant pneumococcal serotypes that were isolated from healthy children and pneumonic cohort were 19, 1, 4, 5, 23, 3, 6, 22, 7, 9, 14, and 18. While the non-PCV-13 pneumococcal serotypes that were commonly isolated from the study sample were 2, 8, and 15. The coverage rate of the PCV13 was found to be (76%) consistent with that found in neighboring countries.

Furthermore, our findings revealed a considerably higher antimicrobial resistance rate among children of Aden city. The serotypes 19, 1, 5, 4, 3, 6, 7, and 8 that isolated from healthy children had shown resistance to Penicillin, Ceftriaxone, and Cefepime, as determined by testing against the meningitis breakpoint established by CLSI, while the serotypes with multiple resistance for the three β-lactam antibiotics among children with pneumonia were 4,1, 2, 3, 5 and 19. As well the serotypes 1, 2, 4, 5, 6, 7, 8, 15, 18, 19, and 23 were the most multiple resistant strains for Erythromycin, Azithromycin, and Doxycycline among healthy group and also 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 19, 22 and 23 were the common resistant serotypes for Erythromycin, Azithromycin, and Doxycycline among the pneumonic children. Similarly, the pneumococcal serotypes 1, 2, 4, and 19 were the most common multiple resistant serotypes for the six out of seven antimicrobial agents studied in this community.

The evidence of the current investigation serves as a source of motivation and encouragement to:

1. Review and update the empirically prescribing guideline of antimicrobial agents, especially for invasive pneumococcal infections.

2. Establish stringent protocols for distributing antimicrobial agents to reduce the heightened prevalence of antibiotic resistance.
3. Promote research institutions, scholars, and funding agencies to give more attention to and address the research gap in this field.
4. Expanded access to preventives and therapeutics facilities can help tackle the national burden of pneumococci.
5. An urgent need for conducting a national surveillance study in Yemen, to evaluate the impact of vaccine introduction on pharyngeal carriage of pneumococci, along with the patterns of susceptibility to antimicrobials.

Chapter One
Introduction

Introduction

Streptococcus pneumoniae (*S. pneumoniae*, also called Pneumococci) is a gram-positive coccus that usually appears as diplococci, and sometime as short chains especially in enrichment media. This microorganism is linked to a higher morbidity and mortality rates in humanity compared to any other illness. In individuals with a normally functioning immune system, approximately 30-70% of healthy people harbor pneumococci in their upper respiratory tract without experiencing any clinical symptoms. The prevalence rate of colonization increases dramatically from 9% during the second month of life to 43% by the second year of childhood. (Syrjänen *et al*., 2001).

S. pneumoniae is linked with many clinical diseases such as sinus inflammations, ear infections, bronchial inflammation, bacteremia, and meningitis. These infections are more commonly observed in kids, the old people, as well as individuals with specific predisposing factors that increase their susceptibility to pneumococcal diseases (Tille, 2014).

Pneumonia is one of the impactful diseases that linked with death of children worldwide. In 2019, pneumonia caused mortality of 740,180 children below 5, representing 14% of all fatalities in this age cohort. Although pneumonia may be attributable to several infectious agents, but *S. pneumoniae* is still as one of the predominant etiological agent causing bacterial pneumonia in children (WHO, 2021). In addition, pneumococci are associated with bacteremia, particularly in children. it has been reported that this occurs in about quarter of individuals with pneumococcal pneumonia and in over three quarters of those with meningitis (Murray *et al*., 2021). Furthermore, the alarming reports that pointed out the multiple drug resistance in *S. pneumoniae* make the situation more worsen in resource-limited countries (Weiser *et al*., 2018).

The Pneumococcal Conjugate Vaccine (PCV) play critical role in saving the lives of offspring by decrease the impact of infections (Tvedskov *et al*., 2022). The drawback of PCV is the pneumococcal serotypes replacement for vaccine serotypes with non-vaccine serotypes. This replacement has been identified as more commonly associated with Invasive and noninvasive pneumococcal infections in children. As well, these serotypes

are among the most frequently identified serogroups that are resistant to Penicillin and Macrolides (Méroc *et al*., 2023).

Even though the Yemeni authorities initiated the vaccination program early with the assistance of the World Health Organization, many reports that deal with the health situation of Yemeni children indicate that conditions have worsened because of the ongoing conflict. This includes high rates of children malnutrition and low immunization rates, as well as outbreaks of communicable diseases in Junior age.

UNICEF released a statement in January 2015 highlighting the severity of the situation in Yemen (at the beginning of the civil war), two-thirds of the population of Yemen, 16 million people, require humanitarian aid, 13.5 million do not get potable water; 12 million lack adequate clean-up, 8.4 million missed medical care, 400,000 kids demand emotional and social assistance, as well as 75,000 kids are anticipated to fall victim to diseases that can be prevented by vaccination. Under such conditions, it is improbable that the prolonged malnutrition epidemic will diminish. It is estimated that 850,000 children are suffering from severe malnutrition, while another 2 million are suffering from ongoing malnutrition (Burki, 2015).

Moreover, in 2017, a significant number of meningitis cases were documented throughout Yemen, primarily affecting young children (WHO, 2017). Furthermore, we should keep in mind that Yemen is one of the Middle Eastern countries that has antimicrobial abuse (Halboup *et al*., 2020).

Accurate and timely data are becoming increasingly important in informing humanitarian and development responses amidst the escalation of long-lasting conflicts. There has been a growing call to enhance data collection and strengthen public health information systems in recent years. While these initiatives are crucial, improving data collection must be complemented by more rigorous analysis techniques to foster greater accountability within organizations and among communities (Garber *et al*., 2020).

The ongoing conflict in Yemen exacerbates the vulnerability of one of the world's poorest countries. Therefore, identifying areas that call for assistance and ensuring accountability for those in need, especially children, is of utmost importance.

Few studies have analyzed the impact of *S. pneumoniae* infections among Yemeni children, and there are no recent data available since the inclusion of PCV in Yemen's vaccination plan. Therefore, this study investigated the prevalence of the nasopharyngeal carriage of *S. pneumoniae*, its antimicrobial susceptibility, the pneumococcal serotypes, and the relative risk factors linked with the carriage rate in children of Aden City, given the limitations of available resources. The findings will elucidate the common circulating resistant serotypes, assisting in understanding the infection among children and informing updates to the local empirical prescribing guidelines. This thesis is formed in five chapters including introduction, review of literature, materials, and methods, result and discussion, and conclusion, future scope, and recommendation.

Objectives of the study

The current Study has the following objectives:

1. To determine the rate of Nasopharyngeal Carriage of *S. pneumoniae* in Yemeni Children.
2. To create a warning alarm for the Public Health Service and Health Care about Multi Drug-resistant strains of pneumococci to improve the national surveillance and public awareness program.
3. To Identify the most common circulating pneumococcal serotypes in the Yemeni children after introduced the Pneumococcal Conjugate Vaccine.
4. To Praise the patterns of Penicillin alternative Antibiotics by measuring their breakpoint concentration of those antimicrobial agents.

Chapter Two
Review of Literature

2. Review of Literature

2.1 Historical overview

S. pneumoniae was formerly known as *Diplococcus pneumoniae*, was discovered by George M. Sternberg in 1880, and its isolation for the first time was confirmed by Louis Pasteur in 1881(Kauffmann *et al*., 1960; Gierke *et al*., 2021; Murray *et al*., 2021). Subsequently, Carl Friedlander delineated the function of capsulated pneumococci in the etiology of lobar pneumonia among individuals who had succumbed to pulmonary infections (Nature, 1947). Since its isolation in 1881, pneumococci have been extensively studied microorganisms. Over the years, research on these bacteria has significantly supported our acquaintance with molecular genetics, antibiotic resistance, as well as the development of vaccines for prophylaxis. In the early twentieth century, the German bacteriologist Fred Neufeld was the first to classify *S. pneumoniae* into serogroups using serological techniques (Eichmann & Krause, 2013).

In 1928, Griffith discovered that when mice were implanted with dead capsulated *S. pneumoniae* beside non-capsulated pneumococcal species, the naked strain could be transformed into the capsulated form similar to that of the dead capsulated strain (Watson *et al*., 1993). Subsequently, from 1915 to 1940, over 80 serotypes of pneumococcus were described (Gierke *et al.*, 2021).

In 1953, James Watson and Francis Crick presented the double-helix configuration of DNA, resulting in their joint receipt of the Nobel Prize in Physiology and Medicine in 1962, alongside Maurice Wilkins. This groundbreaking discovery confirmed Griffith's observation regarding genetic transformation between strains of closely related organisms (Kumar *et al*., 2019).

Although the correlation between pneumococcus and lobar pneumonia had been established in1883 by Friedlander and Talamon (Gierke *et al.*, 2021), the pneumococcal disease remains a significant contributor to morbidity and mortality (Murray *et al*., 2021). At the Seventh International Microbiology Congress in 1958, the Bacteriological Nomenclature Committee recommended the inclusion of pneumococci within the Streptococcal genus (Kauffmann *et al*., 1960).

Early vaccine development for pneumococcus began in the gold mines of South Africa, where immunization with dead diplococci showed remarkable effectiveness. Remarkably, this occurred before the identification of the antigenic distinctions between different pneumococci (Musher *et al*., 2022). In the historical context, the year 1977 proved to be significant with the approval of the pneumococcal vaccine initially in USA. This initial vaccine was formulated from 14 serotypes. Subsequently, in 1983, a vaccine containing 23 different types of polysaccharides was officially approved. However, it failed to be effective in providing protection to children. The children's suffering continued until the beginning of second millennium when the first 7-valent Pneumococcal Conjugate vaccine (PCV) was authorized specifically for children. Building upon this progress, in 2010, the PCV 13 was introduced (Gierke *et al*., 2021).

Unfortunately, the first reports of non-Penicillin susceptible Pneumococcal strains were documented during 1965 in the USA, followed by Australia in 1967, and Papua New Guinea in 1977. Since these initial reports, strains of *S. pneumoniae* from various regions worldwide have shown an escalating trend of Penicillin resistance (Appelbaum, 1992).

2.2 *Streptococcus pneumoniae* Characteristics

Pneumococci are gram-positive, oval cocci that are often placed end to end in pairs, giving the cells a bullet-like form (Ryan *et al*., 2018). The phenomenon of brief clusters is observed, particularly when cultivation has taken place in an enriching broth culture. *S. pneumoniae* exhibits immobility, and many pneumococci are marked by the presence of a capsule. When examining Gram-stained specimens under a microscope, it is often possible to observe the capsule as a transparent area surrounding the paired cocci (Cheesbrough, 2006).

2.2.1 Serotyping of *Streptococcus pneumoniae*

Despite the pneumococcal serotyping is typically not required for a clinical response, it remains a valuable technique for searching pneumococcal epidemiology and generating valuable data for studies focused on the efficacy and impact of vaccinations (Habib *et al*., 2014).

The conventional approach to serotyping pneumococci involves employing polyclonal antibodies obtained from sera of rabbits immunized with *S. pneumoniae*. Pneumococcal serotypes are classified using two systems. The American system ranks serotypes based on the chronological sequence in which they were identified, while the Danish system, which is more widely used, categorizes cross-reactive serovars into serogroups. In the Danish classification, 46 serotypes/groups are assigned numbers (1 to 48) according to their structural characteristics and immunogenicity. Notably, the numbers 26 and 30 are excluded from the system (Leung, 2012).The numbers are used to label the serotypes, and those that share a structural characteristic are categorized together in one group and given a letter such as 1, 2, 14, 11A, 12F, 19 F and so on (Kumar, 2016).

2.2.1.1 Quellung Reaction

The pneumococcal quellung reaction was initially outlined by the scientist Fred Neufeld in 1902. It was specifically implemented for *S. pneumoniae*, involving the investigation of capsule swelling under the microscope and agglutination, confirmed by visible clumping with the naked eye (Neufeld, 1902). It became the gold standard approach for pneumococcal capsular serotyping, in which a pneumococcal cell suspension is tested with a pool of antisera directed against the capsular polysaccharide antigens (Habib *et al.*, 2014). This serological test is utilized to gain insights into the epidemiological aspects of pneumococci, evaluate vaccines, and monitor antimicrobial activity. The test procedure can be carried out in three distinct stages. Firstly, a loopful of a lightly suspended organism in saline is prepared on a glass slide. Afterwards, it is combined with a drop of antiserum and a loopful of methylene blue. The resulting drop is covered with a coverslip then incubated for 10 minutes at room temperature. Finally, the reaction is observed under a high-power field to identify the presence of bulging around bacteria, indicating a positive result (Procop, 2017).

2.2.1.2 Latex Serotyping Test

The quellung response is often performed in specialized reference or research laboratories, partly due to its time-consuming nature and the need for competence. Nevertheless, in some situations when performing the quellung test is impractical, the Pneumotest-Latex

approach has emerged as a viable substitute with high sensitivity for serotyping or classifying the majority of pneumococcal isolates (Kuch *et al*., 2014).

A straightforward latex agglutination test for serotyping pneumococci was formulated at Statens Serum Institute. The Pneumotest-Latex technique includes 14 distinct pooled pneumococcus antisera, covering pools A–I and P–T, which are fixed on latex particles. In a randomized study involving 352 pneumococcal strains comprising 90 serotypes, the Pneumotest-Latex accurately categorized or grouped 95.5% of the 336 isolates. Furthermore, two out of 30 strains initially considered non-typeable, or rough strains were successfully identified by using the Pneumotest-Latex. These rough strains were subsequently verified by conducting the capsule agglutination assay using type-specific antisera (Slotved *et al*., 2004).

Antibody-coated latex reagents can be utilized to detect the capsular antigen in colonies of presumptive pneumococci that thrive on blood agar. Additionally, these reagents can detect the soluble capsular antigen that is found in the patient's body fluids. The intensity of the reaction can provide insights into the severity or concentration of the antigen, allowing for assessment of the condition (Sanz *et al*., 2010).

The Latex test method for pneumococcal serotyping involves several steps. Firstly, the pneumococcal isolates are cultured on a sheep blood agar medium and incubated at an optimal temperature [“35–37°C”], with a 5% concentration of carbon dioxide, for a duration of 18–24 hours to promote their growth. Following this, a cell suspension of light to moderate density, equivalent to a 0.5 McFarland standard, is prepared in 0.5 ml of 0.85% normal saline using a sterile loop. Subsequently, 10 µl of the bacterial suspension and 10 µl of latex reagent are combined on a glass slide or reaction card, and the two suspensions are thoroughly mixed. The agglutination reaction is then examined within 10 to 30 seconds utilizing an adequate angle of oblique illumination (Swarthout *et al*., 2020).

2.2.1.3 Determination of *S. pneumoniae* Serotypes Using Polymerase Chain Reaction

The development of PCR-based serotyping systems has arisen as a solution to the challenges posed by the high costs of antisera, subjective interpretation, and the specialized technical requirements inherent in traditional serologic methods. In the early years of the

new millennium, primers were designed for a multiplex PCR serotyping technique, incorporating the genetic sequences of several clinically significant capsular types. This innovative approach has provided a promising alternative, offering a more cost-effective and objective means of serotyping while preserving the clinical utility of the traditional methods (Brito *et al.*, 2003).

Depending on the circulating serovars observed during their ongoing surveillance of Invasive Pneumococcal Diseases strains, Velusamy and colleagues have developed an expanded sequential multiplex technique specifically intended for implementation in the United States. They recommend utilizing this method in any geographical region equipped with a real-time PCR (RT-PCR) procedure for *S. pneumoniae* serotyping of culture or clinical samples. By considering the local serotype landscape, this technique can provide valuable insights and enhance serotyping efforts at any geographical region of planet (Velusamy *et al*., 2020).

2.3 Nasopharyngeal Colonization and Carriage State of Pneumococci

The incidence of asymptomatic nasopharyngeal colonization of pneumococci is characterized by a dynamic process involving multiple episodes of acquisition, colonization, and clearance of the bacteria. Individuals may experience periods of acquiring the pneumococci, carrying it in the nasopharynx without exhibiting symptoms, and subsequently eliminating it from the respiratory tract. This dynamic nature underscores the complex interplay between the host and the bacterium, as well as the intricate mechanisms involved in the colonization and clearance processes (Syrjänen *et al*., 2001).

S. pneumoniae efficiently adheres to respiratory epithelial surfaces using pilus 1, with attachment enhanced by the naturally acquired secretory IgA (sIgA) recognizing the pilus shaft protein, RrgB. The binding of sIgA to the RrgB protein on pilus-1 producing bacteria causes cohesive association, resulting in bacterial aggregation or clumping. Although agglutination occurs, the bacteria have not fully adhered to the mucus. Successful attachment and infection require agglutinated bacteria to be trapped within respiratory mucus particles. The successful infection caused by *S. pneumoniae* depends on the entrapment of agglutinated bacteria within mucus particles (Binsker *et al*., 2020). This

process is affected by a multitude of variables, involving age, overcrowding in sleeping areas, co-sleeping with parents in the same room, and geographical location. These elements participate in the dynamics of asymptomatic nasopharyngeal colonization by *S. pneumoniae* (Ueno *et al*., 2013). Furthermore, asymptomatic carriages are considered a potential source of transmission within the community, particularly in settings such as day-care institutions. The interaction between these factors and the transmission dynamics of pneumococci highlights the importance of understanding and addressing the risk factors associated with carriage, as well as implementing appropriate preventive measures to mitigate the spread of the bacteria in community settings (Abaye *et al*., 2019; Chang *et al*., 2020). While contaminated hands have a relatively minor role, it still contributes to both direct and indirect transmission of pneumococci through contact with contaminated things. This underscores the role of practicing handwashing to inhibit the dissemination of pneumococci. Proper handwashing approach such as employing Sanitary practices [“ soap and water or alcohol-based hand sanitizers”], can help reduce the risk of transmission and reinforce the overall efforts in infection control and prevention (Smith-Vaughan *et al*., 2008). In addition, Pneumococci transmission within and between hosts is influenced by many factors like viral infections, activating Toll-Like Receptors (TLRs) and affecting innate immune responses. The interaction of those factors may weaken the respiratory epithelium, heightening susceptibility to secondary bacterial infections, including pneumococci. TLR-triggered responses shape immune environments, impacting pneumococcal growth and spread. During transmission, pneumococci navigating bottlenecks may interact with the host's immune system via TLR-dependent reactions, influencing infection initiation. The absence of genetic adaptation during bottleneck passages suggests potential alternative bacterial strategies. This complex interplay underscores the multifaceted nature of pneumococcal disease dynamics (Kono *et al*., 2016).

Approximately 30-70% of healthy individuals harbor *S. pneumoniae* in their upper respiratory tract without exhibiting any clinical symptoms under normal immune system conditions. The prevalence rate of colonization is higher in childhood compared to

adulthood, with children display 2 to 6-fold the of rate mature individuals (Mosser *et al*., 2014). Furthermore, the acquisition proportion of pneumococci is particularly high in kids aged five and under (Syrjänen *et al*., 2001; Ibrahim *et al*., 2017).

In countries with poor and low-middle incomes, colonization rates among children are significantly higher. Before the applied of PCVs, the prevalence of colonization was estimated to reach as high as 64.8% in low-income nations and approximately 47.8% in low-middle-income countries (Adegbola *et al*., 2014).

The adhesion of pneumococcal surface antigen-A or choline binding proteins to nasopharyngeal cells is influenced by several variables. The key interaction is the bridging effect of choline-binding proteins on cell wall cholines and carbohydrates that are either covered or present on the host surface of mucosal cells. This attachment process can potentially be facilitated by neuraminidase action, viral infection, or cytokine generation in response to pneumolysin activity. Examples of epithelial binding sites include glycoconjugates containing the disaccharide GlcHAc1-4 Gal or the glycolipid assialo-GM1 (Gupte, 2010; Ryan *et al*., 2018).

Successful colonization and thriving of *S. pneumoniae* on mucosal surfaces require access to various bacterial components. Two notable examples of these components within pneumococci are peptidoglycan-N-acetylglucosamine deacetylase, as well as enzymes related to resistance against antimicrobial agents. These enzymes alter peptidoglycan, conferring resistance against lysozyme's effects, which is plentiful in the outer layer of the upper part of airway system (Davis *et al*., 2008).

2.4 Pneumococcal Virulence factors

The association between pneumococci and its obligatory human carrier is very complicated. Pneumococci have a remarkable ability to coexist harmoniously as specialized symbionts, primarily residing on the epithelial lining of the upper part of airway system in carriers, thereby facilitating their transmission. Conversely, under certain conditions involving both bacterial and host factors, these pneumococci can induce severe

illnesses by infiltrating sterile anatomic loci, such as lungs, bloodstream, and meninges (Weiser *et al*., 2018).

S. pneumoniae possesses a broad array of pathogenic determinants that facilitate its attachment, invasion of internal organs, and evasion of the host immune system (Brooks & Mias, 2018). Although over one hundred serotypes of pneumococci are determined thus far. Only 23 of them are known to be pathogenic to humans, exhibiting varying degrees of virulence. Specific Serovars ["3, 4, 6B, 9V, 14, 18C, 19F, and 23F"] of pneumococci are primarily in charge of the majority of invasive illnesses (Parija, 2012; Ganaie *et al*., 2020).

The study of pneumococcal microbial physiology has yielded significant discoveries in various areas, involve genetic material, the composition and synthesis of Gram-positive peptidoglycan, quorum sensing, autolysis, transformation mechanisms, as well as the pioneering sequencing of a Gram-positive genome. These advancements have resulted in improved immunization strategies as well as an enhanced comprehension of antibacterial resistance. Importantly, research on *S. pneumoniae* has elucidated the fundamental relationship between innate invasion and innate immunity, providing valuable insights into major pediatric illnesses (Henriques-Normark & Tuomanen, 2013).

The significant role of the pneumococcal capsule in virulence has long been acknowledged. Yet, beyond the capsule, diverse proteins whether situated in the cytosol, extracellular space, or on the outer layer of bacteria also contribute to the pathogenicity of pneumococci. Importantly, it should be noted that host immune responses, along with the biological, chemical, and physical attributes of capsular polysaccharides, are interlinked within a serotype-dependent network. These factors, combined with epidemiological trends, collectively contribute to the emergence of pneumococcal diseases (Wyllie, 2016).

2.4.1 Capsule

The polysaccharide capsule of pneumococcus performs a crucial task for the pathogenicity of pneumococci, contributing to the severity of pneumococcal infections that result in millions of deaths annually (Ganaie *et al*., 2020). The pneumococcal capsule is composed

of a complex oligosaccharide repeating unit with a high molecular mass. This unit is chemically linked to the murein layer (Sørensen *et al*., 1990).

The capsule is usually found in freshly isolated pneumococci, encasing the bacterial pairs. It can be observed in Indian ink preparations as a distinct halo or directly stained using specialized techniques. Alternatively, the capsule can be identified using serotyping-specific antiserum through the Quellung reaction (Kumar, 2016).

Research has shown that the choice of carbohydrate utilized as a carbon source has an impact on production of the exopolysaccharide envelope in different pneumococcal serotypes. Upon the consumption of fructose, there was notable decrease in production of capsule, with serotype 7F displaying a thinner capsule layer. This phenomenon can be attributed to the fact that fructose consumption led to the generation of intracellular fructose-1-phosphate, which was not processed into the essential precursors for making up capsule polysaccharides (Troxler *et al*., 2019). Furthermore, A recent investigation has shown that clinical pneumococcal strains exhibit carbon source-specific gene expression patterns, indicating that *S. pneumoniae* may adapt its lifestyle and capsule production in response to the varying monosaccharides present in different host environments (Werren *et al*., 2023).

The heavily encapsulated serotypes of pneumococci exhibited greater resistance to neutrophil-mediated killing. The level of encapsulation directly correlates with the susceptibility of pneumococci to be eliminated by phagocytes (Weinberger *et al*., 2009). At the same time, a negative charge of the pneumococcal capsule plays an essential role in creating electrostatic repulsion that prevents phagocytes from adhering to pneumococci, thus promoting colonization. Moreover, *S. pneumoniae* with highly negatively charged envelope are less probably to stick to mucus of upper respiratory tract, leading to impaired mucus clearance (Nelson *et al*., 2007). In an animal model research, Sanders and his team consistently associated the capsule of pneumococci as the most critical virulence component in pneumococcal endophthalmitis (Sanders *et al*., 2011). Additionally, Oliver and colleagues conducted a study on *S. pneumoniae* serotype 11D capsular polysaccharide, revealing that minimal genetic changes in the capsular polysaccharide synthesis locus(cps)

can enable pneumococci to encode specific enzymes for assembling polysaccharides with different repeating units (Oliver *et al*., 2013).

Despite sharing 60-80% orthologous genes between *S. pneumoniae* and *S. mitis*, they exhibit significant differences in their pathogenicity towards the human host. Notably, antibiotic-resistant genes in pneumococci are believed to have originated from *S. mitis*. Additionally, *S. mitis* is thought to contribute to the pneumococcus ability to evade vaccines by undergoing changes in its capsule polysaccharide. Interspecies gene exchange, facilitated by competence-associated killing mechanisms like fratricide, is considered a key factor, as both species naturally possess the ability to become competent (Salvadori *et al*., 2019).

Several studies have demonstrated that streptococcal microbiota, including *S. mitis*, share a similar capsular polysaccharide synthesis pathway known as Wzy/Wzx pathway with pneumococci. This similarity suggests that genetic variations observed in pneumococcal strains may result from the transfer of cps locus gene fragments from commensal streptococcus species like *S. mitis*. These commensal streptococci exhibit a diverse range of cps loci, and their genetic material can potentially contribute to the emergence of novel pneumococcal serotypes. This can occur through interspecies recombination, which is facilitated by the widespread usage of PCV (Skov Sørensen *et al*., 2016). The appearance of novel pneumococcal capsule serotypes raises concerns as it has capability to diminish the efficacy of vaccines and complicate serotype transitions. One such novel strain, designated as 10D, has been identified, displaying unique chemical and serological characteristics. Genetic investigations have revealed that the emergence of the 10D capsule type in *S. pneumoniae* can be attributed to the acquisition of a substantial genetic fragment from an oral streptococcus. This finding underscores the capacity of interspecies genetic exchanges to enhance the diversity of pneumococcal capsules (Ganaie *et al*., 2020).

2.4.2 Pneumolysin

Pneumolysin, a cholesterol-dependent cytolysin, is an important virulence factor that significantly contributes to the pathogenicity of *S. pneumoniae* in humans (Mitchell & Dalziel, 2014). It has detrimental effects on the human respiratory system, disrupting the

ciliated epithelium and impairing mucociliary clearance. Furthermore, pneumolysin provokes the generation of signaling molecules of immunity as well as induces inflammation, leading to fluid accumulation in the alveoli. The inflammatory response may manifest as rust-colored sputum, as coughing from the lungs may result in the expulsion of blood (Ryan *et al*., 2018).

Pneumolysin performs a critical duty in every stage of *S. pneumoniae* pathogenesis and its impact on the infection outcome is contingent on the specific context. Depending on the specific circumstances of the infection, pneumolysin can either favor the pathogen or the host, influencing the delicate balance between the two (Pereira *et al*., 2022). This factor exhibits a range of biological roles in pneumococcal infection. Its ability to transition from a monomer to a large oligomeric complex upon interaction with host cell membranes contributes to these effects. While it lacks traditional pores, this factor can function as a mucosal adjuvant. The toxin interacts with membrane cholesterol, actin, and Toll-like receptors, thereby altering cellular signaling and behavior. Pneumolysin also serves as a protective antigen that influences the immune system and affects the host's response to other bacterial products. Modulation of cell signaling or access of pneumolysin pores to intracellular receptors may contribute to these effects (Mitchell & Dalziel, 2014; Vögele *et al*., 2019; Pereira *et al*., 2022). It serves a vital function in enabling *S. pneumoniae* to evade the phagocytic killing process. One of its mechanisms is the suppression of the oxidative burst, which is an important antimicrobial defense mechanism mediated by phagocytes. By inhibiting the oxidative burst, pneumolysin helps the bacterium to evade the bactericidal activity of phagocytes, contributing to its ability to survive and persist in the host (Tille , 2014).

In a study conducted by Subramanian and colleagues, it was discovered that pneumococci utilize the interaction between pneumolysin and mannose receptor C type 1 to not only reduce inflammation but also promote microbial viability at respiratory tract (Subramanian *et al*., 2019). In addition, it was found that pneumococcal pneumolysin plays critical function in inducing the macrophages to make special products [“an inducible nitric oxide synthase (iNOS) and nitric oxide (NO)”], even in absence the exogenous gamma interferon

signaling. This induction of iNOS and NO production by pneumolysin promotes macrophages to generate inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-6, as well as the expression of cyclooxygenase-2 (COX-2). COX-2 catalyzes the formation of prostaglandins, further contributing to the inflammatory response (Braun *et al*., 1999).

In a survey performed by Letsiou and his team, they have shown that pneumolysin activates neutrophils and platelets, resulting in the release of extracellular vehicles (EVs). This data underscore the significant role of neutrophil derived EVs in modulating platelet functions during pneumococcal infections, which is associated with platelet activation (Letsiou *et al*., 2021).

2.4.3 Autolysin

Pneumococcal autolysin, also referred to as *lytA*, is a protein synthesized by Pneumococci. It serves as the primary autolysin within the bacterial cell. Autolysins are endogenous lytic enzymes responsible for breaking down the peptidoglycan components of bacterial cells. Thire enzymatic activity facilitates the separation of daughter cells after cell division. *lytA* plays an important role in autolysis, as well as in fratricidal and Penicillin induced lysis processes. These functions highlight the significance of *lytA* in the life cycle and physiology of pneumococci (Mellroth *et al*., 2012; Zahlten *et al*., 2015).

The activity of the pneumococcal autolysin toxin has been initiated in the presence of specific surface agents and bile. The enzyme is involved in disease-causing ability of pneumococci due to its ability to release cell wall fragments. The disintegration of cell wall components mediated by autolysin is pivotal in shaping and advancing of immune response, underscoring its significance in the overall pathogenicity of pneumococci (Sastry & Bhat, 2019). One of the released fragments due to the action of autolysin, is the pneumococcal triosephosphate isomerase (TpiA). TpiA engages in binding with host plasminogen, utilizing its influence by promoting the conversion of plasminogen into its active state, plasmin. This mechanism implies that TpiA significantly contributes to the process of entering host tissues, as it facilitates the activation of plasmin, which in turn enables the degradation of the extracellular matrix. Ultimately, TpiA's ability to bind to

plasminogen and enhance plasmin activation serves as a pivotal factor in promoting tissue invasion by facilitating extracellular matrix degradation (Hirayama *et al*., 2022).

Studies showed that encapsulated pneumococci quickly survive antimicrobial peptide exposure by shedding their capsule, facilitated by *lytA* autolysin. Unlike typical autolysis, *lytA* promotes survival, distributing circumferentially during capsule shedding. Both autolysis and shedding rely upon the activity of *lytA* in lysis of wall component. Capsule elimination is essential for epithelial cell invasion and reduces surface-linked capsule in initial phase of alveolar invasion in mice. The presence of *lytA* in clinical isolates under antibiotic pressure may be attributed to its involvement in removing the capsule and defending against antimicrobial peptides. This phenomenon providing insights into pneumococcal survival and adaptation to antimicrobial defenses (Kietzman *et al*., 2016).

On the other hand, the *lytA* protein exhibits high immunogenicity, making it a strong candidate for immunization against pneumococci. Due to its immunogenic properties, *lytA* has the potential to elicit a robust immune response in vaccinated individuals. Targeting *lytA* as an antigen in vaccines holds promise for enhancing protective immune responses against pneumococcal pathogens. This approach can bolster the immune system's ability to recognize and neutralize pneumococcal infections, contributing to the development of effective preventive strategies against pneumococcal diseases (Afshar *et al*., 2020).

2.4.4 IgA1 proteases

IgA1 proteases, also referred to as metalloproteases, have a molecular weight of 200 kilodaltons and are encoded by the *iga* gene. Their primary function is to cleave the hinge region of IgA1 by selectively targeting the bond between Pro-227 and Thr-228 (Wani *et al*., 1996). *S. pneumoniae* produces IgA1 protease as a vital component of its pathogenicity, allowing it to counteract the protective effects of IgA1, the predominant secretory antibody found in mucous membranes, constituting over 90% of mucosal antibodies. By targeting and cleaving IgA1, the IgA1 protease produced by *S. pneumoniae* helps the bacterium escape the immune surveillance as well as establish colonization on the mucosal layer (Riedel *et al*., 2019).

Experimental evidence has demonstrated that the N-terminal site of pneumococci IgA1 protease serves as a principal attachment site for IgA1. However, it has also been established that the C-terminal of pneumococci IgA1 protease is crucial for the splitting of antibody subclass A 1. In other words, while the N-terminal region facilitates the initial interaction between *S. pneumoniae* IgA1P and IgA1, the C-terminal region that carries out the enzymatic cleavage of the IgA1 molecule (Chi *et al*., 2017).

2.4.5 Pneumococcal surface adhesion

Pneumococcal surface adhesion refers to the capacity of *S. pneumoniae* to attach to and bind with host cells and tissues. This ability allows the bacterium to establish colonization and initiate infection in many anatomical regions, including the respiratory system. Pneumococcal surface adhesion is facilitated by specific proteins or adhesins expressed on the surface of the bacterium, which interact with host cell receptors, extracellular matrix components, or other molecules to promote adherence. This initial attachment is an important step in the disease development of *S. pneumoniae* as well as assist in its ability to invade and enhance illness in the host (Rajam *et al*., 2008).

Furthermore, Pneumococcal surface adhesion aids immune evasion by inhibiting the bactericidal activity of lactoferrin and preventing the deposition of complement proteins on invading pneumococci. These mechanisms contribute to pneumococcal ability to establish colonization, evade immune responses, and enhance their survival within the host (Shaper *et al*., 2004).

Pneumococcal surface adhesin A (PsaA) is a lipoprotein with a molecular weight of 37 kilodaltons that is often seen on the outer layer of all detected strains of pneumococci. The role of PsaA in stimulating an immune response in the host has been demonstrated, which is very promising for the development of a vaccine against pneumococci. PsaA demonstrates remarkable conservation across various serotypes, highlighting its crucial role as a conserved virulence factor of pathogenicity of pneumococci (Rajam *et al*., 2008).

Recent evidence indicates that human Annexin A2, a protein commonly found on airway epithelial cells, serves as the primary receptor facilitating pneumococcal colonization.

Annexin A2 interacts with a highly conserved PsaA. This interaction between Annexin A2 and PsaA plays key function in the initial attachment as well as subsequent colonization of *S. pneumoniae* within the airway epithelium. By engaging with Annexin A2, PsaA enhances the adhesion and colonization capabilities of *S. pneumoniae*, promoting its persistence and pathogenicity in the host respiratory tract (Hu *et al*., 2021).

Other proteins of interest are the choline-binding proteins (CBPs). The CBPs constitute a family of polypeptides that are present in pneumococci as well as similar bacteria. They are also found in certain associated bacteriophages. The presence of CBPs in bacteriophages associated with these bacteria suggests their importance in the viral life cycle and their potential involvement in horizontal gene transfer between bacteriophages and bacteria. These proteins have vital functions in numerous biological processes, including cell adhesion, colonization, and pathogenicity, highlighting their significance. The CBPs derive their name from their ability to specifically bind to choline moieties existing in the teichoic acids of bacterial cell walls (Maestro & Sanz, 2016).

The CBPs have the ability to inhibit the complement system and promote adhesion. Pneumococcal surface protein C (PspC) is an example of CBPs that bind to essential host proteins, notably Vitronectin and Factor H. The binding of PspC to Vitronectin, promotes the attachment of pneumococci to host cells. Additionally, the interplay of PspC with Factor H helps to inhibit the complement system, which is a critical immune defense mechanism. These activities of PspC aid in the escaping from immune surveillance and aid in the successful colonization of pneumococci (Voss *et al*., 2013). Indeed, there are six PspC variants, also known as clades or families, designated as PspC1, PspC2, PspC3, PspC4, PspC5, and PspC6. Each variant has a unique amino acid sequence and structural characteristics. The variation in PspC contributes to the heterogeneity observed among different strains of *S. pneumoniae*, influencing their virulence, colonization capabilities, and interactions with the host immune system (Du *et al*., 2021).

CBPs have a distinctive two-unit structure; a functional module (FM) and a choline-binding module (CBM). The FM carries out specific protein functions like adhesion,

colonization, or immune evasion, while the CBM anchors the protein to the bacterial cell wall by non-covalently interacting with choline residues. This dual-module organization allows CBPs to perform specific functions while remaining attached to the bacterial cell wall (Maestro & Sanz, 2016).

2.4.6 Pneumococcal Glycosidases

Pneumococcal glycosidases are key virulence factors for *S. pneumoniae*, aiding in nutrient acquisition, promoting adherence, and impairing the immune response. These enzymes modify various glycan moieties in the respiratory tract, including N-linked glycans, O-linked glycans, and glycosaminoglycans, to obtain essential nutrients. By breaking down complex carbohydrates into simpler forms, they support bacterial growth and survival. Additionally, pneumococcal glycosidases enhance pathogenesis by facilitating adherence to host cells through the modification or cleavage of host glycan structures on epithelial cell surfaces (Mathew *et al*., 2023). The adhesive property of the bacterium is essential for establishing infection and evading clearance mechanisms, enabling it to escape immune surveillance, extend its persistence within the host, and contribute to its virulence (King, 2010).

It has been demonstrated that glycoconjugates containing the disaccharide unit GlcNAcβ1 play a crucial role as the primary receptor for pneumococcal binding to epithelial cells in the oropharyngeal region during colonization. The presence of these glycoconjugates on host cell surfaces facilitates pneumococcal attachment and adherence, leading to successful colonization. The specific interaction between pneumococcal surface components, such as adhesins or proteins, and GlcNAcβ1-containing glycoconjugates on host epithelial cells is essential for establishing a stable association between the bacterium and host tissue. This interaction allows pneumococci to evade clearance mechanisms and establish a niche for colonization within the oropharyngeal region (Andersson *et al*., 1983).

2.4.7 Phosphorylcholine

Phosphorylcholine, found on the surface of various respiratory microbes, including *S. pneumoniae,* plays a critical role in their colonization and invasion of the respiratory tract (Iuchi *et al.,* 2019). The phosphorylcholine interacts with thrombocyte activating factor (TAF) promoting respiratory tract infiltration and triggering inflammation and immune responses. The recruitment of immune cells and release of inflammatory mediators can be regulated by C-reactive protein (CRP) and surfactant. CRP binds to phosphorylcholine, inhibiting its interaction with TAF and limiting inflammation. Surfactant directly interacts with phosphorylcholine, reducing microbial infiltration and inflammation in the respiratory tract. These interactions are crucial in regulating immune responses as well as inflammation during respiratory microbial infections (Henriques-Normark & Tuomanen, 2013).

Phosphorylcholine enables pneumococci to adhere to receptors associated with TAF in various anatomical locations, including endothelial cells, white blood cells, platelets, lung cells, and meningeal cells. This molecule plays an important role in initiating colonization following invasive *S. pneumoniae* infection. By binding to TAF receptors, pneumococci gain the ability to adhere to and establish themselves in different tissues and cell types throughout the body. This adhesion is an important stage in the colonization process following invasive pneumococcal infection (Tille , 2014). Additionally, pneumococcal choline-binding proteins were shown to function as vitronectin-binding proteins. Specifically, when these proteins attach to certain carboxy terminus of vitronectin [“the C-terminal heparin-binding domain of vitronectin”], they can inhibit the complement system. This interaction is important in pneumococcal pathogenesis, as it assists in evading the host's immune response by preventing complement cascade activation. The complement system is an essential component of the innate immune system responsible for eliminating invading pathogens. However, by inhibiting complement activation, choline-binding proteins enable pneumococci to evade immune detection and clearance, thereby promoting their survival and the establishment of infection (Voss *et al*., 2013).

2.4.8 Neuraminidases

Neuraminidase, also known as sialidase, is an important enzyme found in pneumococci. This class of glycoside hydrolase enzymes plays a significant role in pneumococcal pathogenesis by cleaving the glycosidic linkages of neuraminic acids. *S. pneumoniae* isolates have been found to express up to three different neuraminidases which are designated as NanA, NanB, and NanC (Pettigrew *et al*., 2006).

The pneumococcal neuraminidase acts on neuraminic acids, which are a type of sugar molecule commonly found on the surface of host cells and in secreted glycoproteins. By cleaving these glycosidic bonds, neuraminidase allows pneumococci to modify the host cell surfaces and manipulate host-pathogen interactions (Gut *et al*., 2008).

The participation of neuraminidase in the infectiousness of *S. pneumoniae* has been demonstrated through animal infection models. The studies have provided evidence that neuraminidase plays a critical role in the colonization of host tissues by pneumococci (Parker *et al.*, 2009). The most important function of pneumococcal neuraminidase is its involvement in the colonization and invasion of host tissues. By removing neuraminic acids from the surface of host cells, the enzyme facilitates the adhesion and penetration of pneumococci into the respiratory tract and other anatomical sites. Moreover, pneumococcal neuraminidase plays a role in immune evasion. By removing neuraminic acids from the surface of host glycoproteins, the enzyme can interfere with the recognition and response of the host immune system, making it more challenging for the immune system to detect and eliminate the bacteria (Syed *et al*., 2019).

2.4.9 Lipoteichoic Acid and Teichoic Acid

Lipoteichoic acid (LTA) is an adhesion molecule characterized by dual-affinity properties, with some parts attracted to water and others repelling water. It is present in the cell wall of Gram-positive bacteria. It functions as a regulator for autolytic wall enzymes, particularly muramidases. Upon bacterial lysis caused by lysozyme, leukocytic cationic peptides, or beta-lactam antibiotics, LTA is one of the primarily released from bacterial cells. It can interact with target cells through non-specific binding, attaching to membrane

phospholipids, or through selective adhering, attaching to CD14 and Toll-like receptors (Ginsburg, 2002).

LTA is pivotal in the pathogenesis of systemic pneumococcal infections. Studies show that pneumococcal mutants lacking the enzyme lipoteichoic acid ligase (TacL) exhibit reduced pathogenicity among pneumonic and invasive animal trials. This decrease in virulence is attributed to the absence of lipoteichoic acid, which is affected by the absence of TacL (Heß *et al.*, 2017).

In vivo, LTA induces a profound and robust inflammatory response in the lungs, activating the coagulation pathway. This inflammatory cascade is mediated through a TLR2-dependent route, indicating the involvement of Toll-like receptor 2 in recognizing and responding to Pneumococcal LTA. Additionally, the presence of endogenous ligands for TLR4 likely potentiates the inflammatory effects of LTA, contributing to the amplification of the immune response (Dessing *et al*., 2008).

2.4.10 Hydrogen peroxide

Hydrogen Peroxide (H_2O_2) is a potent peroxide compound and oxidizing agent known for its antiviral and antibacterial properties. It has been documented that pneumococci generates significant amounts of H_2O_2 as a byproduct of its metabolism under aerobic conditions, which contributes to its virulence (Rai *et al*., 2015). The presence of the pyruvate oxidase enzyme encoded by the SpxB gene enables the production of H_2O_2, leading to oxidative stress within cells. This oxidative stress can result in potential DNA damage and subsequent cell death, particularly in lung cells (Yesilkaya *et al*., 2013). As well, pneumococci utilize this strategy to impede the growth of potential competitors (Pericone *et al*., 2000). Furthermore, studies have shown that the SpxB gene does more than just generate H_2O_2; it also significantly improves the cell's ability to withstand its own production, enhancing the bacterium's survival strategy (Pericone *et al*., 2003). Similarly, H_2O_2 produce by pneumococci stimulate a naive immune reaction by promoting the elaboration of cytokines inducing inflammation, while also it effects the stress adaptations of cells (Loose *et al*., 2015).

2.4.11 Pili

Pili are hair-like protrusions that many bacteria have on their surfaces. Among the various kinds of fimbriae presented on the surface of pneumococci, Pilus-1 and Pilus-2 are the two most common. Pilus-1 is present in approximately 30% of pneumococcal strains, while Pilus-2 is encountered in only about 16% of symptomatic infections (Bagnoli *et al*., 2008; De Angelis *et al*., 2011; Iovino *et al*., 2020).

The studies have provided evidence regarding the role of pili in colonization where it was demonstrated that they facilitate initial microbial adherence and subsequently increase the capacity to induce invasive illness and antibiotic resistance (Dzaraly *et al*., 2020). The pneumococcal rlrA islet, existing in a bit of clinical isolates, is responsible for encoding these adhesive pili appendages. During pneumococcal infection, piliated pneumococci have been demonstrated to elicit stronger tumor necrosis factor responses than non-piliated (Barocchi *et al*., 2006). Additionally, pili assist the bacteria in evading phagocytosis by the host's immune cells (van der Poll & Opal, 2009).

A recent study in Japan found that serotype 35B contained 80.2% pilus genes. Among serotype 35B isolates, 77.9% belonged to clonal complexes 558, which exhibited a high positivity rate (98.5%) for *rrgC* and *gPRSP* genes. Furthermore, 19.8% of CC2755 isolates were rrgC-negative and resistant to gPISP. The combination of CC558, possessing pili as adhesins and Penicillin resistance, may have contributed to the increased prevalence of serotype 35B post-vaccination era in many reigns (Miyazaki *et al*., 2020).

2.4.12 DNase

The pneumococcal DNase, also known as pneumococcal endonuclease, is an enzymatic protein synthesized by microorganisms. This specific endonuclease exhibits specificity towards DNA molecules. Beiter and colleagues demonstrated how pneumococci can break down the neutrophil extracellular traps DNA scaffold and escape with the help of an endonuclease. The spread of pneumococci during pneumonia, from the upper airway to the lung and from the lungs into the bloodstream, is promoted by escaping the neutrophil extracellular traps DNA scaffold (Beiter *et al*., 2006).

Jhelum and colleagues discovered a novel DNase named TatD, exhibiting conserved DNase activity in the pneumococcal secretum. TatD, belonging to the TatD_DNase family associated with the extracellular vesicle compartment, is sensitive to proteinase K and heat. Their study demonstrated that TatD's DNase activity contributes to *S. pneumoniae* evasion from human neutrophil extracellular traps. Deleting TatD in a murine sepsis model resulted in reduced bacterial burden, lung tissue pathology, and increased survival compared to wild-type *S. pneumoniae,* highlighting TatD's role as a virulence factor. TatD holds potential as a drug target against pneumococcal infections, but a more thorough understanding of its precise role in human infection is needed (Jhelum *et al*., 2018).

2.4.13 Trigger Factor protein

The Trigger factor protein (TF) serves as a molecular chaperone in bacterial organisms, facilitating proper protein folding and assembly. Cohen and his team demonstrated that pneumococci lacking the amino acid sequence of TF exhibited significantly reduced virulence in a mouse model. Additionally, they found that the recombinant Trigger Factor protein (rTF) provided immunological protection. Both rTF and anti-rTF antisera were observed to inhibit pneumococcal adhesion to human lung epithelial cells. Based on that conclusion, they proposed considering rTF for inclusion in future pneumococcal vaccine formulations. Notably, this protein lacks human homology, stimulates a potent immune response, and is present among various pneumococcal strains (Cohen *et al*., 2019).

2.4.14 Biofilms

Biofilms are colonies of microorganisms that adhere to surfaces and consist of aggregated cells embedded in a polysaccharide extracellular matrix. This structure is produced when bacteria live under extreme conditions and helps them survive (Donlan, 2002; Chao *et al*., 2015). The close proximity of bacterial cells within biofilms increases the likelihood of gene exchange, contributing to their survival and adaptability under various conditions (De la Fuente-Núñez *et al*., 2013).

Many studies demonstrated that antimicrobial therapies are ineffective at eradicating *S. pneumoniae* biofilms as a result of increasing antimicrobial resistance (Wu *et al*., 2015).

Moreover, *S. pneumoniae* biofilms are capable of evading host immune responses such as mucociliary clearance (Fliegauf *et al*., 2013).

When the host experiences an attack, such as a viral infection, it can lead to changes within the colonizing environment of *S. pneumoniae*. These changes can trigger a response known as biofilm dispersion. In the context of *S. pneumoniae*, biofilm dispersion refers to a process where some bacteria within the biofilm undergo physiological changes that enable them to detach from the biofilm structure. Once detached, these bacteria can disseminate to other sites within the respiratory tract or even reach distant locations, such as the middle ear or bloodstream, leading to subsequent infections in that areas (Chao *et al*., 2019).

2.5 Clinical manifestations

The respiratory system is one of the anatomical sites associated with the accessibility of many microbes to the human body. Acute respiratory illnesses are primarily linked to viruses as well as bacteria. It is very tough to predict the causative agent of pneumonia due to the overlapping clinical presentations and controversies about the reliability of laboratory tests and radiographic findings (Schrag *et al*., 2001). Pneumococci are considered the main bacterial agents associated with these clinical manifestations, and this microbe deserves special consideration due to its increasing antimicrobial resistance (Örtqvist *et al*., 2005, Cowan & Smith, 2018).

Pneumococci have indeed developed intricate mechanisms to engage with their host and induce illness. Their capacity to effectively inhabit the human nasopharynx depends on the interplay between surface-exposed adhesins and pili of the bacteria with specific receptors or proteins on host cells. This engagement facilitates the attachment of *S. pneumoniae* to mucosal epithelial cells. Moreover, binding to human host cells can initiate the activation of further virulence factors linked to infiltration, adaptability, and evasion of the host's immune surveillance by pneumococci (Hilleringmann *et al*., 2015).

Pneumococcal infections can affect individuals of all ages, but they are most common in toddlers and infants aged two and below and elder people over 60 (Ryan *et al*., 2018).

The existence of *S. pneumoniae* in the upper airway, where the microbe may be eliminated by immunity or spread to other host, is the first and most important stage in the

development of pneumococcal illness. Sometimes, it causes localized mucosal pneumococcal infections, such as middle ear infection or lung infection. Alternatively, it may enter the mucosa, leading to serious conditions known as invasive pneumococcal illnesses (Navne, 2014).

Invasive pneumococcal disease (IPD) is defined as the detection of *S. pneumoniae* in specimens from internal anatomic sites, including interstitial fluids. It typically occurs due to penetration of epithelial layer of the upper airway by the pneumococci (Dockrell *et al*., 2012).

The ability of pneumococci to evade phagocytosis enables them to thrive, proliferate, and disseminate into several systems. The capsular resistance to phagocytosis is enhanced with Pneumolysin, which inhibits the oxidative burst of phagocytic cells, a process essential for intracellular bacterial elimination (Parija, 2012).

In developing countries, the mortality rates for IPD may reach up to 20% for sepsis and 50% for meningitis (WHO, 2018). There are significant differences observed between pneumococcal serotypes isolated from adults and those found in children. The most commonly isolated strains associated with pediatric infections are serotypes 6, 14, 19, and 23 (Riedel *et al*., 2019).

There are several conditions that can reduce the cough reflex, increase secretion aspiration, lower resistance, and predispose individuals to pneumococcal infection. These conditions may include respiratory tract abnormalities such as viral infections, mucus pooling, bronchial obstruction, respiratory tract injury caused by irritants, and abnormal circulatory conditions (Warren, 2016).

2.5.1 Otitis Media

Otitis media is defined by the accumulation of serous fluid in the middle ear, if this infection accompanies by the absence of indications of acute inflammation, then it should be considered a noncontagious disease; if it occurs with signs and symptoms of acute inflammation, it should be referred to as acute Otitis media (AOM). In case of the infection remains ongoing for a duration exceeding three months, the label should be changed to

chronic Otitis media. The recurrent infection of Otitis media may occur three or more times in a period of half-year span, or four or more episodes annually (Domachowske, 2019).
AOM involves an infection of the space behind the eardrum. It is usually affecting children and characterized by symptoms like ear pain, fever, and sometimes difficulty hearing. It was demonstrated that about 80% of children had at least one time of AOM by their third year, and 40% had at least seven episodes by the age of seven (Pommerville, 2018).
Approximately 50% to 90% of AOM cases are bacterial in origin, with the remainder caused by viruses. Among bacterial Otitis Media cases, 30–40% are attributed to pneumococci, primarily serotypes 6, 14, 19F, and 23F. Non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* are the second and third most common bacterial agents associated with middle ear infections (Van Dyke *et al.*, 2017; Domachowske, 2019).

2.5.2 Sinusitis

Bacterial sinusitis is an inflammation of one or more of the four pairs of sinuses, which are air-filled spaces around the nose and nasal passages (Pommerville, 2018). This condition can be caused by allergic problems and, less commonly, by infectious agents. The primary symptom of sinusitis is the production of excess fluid, which provides an environment for microbial growth. Pneumococci are among the important pathogens associated with sinus infections following viral infections or allergic episodes. During a bacterial infection, the mucoid fluid can change to a green or yellow color. Patients typically experience nasal congestion and pain around the nose and forehead. Some individuals may also have headaches, toothaches, or facial swelling (Cowan & Smith, 2018).
It is a common condition among children, often affecting the maxillary and frontal sinuses. While medical intervention is preferred in most cases, surgery may be necessary in a small number of cases, notably in kids with asthma (Alshehri *et al*., 2021).

2.5.3 Pneumonia

Pneumonia is a pathological condition featured by inflamed pulmonary tissue, which is linked to significant morbidity as well as mortality on a global scale. The etiology of this

condition can be attributed to various microorganisms such as bacteria, viruses, fungi, or other pathogens (Kim *et al*., 2017).

S. pneumoniae is a fundamental cause of pneumonia among toddlers, especially those under 5 years old. The main serotypes responsible for the majority of pediatric pneumonia cases are 6, 14, 18, 19, and 23. In contrast, among adults, there are other serotypes ["1, 3, 4, 7, 8, and 12"] associated with pneumonia, resulting in a fatality rate exceeding 5–10% (Parija, 2012). More children die from pneumonia than from any other illness, including AIDS, malaria, and measles combined. Each year, a significant number of children die from pneumonia, contributing to nearly 1 in 5 deaths among youngsters below five years old on a global scale. Surprisingly, the impact of pneumonia on children's lives is rarely highlighted in headlines (UNICEF & WHO, 2006). Pneumococci are one of main agents of pneumonia of community origin, which is a major contributor to mortality in younger kids (Amann *et al*., 2014).

Since its discovery in 1881, pneumococcus has remained a significant human pathogen. In the past, *S. pneumoniae* played a major role in deaths, particularly during the Spanish influenza pandemic of 1918–1919, where it caused secondary bacterial infections and was associated with the deaths of many infected cases (Morens *et al*., 2008). In the late 19th and early 20th centuries, pneumonia was the primary infectious culprit of death. It was ranked as the third leading factor of mortality overall (Chow, 2021). During a brief period in the 1930s and early 1940s, public health advocates brought pneumonia to the forefront of public health concerns (Podolsky, 2005). In 2019, lower respiratory infections were the most problematic infectious syndrome, contributing to over 1.5 million deaths of children, with *S. pneumoniae* being the third pathogen associated with that mortality rate due to antibacterial resistance (Murray *et al*., 2022).

In cases when a child exhibits persistent or recurring fever over 38.58 degrees Celsius, chest recession, and an increased respiratory rate, it is advisable to investigate the possibility of bacterial pneumonia (Harris *et al*., 2011). In addition to fever and an increased respiratory rate, patients with pneumococcal pneumonia may experience chills,

shivering, chest pain, cyanosis due to inadequate oxygen intake, a cough producing rusty-colored (bloody) sputum, and irregular breathing (Talaro & Chess, 2018).

The initial stage of pneumococcal pneumonia involves the aspiration of respiratory secretions containing *S. pneumoniae* (Ryan *et al*., 2018). While pneumococcal infection is primarily transmitted endogenously through one's own microbiota, it can also occur through direct contact with carriers through respiratory secretions or droplets (Talaro & Chess, 2018).

Babies are at more threat to develop pneumonia during or shortly after birth when they come into contact with microbes in the birth canal of infected mothers or contaminated materials while being delivered (UNICEF & WHO, 2006). Furthermore, the children may face a higher risk of pneumonia and its severe consequences due to associated risk variables, such as living in overcrowded environments, exposure to nicotine, or contaminated indoor air (Ueno *et al*., 2013; Wada *et al*., 2019).

The pneumococcal virulence factors operate in two phases after organisms reach the alveolus. In the first phase, the capsule and certain surface proteins of intact organisms inhibit phagocytosis, allowing the organisms to replicate and spread. The second phase occurs when the organisms start to disintegrate, releasing various substances either generated by the pneumococcus or part of its structure, causing harm. These substances include pneumolysin, autolysin, and cell wall components (Ryan *et al*., 2018).

The initial crucial step in reducing child mortality under the age of five involves recognizing the signs of pneumonia. It is essential for healthcare professionals to identify these signs promptly, ensuring that sick children receive timely and appropriate care. Alarmingly, it has been observed that only one out of every five caregivers in developing countries is familiar with the primary indicators of pneumonia (UNICEF &WHO, 2006).

Bergeron and colleagues have comprehensively fractionated pneumococcal pneumonia into five stages based on animal experimental study from infection through model mortality. The first phase (0–4 hours) is when pneumococci establish themselves in

alveolar loci and alveolar macrophages fail to clear infection due to the antiphagocytic process of capsule and Pneumolysin. The ineffective phagocytosis releases cytotoxins, such as TNF, IL-6, and NO, which are more prevalent in bronchoalveolar. Second Phase (4 to 24 hours), in which pneumococci multiply in alveoli with polymorphonuclear neutrophil chemotaxis from blood circulation to lung tissue, TNF, IL-6, IL-1, and LTB4 are markedly increased in alveolar tissue and bronchoalveolar lavage (BAL), along with transient elevation of serum IL-1. Pneumolysin complement-activating properties attract neutrophils to alveoli. Pneumolysin cytolytic actions support alveolar damage and fluid buildup in the third phase (24–48 hours). In this period, type II pneumocytes multiply and attempt to heal damage by increasing surfactant production. Bacteremia occurs when bacteria from the alveoli enter the circulation. The patient may lose weight. In the fourth phase (48–72 hours), many Monocytes mobilize from blood to alveoli and high Macrophages and Neutrophiles activities liberate NO in lung tissue and alveoli. Leucopenia and thrombocytopenia caused by TNF and IL-6 rise are the primary complications of bacteremia. Fifth Phase (72 to 96 hours) in which bacteria multiply and emit more NO, causing severe and lipid peroxidation in BAL fluid and massive tissue damage with a high death rate (Bergeron *et al*., 1998).

2.5.4 Bacteremia

Despite advancements in antibiotic and vaccine technology, patients with pneumococcal bacteremia still face a significant risk of mortality (Balakrishnan *et al*., 2000). This illness predominantly affects children rather than adults, impacting over 66% of meningitis patients and 25% of individuals with pneumococcal lung diseases. However, it rarely occurs in individuals with sinusitis or otitis media (Parija, 2012).

S. pneumoniae remains a significant global cause of bacteremia. It affects approximately quarter of individuals with pneumococcal lung infection and over 80% of cases with meningeal inflammation (Murray *et al*., 2021).

It was demonstrated that certain serotypes [“6, 14, 19, and 23”] are the prominent pneumococcal serovars associated with pediatric bacteremia (Riedel *et al*., 2019).

In all cases of IPD, immediate hospitalization is essential, and treatment with intravenous (or intramuscular) antibiotics, effective against the bacteria involved, should commence promptly. Supportive care, including the administration of fluids, oxygen, and potentially mechanical ventilation, may be necessary (WHO, 2018).

2.5.5 Meningitis

Meningitis commonly occurs as a complication of another pneumococcal illness, such as pneumonia, bacteremia, ear, sinus infections or after traumatic injury of the head. it seems that the Pneumolysin may empower the passage of pneumococci into the brain by a disability of the Brain endothelial barrier. This factor has ability to induce cerebral endothelial cells of brain barrier defect that associate with dysfunction in the activation of a protein synthesis-dependent pathway, tyrosine phosphorylation, and caspase (Zysk *et al*., 2001; Parija, 2012, Murray *et al*., 2021). Additionally, the organism contains phosphorylcholine within its cell wall. This component binds to the receptors of platelet-activating factor found on many cells of endothelial types, white blood cell, thrombocytes, and cellular structure of lungs as well as meninges. This interaction enables the organism to enter and disseminate within the body (Tille, 2014).

S. pneumoniae is among the bacterial agents that can cause meningitis and is linked to a significant risk of fatal outcomes. Survivors of pneumococcal meningitis may experience long-term residual symptoms, including hearing impairment, learning disorders, intellectual disability, motor sensory deficits, and even seizures, as a result of neurological sequelae (Grimwood *et al*., 1995; Amann *et al*., 2014).

2.5.6 Other pneumococcal infections

Pneumococci can also cause pyogenic infections in other anatomic sites of the body, such as empyema, pericarditis, conjunctivitis, suppurative arthritis, and peritonitis as outcomes of pneumonia (Kumar, 2016).

2.6 Immunity

S. pneumoniae hold a multiple virulence elements which facilitate their adhesion and invasion the targeted tissue, alongside evasion of the host's immune system (Brooks & Mias, 2018). The quality and efficiency of the immune system are closely associated with

age, which plays a significant role in determining an individual's ability to eliminate microbes before they can potentially cause an infection (Bandaranayake & Shaw, 2016; Pinti *et al*., 2016).

Pneumococcal disease is rare in newborns as well as babies in their early months of life. However, it begins to rise rapidly before gradually decreasing after the age of 2 years. This pattern may be attributed to the protection provided by maternal IgG antibodies, which are transported from the mother to her fetus through the placenta or via breastfeeding. Although, these antibodies have a relatively short half-life and typically fade away from the infant's body fluids within 23 to 27 days, they still provide the production for kids under 2 years old (Infante *et al*., 2015).

Respiratory epithelial cells possess the remarkable ability to synthesize a wide range of biophysical scaffolds, host-defense molecules, and barriers. They also play a crucial role by employing cytokines, chemokines, and damage-associated molecular patterns for communication among themselves and with professional immune system cells. This intricate coordination allows the lungs to maintain a near-sterile environment throughout an individual's lifetime (Whitsett & Alenghat, 2015). Likewise the epithelial cells have the capability of directly destroying *S. pneumoniae* by releasing host defense peptides such as apolactoferrin, lysozyme, and defensins into the surrounding environment (André *et al*., 2015; Whitsett & Alenghat, 2015).

Furthermore, mucociliary airway clearance is another innate protective mechanism that plays an essential role in safeguarding the lungs against the damaging effects of inhaled infections. However, to evade this mechanical clearance, certain airway pathogens, including *S. pneumoniae*, employ strategies to disrupt mucociliary clearance. They accomplish this by impairing ciliary beating, thereby slowing down the movement of mucus, and by causing lytic destruction of epithelial cells. Pneumolysin and other pore-forming toxins produced by pneumococci are thought to play a role in these processes, contributing to the evasion of mucociliary clearance (Fliegauf *et al*., 2013).

Although the strategy of passive maternal anti-capsular antibodies may be protective against carriage acquisition at higher concentrations induced by vaccination, it was not detected at naturally occurring concentrations in infanthood (Ojal *et al*., 2018).

The capsular antigens of *S. pneumoniae* are T-cell-independent type 2 (TI-2) immunogens, binds instantly to B lymphocytes via its repeating sugar epitopes under effects co-stimulation by CD21. They promote production of specific IgG antibodies, the co-stimulation of CD21, which is expressed at low levels in infants, is necessary for the immune response to TI-2. This explain the poor response of unconjugated vaccine in childhood (Rijkers *et al*., 1998). The human who has predisposing factors can get pneumococcal infections many times during his life because the type-specific immunity against pneumococci is linked to antibodies produced under effect of exposure to the capsular polysaccharide (Kumar, 2016). These antibodies, which develop in body within 5 to 8 days later on of invasion, offering specific protection for only serotype that stimulate their production (Parija, 2012).

The impressive capacity of pneumococci to escape or exploit the process of inflammation as well as defense mechanisms of the host is vital for its transmission, colonization, and invasion. This ability allows the bacterium to adapt and thrive within its host environment (Weiser *et al*., 2018). As a result of being exposed to an active upper respiratory tract carrier, the generation of naturally acquired IgG antibodies against PsaA rises with aging. It is unclear what function PsaA-specific naturally acquired immunity plays in protecting against pneumococcal illness (Francis *et al*., 2016).

Animal model study found that Plasma gelsolin (pGSN) promotes macrophage function by triggering the endothelial nitric oxide synthase synthetic system, and phagocytoses by removing actin from macrophage scavenger receptors, this provides protection for the lung host against pneumonia (Yang *et al*., 2015). Furthermore, the gene encoding gelsolin produces a differential splicing that codes for a protein known as pGSN. This isoform contains an additional 25 amino acids and is released into extracellular fluids. Being one of the most prevalent plasma proteins in mammals, pGSN circulates at concentrations ranging from 200 to 300 μg/mL. Notably, even in the presence of antibiotic resistance,

administration of recombinant human plasma gelsolin has shown improved outcomes in a highly lethal model of pneumococcal pneumonia. Remarkably, these positive effects were observed when the treatment was initiated after a clinically relevant delay, suggesting the potential therapeutic value of pGSN in combating severe pneumococcal infections (Yang *et al*., 2019).

The research survey has demonstrated that the deficiency of dietary zinc significantly impacts the abundance and mobilization of zinc within host tissues during infection caused by many primary respiratory pathogens such as *S. pneumoniae* (Eijkelkamp *et al*., 2019). Phagocytes, including neutrophils as well as macrophages, contribute a central duty in recognizing and engulfing the pathogen as an integral component of the host's immune protection. Activation of these phagocytic cells triggers a complex system wherein zinc is mobilized. This mobilization process involves the activation of specific zinc transporters, such as ZIP8 and ZIP14, over the outer layer of the phagocytes. These upregulated transporters promote the uptake of extracellular zinc, thereby increasing the intracellular zinc concentration (Hall *et al*., 2021). Zinc ions exhibit direct antimicrobial properties by disrupting essential microbial processes, including enzymatic reactions and DNA synthesis, thereby inhibiting the growth and survival of the pathogen. Additionally, zinc ions contribute to the formation of reactive oxygen intermediates (ROIs) within the phagocytic cells, further enhancing the host's defense against the pathogen. Altogether, the mobilization of zinc by phagocytic cells represents a sophisticated system orchestrated by the host's immune response, involving transporter upregulation, zinc influx, and subsequent antimicrobial effects mediated by zinc ions and ROIs. This system serves as a key component in the host's defense strategy against infections caused by *S. pneumoniae* (Sapkota & Knoell, 2018).

The Role of the complement system in defending against pneumococcal infections was proved. Various mechanisms activate the complement system in response to pneumococci. Pathogen recognition by components like CRP, serum amyloid P, C1q, SIGN-R1, or antibodies triggers the classical complement pathway. Certain strains of pneumococci are recognized by ficolins, activating the lectin pathway. Pneumococci can also instantly

stimulate the alternative complement cascade. Once activated, the complement forms covalent bonds between opsonic complement factors (C3b and iC3b) and pneumococcal surfaces. This promotes bacterial clearance through phagocytosis, enhances immune adherence to erythrocytes, and protects against septicemia development (Gil *et al*., 2022).

2.7 Pneumococcal Resistance to Environmental Conditions

Pneumococci are delicate microorganisms; subjecting them to a temperature of 52°C for a duration of 15 minutes or using typical disinfectant concentrations results in their destruction. Prolonged incubation periods lead to the demise of pneumococcal colonies (Parija, 2012).

2.8 Laboratory Diagnosis

The identification of the pneumonia etiologic agent is very important for determining the most successful and efficient course of treatment for each patient, as well as for directing the creation of treatment and prevention plans. Nevertheless, due to the relatively inaccessible nature of the diseased tissue and the challenge of obtaining samples without contaminating them with secretions from the upper respiratory channels, determining the agent of pneumonia remains challenging (Hammitt *et al*., 2012). Additionally, it should consider that the total nasopharyngeal concentration of *S. pneumoniae* is comparable in both healthy and symptomatic children, while the concentration of a particular serotype varies significantly among and within children (Morpeth, 2015).

While blood culture is the most critical tool for diagnosis, experienced clinicians can also find Gram's stain and sputum culture useful. Likewise, adults with pneumococcal pneumonia may benefit from urine antigen test (Örtqvist *et al*., 2005).

Pneumococci grow better in the enriched environment with 5% to 10% Carbon dioxide, but bacteria die in the presence of oxygen due to a lack of catalase and peroxidases (Talaro & Chess, 2018).

2.8.1 Specimen

The World Health Organization advised to use the specimen of nasopharynx for the investigation and diagnosis of *S. pneumoniae* carriage among newborns and offspring and

noted that multiple sampling at the same time point did not enhance the exceptional sensitivity of microbiological isolation (Satzke *et al*., 2014).

Indeed, the specimens gathered for examination can differ based on the precise site of infection, typically encompassing samples such as cerebrospinal fluid (CSF), exudate, blood, sputum, and Bronchial lavage (Cheesbrough, 2006).

It was found that flocked swabs are superior in collecting pneumococcal nasal and nasopharyngeal specimens compared to Dacron and Rayon swabs. It was also established that this type of swab has a higher bacterial load during pneumococcal quantitative PCR detection compared to Dacron swabs (Dube *et al*., 2013).

2.8.2 Transport Media

S. pneumoniae is a fragile microorganism that needs to reach the laboratory urgently for immediate inoculation; otherwise, it requires special treatment to keep it alive if delay is unavoidable (Cheesbrough, 2006).

O'BRIEN and colleagues recommend using ["Skim milk Tryptone Glucose Glycerin Broth (STGGB)"] for shipment as well as increase the chance of pneumococci isolation from oropharyngeal specimens collected for epidemiological research (O'Brien *et al*., 2001).

On other hand, Charalambous and his team suggested to using the Skim milk Glycerol Glucose (SGG), and STGGB for better recovery of nasopharyngeal carriage of pneumococci before direct plating on sheep blood agar, and for transportation and storage of pneumococci. They established that the specimens could potentially be preserved in STGGB and in SGG for 96 and 60 hours at ["6 – 8 °C"], 28 and 17 hours at ["21 °C"], as well as 15 and 7 hours at ["30 °C"] in broths media respectively. STGGB was preferred for the preservation of the organism at ["21 °C 30 °C"] (Charalambous *et al*., 2003).

Furthermore, Siberry and colleagues in their study showed that lyophilization and storage at 4°C or freezing at -70°C are the best preservation methods for pneumococci. Additionally, they demonstrated that the ["sand desiccation and storage at 4°C"] provides a cost-effective approach for the prolonged conservation of pneumococci in laboratories where lyophilization is not practical (Siberry *et al.*, 2001).

Moreover, Kaltoft and his team suggested using beef infusion serum broth as transport and primary media for isolation, serotyping, and increasing the chance of growth for nasopharyngeal carriage of *S. pneumoniae* before plating on gentamycin blood agar (Kaltoft *et al*., 2008).

However, some researchers have recommended avoiding broth culture amplification for nasopharyngeal samples before serotyping because this technique may lead to differences in isolation rates (Slotved & Satzke, 2013).

Likewise, Quintero Moreno and colleagues suggested using ["Todd-Hewitt Hemoglobin Yeast Charcoal Agar and Todd-Hewitt Skim Milk Yeast Charcoal Agar"] to maintain the viability of *S. pneumoniae* for a minimum of 6 months at ambient temperature (Quintero Moreno *et al*., 2017).

Despite the variation in researchers opinions on the best method for transporting and preserving pneumococci, the World Health Organization protocols recommend using STGGB as primary transport as well as storing the *S. pneumoniae* in freezing conditions (Satzke *et al.*, 2014).

2.8.3 Pneumococcal Morphology and Gram Reaction

S. pneumoniae is a Gram-positive, elongated (lanceolate) diplococcus. It can also cluster into short chains, especially during culture. Pneumococci lack motility and most of their serotypes possess a capsule, although this capsule may not be present in cultures. When examining Gram-stained smears from specimens, the capsule is often recognizable as an unstained, empty region surrounding the diplococcus (Cheesbrough, 2006).

The presence of *S. pneumoniae* in gram stain prepared from sterile body fluids give highly suggestive result for pneumococcal infection, but this criterion is not applicable for sputum because nasopharyngeal carriage for this organism in many cases of healthy people. In those instances, the result interpretation depends on the presence less than 10 epithelial cells and more than 25 leukocytes per high power field of sputum gram stain is considered to confirm the infection in lung (Gierke *et al*., 2021).

2.8.4 Culture Characteristics

S. pneumoniae are facultative anaerobes that prefer to grow in capnophilic conditions (5–10% carbon dioxide-enriched atmosphere). They exhibit alpha-hemolytic activity on blood agar, resulting in green pigmentation because of the elaboration of H_2O_2 as well as pneumolysin. These factors collectively contribute in the lysis of erythrocytes as well as in the oxidation of hemoglobin into methemoglobin (Murray *et al*., 2021).

However, under anaerobic conditions, an oxygen labile pneumolysin O creates a zone of beta hemolysis around the colony; and it appear as a consistent turbidity in liquid media like glucose broth (Kumar, 2016).

Encapsulated pneumococci typically appear as translucent to mucoid raised colonies spanning 0.5 to 1.2 mm in diameter. Gradually, these colonies can take on a droplet-like appearance. In contrast, non-capsulated colonies are smaller and flattened in shape (Cheesbrough, 2006).

The gentamicin sheep blood agar was confirmed to be more sensitive and accurate by 40% than plain sheep blood agar in isolation of *S. pneumoniae* from contaminated specimens (Sondag *et al*., 1977). Similarly, Crystal Violet-Nalidixic Acid-Gentamicin agar was developed as a selective medium with a high specificity for isolating *S. pneumoniae* from specimens containing normal flora (Nichols & Freeman, 1980).

2.8.5 Biochemical Tests

2.8.5.1 Optochin Sensitivity Test

The optochin sensitivity test involves using an optochin disc impregnated with 5 gm of ethylhydrocupreine hydrochloride to differentiate between pneumococci and other alpha-hemolytic streptococci. Growth that produces an antibacterial zone of 14 millimeters or higher is presumed to be *S. pneumoniae*, while growth with inhibition zones less than 14 mm requires further confirmation (Carvalho *et a*l., 2010; Riedel *et al*., 2019).

2.8.5.2 Inulin Fermentation Test

The inulin fermentation test is a microbiological assay used to ascertain an organism's capacity to undergo fermentation of inulin, a specific kind of carbohydrate. The practice of distinguishing certain bacterial groupings, especially those belonging to the streptococcus genus, is widely used. *S. pneumoniae* ferment inulin and produces acid and no gas. Pneumococcal ability to ferment inulin is a valuable test for separating them from streptococci since the latter do not. In usual circumstances, this test is conducted on Hiss's serum water or serum agar slopes (Kumar, 2016).

2.8.5.3 Bile Solubility Test

The principle of the bile solubility test relies on the existence of an enzyme called amidase which exists in *S. pneumoniae* but not in viridians and other streptococci. This enzyme breaks the bonds that link alanine with muramic acid within the outer covering of pneumococci causing the lysis of cell under effect of external stimulating factors like bile or bile salts or sodium deoxycholate solution (Kumar, 2016).

Recently, it has been indicated that the measurement of bile optical density value using a spectrophotometer was an accurate, fast, and easy method for discriminating between optochin-resistant variants of pneumococci as well as the *S. mitis* species. Furthermore, the existence these strains in the community can be detected and brought to the attention of clinical microbiologists through the utilization of molecular techniques, which confirm the bile test and enable proactive prevention of these atypical pneumococcal strains (Ktari *et al*., 2021).

2.8.5.4 Pneumococcal Antigen Detection Test

The immunological techniques cannot be fully utilized in the clinical management of pneumonia because their low sensitivity in detecting pneumococcal antigen in serum samples. However, they might be used as a backup strategy in exceptional cases where gathering a sizable amount of urine is impractical and they might be useful in hastily diagnosing pneumococcal pneumonia (Domínguez *et al*., 2006).

This test depends on the identification of pneumococcal capsular antigens within various interstitial fluids including urine, CSF, as well as sputum in acute and convalescent states of pneumococcal infection (Venkatesan & Macfarlane, 1992).

The urinary pneumococcal antigen detection test is not typically recommended for young children because it tends to be less reliable in this age group. Young children can have transient pneumococcal carriage without an active infection, leading to potential false-positive results. Additionally, their immune response and bacterial shedding patterns may differ from those of adults, making interpretation of test results more challenging (Harris *et al*., 2011).

2.8.5.5 Molecular Detection of *Streptococcus pneumoniae*

Rapid quantitative PCR technology has been developed, offering the potential for faster results and improved management of infected individuals (Michelow *et al*., 2002).

The real-time PCR targeting the *lytA* gen has been recommended as a reliable non-culture assay that can deliver results in less than one hour, serving as a valuable complement to the gold standard technique for detecting *S. pneumoniae* (Carvalho *et al*., 2007). It has been demonstrated that Real-Time PCR on plasma and other samples outperforms culture for detection of *S. pneumoniae* in all age groups (Cvitkovic *et al*., 2013).

Additionally, in resource-constrained situations, the *lytA* PCR approach in body fluids not only delivers fast and accurate outcomes for detecting pneumococci but also adaptable for quantification and serotyping (Kabir *et al.*, 2017).

Tavares and his colleagues hypothesized that the simultaneous detection of both *lytA*-CDC and SP2020 is an effective technique for identifying pneumococci, whether from clinical specimens or pure cultures (Tavares *et al*., 2019).

At present, there is a rising consensus in favor of adopting quantitative PCR technology for future research on *S. pneumoniae* carriage, both for detection and serotyping, as an alternative to traditional culture and Quellung techniques. The preference is particularly strong if it allows for the inclusion of additional serotypes in existing PCR assays. This

shift is driven by the observation that the carriage rates of vaccine serotypes have not significantly decreased, as suggested by research relying solely on conventional techniques like the quellung reaction. In contrast, results obtained through quantitative PCR provide a more accurate assessment of serotype carriage levels (Ricketson *et al*., 2021).

2.8.5.6 Antibiogram

The Clinical Laboratory Standards Institute recommends using Mueller-Hinton medium enriched with 5% sheep blood or Mueller-Hinton Fastidious medium, which contains 5% defibrinated horse blood and 20 µg/mL Nicotinamide Adenine Dinucleotide, for the antibiogram of *S. pneumoniae* (CLSI., 2021).

The implementation of appropriate antibiotic therapy for pneumococci can be achieved by applying the disc diffusion technique with Oxacillin at a concentration of 1 µg/mL on Mueller-Hinton blood agar. This method is applied as a screening approach to predict the sensitivity of pneumococci to Penicillin, Ceftriaxone, and other β-lactams. However, Oxacillin-resistant strains need further confirmation of their β-lactam resistance by the Minimum inhibitory concentrations (Sastry & Bhat, 2019).

MICs are not routine in pneumococcal infections. It can be conducted by the dilution method which is an essential laboratory technique used to evaluate the efficacy of antibiotics against *S. pneumoniae* by measuring their MICs. However, it can pose challenges in terms of time, resources, and interpretation of results (Cheesbrough, 2006).

The Epsilometer test (E test) is a simple and an advanced method for assessing antimicrobial susceptibility, applicable to various drugs and microorganisms. It offers quantitative wide-range MICs in a straightforward and repeatable manner, similar to the simplicity of the agar disc diffusion procedure (Sader & Pignatari, 1994).

Furthermore, antimicrobial sensitivity assay may be conducted rapidly with applied molecular techniques targeting pbp2b to identify pneumococcal Penicillin sensitivity. This technique presents the potential to shift patients from expensive broad-spectrum antibiotic treatment to more focused and economically efficient treatments. Additionally, the test aids in assessing the true extent of pneumococcal illness (Kearns *et al*., 2002).

The tremendous progress in Real-Time PCR technology opened the door wide for a lot of methods to be applied in the field of pneumococcal detection and evaluate its antimicrobial susceptibility. One of these techniques is a quadriplex real-time PCR that is used for pneumococcal identification and to determine pneumococcal susceptibility to penicillin, macrolides, and lincosamides (Srinivasan *et al*., 2011).

2.9 Treatment

S. pneumoniae presents challenges in treatment and prevention through vaccinations due to its rapid evolution of antibiotic resistance and vaccine escape serotypes. (Chewapreecha, 2014). Presently, there are many attempts for making an effective program that targeting the proper use of antibiotics, monitoring of antibiotic resistance, and adopt antimicrobial stewardship, especially in resource-constrained nations in which antibiotic abuse is very common (Sharew *et al*., 2021).

Penicillin (Penicillin G/amoxicillin) is the primary or preferred option against strains that are completely susceptible to Penicillin or have a modestly lowered susceptibility, while Amoxicillin/clavulanic acid, Cefaclor, Cefotaxime, Ceftriaxone, Erythromycin, azithromycin, and clarithromycin are the alternative choices in cases of increased resistance (Örtqvist *et al*., 2005, Harris *et al*., 2011).

Research pointed out that passive immunization gives promising results in the clinical field of pneumococcal infections. Recombinant human plasma gelsolin (rhu-pGSN) has shown positive outcomes on various laboratory animals of inflammation and injury. In a murine model infected with *S. pneumoniae* of serotypes 3 and 14, whether sensitive or resistant to penicillin, rrhu-pGSN administration led to enhance survival rates, decreased fatality, as well as less body mass loss, all without the use of antibiotics. Such observations underscore the prospective efficacy of Rhu-pGSN, even in the presence of antimicrobial non susceptibility. Notably, in a highly lethal pneumococcal pneumonia model, it was demonstrated that Rhu-pGSN, when administered after a clinically relevant delay, significantly improved outcomes (Yang *et al*., 2019).

2.9.1 Antimicrobial resistant of *Streptococcus pneumoniae*

Multiple drug resistance (MDR) in *S. pneumoniae* refers to the condition where the bacterium demonstrates resistance to one or more drugs from multiple categories of antibiotics. This non-susceptibility may include simultaneous resistance to Penicillin, Tetracycline, Erythromycin, Sulfonamides, as well as Clindamycin (Sastry & Bhat, 2019). AMR is currently a significant threat to public health, and the prevalence of bacteria that are resistant to antimicrobials is rising at an alarming rate (Al-Shami *et al*., 2021).

The uncontrolled utilization of antibiotics within medical and animal treatment, along with food production, has resulted in the creation of antimicrobial non-susceptible pathogen in clinical practice (Chereau *et al*., 2017).

Over the past two decades, there has been a notable and concerning escalation in rate of drug-resistant pneumococcal strains across multiple countries. Consequently, these nations have now affiliated to the category of nations where Penicillin-resistant pneumococci is particularly widespread (Mokaddas *et al*., 2007; Koliou *et al*., 2018).

Among pneumococcal Penicillin-resistant isolates, resistance to other antimicrobial classes is very common. The development of multiple drug-resistant bacteria is considered one of the major challenges to world public health because it limits medical staff's options for managing infectious diseases (Amann *et al*., 2014; Shadi, 2019).

Development of resistance pneumococci illuminates critical necessity for effective approach to combat proliferation of antimicrobial resistance and underscores the significance of vaccination as a crucial preventive measure (Koliou *et al*., 2018).

According to surveillance studies conducted in industrialized nations like America in the previous two decades, the proportion of Macrolide non susceptibility in *S. pneumoniae* have been estimated to be as much as 31%, while the occurrence of *S. pneumoniae* that was resistant to Penicillin varies from 25% to greater than 50% (Appelbaum, 2002).

In addition, it was documented that bacterial drug resistance caused 4.95 million deaths in 2019, with pneumococci ranked as the third most common causative agent among six microbes associated with this fatality rate (Murray *et al*., 2022).

Unfortunately, there are an alarming number of antibiotic-resistant species in Asia, one of the hotspots of antimicrobial drug resistance, including *S. pneumoniae,* which is resistant to both Penicillin and Erythromycin (Jean & Hsueh, 2011).

The gaining of jumping genes, mutations, and occasionally gene amplification ["gene duplication and overexpression"] are examples of intrinsic and acquired mechanisms that influence susceptibility of *S. pneumoniae* to a wide range of antibiotics (El Moujaber *et al*., 2017). Moreover, pneumococci can develop antimicrobial resistance under the effect of genetic transformation and conjugative transposons. In addition, a single-point genome mutations may paly week role in resistant of clinical isolates of pneumococci (Dowson *et al*., 1997).

S. mitis has long been recognized as a storehouse of antimicrobial non-susceptibility factors for pneumococci, in addition to being a wellspring of variability in capsule structure, which may contribute to evading vaccine action and resistance (Salvadori *et al*., 2019).

Many of the non-vaccine serovars which are not part of PCV demonstrate significant resistance to conventional therapies, thereby posing challenges in their management and control. Mitigating the risk posed by these resilient serotypes necessitates a collective effort involving healthcare practitioners, researchers, and policymakers to protect the well-being of the general population and curb the exacerbation of antimicrobial resistance (Higgs *et al*., 2023).

It is crucial to prioritize regular surveillance of antimicrobial resistance in pneumococci as this allows for the updating of therapeutic policies in response to newly emerged resistant strains in the surrounding environment (Moyo *et al*., 2012).

2.9.1.1 Non-susceptibility of pneumococci to Beta lactam Agents

In spite of introduction of the Penicillin into clinical practice, associated with dramatically shifting in the mortality rate of pneumococcal pneumonia from 90% to a survival rate of about 90% (Amann *et al*., 2014). The first report of Penicillin-resistant pneumococci was in Boston during 1965, followed by a similar report in Australia in 1967 (Appelbaum, 1992). While the first report among symptomatic children was in 1977, in South Africa.

Since these initial reports, Penicillin non-susceptible pneumococci are frequently discovered throughout countries around the world (Appelbaum *et al*., 1977).

Dowson and his colleagues demonstrated that *S. pneumoniae* can transfer genetic material encoding low-affinity Penicillin Binding Protein (PBPs) genes to viridians streptococci (Dowson *et al*., 1990). Furthermore, the selective pressure of Penicillin abuse on pneumococci may lead to favorable point mutations, resulting in a lowering in PBPs affinity and consequently a rise of MICs (Negri *et al*., 1994; Amann *et al*., 2014).

The Penicillin-resistant *S. pneumoniae* strains exhibit alterations in their cell wall proteins, specifically the PBPs. These alterations are associated with a reduced affinity of beta-lactam drugs to bind to their primary cellular target protein, mainly PBP 2b. Furthermore, modifications in PBP 1a and PBP 2x can lead to resistance against extended spectrum cephalosporins ["such as Cefepime, Cefotaxime, and Ceftriaxone]" (Cavalieri *et al*., 2005).

Pneumococci obtained from immunized kids exhibited high Penicillin sensitivity as well as a decreased prevalence of resistance to remaining antimicrobial agents in parallel with isolates from non-immunized Offspring. As well as, the incidence of multi-resistant was reduced by 56%, and the presence of pneumococci that were non-susceptible to all tested antimicrobials decreased by 94% (Sigurdsson, 2018).

In the era following the use of PCV, there is a shift in the trend of Penicillin-resistant strains from vaccine serotypes to non-vaccine serotypes. Manenzhe and colleagues pointed out that serotypes 15B/15C, 15A, and 21 were among the most commonly found Penicillin-non-susceptible strains, in addition to serotypes 19A and 19F. Furthermore, their research demonstrated that serotypes 23A, 34, and 17A were isolated as MDR strains (Manenzhe *et al*., 2019).

2.9.1.2 Resistance of *Streptococcus pneumoniae* to Macrolides, and Lincosamide

Macrolides are a class of antibiotics that work by inhibiting protein synthesis in bacteria. Unfortunately, their overuse has contributed to a rise in resistance among *S. pneumoniae*, diminishing their effectiveness in treating infections caused by this bacterium. This resistance is primarily driven by specific genetic changes within the bacteria. While

vaccines have succeeded in reducing the occurrence of macrolide-resistant infections, the emergence of new resistant strains remains a significant concern (Schroeder & Stephens, 2016).

S. pneumoniae can produce a ribosomal methylase enzyme, which is mediated by the *ermB* gene. This enzyme modifies the ribosomal RNA, reducing the binding affinity of Macrolides, Lincosamide, and Streptogramins to the bacterial ribosome, making them less effective. Moreover, the *mefA* gene in *S. pneumoniae* can facilitate the efflux of Macrolides. Efflux pumps are cellular mechanisms which promote antibiotic expulsion from the pathogen, reducing their concentration inside the cell and, consequently, their effectiveness. Resistance can also arise from mutations in ribosomal RNA genes. These mutations can alter the structure of the ribosome, making it less susceptible to the action of Macrolides, Lincosamide, and Streptogramins. These mechanisms collectively enhances pneumococcal resistance to these antibiotics, posing challenges for treatment (Cavalieri *et al*., 2005).

2.9.1.3 Fluoroquinolone resistance in *Streptococcus pneumoniae*

The utilization of quinolones is widespread across various cases of respiratory illness, primarily because of their effectiveness in combating pneumococci and many bacteria of frequently implicated pathogens in the respiratory diseases. However, the emergence of pneumococcal isolates resistant to quinolones has become evident. The non susceptibility is attributed to modifications in the genetic code that impacting the antibiotic targeting units, namely topoisomerase IV and DNA gyrase (Eliopoulos, 2004). The mutations occurred in two key genes, *parC* and *gyrA*, which encode enzymes known as topoisomerases and DNA gyrase essential for regulating DNA processes like replication and repair. Normally, they ensure accurate DNA replication and transcription. Fluoroquinolones disrupt these enzymes, causing DNA breaks and bacterial cell death. Mutations in *parC* and *gyrA* alter the enzymes, reducing fluoroquinolones' effectiveness by decreasing their binding affinity. This resistance hampers the antibiotic's ability to disrupt bacterial DNA processes (Cavalieri *et al*., 2005).

Also, some strains of *S. pneumococci* use a drug efflux system that makes them less sensitive to quinolones. The prevalent pneumococci that are totally non susceptible to many quinolones possess single or multiple genetic modifications impacting DNA gyrase and topoisomerase IV (Eliopoulos, 2004).

It was demonstrated that *S. pneumoniae* exhibited high-level resistance to Ciprofloxacin, with a cutoff of ≥16 µg/ml. Non susceptible pneumococci displayed alterations in the *ParC*, *ParE*, or *GyrA* genes. This non-susceptibility was obtained via specific mutations or genetic exchange with alpha hemolytic streptococci in ["the topoisomerase II genes"] (De La Campa *et al*., 2004).

2.9.1.3 Resistance of *Streptococcus pneumoniae* to Trimethoprim Sulfamethoxazole

The first identification of Trimethoprim-sulfamethoxazole-resistant pneumococcus occurred in 1972, in a female patient afflicted with chronic bronchitis. Trimethoprim-Sulfamethoxazole also known as Co-trimoxazole, functions as an inhibitor of the enzymes dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS). These proteins encode by *folA* and *folP* genes, respectively. The two important enzymes, DHFR and DHPS, serve a fundamental function in the bacterial folate biosynthesis pathway, which is essential for the synthesis of purines (building blocks of DNA) as well as amino acids (Howe & Wilson, 1972). Excessive, highly conserved changes in nucleotide sequencing have been observed as characteristic of resistance in DHFR genes of pneumococci. Additionally, genes encoding resistance in DHFR may evolve through transformative interchanges between or within relevant species (Adrian & Klugman, 1997).

Unfortunately, the use of Fansidar in the treatment of malaria cases is demonstrated as a potential risk for the onset of *S. pneumoniae* resistance to co-trimoxazole, so it important to take that in consideration in endemic area of malaria infections (Cavalieri *et al*., 2005).

2.10 Vaccine

The pneumococcal capsule has been used as the most effective target for vaccination in the fight against the morbidity and mortality caused by pneumococcal illness (Wyllie, 2016). Regardless of the effectiveness of antibiotic therapy, immunocompromised patients, and

kids aged five and below facing a significant hazard from pneumococcal infections. Therefore, it is essential for individuals in these high-risk groups to receive the PCV (Warren, 2016).

The selection of pneumococcal serotypes included in these vaccines and the corresponding usage recommendations are primarily informed by epidemiological investigations conducted on the serotypes of IPD in the America and other Western nations (Frayha & Al Mazrou, 2005).

Immunization with PCV has led to decline in the prevalence of vaccine-type pneumococcus carriage in many countries. However, vaccine coverage remains moderate in some areas, and the rate of *S. pneumoniae* colonization may differ markedly among countries (Weinberger *et al*., 2013; Tvedskov *et al*., 2022).

Polysaccharide and polysaccharide-protein conjugate vaccines have been effective against pneumococcal disease, but challenges remain in achieving immunity independent of capsule type. These challenges include serotype replacement and the incidence of naked pneumococci (NESp), that make up 3% to 19% of asymptomatic carriage isolates. NESp strains, despite lacking a capsule, possess unique surface proteins that impact colonization and virulence. Some NESp isolates replace the capsule with surface proteins, leading to conditions like otitis media. Furthermore, the NESp strains are found in both invasive and noninvasive pneumococcal diseases, with noninvasive conditions like conjunctivitis (85%) and AOM (8%) being more common (Keller *et al*. 2016). Hence, researchers are actively seeking novel pneumococcal vaccines that have been started in clinical trials to address this constraint (Kim *et al*., 2017).

The researchers are exploring innovative strategies for highly efficient pneumococcal vaccines, including live-attenuated vaccines with genetic modifications, peptide-based vaccines with conserved antigenic regions, and the use of vesicles displaying several pathogenic factors like [“PspA2, PspA4, and PsaA”] (Masomian *et al*., 2020).

The continuous survey for the asymptomatic carriage rate in children highlights the importance of ongoing surveillance of pneumococcal carriage to assess the PCV effectiveness. Additionally, this monitoring will help identify any potential changes in the

circulating serotypes, which could guide future vaccine strategies to detect the emergence or expansion of serotypes not targeted by vaccination or changes in serotype-associated invasiveness (Koliou *et al*., 2017)

2.10.1 Pneumococcal Conjugate Vaccine

The initial version of PCV contained 7 serovars, while subsequent versions included either 10 or 13 serotypes, with the selection of serotypes based on their prevalence as common causes of invasive infections in children. The World Health Organization recommends a three-dose schedule for PCV, which can consist of either two doses during infancy with a subsequent booster or three doses during infancy without a booster dose (WHO, 2018).
The PCVs differ from earlier non conjugate immunization, like the PPV23 ["23-valent pneumococcal polysaccharide vaccine"], in their capacity to induce immunity in kids aged two years or younger. This age group, along with individuals over 65, experiences the highest morbidity and mortality rates from pneumococcal diseases (Navne, 2014).

PCVs, unlike antibiotics, are highly specific to the diseases they target, reducing the likelihood of resistance development. Studies have already demonstrated that vaccinations against contagious diseases, such as *S. pneumoniae* and *H. influenzae*, can reduce antimicrobial drug tolerance (Alghamdi, 2021).

The existing pneumococcal vaccines are formulated to stimulate the generation of immunoglobulins for capsular polysaccharides of common disease-causing pneumococcal isolates. Nevertheless, these antibodies provide protection only against pneumococcal strains expressing the specific capsule types targeted by the vaccine. Given the pneumococcal capacity to produce various capsule polysaccharides, there exists a potential for reduced vaccine effectiveness (Ganaie *et al*., 2020).

The era of PCV began with PCV7 and subsequently advanced to PCV10 and PCV13. PCV15 and PCV20, representing the next generation, are currently under trial investigations. However, these vaccines are limited by their serotype specificity, creating the challenge of potential replacement by new serotypes (Musher *et al*., 2022; Kim *et al*., 2023).

The PCV is made up of purified polysaccharide antigens from common disease-causing serotypes that have been bound to a nontoxic mutant of diphtheria toxoid ["called CRM197"], with a goal of transforming the antigen into a form that elicits a T-dependent response in children (WHO, 2013).

The PCV13 vaccine comprises 13 different serogroups, seven of them are shared with the PCV7 ["serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F"]. The remaining six serovars, inclusive of ["1, 3, 5, 6A, 7F, and 19A"], are exclusive to PCV13 (Mokaddas *et al*. 2018). Pneumococcal vaccinations have demonstrated a favourable safety profile, with no notable safety issues reported for any of the vaccines. All permitted PCV generations are considered safe when administered alongside standard vaccinations in infants and young children. Similarly, PCV-13 was safe for older individuals and adults (Petousis-Harris *et al*., 2014).

The introduction of these multicomponent PCVs should be given top priority, especially in nations with high childhood mortality rates (i.e., >50 deaths/1000 births for children under 5). Planning for the widespread use of pneumococcal vaccines should take into account the estimates of disease burden that are already available locally or regionally, the age distribution of cases, and the distribution of pneumococcal serotypes in various age groups (WHO, 2012).

Nevertheless, since the launch of the PCV13 vaccine into national programs, several findings have displayed a decrease in PCV13 serovars compared to a pre-PCV era, leading to a rise in the number of serovars not covered by the vaccine. Additionally, a rate of isolation of PCV13 strains remains high, regardless of immunization (Yüksek *et al*., 2020; Mokaddas *et al*., 2021).

Recently there are alarming reports about the serotypes ["8, 10A, 11A, 12F, 15B/C, 22F, and 33F"] that have been identified as more commonly linked with meningitis as well as AOM in offspring. Furthermore, these serotypes are among the most frequently identified species that are resistant to Penicillin and Macrolides. Importantly, they are not covered by the PCV13 vaccine but may potentially be targeted by the PCV20 vaccine (Méroc *et al*., 2023).

In a Japanese investigation, the isolation percentage of non-PCV13 and non-PCV20 pneumococcal serotypes was found to be 94.0% and 73.7% respectively among children under 15 years old. The most common non-PCV13/PCV20 serotypes ["15A, 35B, and 23A"] were largely resistant to multiple drugs (≥80.5%), including Penicillin (Kawaguchiya *et al*., 2023).

In the time following PCV implementation, pediatric IPD has been correlated with a broad range of serotypes. During the initial period of the first PCV generation, serovar 19A was the commonly identified serotype across various world regions. However, higher-valent PCVs are associated with the expected distribution of serotypes such as 22F, 12F, 33F, 24F, 15C, 15B, 23B, 10A, and 38, which are non-PCV13 serotypes and significant causative agents of childhood IPD. This highlights the requirement for development of novel vaccines including supplementary serotypes to decrease the impact of pneumococcal illness (Balsells *et al*., 2017).

Certainly, evaluating the advantages of vaccination involves tracking its efficacy throughout the nation or in specific area. While strategy of Off-the-shelf approach in pneumococcal immunization, using the western version regardless of serotype prevalence, has its drawbacks, it's important to consider custom vaccines tailored to the predominant serotypes causing IPD in specific nations or regions (Mokaddas *et al*., 2021).

Many researchers have worked on the approach of expanding the covering spectrum of PCV, exemplified by research conducted to create a PCV 15 by adding polysaccharides antigen from 22 F and 33 F to PCV 13. These polysaccharides are linked to a non-toxic variant of diphtheria toxin ["CRM197"] and combined with an aluminum phosphate adjuvant (Skinner *et al*., 2011). PCV20 is one of the PCV formulations that are expected to be granted approval for administration to children. It is a next-generation PCV that includes protection against 20 different serotypes of *S. pneumoniae*, which is an increase from the 13 serotypes covered by previous PCV generations. By including protection against more serotypes, PCV20 could potentially provide greater herd immunity and further decrease the prevalence of carrier cases and illness. However, it is important to note

that the introduction of a new vaccine, such as PCV20, would need to be carefully evaluated through clinical trials and ongoing surveillance to determine its safety, efficacy and impact on the epidemiology of pneumococcal disease (Tiley *et al*., 2022).

One primary impact of PCV is the replacement of serovars included in the immunization regime with those not incorporated in its composition. Additionally, other pathogens were found to be more abundant in vaccinated children compared to the same group of children before receiving the vaccine. It's important to note that vaccine serotypes were not completely eradicated; some, like serotype 3, remained and were associated with poor clinical outcomes and a weak antibody response (Littorin, 2020).

An innovative serotype-independent immunization strategy is required to continue lowering the prevalence of pneumococcal disease. One such strategy is the use of pneumococcal surface PNPsaA. This vaccine includes a full-cell, non-encapsulated pneumococcal antigen that has undergone gamma irradiation for inactivation. It triggers a serotype-independent immune reaction targeting highly conserved sub-capsular immunogens (Laan, 2020).

2.10.2 Mucosal Vaccines Trails

Several studies indicate that individual administration of pneumococcal protein antigens fails to effectively control and broadly protect against pneumococcal colonization. To achieve comprehensive protection and activate both innate and adaptive immune responses, it is recommended for novel vaccines to combine conserved protein antigens. This approach, particularly when administered intranasally, is expected to optimize efficiency by stimulating mucosal immunity and reducing pneumococci in the nasopharyngeal area. While not aiming for complete elimination, this strategy seeks to decrease transmission rates and provide protection against associated illnesses (Lagousi *et al*., 2020).

Recently, the Pneumococcal surface adhesin (especially lipidated form rlipo-PsaA, rPspAΔC, and rPspCΔC which known as LAAC) was studied in mice model. The mice immunized intranasally with LAAC vaccine formulation had generated substantially higher IgG antibody levels compared to mice given the non-lipidated versions of the

proteins (AAC). Furthermore, the LAAC vaccine had stimulated increased IgA antibodies in vaginal washes, faeces, and blood of mice, signalling that LAAC could provoke systemic mucosal defence. LAAC had also promoted Th1/Th17-type immune responses and enabled opsonic phagocytosis of *S. pneumoniae* strains of multiple serotypes, suggesting the immunogenicity of LAAC provided protection against pneumococcal diseases (Chiu *et al*., 2023). Moreover, randomised controlled research demonstrated that nasal colonisation with genetically attenuated pneumococcal strains was both safe and efficacious in eliciting homologous protection. This suggests that the utilisation of live attenuated intranasal vaccinations may serve as a viable strategy for preventing pneumococcal illness (Hill *et al*., 2023).

Pneumococci are natural residents of respiratory microbial communities, and the consequences of selectively removing them remain uncertain. This may lead to nonvaccine strains thriving, and other pathogens finding new niches. Integrating mucosal immunology with microbiota and microbiome research is essential to reduce pneumococcal disease without unintended consequences (Swiatlo & McDaniel, 2020).

2.11 Prevention and Control

The ability of *S. pneumoniae* to exhibit resistance to antimicrobial drugs and evade immune surveillance underscores the tremendous challenge in eradicating this infection (van der Poll & Opal, 2009). Reports from developed countries have revealed significantly lower serotype prevalence of PCV13 and reduced Penicillin resistance among children (Wouters *et al*., 2018). On the contrary, the carriage rate remains substantially high in many regions of Middle Eastern countries, as well as across the resource-constrained nations in Asia and Africa (Sutcliffe *et al*., 2019; Wada *et al*., 2019; Al-Lahham, 2021).

This situation urgently requires antimicrobial custodianship, national surveillance, and public awareness programs to fight the establishment of multidrug-resistant pneumococci (El Moujaber *et al*., 2017).

The PCV has proven to be effective in preventing the spread of pneumococcal vaccine strains to other members of the community, as it breaks the chain of transmission, resulting in a herd immunity effect. However, carriage of non-vaccine strains has markedly risen. This has resulted in slightly or no overall alteration in carriage proportion. Moreover, the predominance of other co-colonizing bacteria may shift following the introduction of PCVs, potentially leading to different illness patterns with widespread usage of pneumococcal vaccines (Navne, 2014).

2.13 Pneumococcal Carriage Rates and Infections Among Children in Resource-constrained nations in Asia and Africa

Pneumococcal infections have a higher prevalence in developing countries due to challenges related to hospital accessibility and underfunded diagnostic facilities. These factors, among others, contribute to the persistent burden of pneumococcal disease in these regions. Notably, pneumococcal carriage rates vary across income groups, with rates of 51%, 38.5%, 31.5%, and 28.5% observed in low-income group, middle income cohort, upper middle earning category as well as high-earning classes respectively. This discrepancy in carriage rates highlights the influence of socioeconomic factors on pneumococcal prevalence (Neal *et al*., 2022).

During last two decades, the burden of pneumococcal mortality was high in several countries in Asia and Africa, including India, China, Pakistan, Bangladesh, Nigeria, Uganda, Ethiopia, Congo, Angola, and Afghanistan. Recognizing the urgency of addressing this issue, the Global Alliance for Vaccines and Immunization approved support for vaccine introduction in these regions. This support aimed to combat the significant burden of pneumococcal mortality in Asia and Africa and initiate efforts to reduce the impact of this disease. Collectively, these countries accounted for approximately 95% of all pneumococcal deaths, underscoring the concentrated need for targeted interventions and emphasizing the importance of vaccine support in combating pneumococcal-related fatalities (O'Brien *et al*., 2009).

Moreover, in 2019, pneumonia claimed the lives of 740,180 kids aged five years and below, representing 14% of total pediatric fatalities, where 22% of all victims aged one to

five years. Pulmonary infection impacts kids and families everywhere, but southern part of Asia as well as Sub-Saharan Africa undergo the highest mortality rate (WHO, 2021).

In India, prior to the PCV era, the occurrence rate of *S. pneumoniae* among 91 pneumonic children and 510 healthy children under five years old was reported as 74.7% and 54.5% respectively. Among these children, ["serotypes 6A, 6B, 14, 19A, 19F, and 23F"] were identified as the prominent colonizing serovars (Sutcliffe *et al*., 2019). Another study conducted in the state of Gujarat found that 19.2% of 125 children under 12 years old, who presented with acute lower respiratory symptoms and sought treatment at Baroda hospital, tested positive for *S. pneumoniae* (Barve *et al*., 2019). Moreover, the prevalence rate of pneumococci has been found to be 33.5% among 200 asymptomatic Bangladeshi kids (Shormin *et al*., 2022).

On the other hand, the prevalence was documented at 20% among Kenyan children (Walekhwa *et al*., 2018). Likewise, it was 54% among Ghanaian children under 5 years of age (Dayie *et al*., 2019).

In Morocco, the carriage rates of *S. pneumoniae* were investigated among 697 pediatric patients and 195 children under the age of five, revealing a prevalence of 40.5% among asymptomatic cases and 22.8% among patients. The prominent circulating serotypes observed were 6A, 6B, and 19F (Jroundi *et al*., 2017). Similarly, in Ethiopia, a study among healthy young kid documented a prevalence at 43.8% (Gebre *et al*., 2017), another study conducted among Ethiopian children aged 3-13 years, with a sample size of 710, revealed a prevalence rate of 43.8%. Among children aged 3-5, the carriage rate was 62.5%, which was higher compared to children aged 6-13, where the carriage rate was 38.6% (Wada *et al*., 2019).

In Tanzania, a study focused on Pneumococcal Nasopharyngeal carriage among 142 HIV-positive children aged 1 to 14. The carriage rate was found to be 81% among the study group, with a rate of 76% among children aged 5 to 14. The study also reported covering rates of 37% for PCV7, 37% for PCV10, and 49% for PCV13 in this group. Caregivers who had experienced respiratory symptoms in the previous week have been reported as potential source for carriage (Anthony *et al*., 2012).

These findings collectively highlight the significant presence of pneumococcal colonization and infection among children in these regions, underscoring the need for effective preventive strategies such as increasing vaccination rate and continued surveillance to address pneumococcal-related morbidity and mortality.

2.14 Pneumococcal carriage in Middle Eastern pediatric population

The occurrence of pneumococci in the nasopharynx among children under 5 years old displays a significant variation, ranging from 42% to 90% (Gordon *et al*., 2003; Hill *et al*., 2008) .

Identifying both the colonization rate and the prevalence of pneumococcal serovars in a pediatric population can provide valuable information on the occurrence of severe illnesses, antibiotic resistance, and the potential consequences of implementing a national pneumococcal conjugate vaccine (PCV) program in the future (El-Nawawy *et al*., 2015; Taha & Ali, 2019).

The overall pneumococcal cumulative rate in Middle East was found to be 35%. Among the isolates, the commonly prevalent pneumococcal serovars were [“19, 19F, 6, 23, and 6A/B”], accounting for 19%, 12%, 11%, 10%, and 10% of the isolates, respectively (Karimaei *et al*., 2021).

Several studies conducted in different Middle Eastern countries from 2005 to 2019 (Table 2.1) assessed the colonization rates as well as serotypes of pneumococci in a pediatric population. These studies reported varying carriage rates in different countries, highlighting the variability in carriage rates among the kids of the Middle East.

In Jordan, six studies involving kids aged 4 years, reported varying carriage rates between 19.5% and 56.2% among 3,720 healthy participants for the period from 2005 to 2019 (Al-Lahham & Van der Linden, 2014; Al-Kayali *et al*., 2016; Al-Lahham *et al*., 2018; Al-Lahham, 2020; Al-Lahham, 2021; Al-Lahham *et al*., 2021). This made Jordan the country with the highest rate of pneumococcal carriage among Middle Eastern children according to the available data. Whereas, in the State of Palestine, the prevalence ranging from 30% to 55.7% was observed between 3,531 children aged below 5 years old from 2009 to 2013

(Regev-Yochay *et al*., 2012; Nasereddin *et al*., 2013; Daana *et al*., 2015). While pneumococcal rates within Cyprus and Egypt were comparable, ranging between 25.3% to 35.3% among 1,507 asymptomatic Cypriot kids aged below 5 years from 2007 to 2014 (Hadjipanayis *et al*., 2016; Koliou *et al*., 2017), and from 29.2% to 32.9% among 534 children under 5 years old in Egypt from 2012 to 2017 (El-Nawawy *et al*., 2015; El-Kholy *et al*., 2020).

In Iran, the immunization program has been exclusively targeted at the high vulnerability subgroup , the prevalence rate in 2,432 children aged 6 months to 14 years varied from 2.2% to 44.1% (Sanaei *et al*., 2012; Karami *et al*., 2019; Sayyahfar *et al*., 2021). On other hand, the carriage rate in Turkey among kids aged below 18 years was varied between 6.4% to 21.9% throughout 4,346 asymptomatic individuals from 2011 to 2021 (Özdemir *et al*., 2014; Soysal *et al*., 2016; Yüksek *et al*., 2020; Ceyhan *et al*., 2021).

In Iraq, the nasopharyngeal rate was found to be 20.5% among 1,092 healthy children aged 6 to 13 years (Taha & Ali, 2019). In contrast, it is worth noting that the prevalence of carriage in Yemen during the year 2006 was quite low, as shown by 5.6% among a cohort of 602 children who were deemed to be in good health (Al-Shamahy *et al*., 2008).

2.15 Impacts of Pneumococcal Infections in Middle Eastern Children.

Despite the availability of vaccines, IPD caused by pneumococci still a major reason for death in children universally. The effectiveness of PCVs has been observed, vaccine serotype illnesses continue to occur, even among 82% of US children aged three who have received four or more PCV immunizations. In the US, non-vaccine serotypes account for over 70% of invasive pneumococcal illnesses, with higher vulnerability observed in healthy young children and those with underlying medical conditions (Olarte & Jackson, 2021). Moreover, an investigation conducted in the America revealed that despite the availability of vaccines, the highest incidence rates of acute otitis media linked to pneumococcal bacteria were reported at a frequency of 2,756 cases per 100,000 people

(Talbird *et al*., 2022). It is crucial to observe that America characterizes by the significantly higher immunization rate in contrast to Yemen and the other countries of Middle East.

In point of fact, the practical implementation of PCV-13 did not completely eliminate the circulation of PCV-13 covered serotypes in vaccinated children (Dayie *et al*., 2019). Additionally, the rising occurrence of IPD throughout developing countries poses a significant challenge across all age groups, primarily due to limited comprehensive surveillance (Hanna-Wakim *et al*., 2012). Although PCV has been included in the vaccination programs for the majority of children in the Middle East for an extended period, the ongoing high mortality rate associated with IPD remains a cause for concern (Al-Jardani *et al*., 2019).

The annual mortality rate of pneumococcal illness among kids in Egypt had been reported as 33.3%, with a higher rate observed in cases of IPD at 75% compared to non-IPD cases at 12.5% (Draz *et al*., 2015). While in Lebanon, the death rates increased considerably, rising from 12.5% in the PCV7 period to 24.8% in the PCV13 period (Reslan *et al*., 2022). Furthermore, in Oman, the case fatality rate of IPD was prevalent in kids below 5 years, attaining 14.2% (Al-Jardani *et al*., 2019).

On other hand Saudi Arabia (KSA) reported a death rate of 12% for invasive illness resulted from pneumococci during the period from 2001 to 2007 (Al Ayed & Hawan, 2011). Additionally, a recent study conducted in KSA revealed that pneumococci was linked to 45.6% of sinus inflammation among Saudian kids (Alshehri *et al*., 2021). Despite the implementation of PCV, pneumococci remain the predominant causative agent of meningeal infection in Kuwait, accounting for 40.4% of reported cases. It is often linked to the most elevated rates of morbidity and death, particularly among the pediatric population (Sadeq *et al*., 2017). While in Bahrain, the mortality rate of pneumococcal infections among individuals aged 14 years and older was 14.4% during the period of 2010 to 2014 (AlSalaman *et al*., 2017).

During the period from 2017 to 2019, the serovars of third generation of pneumococcal immunization [“PCV13”] were found to be more prevalent in kids who were suffering from IPD and non-IPD in Iran. These serotypes accounted for 83% and 84% of *S. pneumoniae*

that were identified, respectively (Esteghamati *et al*., 2022). It is important to note that the PCV13 vaccine is currently not incorporate into the routine immunization plan in Iran. As a result, pneumococcal meningitis is a substantial public health concern in Iran, with an occurrence ratio of 25% (Tabatabaei *et al*., 2019). Regrettably, due to not involve PCV in routine practice, approximately 18,713,211 Iranian kids aged under 5 have been affected by pneumococcal illness in the past decade ["2014 and 2023"]. The reported data consist of 519,412 instances of pneumonia, 18,148,116 instances of acute otitis media, 6,884 instances of meningitis, and 38,799 instances that do not fall within the categories of pneumonia or meningitis (Ezoji *et al*., 2019). In addition, meanings inflammation may link to various strains of pneumococci, that might go undetected if comprehensive testing for all serotypes is not conducted on cerebrospinal fluid samples (Tabatabaei *et al*., 2022).

Similarly, recent reports from Lebanon have raised alarms about the incidence of serotype 24F in clinical practice of invasive illness. This serotype has demonstrated significant pathogenicity as well as resistance to antimicrobial agents, posing a notable concern. It is crucial to emphasize that the current PCV vaccination does not provide coverage for serotype 24F (Reslan *et al*., 2021). Due to the substantial occurrence of IPD in toddlers across third world countries, the uptake of second-generation PCVs has been swift. This generation targets a wider range of serotypes and started to incorporate into the national plans of many regions of third world (CDC., 2013). Furthermore, there are upcoming initiatives to introduce a broader version of PCV ["PCV15 and PCV20"] for the purpose substantial coverage level by transitioning from PCV-13 to next version that will respond to the prevalent of non-PCV 13 serotypes (El-Beyrouty *et al*., 2022).

Moreover, the persisting internal conflict across certain Middle Eastern regions, further worsened the circumstances. This phenomenon is particularly apparent in Yemen, in which the detrimental impact on immunization levels has corresponded with an increasing prevalence of acute bacterial meningitis among population. *S. pneumoniae* has been established to be the primary microbe responsible for these infections (Al-Samhari *et al*., 2023).

Unfortunately, Yemen has never conducted a national survey on pediatric respiratory diseases. The respiratory diseases was demonstrated as the most prevalent type of illness (44.3%) among Yemeni children for the period from 1978 to 2018 (Al-Shamahy & Ishak, 2021). Moreover, the isolation rate of *S. pneumoniae* was ranging from 30.1–34.9 % among Yemeni children whom had meningitis (Al Khorasani & Banajeh, 2006; Alshehari *et al*., 2018). As well, Al-Ofairi and colleagues demonstrated that the pneumococci constituted 27% of the isolated gram-positive bacteria collected from 100 Yemeni children with otitis media (Al-ofairi *et al*., 2017). Another study found that recurrent pneumonia affected 1 in 12 children under the age of 15 who visited Mukalla City Hospital in Yemen between 2014 and 2016 (Bahwal *et al*., 2018). Additionally, pneumococci was linked to associate with 40% of acute meningoencephalitis among 60 patients aged below 14 years who were visited Al-Sadaqa General Teaching Hospital, Aden, in 2010 (Al-Qubati & Bawazeer, 2020).

Even though the diverse data that gathered from various Middle Eastern countries, including Yemen, the results pertaining to serotypes highlight the significance of ongoing surveillance. Continuously checking the changes in serotype prevalence through monitoring is essential for guiding the improvement of vaccines.

Table 2.1: Epidemiology and Predominant Serotypes of Pneumococci in Middle Eastern Kids

Countries	Total Study Population	Carriage rate	Common Serotypes	PCV Covering Rate	References
Bahrain	100 serotypes collected from invasive and non-invasive samples [children and elderly]	Not available	19, 6, 23, 3, &14	It was 66.7% in children < 2 years old.	(Al-Muhtaresh & Bindayna, 2020)
Cyprus	1507 healthy children <5years old	25.3-35.3%	1, 6BC, 5AB, 19A & 23AB.	PCV7 was 2.1-22.2%. PCV10 was 23.8%. PCV13 was 10.4-28.6%.	(Hadjipanayis *et al*., 2016; Koliou *et al*., 2017)
Egypt	1143 samples from healthy children under 5 years	56.5- 65% (RT-PCR). 29.2- 32.9% (culture).	1, 6ABC, 5, 18ABC,19 A, 19F, and	PCV10 was 65.5%. PCV13 72.4-77.4%.	(El-Nawawy *et al*., 2015; Badawy *et al*., 2017; El-Kholy *et al*., 2020)

Iran	2432 carriage cases aged from 6 months to 14 years in 4 studies. 130 infected samples aged from 10 days to 92 years. 2049 clinical cases from children younger than 18 years.	2.2-44.1%	1, 3, 6, 9V, 14, 17, 19, 20, 21., and 23	PCV7 was 33.7-38.56%. PCV10 was 34.9 -65.1%. PCV13 was 66.2- 83.5%.	(Sanaei *et al*., 2012; Houri *et al*., 2017; Karami *et al*., 2019; Ghahfarokhi *et al*., 2020; Abdoli *et al*., 2020; Sayyahfar *et al*., 2021; Tabatabaei *et al*., 2021; Esteghamati *et al*., 2022)
Iraq	1092 carriage cases aged 6-13 years.	20.5%	Not available	Not Available	(Taha & Ali, 2019)
Jordan	3720 carrier children under 1 month to 4 years.	19.5 -56.2%.	6A, 6B, 14, 9V, 11A, 15A/B, 19F, 23A,23F, & 35B	PCV7 was 27.8-52.3%. PCV10 was 48.2-62.2%. PCV13 was 49.4-73.2%.	(Al-Lahham & Van der Linden, 2014; Al-Kayali *et al*., 2016; Al-Lahham *et al*., 2018; Al-Lahham, 2020; Al-Lahham *et al*., 2021; Al-Lahham, 2021).
Kuwait	397 isolates from different infected patients, only 193 for children ≤ 15 years	Not Available	23F, 19F, 6B, 14, & 9A	Pre-vaccination period: PCV7 was 53.26%. PCV13 was 72.6%. vaccination period: PCV7 decreased to 32.7% and 6.6%.	(Mokaddas *et al*., 2008; Mokaddas & Albert, 2012; Mokaddas & Albert, 2016; Mokaddas et al., 2021).
Lebanon	850 pneumococcal isolated from symptomatic children and adults	Not Available	1, 3, 5, 6, 14, 19F,	PCV7 was 50-51%. PCV10 was 45.6-74%. PCV13 was 63-80%.	(Hanna-Wakim *et al*., 2012; Reslan *et al*., 2022)
Oman	252 strains isolated from symptomatic children and adults	Not Available	3, 6B, 9A, 12, 14, 15, 19A, 19F& 23F.	PCV7 was 46.1% then become %15.9. PCV10 was 24.2%. PCV13 was 37.1%.	(Al-Yaqoubi, 2011; Al-Jardani *et al*., 2019)
Palestine	3531 pneumococci isolated from carriers ≤ 5 years	30-55.7%	6A/B, 14, 19F, and 23F.	PCV7 was 34.4%. PCV10 was 36.5%.	(Regev-Yochay *et al*., 2012; Daana *et al*., 2015;

				PCV13 was 49.2%.	Nasereddin *et al*., 2013).
Qatar	282 strains isolated from children and adult who have IPD and NIPD	Not Available	1, 3, 6A, 6B, 9V, 11 A, 14, 15B, 19A, 19F and 23 F	PCV7 was 43-52% PCV10 was 52-679%. PCV13 was 75-78.3%	(Al Khal *et al*., 2007; Taj-Aldeen & Elshafie, 2016).
Saudi Arabia	- 78 strains isolated from children and adult who have IPD and NIPD - 1051 children aged less than 15 years with IPD and NIPD.	Not available	1, 5, 6 7, 14, 15, 19, 18, 22, and 23	- PCV7 was 30-77% - PCV10 was 81% PCV13 was 90%	(Al-Mazrou *et al*., 2005; Shibl, 2008; Al-Sherikh *et al*., 2014; Almazrou *et al*., 2015)
Syria	Data not available				
Turkey	4346 healthy children <18 years.	6.4% - 21.9%	19F, 6A, 6B, 9V, 11A, 12F, 15ABF, 19F, 22AF, 23A, and 23F	PCV7 was 46.2 %. PCV13 was 27.2 -60%	(Özdemir *et al*., 2014; Soysal *et al*., 2016; Yüksek *et al*., 2020; Ceyhan *et al*., 2021)
Emirates	Data not available				
Yemen	602 healthy children (Nasopharyngeal swabs)	5.6%	1, 2, 4, 5, 15, 19, and 22	Data not available	(Al-Shamahy *et al*., 2008).

2.16 Antimicrobial resistance of *Streptococcus pneumoniae* in Resource-constrained nations of Asian and African countries.

AMR is a major concern for pneumococci in resource-constrained nations in Asia and Africa, where its prevalence exceeds that of developed nations. This is due to antibiotic misuse, easy availability without prescriptions, and their ineffective use against viral infections. Limited healthcare access and diagnostics compound the problem. Antibiotic resistance is a pressing issue in the 21st century, particularly in developing countries. In an early 2000s report from the Asian Network, it was revealed that the Penicillin non-susceptibility rate of pneumococci in Asia reached 35.8%. Taiwan had the highest resistance rate among Asian countries, reaching 91.3% (Lee *et al*., 2001). Additionally, in another study conducted in Asia between 2000 and 2001, 52.4% of pneumococci checked across 14 medical facilities in 11 Asian regions exhibited Penicillin resistance, with Vietnam recording the highest resistance rate at 92.1% (Song *et al*., 2004).

In India, studies have reported the antibiotic resistance rates; 43.7% for Penicillin, 14.9% for cefotaxime, 32.3% for Erythromycin, 65.2% for cotrimoxazole, and 3.2% for Chloramphenicol (Verghese *et al*., 2017; Sutcliffe *et al*., 2019). Another Indian investigation conducted in the state of Gujarat revealed the resistance rates in *S. pneumoniae* isolates 66.6% against Tetracycline, 8.3% against Oxacillin, 3.8% against Co-trimoxazole. Furthermore, an absolute of sensitivity (100%) was observed for Ciprofloxacin, Cephalexin, and Amoxiclav, respectively. The study also found that MDR was present in 33.4% of the microorganism (Barve *et al*., 2019). Additionally, the resistance rate was 92.7% for Penicillin, 84.3% for Erythromycin, 79.3% for Ceftriaxone , and 77.7% for Tetracycline according to meningitis breakpoints among Sri Lankan children (Vidanapathirana *et al*., 2020). In Bangladesh, a study involving 67 *S. pneumoniae* isolates obtained from 200 healthy children under the age of five revealed high sensitivity to Penicillin, cephalosporin, glycopeptide, and carbapenems. However, resistance rates were 7.5% for Ciprofloxacin, 47.8% for Erythromycin, 47.8% for Azithromycin, 88.1% for Gentamicin, and 76.1% for Co-trimoxazole (Shormin *et al*., 2022). As well as the MDR was 18% among isolated pneumococcal strains from Indonesian children under five years old. The resistance rates were 40% for Penicillin, 36.8% for Tetracycline, 29.7% for Trimethoprim/Sulfamethoxazole, 16.8% for Erythromycin, 9.7% for Chloramphenicol, and 8.6% for Clindamycin (Salsabila *et al*., 2022).

Antimicrobial resistance of pneumococci is also prevalent in African countries. In a study among healthy Ethiopian children under 5 years old the resistance rates were 53.2% for Tetracycline, 43.7% for Co-trimoxazole, 36.1% for Penicillin, 13.3% for Chloramphenicol, and 8.9% for Erythromycin. 17.7% of the isolates exhibited MDR (Gebre *et al*., 2017). Furthermore, Wada and colleagues discovered that the Tetracycline resistance rate was 48.9%, the Co-trimoxazole resistance rate was 45.3%, and the Oxacillin resistance rate was 28.3%. Chloramphenicol exhibited the lowest resistance rate at 12.5% among school children in South Ethiopia (Wada *et al*., 2019). Another Ethiopian study, carried out during 2018-2019, found that 17.5% of 57 *S. pneumoniae* gathered from both kids and adults were non-susceptible to Penicillin. While 1.8% showed non-susceptibility to Cefotaxime and

Ceftriaxone. Additionally, there were disparity in the rates of non-susceptibility among these isolates for the antibiotics included in the study ["Erythromycin, Clindamycin, Tetracycline, Chloramphenicol, and Trimethoprim-Sulfamethoxazole were 59.6%, 17.5%, 38.6%, 17.5%, and 24.6%, respectively"]. The MDR was detectable in 33.3% of the microbe (Sharew *et al*., 2021).

In a Tunisian children, the rate of isolation for Penicillin-resistant strains was 75.3%, and the rate of Erythromycin resistance was 71.4% (Ktari *et al*., 2017). While it was found a significant increase in the isolation rate of non-penicillin susceptible strains from 31% in 2013 to 47% in 2014 among Tanzanian children (Emgård *et al*., 2019).

Nevertheless, the prevalence of Penicillin-non-susceptible carrier isolates among Ghanaian children decreased from 45% before the introduction of PCV-13 to 22.3%. Resistance rates were 63% for Tetracycline, 61.4% for Trimethoprim-Sulfamethoxazole, and 11% for Erythromycin, with 20% showing multidrug resistance (Dayie *et al*., 2019). In contrast, the rates among invasive Nigerian strains of *S. pneumoniae* were 83.3% for Penicillin (resistant + intermediate), 69.6% for Oxacillin, 65.2% for Cefuroxime, and 47.8% for Ceftriaxone (Iliyasu *et al*., 2015; Suleiman *et al*., 2018).

The rates of antibiotic resistance exhibit significant variations across different geographical locations of Asia and Africa, which give rise to concerns and emphasize the necessity for improved monitoring and availability of novel approaches to combat the potential dissemination of drug-resistant bacteria.

2.17 Antimicrobial resistance of *Streptococcus pneumoniae* in Middle East

Although there are several variables that have an impact on antibiotic resistance, rise in antibiotic utilization is one of the most significant factors linked to elevate the non-susceptibility among *S. pneumoniae* in the general clinical practice. Rationalizing antibacterial use still the key effective way to decrease the rise of antimicrobial non-susceptibility (Schrag *et al*., 2001; English & Gaur, 2010). The public health sector remains under a substantial threat due to the drug tolerance displayed by pneumococci. The Centers for Disease Control and Prevention (CDC) estimates that over 2 million cases of antibiotic-

resistant infections happen annually within America, resulting in approximately 23,000 individuals losing their lives due to these bacteria (CDC., 2017).

The remarkable capacity of *S. pneumoniae* to reconstruct its genetic material by incorporating external DNA pieces gained from similar species of the pneumococcal genus has enabled the dissemination of antimicrobial non-susceptibility, as well as the avoidance of immunization-triggered immune responses (Weiser *et al*., 2018).

Despite various ongoing AMR initiatives in some regions of the Middle East, such as Saudi Arabia, including 2017 National Action Plan, AMR is rising among physicians and public knowledge gaps about appropriate antibiotic use (Torumkuney *et al*., 2022).

Over the past two decades, pneumococcal isolates from children in Middle East countries have shown a high rate of antimicrobial resistance, as illustrated in Table 2.2.

Moreover, the CDC suggested that approximately one-third of prescribed antibacterial agents in outpatient clinics are considered to be unnecessary (CDC., 2017). Unfortunately, the prevalence of acquiring antibiotics without a medical prescription is seen in some locations of Middle East, where rates of self-medication range from 19% to 82% (Alhomoud *et al*., 2017).

In Yemen, an investigation revealed that 60% of non-hospitalized children aged under fifteen years received non-prescribed antimicrobial drugs (Mohanna, 2010). Likewise, research conducted in 2015 at Sana'a City found that out of 200 local pharmacies, 73.3% supplied antimicrobial agents to people and did not ask for a medical prescription. The dispensed medications encompassed a spectrum of antimicrobial agents ["Penicillin (48.5%), Sulphonamides (12.5%), Macrolides (10.6%), Fluoroquinolones (8.8%), Metronidazole (7.8%), Cephalosporins (6%), and B-Lactam and B-Lactamase inhibitors (5.8%)"]. The distribution rates of antibiotics differed based on the specific disease conditions. Tonsillitis exhibited peak frequency, followed by cough, diarrhea, and otitis media. In contrast, urinary tract infection had the minimum distribution rate (Halboup *et al*., 2020). Similarly, an investigation revealed a substantial prevalence of non-susceptibility of antibiotics in Yemen. It indicated that three quarters of physicians felt compelled to recommend the wide-spectrum antibiotics. Furthermore, the survey found

that four fifths of patients had not undergo antibiogram approach to guide drug selection. Moreover, a significant number of pharmacies (67%) dispensed antibiotics without requiring a prescription. The prominent specified (63%) and distributed (82%) agent was Amoxicillin, including Amoxicillin-clavulanate (Orubu *et al*., 2021).

The highest level of Penicillin non-susceptibility (ranging from 80% to 95.8%) was noted in Jordan among a group of 3,720 healthy children over the years spanning from 2005 to 2019 (Al-Lahham & Van der Linden, 2014; Al-Kayali *et al*., 2016; Al-Lahham *et al*., 2018; Al-Lahham, 2020; Al-Lahham, 2021; Al-Lahham *et al*., 2021).

Likewise, in Yemen, the observed rate of resistance to Penicillin fell within the range of 85% to 93.3% among the population under study (Al-Shamahy *et al*., 2008, Al-Ofairi *et al*., 2017). Additionally, a comparable rate was identified in Iran, where Penicillin non-susceptibility fluctuated from 9.2% to 95.3% throughout 2,165 kids during the period from 2008 till 2016 (Sanaei *et al*., 2012; Houri *et al*., 2017, Gharibani *et al*., 2019).

In Turkey, the range of Penicillin resistance among 3,266 children aged below 18 years during 2011 to 2013 extended from 62% to 73% (Özdemir *et al*., 2014, Soysal *et al*., 2016).

A notable rise in non-susceptibility of pneumococcal to Ceftriaxone and Cefotaxime in some regions of the Middle East. In Iraq, Ceftriaxone resistance was recorded at 45.1%, while Cefotaxime resistance varied from 13% to 87% between 2014 and 2016 (Saadi *et al*., 2017; Taha & Ali, 2019). While, Turkey reported a Cefotaxime resistance rate of 47.7% from 2008 to 2011 (Özdemir *et al*., 2014; Soysal *et al*., 2016).

There were notable levels of Erythromycin resistance in many regions of Middle East ["Iran, Iraq, Jordan, and Saudi Arabia"] as mentioned in table 2.2. Furthermore, there were significant disparities in the occurrence of resistance to Tetracycline and Trimethoprim-Sulfamethoxazole across various groups, as seen in Table 2.2.

The occurrence of MDR pneumococcal strains showed discrepancies across various countries in the region. In Saudi Arabia, the MDR varied between 63-75% according to three studies (Al Ayed & Hawan, 2011; Al-Sherikh *et al*., 2014; Almazrou *et al*., 2015). In Oman, it was 18.9% (Al-Jardani *et al*., 2019), while in Qatar, it was 32.46% (Taj-Aldeen & Elshafie, 2016).

Cyprus reported a MDR prevalence of 24.1% (Koliou *et al*., 2017), and Egypt reported a prevalence at 41% (El-Nawawy *et al*., 2015). In Iran, the MDR prevalence varied between 51% and 69.4%.(Sanaei *et al*., 2012; Ghahfarokhi *et al*., 2020). Whereas Jordan indicated that the MDR varied between 14.6-56.9% (Al-Lahham & Van der Linden, 2014; Al-Kayali *et al*., 2016; Sallam, 2019). On other hand the prevalence of MDR in the State of Palestinian varied between 30-34.1% in *S. pneumoniae* collected from kids (Regev-Yochay *et al*., 2012; Nasereddin *et al*., 2013).

Although antibiotic resistance rates vary significantly between studies included in table 2.2, they are a cause for concern. These variations underscore the necessity for improved surveillance and the development of contemporary plans to combat the potential expansion of multiple drug-resistant microbes, especially in economically vulnerable countries within the Middle East area. When formulating and executing effective containment initiatives, it is essential to take into account a comprehensive range of factors, including those at the individual, social, and national levels, that contribute to antimicrobial resistance.

Table 2.2. Antimicrobial Resistance of *S. pneumoniae* among children of Middle Eastern Countries

Antibiotics/ Countries	PNC (%)	CTX (%)	CRO (%)	ERY (%)	TET (%)	TMX (%)	References
Bahrain	32.7	NA	NA	40.8	34.6	NA	(Al-Muhtaresh & Bindayna, 2020)
Cyprus	27.9 -40.8	0	5.8	28.2-39.6	12.9-31.9	17.1-58.2	(Hadjipanayis *et al*., 2016; Koliou *et al*., 2017)
Egypt	15.8-55	1-16	0	20.1- 50	49-72.6	55-96.7	(Badawy *et al*., 2017; El-Kholy *et al*., 2020)
Iran	20.8-95.3	2.9-69.8	4.5	71.4-74.4	41.9-69.9	11.8-81.3	(Houri *et al*., 2017; Gharibani *et al*., 2019; Ghahfarokhi *et al*., 2020)
Iraq	53-57.6	13-87	45.1	15.6-83		89	(Saadi *et al*., 2017; Taha & Ali, 2019)
Jordan	80-95.8	3.6-29.2	NA	46-78.2	32.3-53.8	61.4-86.6	(Al-Kayali *et al*., 2016; Al-Lahham *et al*., 2018; Al-Lahham, 2020; Al-Lahham, 2021;

							Al-Lahham *et al*., 2021)
Kuwait	64	NA	NA	NA	NA	NA	(Mokaddas & Albert, 2012; Mokaddas & Albert, 2016),
Lebanon	13.2- 29.1	12.8-18.3		30.8-40.7	NA	NA	(Hanna-Wakim *et al*., 2012)
Oman	40.9-44	0.8	0.8-1	25.8	NA	26.5	(Al-Yaqoubi, 2011; Al-Jardani *et al*., 2019)
Occupied Palestine Territory	12.6-70.1	0	NA	30.3	NA	45.9	(Regev-Yochay *et al*., 2012; Nasereddin *et al*., 2013; Daana *et al*., 2015)
Qatar	27-43.8	2-16.6	NA	22.8-30	38.14	56.78	(Al Khal *et al*., 2007; Taj-Aldeen & Elshafie, 2016)
Saudi Arabia	30-46	6-10	NA	26-77	NA	43.5-100	(Al-Mazrou *et al*., 2005; Shibl, 2008; Al-Sherikh *et al*., 2014; Almazrou *et al*., 2015)
Syria	NA						
Turkey	62-73	47.7	13	43	NA	NA	(Özdemir *et al*., 2014; Soysal *et al*., 2016)
UAE	40.2-41.3	NA	6.7-18.8	52.6	37	45.5	(Tariq *et al*, 2016)
Yemen	85-93.3	NA	NA	0	NA	20	(Al-Shamahy *et al*., 2008; Al-Ofairi *et al*., 2017)

Keywords of table 2.2: *PNC penicillin, CTX cefotaxime, CRO ceftriaxone, ERY erythromycin, TET tetracycline, TMX trimethoprim-sulfamethoxazole, NA not available*.

Pneumococcal Conjugate Vaccine Coverage among Children in the Middle East

The Global Alliance for Vaccines and Immunizations has contributed a significant task in improving the availability of immunizations for life-threatening infectious illnesses, such as pneumococcal disease, in several nations, including those in the Middle East region (Gandhi, 2020).

There is abundant evidence that PCV has a crucial role in decreasing the prevalence of non-Penicillin susceptible pneumococci in clinical environments (Sutcliffe *et al*., 2019). It was discovered that implementing PCV13 in specific Middle East regions has the power to inhibit a multitude of instances of pneumococcal illness, leading to a potential decrease

of 38% of overall fatalities of *S. pneumoniae* (Ezoji *et al*., 2019). Consequently, the PCV has resulted in the development of several pneumococcal strains that are not included in the vaccine's formulation. These emerging strains require ongoing surveillance to monitor their transmission and sequelae (Hjálmarsdóttir *et al*., 2020). Additionally, the interaction between pneumococci and other coexisting bacteria can impact the effectiveness of vaccines. There exists a natural equilibrium among these microorganisms that plays a role in determining vaccine efficacy (Bogaert *et al*., 2004).

In most Middle Eastern nations, the implementation of PCV occurred without previous awareness of the dominant serotypes (Mokaddas *et al*., 2008). Furthermore, the majority of nations in this region promptly implemented the PCV upon its adoption (Figure 1).

Unfortunately, the mortality rate due to acute lower respiratory diseases among Yemeni children is currently unclear due to the absence of updated data. However, it was found to be 9.8% between 1991 and 1995, with the highest rate observed in those who were under one year old (Banajeh, 1998). According to a report published in 2020, the pneumococcal vaccination rate in Yemeni kids aged below two years was 71.4% (Al-Tarbi & Ghouth, 2020). Furthermore, there is no national surveillance study has been conducted in Yemen. Moreover, the ongoing civil war has significantly affected the country's healthcare system, including its vaccination programs. As a result, the impact of PCV on each serotype and the trends in antimicrobial susceptibility remain unknown among Yemeni children. The absence of data is threatening the lives of several kids (Table 2.2).

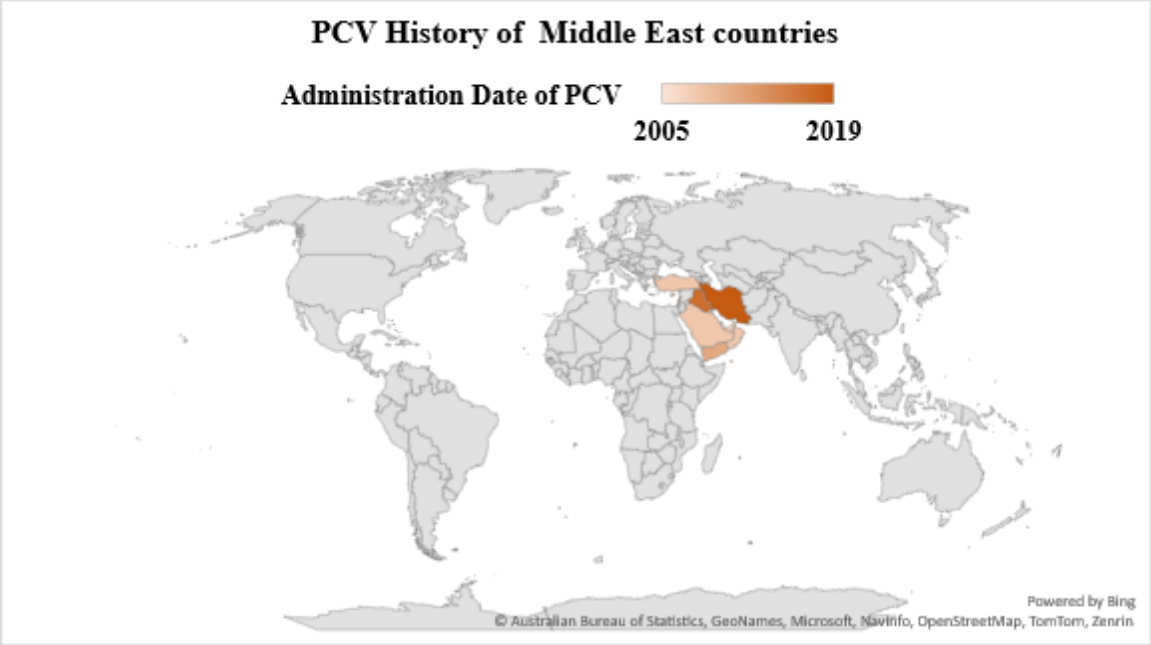

Figure 2.1: The timing of PCV administration in the public immunization plans of Middle Eastern countries, the Gulf Cooperation Council, Cyprus, and Turkey was originally used PCV7 in between 2005 and 2008, then they substituted it with either PCV10 or PCV13. Yemen implemented the introduction of PCV13 in 2011, with financial assistance provided by the WHO. Lebanon, Iraq, and Iran included PCV into their programs in 2005, 2017, and 2019, respectively. Notably, PCV is not integrate into the Egyptian and Jordanian immunization plan as per the WHO guidelines. Furthermore, the WHO website indicates a scarcity of data pertaining to Syria and the Palestinian state.

2.19 Future Challenges of Pneumococcal Diseases in Yemen

In 2017, a concerning number of meningitis cases were reported across Yemen, predominantly in children under 5 yours old. Testing of cerebrospinal fluid samples pointed to *S. pneumoniae* as the main causative agent. Although introduction of PCV to minimize pneumococcal disease but humanitarian crisis may be limited availability for many children (WHO, 2017).

Furthermore, the prevalence of systemic infection of *S. pneumoniae* and the rise of pan-resistant strains are often seen in Middle Eastern nations in which Yemen is included (Almazrou *et al*., 2015; Abdoli *et al*., 2020; Tabatabaei *et al*., 2021). The problem is compounded by the emergence of the PVSR and the presence of particularly aggressive

pathogenic and drug-resistant serotypes of *S. pneumoniae*, such as 15A as well as 24F, in this specific geographical area (Taj-Aldeen & Elshafie, 2016; Reslan *et al*., 2021).

A research investigation pertaining to immunized children revealed a substantial lowering in the quantity of vaccine serotypes. Among the whole of pneumococcal strains that were recovered, the serotypes associated with PCV10 and PCV13 constituted 2.1% and 10.4% respectively. In contrast, Non Vaccine Serotypes (NVS) comprised the majority, accounting to 76.8% (Hadjipanayis *et al*., 2016). Additionally, a case report study conducted in Turkey highlighted the possible consequences of the ineffectiveness of the PCV13 vaccination, which include the development of a complicated kind of pneumonia accompanied by empyema (Sütçü *et al*., 2017). Comparatively, a recently Japanese study designed for admitted children aged 5 years with ordinary pneumonia, established increase in Penicillin G non-susceptibility for serotypes 15A and 35B during 2016 -2018 (Takeuchi *et al*., 2020).

Unfortunately, it is worth noting that the vaccination rate in a majority of Middle Eastern nations remains below the expected threshold as reported among Yemeni children aged below two years was at 71.4% (Al-Tarbi & Ghouth, 2020). Moreover, as of the year 2021, the segment of the community in the Middle East peoples who have completed the full course of the PCV stands at only 54%. Although some improvement has been made, there is still a significant gap in achieving optimal vaccination coverage (WHO, 2022).

According to this data, many Yemeni children will be under the risk of mortality and morbidity of pneumococcal infections. In order to address the forthcoming challenges, it will be imperative for authorities, global entities, medical practitioners, and societies to collaborate in prioritizing the prevention of pneumococcal disease, enhancing healthcare infrastructure, augmenting vaccine coverage, promoting appropriate antibiotic usage, and fortifying health systems holistically.

Chapter Three
Methods
and
Materials

3. Methods and Materials

3.1 Sample size

The sample size for the study was stratified by age and population size, calculated according to the Thompson equation resulting in 385 children chosen as representatives of the children's population in Aden City. The questionnaire was utilized to gather information from the participant's parents during sample collection.

3.2 Project implementation plan

3.2.1 Inclusive and Exclusive Criteria

A sum of 385 kids participated in the current research, all of them under 17 years old. The children were categorized into two groups depending on whether they had clinical signs and symptoms of pneumonia or not. The symptomatic group consisted of 185 children who were selected from those visiting the pediatric clinics at Al-Sadaqa General Teaching Hospital and Pediatric Clinic of Al-Basatin Healthcare Center. They were clinically diagnosed by physicians for pneumonia, in accordance with the recommendations of the British Thoracic Society (Harris *et al*., 2011).

The healthy cohort comprised 200 children who were selected from primary schools and immunization centers. Children who had taken antibiotics in the two weeks preceding the sample gathering were left out of the study.

3.2.2 Sample collection

The samples were gathered by using Nylon Swab (Himedia, India) in accordance with the WHO conventional technique for identifying the existence of pneumococcal nasopharyngeal carriage. One swab was collected per child (Satzke *et al*., 2014).

The swabs were directly inoculated into STGGB medium, then kept on ice in vaccine transport box and transported into the department of microbiology laboratory at the National Center of Public Health Laboratories in Aden City for further processing.

3.2.3 Specimen Processing

The specimens were streaked over sheep blood agar plates supplemented with 5 µg/ml of Gentamycin (GBA). Afterward, they placed in candle jar and incubated at 37°C. The remaining specimen was kept frozen until processed according to the WHO protocol for pneumococci identification from swabs, and it was subsequently used for a molecular assay (Carvalho *et al*., 2010).

3.2.3.1 Isolation of *Streptococcus pneumoniae* by conventional Method

3.2.3.1.1 Requirements

1. Sheep blood, which was aseptically collected from farm animals at Al-Naqib Farm in Al-Shaik Salem, Al-Basatein district, Aden City, was used for the preparation purposes.
2. Soyabean Casein Digest Agar (Himedia, India)
3. Gentamicin Sulfate Powder (Himedia, India)
4. Sterile distilled water
5. Autoclave
6. Sterile Plastic Petri plates
7. Bunsen burner.

3.2.3.1.2 Preparation of Gentamicin Stock Solution

The final concentration of Gentamicin Stock Solution was prepared at 2.5 mg/ml per each one milliliters of stock solution according to the following steps:

1. The 50 milligrams of Gentamicin Sulfate powder were accurately weighed.
2. The following formula was applied to calculate the amount of sterile distilled water that was added to prepare a stock solution with a strength of 2.5 mg/ml:

   Volume (ml) = Weight of Gentamicin (mg) / Desired Concentration (mg/ml)

Volume (ml) = 50 mg / 2.5 mg/ml = 20 ml

3. The solution was mixed thoroughly until the powder was completely dissolved in the water.
4. The stock solution was transferred to a clean, sterile container and labeled with the name of stock solution, concentration (2500 µg/ml), and date of preparation, after that it was kept in the refrigerator till using in the preparation procedure.

3.2.3.1.3 Preparation of Gentamycin Blood Agar

The Soybean Casein Digest Agar has been prepared in accordance with the manufacturer's instructions as following:

1. 20 grams of Soyabean Casein Digest Agar was suspended in 500 milliliters of distilled water.
2. After preparation the suspended medium was sterilized by autoclave.
3. It was allowed to cool, reaching a temperature ranging from 45°C to 50°C, then 5% Sheep blood was added.
4. 1 ml of prepared gentamicin sulfate solution was added into the suspended solution, resulting in a concentration of 5 µg of gentamicin sulfate per milliliter of Sheep Blood Agar.
5. The mixture was shaked thoroughly and poured into sterile plastic petri dishes and allowed to solidify.
6. The batch GBA underwent a Sterility Test and Quality control assessment.

After the culture media went through the preparation procedure and passed the sterility testing process, the present study utilized ATCC 49619 *S. pneumoniae* for a qualitative assessment of transport and culture media, as well as an examination of potential activity of antibacterial discs and strips. This strain was supplied by the Laboratory of the organization of Medecins Sans Frontieres in Aden City.

1. Isolation of *Streptococcus pneumoniae* on Gentamycin Blood agar

The specimen was streaking on GBA and incubation for 18 to 24 hours at 37 °C under carbon dioxide of candle jar technique, the colonies that were typically small, smooth, round, and slightly mucoid with a greenish discoloration (alpha-hemolytic colonies) of the GBA surrounding them and their size about 0.5-1.0 mm in diameter were considered presumptively as *S. pneumoniae* that needed to be confirmed by a Gram stain and an Optochin susceptibility test.

2. Gram Stain

A smear was prepared from GBA for alpha-hemolytic colonies and stained as per the manufacturer's specifications (HIMDIA, India). The bacteria, observed as groups of two cells or short chains with a positive gram reaction and diameters ranging from 0.5 to 1.0 micrometers, were labeled as diplococci after testing negative for catalase activity. These isolates were subcultured on sheep blood agar with an applied Optochin disc to confirm their sensitivity.

3. Optochin Sensitivity Test Procedure

Optochin disks impregnated with 5 µg of ethylhydrocupreine hydrochloride were used to distinguish *S. penumoniae* from other α haemolytic streptococci depending on the sensitivity profile, where any isolated growth and produced 14 mm inhibition zone or greater, considered as *S. pneumoniae*. The gram positive cocci which produced inhibition zone less than 14 mm subjected to further confirmation by bile solubility test (Riedel *et al*., 2019).

The following Steps of Optochin disc diffusion test were done according to manufacturing recommendations (HiMedia India):

1. A Mueller-Hinton Sheep Blood agar plate was prepared and allowed to dry and subjected to quality control as mentioned previously.
2. The plate was inoculated with the test organism by using a sterile swab.

3. The inoculum was allowed to dry for a few minutes.
4. An ethylhydrocupreine hydrochloride disk was installed onto the midpoint of the inoculum using sterile forceps.
5. The disk is pressed gently onto the agar using sterile forceps to ensure proper adherence.
6. The medium is kept at 37°C in 5-10% carbon dioxide atmosphere in incubator for 18-24 hours.
7. After incubation, the plate is examined for a zone of inhibition around the optochin disk.

4. Bile solubility Test

These isolated strains which had inhibition zones of less than 14 mm were tested to bile solubility test to confirm it as pneumococci or not.

The following Steps of Bile solubility Test according to manufacturer recommendations (HiMedia, India):

1. A heavy suspension of a pure culture was prepared in 2 ml of 0.85% normal saline.
2. The suspension was divided into two tubes of 1 ml.
3. The turbidity was adjusted to 0.5 according to McFarland standard.
4. 2 drops of 2% sodium deoxycholate were added to the tested organism and mixed properly and 2 drops of sterile distilled water was added to control tube and mixed.
5. The tested organism and control tubes were incubated for 10-15 minutes at 35-37°C in water bath.
6. The turbidity was observed for a clearing of the tube containing 2% sodium deoxycholate by comparing it with control.
7. When a negative result was reported, the tubes were incubated for up to 3 hours and checked for clearing again.

3.2.3.2 Antibiogram Test

Antibiogram Test was conducted by Kirby-Bauer technique as a screening test (Sastry & Bhat, 2019). While Epsilometer Test (E Test) was selected as the easy and proper procedure for measuring the breakpoint of tested antibiotics (Sader & Pignatari, 1994), so

this method used for detecting the concentration break point for resistant strains. The results of the E Test were evaluated based on the recommendation of CLSI on Mueller Hinton agar media enriched with 5% sheep blood. The "*S. pneumoniae* ATCC49619" was utilized as a reference standard every testing session as advised by CLSI.

The confirmed *S. pneumoniae* were spread onto the "Mueller Hinton agar" enriched with blood of sheep, after which the plate was left to dry. Subsequently, 1 µg Oxacillin discs, 10 µg Erythromycin discs, 15 µg Azithromycin discs, and 30 µg Doxycycline discs (Himedia, India) were applied. Resistant strains, characterized by inhibition zones of less than 20 mm for Oxacillin, were subjected to the E test to measure their MIC for Penicillin, Ceftriaxone, Cefepime, and Co-amoxiclav (Himedia, India). Isolates exhibiting resistance to Erythromycin, Azithromycin, and Doxycycline (Himedia, India) underwent MIC testing using the E Test. Both the Kirby-Bauer test and E technique were evaluated in accordance with the CLSI guidelines 2021.

3.2.3.3 Serotyping

The isolated colonies of pneumococci were transferred into "Todd Hewitt broth" supplemented with serum rabbit (Himedia, India) after that incubated overnight at 37 °C (Carvalho *et al*., 2010) then used for serotyping purposes by applying Pneumotest-Latex Kit (Staten Serum Institute, Denmark) This test is a rapid readymade latex agglutination test that is usually used for direct detection of serotypes and serogroups of *S. pneumoniae* in blood cultures or on plate cultures (Otte *et al*., 2014).

In the serotyping process, a sterile inoculation loop, a mixing stick, and a reaction card were used to mix a loopful of overnight bacterial growth with saline and Pneumotest-Latex reagents. This was done after thoroughly shaking the reagent and allowing it to reach room temperature, according to the recommendations of manufacturer as follows:

1. 10 µL of bacterial suspension from overnight growth of Serum Todd Hewitt broth was applied to reaction card.
2. 10 µL of latex reagent was placed on reaction card beside the bacterial suspension.
3. The drops were mixed using a mixing stick, and the resulting suspension was then spread to cover the circular area.

4. Manual agitation was applied to the card for 3-10 seconds, and the process of agglutination was observed. Any reaction occurring after 10 seconds was considered negative.

3.2.3.4 Molecular Detection *S. pneumoniae*

"Real-time PCR targeting the Autolysin-Encoding Gene (*lytA*)" has been recommended as a rapid and reliable non-culture assay to complement the gold standard technique for detecting *S. pneumoniae* (Riedel *et al*., 2019). In this study, the remaining specimens that stored in STGGB tubes at -20°C were used for Pneumococci detection using the TRUPCR *Streptococcus pneumoniae* Detection Kit Version 1.0 (Blackbio Biotech, India) through Applied Biosystem 7500 Fast Real-Time PCR.

TRUPCR *Streptococcus pneumoniae* Detection Kit

The TRUPCR *Streptococcus pneumoniae* Detection Kit (V-1.0) is a stablise molecular amplification assay. It is specifically designed to detect the presence of S. *pneumoniae* DNA in clinical samples using the Real-Time PCR technique. Particularly, this kit incorporates an endogenous internal control that aids in assessing the presence of PCR inhibition and ensuring the accuracy.

The TRUPCR *Streptococcus pneumoniae* Detection Kit Version 1.0 consists of the following reagents:

i. Master Mix reagent, which includes the following ingredients:
   1. Hot-Start DNA Polymerase
   2. Reaction Buffer
   3. dNTPs (dATP, dCTP, dGTP, dUTP)
   4. $MgCl_2$ and Stabilizers

ii. Primer Probe Mix, which is a specific mix for detecting *S. pneumoniae* and an endogenous internal control.

iii. Sterilized water was used as Negative control.

iv. Positive control, which is the *S. pneumoniae* Positive control.

DNA Extraction

The following procedure was conducted to extract DNA from specimens using the TRUPCR *Streptococcus pneumonia* Extraction kit according to the recommendations of the manufacturer (Blackbio Biotech, India) as follows:

1. 1.5 ml microcentrifuge tube was prepared, and 25 µl of Proteinase K was pipetted into it. Prior to use, Proteinase K had been dissolved in the designated amount of proteinase buffer.
2. 200 µl of the specimen was mixed into the microcentrifuge tube.
3. 300 µl of Buffer BAV1, containing carrier RNA at a potency of 28 µg/ml, were introduced into the microcentrifuge tube.
4. The cap was securely fastened, and the contents were agitated using pulse-vertexing for 15 seconds to guarantee complete homogeneity.
5. The microcentrifuge tube which contains the homogeneous solution was incubated at 56°C for 15 minutes using a heating block to facilitate lysis and DNA release.
6. A brief rotation of the 1.5 ml vial was conducted to remove any remaining droplets from the inside surface of the lid.
7. 250 µl of ethanol (96-100%) was incorporated into the specimen in the microcentrifuge tube. The cap was closed, and the contents were thoroughly mixed by pulse-vertexing for 15 seconds. The lysate was then incubated with ethanol for 5 minutes at ambient temperature (15-25°C).
8. Another brief spinning of the 1.5 ml vial was conducted to get rid of any leftover droplets on the inner side of the cap.
9. The entire lysate from step 7 attentively conveyed onto the Spin column. The cap was tightly fastened, then centrifugation was carried out at 10,000 rpm for 1 minute (Fisherbrand accuSpin Micro17R Microcentrifuge).
10. The column was installed into a collection tube, and then carefully opened. Next, 500 µl of Buffer BAW1 was added, ensuring no contact with the edges. The cap was tightly fastened, and centrifugation was performed at 10,000 rpm for 1 minute, after which the filtrate was disposed of

11. The column was carefully unsealed, and 500 microliters of Buffer BAW2 was added. The cap was securely fastened, and spinning was performed at 10,000 rpm for 1 minute, with the liquid portion discarded.
12. The column was cautiously opened, and 500 µl of ethanol (96-100%) was introduced. The cap was fastened again, and centrifugation was performed at 10,000 rpm for 1 minute, with the filtrate being eliminated.
13. The column was moved into a new 2 milliliter collection tube and spun at the highest speed "14,000 rpm" for 3 minutes to completely dry out the silica filter.
14. The column was placed in a sterile 1.5 ml microcentrifuge tube, and the capture vial along with the filtrate was disposed of. The cover of the column was cautiously lifted, and a volume of 50-60 µl of Buffer BRE was precisely introduced into the middle of the membrane. The lid was closed, and the column was kept at room temperature for 1 minute. Afterward, the mixture underwent centrifugation at a speed of 10,000 revolutions per minute for 1 minute.

Reaction Procedure

1. In three separate 0.2 ml PCR tubes, 10 µl of master mix was added to 10 µl of Primer Probe Mix. These tubes were then labeled as Test, Positive Control, and Negative Control and securely closed.
2. For each of the labeled tubes, 5 µl of the previously prepared 20 µl extracted DNA sample was added. Additionally, 5 µl of Positive control and 5 µl of Negative control were introduced into their respective labeled tubes.
3. Each batch included the analysis of a total of 22 samples, which comprised 20 samples under investigation along with the inclusion of both positive and negative control samples. This approach was implemented to maintain the integrity of the testing process, allowing for accurate interpretation of the results while considering the presence of control samples for quality assurance purposes.

Program Set Up

The Applied Biosystem 7500 Fast Real-Time PCR instrument was configured with the following settings for the RT-PCR process:

Steps	Temperature °C	Time	Dye Acquisition	Cycles
1	37	5 minutes	-	1
2	94	10 minutes	-	1
3	94	10 second	-	40
4	60	60 second	Yes	

The Hexachlorofluorescein (Hex) channel was chosen for the detection of *S. pneumoniae* using the reporter, while the orange channel was utilized for the endogenous internal control. In addition, the manufacturer's recommendations were followed for the analysis of the results. According to the recommendations, it was expected that the negative control reactions for each probe/primer set would not display luminescence expansion curves crossing the cut-off limit. The occurrence of a false positive in the primers and probe non-template control reactions could indicate potential sample contamination.

The positive control reactions, the expected Ct values [“Cycle Threshold”] were determined based on the manufacturer's guidelines. Specifically, in the Hex channel, the anticipated Ct value for *S. pneumoniae* was expected to be around 20±3, while in the orange channel, the expected Ct value for the Endogenous Internal Control was also expected to be around 20±3.

Regarding the clinical samples, the manufacturer recommended that all specimens should display endogenous internal control reaction curves crossing the cut-off limit at or prior to 32 cycles. This would indicate the presence of sufficient DNA from human genes and confirm the acceptability of the specimen's quality.

It should be noted, however, that certain samples may exhibit abnormalities potentially caused by errors or contaminants present during the original critical analysis. Moreover,

samples obtained from animals or other species, as well as cell cultures, may either show no endogenous internal control reaction or a weak one.

The failure to detect the endogenous internal control in patient specimens can point out an issue or error in the analysis, as per the manufacturer's recommendations.

3.3 Ethical Declaration

The investigation awarded ethical permission from the Research and Ethics Committee of the University of Aden, Yemen under the Research Regulation REC-98-2021. The researchers collected written informed consent from the children's parents. This form informed them about the sample collection and assured them that information about their children would be kept confidential for research purposes only. They therefore had the right to withdraw their children from the research project if they did not agree.

3.4. Data Analysis

The data analysis was computed by SPSS ["Statistical Package for the Social Sciences version 22 (SPSS V 22)"] and EZ SPSS Tutorials. The data cleaning and categorization tasks were performed using SPSS V 22.

The rates of colonization were determined by using percentage calculations. In the same manner, percentages were calculated for every age group, serotype, and rate of resistance using SPSS Version 22. The EZ SPSS Tutorials were utilized to evaluate the correlation among the rate of *S. pneumoniae* carriage and associated risk variables, and to analyze any significant discrepancies in antibiotic resistance. The evaluation utilized the Chi-square ($\chi 2$) test and Odds Ratio (OR), specifying the Confidence Interval (CI) at 95%. The conducted analysis employed a significant level with a p-value of 0.05 or less, which was considered statistically significant.

Chapter Four
Results & Discussion

4.1. Results

A total of 385 children were enrolled for the entered study. Among them, 185 children exhibited typical signs and symptoms associated with pneumonia, while the remaining 200 were healthy. The mean age of the participants was 8.549 years, with a standard deviation of 4.245 years. Females constituted 51.7% of the overall sample, while males accounted for 48.3%.

The prevalence rate of pneumococci in the total cohort of this study was 44.4% (95% CI, 39.5-49.4%) as detected by the culture method and 57.1% (95% CI, 52.2-62%) as identified by the RT-PCR technique. There wasn't a notable variance in the carriage rate between genders across the entire cohort (Table 4.1).

Table 4.1. Demographic profile of participants of study sample from Aden city

Age group (Years)	Frequency (%)	Female (%)	Male (%)
2 — 6	142(36.8)	68 (47.9)	74 (52.1)
7—11	135(35.1)	73 (54.1)	62 (45.9)
12—17	108(28.1)	58 (53.7)	50 (46.3)
Total	385(100)	199 (51.7)	186 (48.3)
Minimum	2 years	**Maximum**	17 years
Mean	8.549 years	**S.D.**	4.245 years

Among the pneumonic group, the carriage rate was 45.9% (95% CI, 38.9-53.1%) using the culture method and 59.5% (95% CI, 52.3-66.3%) using the RT-PCR technique. In the healthy group, the carriage rate was 43% (95% CI, 36.3-49.9%) with the culture method and 55% (95% CI, 48.1-61.7%) with the RT-PCR technique (Table 4.2 and 4.3).

Regarding the prevalence rates within the pneumonic cohort, the culture technique showed rates of 49.3%, 54.8%, and 29.8% among age groups of 2-6 years, 7-11 years, and 12-17 years, respectively. While in the healthy children, the rates were 50.7%, 31.5%, and 48.2% for the corresponding age groups (Table 4.2).

Table 4.2. Prevalence of *S. pneumoniae* carriage in pneumonic and healthy children of Aden City, by age group, using culture method.

Age Group (years)	Nasopharyngeal Prevalence of Pneumococci					
	Pneumonic Children (%)			Healthy Children (%)		
	Negative	Positive	Total	Negative	Positive	Total
2— 6	36(50.7)	35(49.3)	71(38.4)	35(49.3)	36(50.7)	71(35.5)
7— 11	28(45.2)	34(54.8)	62(33.5)	50(68.5)	23(31.5)	73(36.5)
12— 17	36(69.2)	16(29.8)	52(28.1)	29(51.8)	27(48.2)	56(28.0)
Total	100(54.1)	85(45.9)	185(100)	114(57.0)	86(43.0)	200(100)

On the other hand, when the RT-PCR technique was utilized, the prevalence rates of pneumococci among the age groups of 2-6 years, 7-11 years, and 12-17 years in the pneumonic cohort were 59.2%, 67.7%, and 50%, respectively. In contrast, the rates in the healthy group were 64.8%, 43.8%, and 57.2%, respectively (Table 4.3).

Table 4.3. Nasopharyngeal occurrence of Pneumococci in pneumonic and healthy kids in Aden City using RT-PCR

Age Group (years)	Nasopharyngeal Carriage Rate of *S. pneumoniae*					
	Pneumonic Children (%)			Healthy Children (%)		
	Negative	Positive	Total	Negative	Positive	Total
2— 6	29(40.8)	42(59.2)	71(38.4)	25(32.2)	46(64.8)	71(35.5)
7— 11	20(32.3)	42(67.7)	62(33.5)	41(56.2)	32((43.8)	73(36.5)
12— 17	26(50)	26(50)	52(28.1)	24(42.9)	32(57.2)	56(28.0)
Total	75(40.5)	110(59.5)	185(100)	90(45)	110(55)	200(100)

In the present investigation, *S. pneumoniae* was identified using RT-PCR in 220 samples out of 385 nasopharyngeal swabs, including 170 with a positive culture. In contrast, the culture method detected a lower number of positive cases, specifically 171. The RT-PCR

had a greater level of sensitivity (99.5%) and infinite specificity in comparison to the conventional culture method. The true positive detection rate of RT-PCR was determined to be 100%, indicating that a positive test result accurately signifies the existence of the target condition. Furthermore, the negative predictive value of RT-PCR was determined to be 99.4%, indicating the probability of a negative test result precisely excluding the target condition. The data demonstrate the high accuracy of RT-PCR, achieving a 99.7% accuracy rate. This highlights the significant influence of the selected testing methodology on the outcomes of *S. pneumoniae* detection (Table 4.4).

Table 4.4. Performance measures of Real-time PCR

Real-time PCR	
Characteristics	**Results**
True positive	220
False Negative	1
False Positive	0
True Negative	164
Sensitivity	99.5%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	99.4%

Contrary to expectations, the vaccination rate in the total cohort was 76.1% (95% CI, 71.6-80.1%). It is noteworthy that the rate of vaccination was 73.7% in the pneumonic group and 78.5% among the healthy children (Figure 4.1).

During the study of the pneumonic group, several observations were made. Among the participants, 91.4% experienced fever, 82.2% reported sustained breath shortness, 82.2% complained of rapid breathing, 89.7% had a cough, 18.8% exhibited wheezing, 82.7% had a respiration rate greater than 20 Beats Per Minute, 93.5% had a pulse rate greater

than 100 Beats Per Minute, and 17.8% had an oxygen saturation rate less than 95% (Figure 4.2).

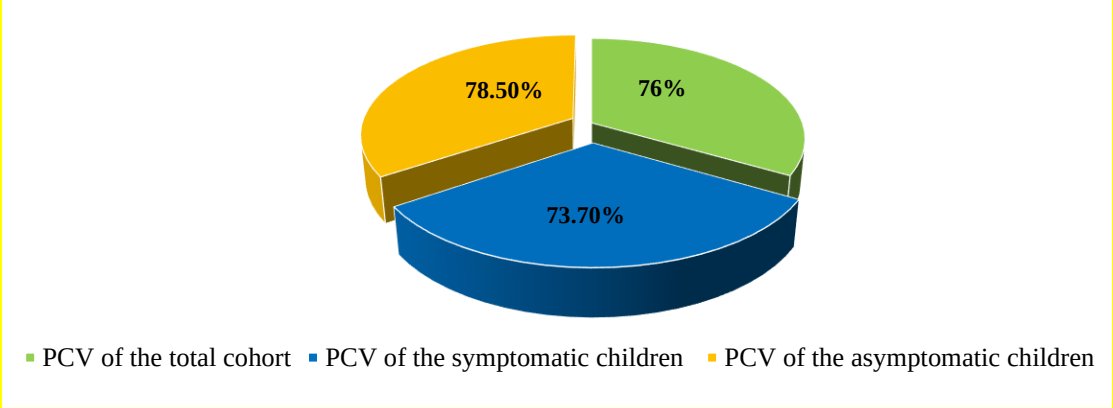

Figure 4.1. Vaccination rate among pneumonic, healthy cohorts, and total children

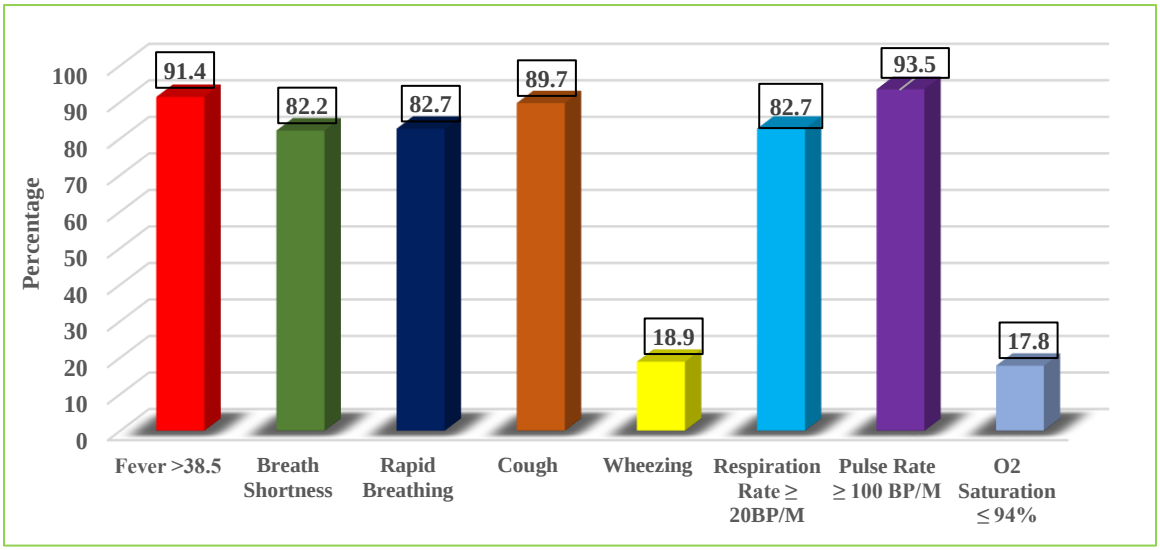

Figure 4.2. Clinical characteristics of pneumonic children in Aden City.

Furthermore, it was found that 40% of the pneumonic cohort had a total white blood cell count greater than 11,000/µl. Among the group, 33% had a differential count of neutrophils gone beyond the normal cut-off (>70%), 29.7% had a lymphocyte count that

exceeded the established normal cut-off point (>40%), and 39.5% of the pneumonic children tested positive for the C-Reactive Protein screening test (Figure 4.3).

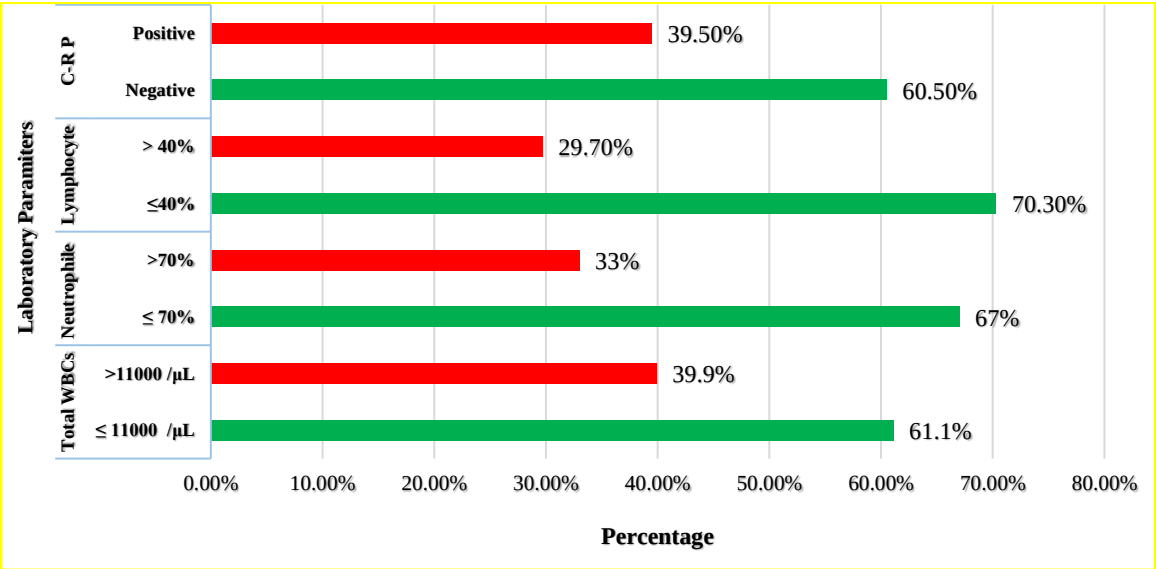

Figure 4.3. Laboratory parameters among pneumonic children in Aden City.

However, in this study, there was no significant association observed between pneumococcal carriage and the associated factors. Although there was a slight association between carriage rate and age less than nine years (OR = 1.6; 95% CI 1-2.4; p = 0.03), the carriage rate among children from families with single rooms showed significance association (OR = 7.9; 95% CI 4.9-12.8; p = 0.00001). Furthermore, the nasopharyngeal carriage was markedly higher in kids who shared sleeping space with their family members (OR = 15.1; 95% CI 7.1-32.2; p = 0.00001). Additionally, there was a statistically significant association (OR = 2.02; 95% CI 1.2-3.4; p = 0.007) between pneumococcal carriage and low monthly incomes of less than 100 US dollars per family (Table 4.5).

It was observed that the covering rate of PCV 13 was 76%. The rate of PCV 13 serotypes were demonstrated in 80.2% of the isolated pneumococcal strains from the healthy. In contrast, the prevalence of PCV-13 serotypes among pneumonic children was 71.8%.

Table 4.5. Associated risk factors and carriage of *S. pneumonia* among total children cohort of Aden City.

Associated Risk Factors		Frequency (%)	Carriage by Culture (%)		P-Value	OR (95% CI)
			Negative	Positive		
Sex	Male	186(48.3)	105(56.5)	81(43.5)	0.74	0.93(0.62–1.4)
	Female	199(51.7)	109(54.8)	90(45.2)		
Age	≤ 9	227(59)	116(51.1)	111(49.9)	0.03	1.7(1–2.4)
	≥ 10	158(41)	98(62)	60(38)		
No. of Siblings <6 Years	0	78(20.3)	28(36)	50(64)	0.088	1.6(0.9–2.6)
	≥ 1	307(79.7)	143(46.6)	164(53.4)		
No. Of Rooms Per Family	1	134(34.8)	33(24.6)	101(75.4)	0.00001	7.9(4.9–12.8)
	≥ 2	251(61.1)	181(75.2)	70(24.8)		
Sleeping in One Room	Yes	286(74.3)	123(43)	163(57)	0.00001	15.1(7.1–32.2)
	No	99(25.7)	91(91.9)	8(8.1)		
Domestic Animals	Yes	40(10.4)	22(55)	18(45)	0.93	1.03(0.5–1.9)
	No	345(89.6)	192(55.7)	153(44.3)		
Smoker in Family	Yes	159(41.3)	81(50.9)	78(49.1)	0.12	1.4(0.9–2.1)
	No	226(58.7)	133(44.3)	93(55.7)		
Using Incense	Yes	168(43.6)	93(55.4)	75(44.6)	0.93	1.02(0.68–1.5)
	No	217(56.4)	121(55.8)	96(44.2)		
Using Mosquito Coil	Yes	171(44.4)	95(55.6)	76(44.4)	0.99	1.0(0.67–1.5)
	No	214(56.6)	119(55.6)	95(44.4)		
Monthly Income ≤ 100$	No	302(78.4)	157(52)	145(48)	0.007	2.02(1.2–3.4)
	Yes	83(21.6)	57(68.7)	26(31.3)		

The predominant pneumococcal serotypes among healthy children and those with pneumonia were 19 (15.1% and 8.2%), 1 (12.8% and 11.8%), 4 (9.3% and 12.9%), 5 (12.8% and 8.2%), 2 (9.2% and 7.1%), 23 (8.1% and 7.1%), 3 (7.0% and 7.1%), 6 (5.7% and 7.1%), 22 (2.3% and 7.1%), 15 (4.7% and 3.5), 7 (2.3% and 3.5%), 14 (3.5% and 2.4%), 9 (2.4% and 2.3%), and 18 (1.2% and 2.3%) respectively. Additionally, the non-PCV-13 serotypes accounted for 19.8% of isolates from healthy children and 28.2% of pneumococci isolated from pneumonic group. The most common non-PCV-13 serotypes

identified from the healthy and pneumonic children were 2 (9.3% and 8.2%), 15 (4.7% and 3.5%), and 8 (1.2% and 1.2%) respectively (Figure 4.4).

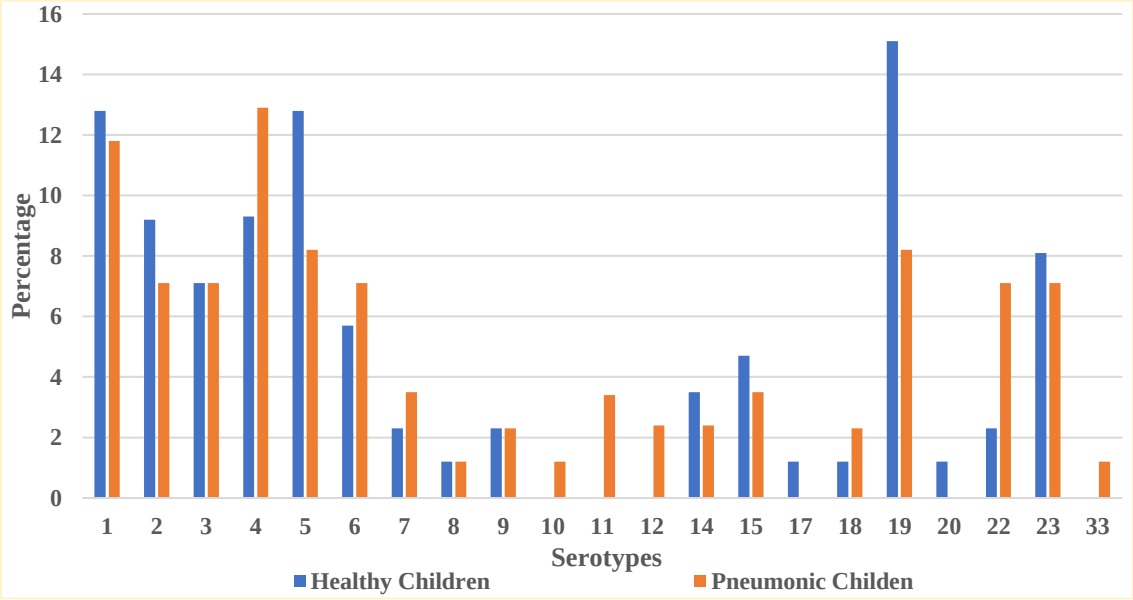

Figure 4.4. The Pneumococcal Serotypes among Healthy and Pneumonic Children in Aden City.

Furthermore, the pneumococci isolated from both pneumonic and healthy children showed low susceptibility rates (SRs) when checked by the disk diffusion method. The susceptibility rates for Oxacillin were 2.4% and 4.7%, for Erythromycin were 29.4% and 25.6%, for Azithromycin were 50.6% and 57.0%, and for Doxycycline were 20% and 24.4% respectively. Nevertheless, there was no notable difference in antimicrobial resistance was observed between the isolates from both cohorts (Table 4.6).

The Penicillin resistance (meningitis breakpoint) was observed in 96.5% of the total cohort, with rates of 95.3% among healthy children and 97.6% in the pneumonic cohort. Conversely, the Penicillin resistance (non-meningitis breakpoint) was found in 13.3% of the total cohort. Additionally, resistance to Cefepime (meningitis breakpoint) was detected in 15.8% of the total cohort, while no resistance was observed for the non-meningitis breakpoint. Similarly, the Ceftriaxone resistance (meningitis breakpoint) was

Table 4.6. Antimicrobial susceptibility patterns of *S. pneumoniae* isolated from pneumonic and healthy cohorts of Aden City (disc diffusion method), and the differences between two groups of study in terms of antibiotic resistance.

Antibacterial Agents	Breakpoints in mm	Pneumonic Frequency (%)	Healthy Frequency (%)	(χ^2)	*P*- Value
Oxacillin	≥20 (S)	2(2.4)	4(4.7)	0.667	0.41
	≤20 (R)	83(97.6)	82(95.3)		
Erythromycin	≥21 (S)	25(29.4)	22(25.6)	1.27	0.25
	16-20 (I)	16(18.8)	26(30.2)		
	≤20 (R)	44(51.8)	38(44.2)		
Azithromycin	≥18 (S)	43(50.6)	49(57.0)	1.46	0.23
	14-17 (I)	12(14.1)	14(16.3)		
	≤13 (R)	30(35.3)	23(26.7)		
Doxycycline	≥28 (S)	17(20)	21(24.4)	0.72	0.40
	25-27 (I)	20(23.5)	22(25.6)		
	≤24 (R)	48(56.5)	43(50.0)		

Keywords of table 4.6. [S = Sensitive, I = Intermediate, R = Resistant, and χ^2 = Chi squire Test].

observed in 16.4% of the total cohort, while it was absent at the non-meningitis breakpoint among the healthy group and only 1.2% among the symptomatic children. In contrast, the pneumococci showed 100% sensitivity to Amoxicillin/clavulanic acid. The results showed no notable disparity in antibiotic resistance between the isolates from both research groups. However, a significant difference (χ^2 = 3.9; p = 0.04) was found in the resistance to Penicillin (Non-Meningitis Break Points) between the isolates of the two cohort groups (Table 4.7).

Table 4.7. Comparison of pneumococcal resistance and antibiotic non-susceptibility rates in two study groups of children in Aden City using E test

Antibacterial Agents		R in the Pneumonic children (%)	R in the Healthy children (%)	χ^2	*p*-value
Penicillin	M	83 (97.6)	82(95.3)	0.667	0.41
	Non-M	7(8.2)	16 (18.6)	3.94	0.04
Cefepime	M	10(11.8)	17 (19.8)	2.05	0.15
	Non-M	0	0	-	-
Ceftriaxone	M	10 (11.8)	18(20.9)	2.62	0.11
	Non- M	1(1.2)	0	-	-
Amoxiclav		0	0	-	-

Keywords of Table 7.4 [M = Meningitis, Non-M = Non- Meningitis, R = Resistance, and χ^2 = Chi squire Test].

The data revealed a significant frequency of Penicillin resistance within pneumococcal isolates from healthy children, reaching a rate of 95.3%. Among the resistant strains, 19.8% exhibited a resistance level ranging between 0.123 ug/ml and the fold of the meningitis CLSI breakpoint for resistance, while the remaining 75.6% had a resistance rate greater than two times the meningitis CLSI breakpoint for resistance. Similarly, among pneumococcal isolates from the pneumonic cohort, the prevalence of Penicillin resistance was 97.6%. Notably, 10.6% of these resistant strains displayed a resistance level falling between 0.123 µg/ml and the fold of the meningitis CLSI breakpoint for resistance, while the majority (87.1%) of the resistant strains showed a resistance exceeding two times the meningitis CLSI breakpoint for resistance. The rates of non-meningitis Penicillin resistance were 18.6% for the healthy cohort and 8.2% for the pneumonic group.

Regarding the resistance rate to Ceftriaxone among pneumococcal serotypes isolated from children with pneumonia, based on the CLSI meningitis cut-off, it was 11.8%.

Table 4.8. Minimum inhibitory concentration of β lactam antibiotics against *S. pneumoniae* isolated from the tow cohorts in Aden City, according to the CLSI meningitis MIC values (E-test)

Antibiotics	MIC Breakpoints in µg/ml		Symptomatic	Asymptomatic
Penicillin	**Meningitis**	≤ 0.06 (Sensitive)	2(2.4%)	4 (4.7%)
		0.12 (Resistant)	9 (10.6%)	17 (19.8%)
		≥0.5	74 (87.1%)	65 (75.6%)
	Non-Meningitis	≤2 (Sensitive)	66 (77.6%)	60 (69.8%)
		4 (Intermediate)	12 (14.1%)	10 (11.6%)
		≥8 (Resistant)	7 (8.2%)	16 (18.6%)
Ceftriaxone	**Meningitis**	≤0.5 (Sensitive)	58 (68.2%)	54 (62.8%)
		1 (Intermediate)	17 (20%)	14 (16.3%)
		2 (Resistant)	9 (10.6%)	11 (12.8%)
		≥4	1 (1.2%)	7 (8.1%)
	Non-Meningitis	≤1(Sensitive)	70 (82.3%	73 (84.9%)
		2(Intermediate)	14(16.5%)	13 (15.1%)
		≥4(Resistant)	1 (1.2%)	0
Cefepime	**Meningitis**	≤0.5(Sensitive)	58(68.2%)	53 (61.6%)
		1(Intermediate)	17(20%)	16 (18.6%)
		2 (Resistant)	10 (11.8%)	17 (19.8%)
		≥4	0	0
	Non-Meningitis	≤1(Sensitive)	71 (83.5%)	73 (84.9 %)
		2(Intermediate)	14 (16.5%)	13(15.1%)
		≥ 4 (Resistant)	0	0

It was observed that 1.2% of the Ceftriaxone non-susceptible strains exhibited a resistance rate double the resistance cut-off. Conversely, for strains isolated from healthy children, the rate was 20.9%, and 9.3% of the resistant strains demonstrated a resistance rate also double the non-susceptible cut-off of CLSI (Table 4.8).

The study found that 51.8% of pneumococcal serotypes isolated from children with pneumonia exhibited resistance to Erythromycin, as determined by the CLSI. Within this set of strains, a proportion of 36.5% exhibited resistance that was either equal to or beyond twice the established threshold of CLSI cut-off. On the other hand, the strains collected from healthy children showed a resistance rate of 44.2%. Among these resistant strains, 40.7% exhibited resistance levels that were equivalent to or greater than double the predetermined threshold. Furthermore, the rate of Azithromycin resistance in pneumococcal serotypes obtained from pediatric pneumonia cases, using the CLSI threshold was 35.3%. Among these strains, 36.5% demonstrated resistance at or above double the specified threshold. Conversely, strains derived from healthy children displayed a resistance prevalence of 44.2%, with 40.7% of these resistant strains indicating resistance equal to or surpassing twice the officially set threshold.

The prevalence of resistance to Doxycycline in pneumococcal serotypes isolated from cases of pediatric pneumonia, as determined by the CLSI guidelines, was found to be 56.5%. 34.1% of isolates had resistance levels that met or exceeded double the recommended threshold. In contrast, strains obtained from children who were healthy had a resistance rate of 50%, while 22.1% of these resistant isolates demonstrated resistance levels that were equivalent to or greater than twice the CLSI set (Table 4.9).

Among the strains isolated from healthy children, the serotypes that exhibited high Penicillin resistance based on the CLSI meningitis cut-off were 19, 1, 5, 4, 2, 23, and 3. Likewise, serotypes 19, 2, 3, 5, 4, and 14 were commonly resistant strains of Ceftriaxone. While serotypes 19, 3 and 5 were the most resistant to Cefepime.

Table 4.9. The Minimum inhibitory concentration of Macrolide and Doxycycline against *S. pneumoniae* isolated from healthy children and pneumonic group in Aden City, according to the CLSI meningitis values (E-Test)

Antibiotics	MIC Breakpoints in µg/ml	Pneumonic	Healthy
Erythromycin	≤0.25 (Sensitive)	25(29.4)	22 (25.6%)
	0.5 (Intermediate)	16(18.8)	26 (30.2%)
	1 (Resistant)	13 (15.3%)	3 (3.5%)
	≥ 2	31(36.5%)	35 (40.7%)
Azithromycin	≤0.5(Sensitive)	43(50.6)	49 (57.0)
	1(Intermediate)	12(14.1)	14 (16.3)
	2 (Resistant)	27 (31.8%)	21 (24.4%)
	≥4	3 (3.5%)	2 (2.3%)
Doxycycline	≤ 0.25(Sensitive)	17 (20%)	21 (24.4)
	0.5(Intermediate)	20(23.5%)	22 (25.6)
	1 (Resistant)	19 (22.4%)	24 (27.9%)
	≥ 2	29 (34.1%)	19 (22.1%)

Additionally, among the isolates from healthy children, the serotypes with multiple resistance to Penicillin, Ceftriaxone and Cefepime, as tested according to the CLSI meningitis breakpoint, were 19, 1, 5, 4, 3, 6, 7, and 8 respectively (Figure 4.5). While the common serotypes which isolated from children with pneumonia displaying high Penicillin resistance according to the CLSI meningitis cut-off were 4, 1, 2, 3, 5, 6, 19, 22, and 23. Also, serotypes 1, 4, 6, 2, 3, 5, and 19 were commonly resistant strains of Ceftriaxone. While serotypes, 1, 4, 6, 2, 3, 5, and 19 were the most resistant to Cefepime. The serotypes with multiple resistance for the three β-lactam antibiotics among children with pneumonia were 4,1, 2, 3. 5and 19 (Figure 4.6).

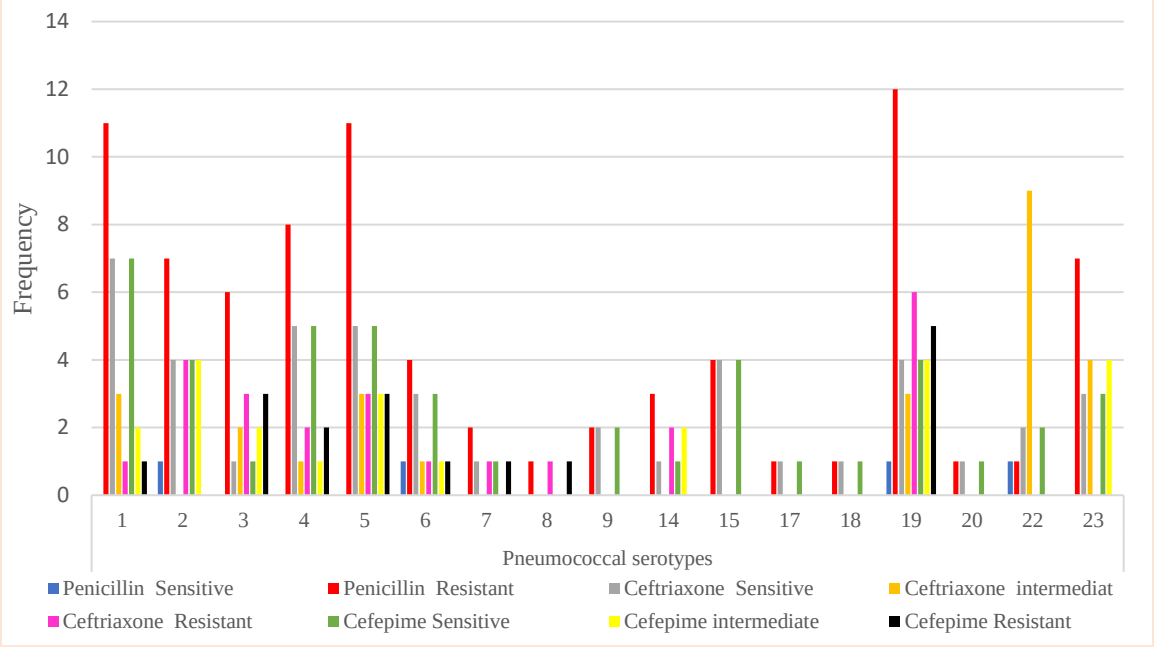

Figure 4.5. Frequency of Beta-lactam-resistant serotypes of *S. pneumoniae* isolated from a healthy cohort in Aden City, according to the CLSI Meningitis MIC values.

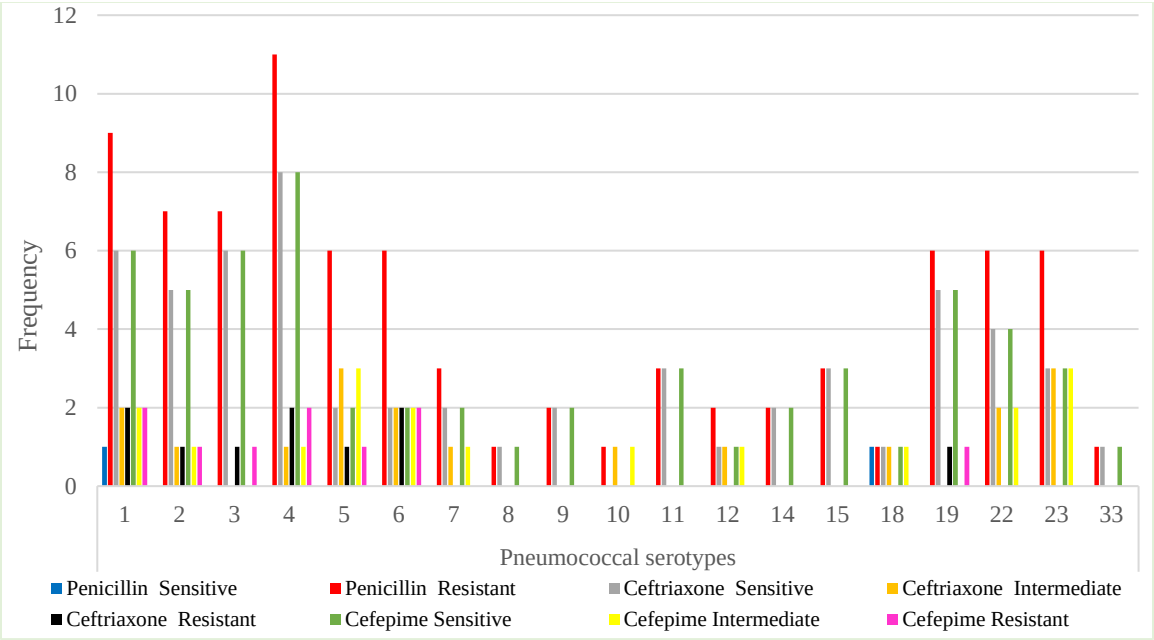

Figure 4.6. Frequency of Beta-lactam-resistant serotypes of *S. pneumoniae* gathered from Pneumonic children within Aden City, according to the CLSI Meningitis MIC values.

The most prevalent serotypes of *S. pneumoniae* that exhibited resistance to Erythromycin within the health group included 1, 2, 4, 5, 19, 15, and 23. Similarly, the serotypes resistant to Azithromycin were 1, 5, 19, 2, 4, 6, and 23. Additionally, resistance against Doxycycline was observed in serotypes 1, 19, 4, 5, 23, 2, and 15. Notably, the most prevalent resistant serotypes for Erythromycin , Azithromycin, and Doxycycline were 1, 2, 4, 5, 19, and 23 (Figure 4.7).

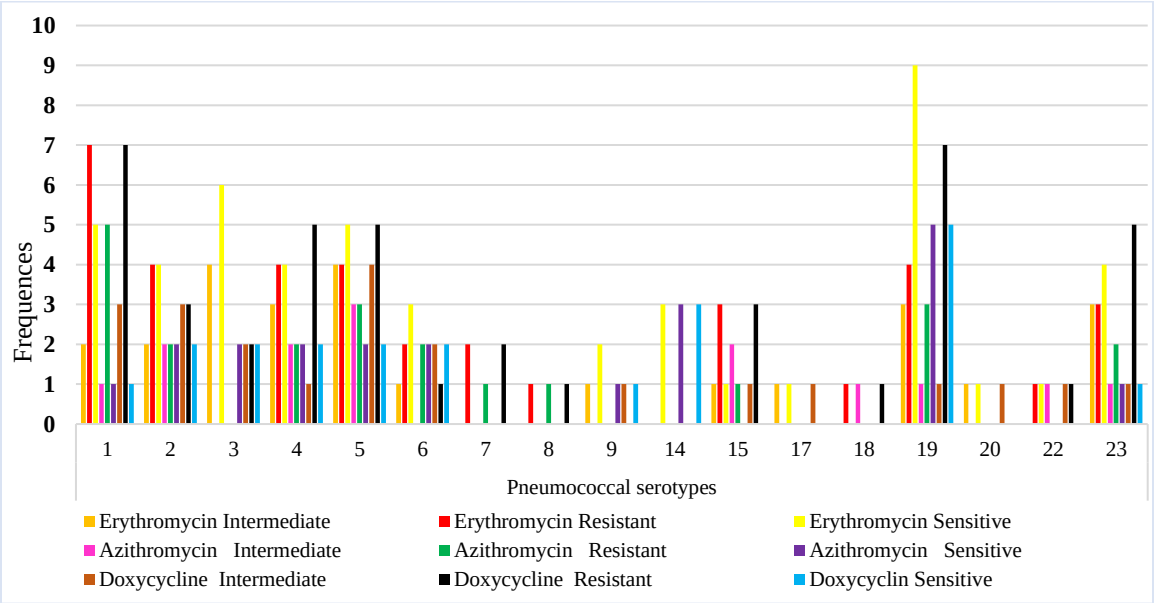

Figure 4.7. Macrolide and Doxycycline Susceptibility Characteristics of pneumococcal Serotypes from Healthy Children in Aden City.

The observed *S. pneumoniae* from pneumonic population exhibited resistance to Erythromycin in many serotypes of *S. pneumoniae*, specifically the common serotypes 1, 4, 3, 5, 2, 6, 7, and 19. In a similar vein, the most serotypes that demonstrated resistance to Azithromycin encompassed serotypes 1, 4, and 19. Furthermore, the existence of non-susceptibility to Doxycycline was seen commonly in serotypes, 1, 4, 6, 3, 5, 11, 19 and 23. it was found that the serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 19, 22 and 23 were the most commonly observed serotypes exhibiting resistance to Erythromycin, Azithromycin, and Doxycycline (Figure 4.8).

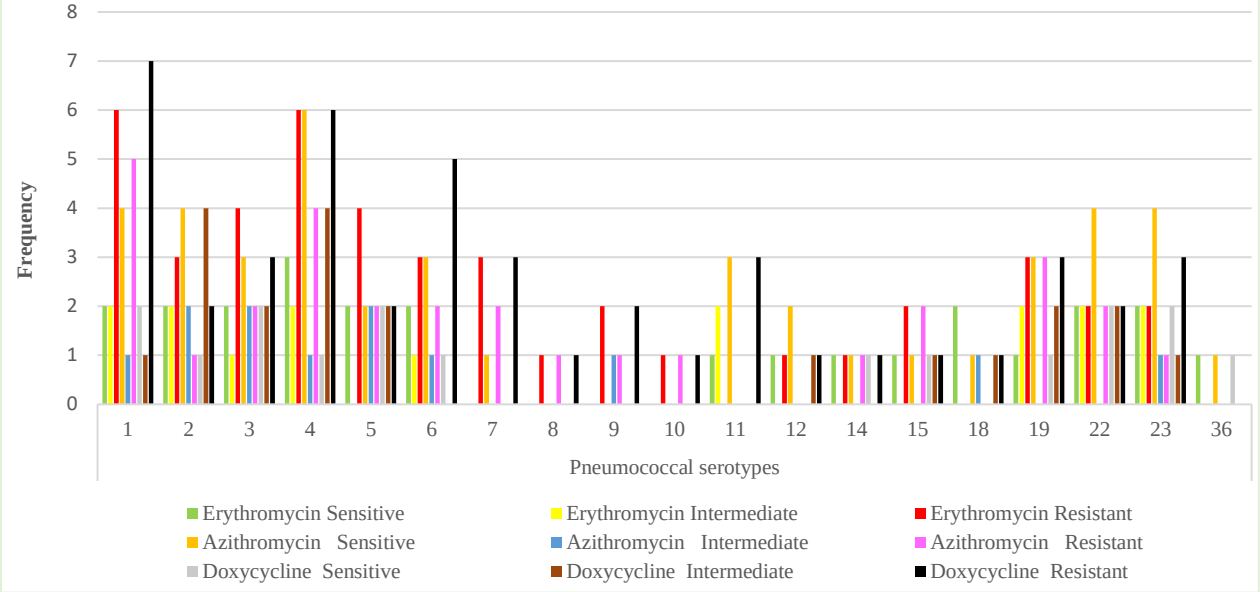

Figure 4.8. Macrolide and Doxycycline Susceptibility characteristics of Pneumococcal Serotypes from Pneumonic kids within Aden City.

The most common resistant serotypes of total cohort for Erythromycin, Azithromycin, and Doxycycline were 1, 4, and 19 respectively. Likewise, the multiple drug resistance was demonstrated at 85.7% and the serotypes 1, 2, 4, and 19 were prominent in this phenomenon.

4.2. Discussion

The current investigation was discovered that the carriage of rate of pneumococci in children of Aden city more than what had been previously documented in Sana'a city (Al-Shamahy, *et al*., 2008). Nevertheless, there are regional `variations in nasopharyngeal carriage, our findings are consistent with those from various resource-limited countries in the Middle East, Asia, and Africa (Nasereddin *et al*., 2013; Al-Lahham, 2020; El-Kholy *et al*., 2020; Wangirapan *et al*., 2020; Karimaei *et al*., 2021). Conversely our result was higher than that has been found in developed countries (Koliou *et al*., 2017; Miguélez *et al*., 2021).

Our study found that Real time-PCR was able to identify *S. pneumoniae* much more frequently (57.1%) compared to culture (44.4%) in nasopharyngeal samples taken from pneumonic and healthy children of Aden city, as demonstrated by Wouters and his colleague (Wouters *et al*., 2018), El-Kholy and his team (El-Kholy *et al*., 2020), Ricketson and colleagues (Ricketson *et al*., 2021) and Abu-Helalah *et al*., 2023. The sensitivity and specificity of our RT-PCR study findings were close to those demonstrated in previous studies (Hasanuzzaman *et al*., 2021). Some researchers recommended incorporating qPCR-guided culture to substantially improve ($p < 0.0001$) the recovery of viable pneumococcus from nasopharyngeal and oropharyngeal samples (Miellet *et al*., 2022).

The findings of present study indicate a high chance of cross-infection when children share a room for sleeping purposes (OR = 15.1; 95% CI 7.1–32.2; $p = 0.00001$). In addition, owning only one room per family (OR = 7.9; CI 95% CI. 4.9–12.8; $p = 0.00001$), and having low income were found to be associated risk factors as well (OR = 2.02; 95% CI. 1.2–3.4; $p = 0.007$). our results were consistent with what was demonstrated in previous findings that reported pneumococci are more effectively transmitted from family members to the child due to overcrowding and direct contact (Koliou *et al*., 2018; Abaye *et al*., 2019).

Although the PCV is well-known for its effectiveness in preventing major infections resulting from vaccine serotypes, along with preventing asymptotic colonization of the nasopharynx (Sutcliffe *et al*., 2019). In Aden City, the vaccination rate among children has been disappointing, falling below the expected levels. This decrease may be linked to the effects of the ongoing war and civil conflict on health services, resulting in an observable increase in the rate of acute bacterial meningitis in Yemen, with *S. pneumoniae* reported as the most common bacterial agent (Al-Samhari *et al*., 2023). Moreover, the result of vaccination was closed to what was found in the rural area of this country (Al-Tarbi & Ghouth, 2020).

Furthermore, we demonstrated that the most common serotypes were 19, 1, 5, 2, 4, 23 among the total cohort as was reported in previous studies in Middle East region (El-Kholy *et al*., 2020; Al-Muhtaresh & Bindayna, 2020; Ceyhan *et al.*, 2021; Esteghamati *et al*., 2022; Abu-Helalah *et al*., 2023). Moreover, the higher prevalence rate of PCV13 serotypes among children, even after twelve years of PCV13 introduction was established in this investigation. However, a significant proportion of young children still exhibited vaccine serotypes despite the implementation of universal childhood PCV13 immunization in different regions of the world (Ricketson *et al*., 2021). Incidentally, this prevalence may be associated with the inadequate response of vaccines, which requires continuous monitoring (Mokaddas *et al*., 2021; Lan *et al*., 2023). The four serotypes (19, 1, 4, 5) of identified strains account for half (50%) of all isolated strains from the healthy children, this finding is consistent with the previous study (Al-Shamahy *et al*., 2008). In contrast, serotype 2 and the four serotypes mentioned above represent 50.3% of the pneumococci isolated from the symptomatic cohort. These strains were demonstrated in various previous research studies conducted in the neighboring countries and Middle East (Al-Yaqoubi, 2011; Al-Sherikh *et al*., 2014; Al-Muhtaresh & Bindayna, 2020; Abu-Helalah *et al*., 2023).

There was a slight replacement of vaccine serovars with non-PCV-13 serovars in our study, as demonstrated elsewhere (Negash *et al*., 2019). About a quarter of the pneumococci in this study were not covered by the vaccine program. A recent report

from the Middle East described an outbreak of invasive *S. pneumoniae* serotype 24F, which is not covered by PCV 13 and has highly virulent and antibiotic-resistant (Reslan *et al*., 2021), this make the NVS to be taking into consideration for public health authorities.

Currently, as a consequence of the serotypes replacement, PCV15 and PCV 20 are being undergo trial process to maintain the high coverage rate of the vaccine after 13 years of applying PCV-13 in the clinical field to overcome the outbreak of common non-PCV13 serotypes (El-Beyrouty *et al*., 2022). The Pneumococcal conjugate vaccine covering rate among children of Aden city (76%) was in line with that found in Arab Gulf countries and other low and middle income countries (Al-Sherikh *et al*., 2014;Taj-Aldeen & Elshafie, 2016; Al-Jardani *et al*., 2019; Arjun *et al*., 2020).

There is universal alarming about the increase of *S. pneumoniae* strains that are non-susceptible to penicillin and other antibiotics. Fortunately, the rate of carriage and distribution of serotypes, along with antimicrobial susceptibility testing among kids in the society, may offer insightful knowledge about invasive illnesses, microbial resistance, and the possible consequences of implementation of PCV across the community (Lee *et al*., 2001; Goyal *et al*., 2007; Sutcliffe *et al*., 2019 ; Lobb *et al*., 2023).

The findings of our study have also shown considerable pneumococcal antimicrobial resistance, which reflects the massive antibiotic misuse in this poor community. The rate of antibiotics intake in Yemen without medical prescription was reported to be 78% (Belkina *et al*., 2014). In addition to that, a survey was done in this third-world community found 73.3% of Pharmacies sold antibacterial agent to people without requiring a physician prescription. The most commonly dispensed agent including different classes of antibacterial agents [“Penicillin (48.5%), Sulphonamides (12.5%), Macrolides (10.6%), Fluoroquinolones (8.8%), Metronidazole (7.8%), Cephalosporins (6%), and β-lactam with 5.8%”] (Halboup *et al*., 2020). Our investigation demonstrated A high penicillin resistance rate at 96.5% of the total cohort (CLSI meningitis breakpoint), it was slightly more than what was reported previously (93.3%) by Al-

Shamahy *et al*., 2008. While this rate is similar to that demonstrated recently in two Jordanian studies (Al-Lahham, 2020; Al-Lahham *et al*., 2021). Furthermore, the data of our study regarding Penicillin resistance was consistent with finding of one study (95.3 %) came from Iran (Gharibani *et al*., 2019). On the other hand, the rate is higher than that found in other neighboring countries (Jamsheer *et al*., 2016; Torumkuney *et al*., 2020). The higher rate of penicillin-resistant (>90%) was reported in many middle-income Asian countries in the last decade and this situation is still a matter of concern to the medical community in these countries (Song *et al*., 2004; Batuwanthudawe *et al*., 2009; Vidanapathirana *et al*., 2020; Guo *et al*., 2023). On other hand, When applying the CLSI non-meningitis breakpoint, the Penicillin resistance was found to be 13.5%, which was in line with the data of a previous study (15%) conducted in Ibb City, Yemen, on isolates from kids with middle ear infections (Al-ofairi *et al*., 2017).

Additionally, the Cefepime and Ceftriaxone non-susceptibility rates (CLSI meningitis breakpoint) of the total children cohort of Aden city (15.8% and 16.4% respectively) were coherent with results of studies in Saudi Arabia, Iran, and India (Al-Sherikh *et al*., 2014; Azimian *et al*., 2020; Arjun *et al*., 2020). In contrast, the sensitivity of pneumococcal isolates to Cefepime and Ceftriaxone (using CLSI Non-meningitis breakpoint) was not similar with previous finding for otitis isolates from Yemeni children (Mohanna & Bahannan, 2016). The observed emergence of resistance to cefepime and ceftriaxone can be attributed to their increased and frequent use in treating many illnesses caused by different bacterial species (Sourav *et al*., 2010). Moreover, in this survey, the sensitivity of Amoxiclav was 100%, which was consistent with the result of study conducted in Yemen during 2015 (Mohanna & Bahannan, 2016).

There was a considerable increase in the resistance of Macrolides in our study than that was found in the previous studies (Al-Shamahy *et al*., 2008; Mohanna & Bahannan, 2016). However this rate was closer to the results demonstrated in the Middle East and some developing countries (El-Nawawy *et al*., 2015; Ghahfarokhi *et al*., 2020; Vidanapathirana *et al*., 2020; Karimaei *et al*., 2021; Sharew *et al*., 2021). While the resistance profile of pneumococci against Doxycycline was consistent with what was

established in income constrained countries (El-Nawawy *et al*., 2015; Vidanapathirana *et al*., 2020; Fallah *et al*., 2021, Sharew *et al*., 2021) .

The level of multiple drug non-susceptibility (85.7%) observed in our study was high. The serotypes 1, 2, 4, and 19 were the most common multiple serotypes resistance for the six out of seven antibiotics studied in this survey. The resistance of those serotypes was confirmed in different previous studies in low and middle income Asian countries (Azimian *et al*., 2020; Sia *et al*., 2021). This situation reflects the intense pressure of antimicrobial agents on pneumococci in the Yemeni community. A recent study revealed a significant level of antimicrobial resistance in Yemen, where 74% of physicians felt compelled to recommend wide-spectrum antibiotics. Furthermore, the survey determined that 81% of the patients did not undergo an antibiogram technique for guide the selection of antibiotics (Orubu *et al*., 2021). This issue of multidrug resistance is a hallmark of many developing countries including Yemen that should be taken into contemplation by public health authorities (Thummeepak *et al*., 2015).

Chapter Five

Conclusion, Future Scope & Recommendation

Conclusion, Future Scope & Recommendation

In summary, our investigation found a high pneumococcal carriage rate in children of Aden city. During the investigation, The RT-PCR achieved a high level of accuracy in detection of *S. pneumoniae* from the nasopharyngeal swabs. In contrast, culture identified fewer cases. RT-PCR exhibited high sensitivity and perfect specificity. Its positive predictive value indicated a positive test reflecting the target condition, while the negative predictive value correctly ruled out the condition. This efficacy underscoring its impact on *S. pneumoniae* detection outcomes. Moreover, the carriage rate of *S. pneumoniae* was significantly higher in kids who slept in a single room with family members, lived in households with only one bedroom, or had a low monthly income. These findings may reflect the consequences of humanitarian crisis for this community class.

In the current investigation, three-quarters of isolated serotypes among a total cohort were included in the PCV13. The most dominant pneumococcal serotypes that were isolated from healthy children and pneumonic cohort were 19, 1, 4, 5, 23, 3, 6, 22, 7, 9, 14, and 18. While the non-PCV-13 pneumococcal serotypes that were commonly isolated from the study sample were 2, 8, and 15. The coverage rate of the PCV13 was found to be (76%) consistent with that found in neighboring countries.

Furthermore, our findings revealed a considerably higher antimicrobial resistance rate among children of Aden city, the serotypes 19, 1, 5, 4, 3, 6, 7, and 8 that isolated from health children had shown resistance to Penicillin, Ceftriaxone, and Cefepime, as determined by testing against the meningitis breakpoint established by CLSI, while the serotypes with multiple resistance for the three β-lactam antibiotics among children with pneumonia were 4, 1, 2, 3, 5 and 19. As well the serotypes 1, 2, 4, 5, 6, 7, 8, 15, 18, 19, and 23 were the most multiple resistant strains for Erythromycin , Azithromycin, and Doxycycline among healthy group as well as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, 19, 22 and 23 were the common resistant serotypes for Erythromycin , Azithromycin, and

Doxycycline among the pneumonic children. Similarly, the pneumococcal serotypes 1, 2, 4, and 19 were the most common multiple resistant serotypes for the six out of seven antimicrobial agents studied in this community.

The findings of current study motivate and encourage to the following:

1. Review and update the empirically prescribing guideline of antimicrobial agents, especially for invasive pneumococcal infections.
2. Establish stringent protocol for distribution antibiotics to reduce the heightened prevalence of antimicrobial resistance.
3. Promote research institutions, scholars, and funding agencies to give more attention and address the research gap in this field.
4. Expand access to preventives and therapeutics facilities can help tackle the national burden of pneumococci.
5. There is an urgent need to conduct a national surveillance study in Yemen to evaluate the consequences of vaccine introduction, the phenomenon of vaccine replacement, as well as drug resistance patterns.

Chapter Six
Bibliography

BIBLIOGRAPHY

Abaye, G., Fekadu, H., Haji, K., Alemu, D., Anjulo, A. A., & Yadate, D. T. (2019). Prevalence and risk factors of pneumococcal nasopharyngeal carriage in healthy children attending kindergarten, in district of Arsi Zone, South East, Ethiopia. *BMC Research Notes*, *12*(1), 1–6. https://doi.org/10.1186/s13104-019-4283-3.

Abdoli, S., Safamanesh, S., Khosrojerdi, M., & Azimian, A. (2020). Molecular detection and serotyping of Streptococcus pneumoniae in children with suspected meningitis in Northeast Iran. *Iranian Journal of Medical Sciences*, *45*(2), 125–133. https://doi.org/10.30476/ijms.2019.45423.

Abu-Helalah, M., Al-Mnayyis, A., Alzoubi, H., Al-Abdallah, R., Jdaitawi, H., Nafi, O., Abu-Sal, K., Altawalbeh, A., Khlaifat, A., Al-Zayadneh, E., *et al*. (2023). Epidemiology of Streptococcus pneumoniae Serotypes in Jordan Amongst Children Younger than the Age of 5: A National Cross-Sectional Study. *Vaccines*, *11*(9), 1396. https://doi.org/10.3390/vaccines11091396.

Adegbola, R. A., DeAntonio, R., Hill, P. C., Roca, A., Usuf, E., Hoet, B., & Greenwood, B. M. (2014). Carriage of Streptococcus pneumoniae and Other Respiratory Bacterial Pathogens in Low and Lower-Middle Income Countries: A Systematic Review and Meta-Analysis. *PLoS ONE*, *9*(8), e103293. https://doi.org/10.1371/journal.pone.0103293.

Adrian, P. V, & Klugman, K. P. (1997). Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of Streptococcus pneumoniae. *Antimicrobial Agents and Chemotherapy*, *41*(11), 2406–2413. https://doi.org/10.1128/AAC.41.11.2406.

Afshar, D., Rafiee, F., Kheirandish, M., Ohadian Moghadam, S., & Azarsa, M. (2020). Autolysin (lytA) recombinant protein: a potential target for developing vaccines against pneumococcal infections. *Clinical and Experimental Vaccine Research*, *9*(2), 76. https://doi.org/10.7774/cevr.2020.9.2.76.

Al-Jardani, A., Al Rashdi, A., Al Jaaidi, A., Al Bulushi, M., Al Mahrouqi, S., Al-Abri, S., Al-Maani, A,. Kumar, R. (2019). Serotype distribution and antibiotic resistance among invasive Streptococcus pneumoniae from Oman post 13-valent vaccine introduction. *International Journal of Infectious Diseases*, *85*(February 2012), 135–140. https://doi.org/10.1016/j.ijid.2019.05.027.

Al-Kayali, R., Khyami-Horani, H., van der Linden, M., & Al-Lahham, A. (2016). Antibiotic resistance patterns and risk factors of Streptococcus pneumoniae carriage among healthy Jordanian children. *Eur Int J Sci Technol*, *5*(1), 55–76.

Al-Lahham, A. (2020). Multicenter study of pneumococcal carriage in children 2 to 4 years of age in the winter seasons of 2017-2019 in Irbid and Madaba governorates of Jordan. *PLoS ONE*, *15*(8 August), 1–14. https://doi.org/10.1371/journal.pone.0237247

Al-Lahham, A. (2021). Prevalence of Pneumococcal Carriage among Jordanian Infants in the First 6 Months of Age, 2008–2016. *Vaccines*, *9*(11), 1283. https://doi.org/10.3390/vaccines9111283.

Al-Lahham, A, Khanfar, N., Albataina, N., Al Shwayat, R., Altwal, R., Abulfeilat, T., Alawneh G., Khurd M., Alqadi Altamimi, A. (2021). Urban and Rural Disparities in Pneumococcal Carriage and Resistance in Jordanian Children, 2015–2019. *Vaccines*, *9*(7), 789. https://doi.org/10.3390/vaccines9070789.

Al-Lahham, A, Qayyas, J. A., & van der Linden, M. (2018). The impact of the 7-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae in infants of Ajlun governorate in Jordan. *Jordan Journal of Biological Sciences*, *11*(2), 155–162.

Al-Lahham, A., & Van der Linden, M. (2014). Streptococcus pneumoniae carriage, resistance and serotypes among Jordanian children from Wadi Al Seer District, Jordan. *The International Arabic Journal of Antimicrobial Agents*, *4*(2), 3–10. https://doi.org/10.3823/752.

Al-Mazrou, A., Twum-Danso, K., Al Zamil, F., & Kambal, A. (2005). Streptococcus pneumoniae serotypes/serogroups causing invasive disease in Riyadh, Saudi Arabia: extent of coverage by pneumococcal vaccines. *Annals of Saudi Medicine*, *25*(2), 94–99. https://doi.org/10.5144/0256-4947.2005.94.

Al-ofairi, B. A., Nagi, N. A., Nagi, S. A., Al-tawil, T. M., & Saif, A. (2017). Otitis Media in Children : Identification and Antibiotics Sensitivity of Bacterial Pathogens in Ibb City , Yemen. *PSM Microbiology*, *2*(3), 51–58.

Al-Qubati, A.-L. M., & Bawazeer, I. M. (2020). In-Hospital Complications of Acute Meningo-Encephalitis among Children Admitted at AL-Sadaqa General Teaching Hospital. *Yemeni Journal of Medical and Health Research*, *9*(1&2). Retrieved from https://www.yjmhr.edu.ye/article/in-hospital-complications-of-acute-meningo-encephalitis-among-children-admitted-at-al-sadaqa-general-teaching-hospital.

Al-Samhari, G. A., Al-Mushiki, G. M., Tamrakar, R., Lin, Y., Al-Shaebi, F., Akroot, M. A., Al-Nahari S. A., Li G-J., Tang, X.-Y. (2023). Prevalence, aetiology, vaccination coverage and spatio-temporal pattern among patients admitted with acute bacterial meningitis to the sentinel hospital surveillance network in Yemen, 2014–20, before and during the civil war. *International Journal of Epidemiology*, *52(4), 1175-1186*. https://doi.org/10.1093/ije/dyad047.

Al-Shamahy, H.A. Jabbar, A. R., Al Nabhi, B., ALBadry, A., & Al Robasi, A. (2008). The prevalence of streptococcus pneumoniae carriage among healthy children in Yemen. *EMJ*, *26*(1), 25–29. Retrieved fromhttps://www.researchgate.net/publication/288804141_The_prevalence_of_Streptococcus_pneumoniae_carriage_among_healthy_children_in_Yemena.

Al-Shamahy, H. A., & Ishak, A. A. (2021). Trends And Causes Of Morbidity In Part Of Children In The City Of Sana'a, Yemen 1978-2018: Findings Of Single Children's Health Center. *Universal Journal of Pharmaceutical Research*, *5(6)*(January), 1–5. https://doi.org/10.22270/ujpr.v5i6.504.

Al-Sherikh, Y. A., Gowda, L. K., Ali, M. M. M., John, J., Mohammed, D. K. H., & Shashidhar, P. C. (2014). Distribution of Serotypes and Antibiotic Susceptibility Patterns Among Invasive Pneumococcal Diseases in Saudi Arabia. *Annals of Laboratory Medicine*, *34*(3), 210–215. https://doi.org/10.3343/alm.2014.34.3.210.

Al-Tarbi, A. M., & Ghouth, A. S. B. (2020). Vaccination coverage in Tarim district, Yemen 2017. *Am J Epidemiol*, *4*(1), 10–15. Retrieved from https://www.researchgate.net/publication/350106427.

Al-Yaqoubi, M., & Elhag K. M. (2011). Serotype Prevalence and Penicillin-susceptibility of Streptococcus pneumoniae in Oman. *Oman Medical Journal*, *26*(1), 43–47. https://doi.org/10.5001/omj.2011.11.

Al Ayed, M. S., & Hawan, A. A. (2011). Retrospective review of invasive pediatric pneumococcal diseases in a military hospital in the southern region of Saudi Arabia. *Annals of Saudi Medicine*, *31*(5), 469–472. https://doi.org/10.4103/0256-4947.84623.

Al Khal, A. L., El Shafie, S. S., Al Kuwari, J., & Bener, A. (2007). Streptococcus Pneumonia Serotypes in Newly Developed State of Qatar: Consideration for Conjugate Vaccine. *Qatar Medical Journal*, *2007*(2), 25–28. https://doi.org/10.5339/qmj.2007.2.11.

Alghamdi, S. (2021). The role of vaccines in combating antimicrobial resistance (AMR) bacteria. *Saudi Journal of Biological Sciences*, *28*(12), 7505–7510. https://doi.org/10.1016/j.sjbs.2021.08.054.

Alhomoud, F., Aljamea, Z., Almahasnah, R., Alkhalifah, K., Basalelah, L., & Alhomoud, F. K. (2017). Self-medication and self-prescription with antibiotics in the Middle East—do they really happen? A systematic review of the prevalence, possible reasons, and outcomes. *International Journal of Infectious Diseases*, *57*, 3–12. https://doi.org/10.1016/j.ijid.2017.01.014.

Al Khorasani, A., & Banajeh, S. (2006). Bacterial profile and clinical outcome of childhood meningitis in rural Yemen: A 2-year hospital-based study. *Journal of Infection*, *53*(4), 228–234. https://doi.org/10.1016/j.jinf.2005.12.004.

Almazrou, Y., Shibl, A. M., Alkhlaif, R., Pirçon, J.-Y., Anis, S., Kandeil, W., & Hausdorff, W. P. (2015). Epidemiology of invasive pneumococcal disease in Saudi Arabian children younger than 5 years of age. *Journal of Epidemiology and Global Health*, *6*(2), 95. https://doi.org/10.1016/j.jegh.2015.08.002.

Al-Muhtaresh, A., & Bindayna, K. M. (2020). The prevalence of antimicrobial resistance and serotypes of streptococcus pneumoniae in the Kingdom of Bahrain. *Journal of Pure and Applied Microbiology*, *14*(1), 133–140. https://doi.org/10.22207/JPAM.14.1.14.

AlSalaman, J., AlShehabi, K., Salah, S., Ahmed, F., Khudhair, H., Sabt, S., *et al*. (2017). Epidemiological and Clinical Characteristics of Streptococcus pneumoniae Infections in a Tertiary Care Center in Bahrain (2010-2014). *The International Arabic Journal of Antimicrobial Agents*, *7*(2), 1–8. https://doi.org/10.3823/0808.

Al-Shami, H. Z., Al-Haimi, M. A., Al-dossary, O. A. E., Nasher, A. A. M., Al-Najhi, M. M. A., Al-Shamahy, H. A., & Al-Ankoshy, A. A. M. (2021). Patterns Of Antimicrobial Resistance Among Major Bacterial Pathogens Isolated From Clinical Samples In Two Tertiary's Hospitals, In Sana'a, Yemen. *Universal Journal of Pharmaceutical Research*, (November). https://doi.org/10.22270/ujpr.v6i5.674.

Alshehari, A. H., Al-Selwi, A. A. M., & Albahloly, M. A. (2018). Epidemiology and Outcome of Acute Bacterial Meningitis Among Children in Saudi Haospital Hajjah, Northwest Territories of Yemen. *American Journal of Pediatrics*, *4*(3), 56. https://doi.org/10.11648/j.ajp.20180403.13.

Alshehri, A. S., Assiri, O., Alqarni, A. S., Alkhairi, M. Y., Alzahrani, M. A., Alshehri, S. A., Alshehri, N.A. A., Abouelyazid, A. (2021). Prevalence and clinical presentation of sinusitis in pediatric age group in Aseer, Saudi Arabia. *Journal of Family Medicine and Primary Care*, *10*(6), 2358. https://doi.org/10.4103/jfmpc.jfmpc_2433_20.

Amann, S., Neef, K., & Kohl, S. (2014). *Antimicrobial resistance (AMR)*. (WHO, Ed.), *World Health Organization* (WHO/HSE/PE, Vol. WHO/HSE/PE). Jenva: WHO. https://doi.org/10.1136/ejhpharm-2018-001820.

Andersson, B., Dahmén, J., Frejd, T., Leffler, H., Magnusson, G., Noori, G., & Edén, C. S. (1983). Identification of an active disaccharide unit of a glycoconjugate receptor for pneumococci attaching to human pharyngeal epithelial cells. *Journal of Experimental Medicine*, *158*(2), 559–570. https://doi.org/10.1084/jem.158.2.559.

André, G. O., Politano, W. R., Mirza, S., Converso, T. R., Ferraz, L. F. C., Leite, L. C. C., & Darrieux, M. (2015). Combined effects of lactoferrin and lysozyme on Streptococcus pneumoniae killing. *Microbial Pathogenesis*, *89*, 7–17. https://doi.org/10.1016/j.micpath.2015.08.008.

Anthony, L., Meehan, A., Amos, B., Mtove, G., Mjema, J., Malahiyo, R., *et al*. (2012). Nasopharyngeal carriage of Streptococcus pneumoniae: Prevalence and risk factors in HIV-positive children in Tanzania. *International Journal of Infectious Diseases*, *16*(10), e753–e757. https://doi.org/10.1016/j.ijid.2012.05.1037.

Appelbaum, PC. (1992). Antimicrobial Resistance in Streptococcus pneumoniae: An Overview. *Clinical Infectious Diseases*, *15*(1), 77–83. https://doi.org/10.1093/clinids/15.1.77.

Appelbaum, P.C., Scragg, J. N., Bowen, A., Bhamjee, A., Hallett, A. F., & Cooper, R. (1977). Streptococcus Pneumoniæ Resistant To Penicillin And Chloramphenicol. *The Lancet*, *310*(8046), 995–997. https://doi.org/10.1016/S0140-6736(77)92892-6.

Appelbaum, PC. (2002). Resistance among Streptococcus pneumoniae: Implications for Drug Selection. *Clinical Infectious Diseases*, *34*(12), 1613–1620. https://doi.org/10.1086/340400.

Arjun, R., Ratheesh, R. S., Mohan, V., Uduman, S., Jalaludeen, S., Prabhakaran, A., Sasidharan, A., Niyas, V. K. M. (2020). Susceptibility and serotypes of streptococcus pneumoniae isolates in invasive pneumococcal disease: A study from Kerala, South India. *Infezioni in Medicina*, *28*(4), 558–564. PMID: 33257631.

Azimian, A., Khosrojerdi, M., Kebriaei, A., Namdarahmadabad, H., & Besharati, R. (2021). Characterization of Blood-isolated, Penicillin-Nonsusceptible Streptococcus pneumoniae From Children Between 2014 and 2018 in Bojnurd, Iran. *Jundishapur Journal of Microbiology*, *13*(11), 1–9. https://doi.org/10.5812/jjm.111147.

Badawy, M., El Kholy, A., Sherif, M. M., Rahman, E. A., Ashour, E., Sherif, H., *et al*. (2017). Serotypes of Streptococcus pneumoniae in Egyptian children: are they covered by pneumococcal conjugate vaccines? *European Journal of Clinical Microbiology and Infectious Diseases*, *36*(12), 2385–2389. https://doi.org/10.1007/s10096-017-3071-z.

Bagnoli, F., Moschioni, M., Donati, C., Dimitrovska, V., Ferlenghi, I., Facciotti, C., Muzzi A., Giusti, F., Emolo, C., Sinisi, A., Hilleringmann, M., Pansegrau, W., Censini, S., Rappuoli, R., Covacci, A., Masignani, V., Barocchi, M. A. (2008). A second pilus type in Streptococcus pneumoniae is prevalent in emerging serotypes and mediates adhesion to host cells. *Journal of Bacteriology*, *190*(15), 5480–5492. https://doi.org/10.1128/JB.00384-08.

Bahwal, S. A., Jawass, M. A., & Gouth, H. S. Bin. (2018). Recurrent Pneumonia in Children Attending Mukalla City Hospital–Yemen. *Hadhramout University Journal of Natural & Applied Sciences*, *14*(2), 137–143.

Balakrishnan, I, Crook, P., Morris, R., & Gillespie, S. H. (2000). Early predictors of mortality in pneumococcal bacteraemia. *Journal of Infection*, *40*(3), 256–261. https://doi.org/10.1053/jinf. 2000.0653.

Balsells, E., Guillot, L., Nair, H., & Kyaw, M. H. (2017). Serotype distribution of Streptococcus pneumoniae causing invasive disease in children in the post-PCV era: A systematic review and meta-analysis. *PLOS ONE*, *12*(5), e0177113. https://doi.org/10.1371/journal.pone.0177113.

Banajeh, S. M. (1998). Outcome for children under 5 years hospitalized with severe acute lower respiratory tract infections in Yemen: A 5 year experience. *Journal of Tropical Pediatrics*, *44*(6), 343–346. https://doi.org/10.1093/tropej/44.6.343.

Bandaranayake, T., & Shaw, A. C. (2016). Host Resistance and Immune Aging. *Clinics in Geriatric Medicine*, *32*(3), 415–432. https://doi.org/10.1016/j.cger.2016.02.007.

Barocchi, M. A., Ries, J., Zogaj, X., Hemsley, C., Albiger, B., Kanth, A., *et al.* (2006). A pneumococcal pilus influences virulence and host inflammatory responses. *Proceedings of the National Academy of Sciences*, *103*(8), 2857–2862. https://doi.org/10.1073/pnas.0511017103.

Barve, S., Nanda, S., & Ritu, & B. (2019). Incidence Of Pneumococcal Lower Respiratory Tract Dr . Ritu Bhatt. *Indian Journal Of Applied Research*, *16*(12), 15–16. https://doi.org/10.36106/ijar.

Batuwanthudawe, R., Karunarathne, K., Dassanayake, M., De Silva, S., Lalitha, M. K., Thomas, K., Steinhoff, M., Abeysinghe, N. (2009). Surveillance of invasive pneumococcal disease in Colombo, Sri Lanka. *Clinical Infectious Diseases*, *48*(SUPPL. 2), 136–140. https://doi.org/10.1086/596492.

Beiter, K., Wartha, F., Albiger, B., Normark, S., Zychlinsky, A., & Henriques-Normark, B. (2006). An Endonuclease Allows Streptococcus pneumoniae to Escape from Neutrophil Extracellular Traps. *Current Biology*, *16*(4), 401–407. https://doi.org/10.1016/j.cub.2006.01.056.

Belkina, T., Al Warafi, A., Hussein Eltom, E., Tadjieva, N., Kubena, A., & Vlcek, J. (2014). Antibiotic use and knowledge in the community of Yemen, Saudi Arabia, and Uzbekistan. *The Journal of Infection in Developing Countries*, *8*(04), 424–429. https://doi.org/10.3855/jidc.3866.

Bergeron, Y., Ouellet, N., Deslauriers, A.-M., Simard, M., Olivier, M., & Bergeron, M. G. (1998). Cytokine Kinetics and Other Host Factors in Response to Pneumococcal Pulmonary Infection in Mice. *Infection and Immunity*, *66*(3), 912–922. https://doi.org/10.1128/IAI.66.3.912-922.1998.

Binsker, U., Lees, J. A., Hammond, A. J., & Weiser, J. N. (2020). Immune exclusion by naturally acquired secretory IgA against pneumococcal pilus-1. *Journal of Clinical Investigation*, *130*(2), 927–941. https://doi.org/10.1172/JCI132005.

Bogaert, D., de Groot, R., & Hermans, P. (2004). Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *The Lancet Infectious Diseases*, *4*(3), 144–154. https://doi.org/10.1016/S1473-3099(04)00938-7.

Braun, J. S., Novak, R., Gao, G., Murray, P. J., & Shenep, J. L. (1999). Pneumolysin, a Protein Toxin of Streptococcus pneumoniae , Induces Nitric Oxide Production from Macrophages. *Infection and Immunity*, *67*(8), 3750–3756. https://doi.org/10.1128/IAI.67.8.3750-3756.1999.

Brito, D. A., Ramirez, M., & de Lencastre, H. (2003). Serotyping Streptococcus pneumoniae by Multiplex PCR. *Journal of Clinical Microbiology*, *41*(6), 2378–2384. https://doi.org/10.1128/JCM.41.6.2378-2384.2003.

Brooks, L. R. K., & Mias, G. I. (2018). Streptococcus pneumoniae's virulence and host immunity: Aging, diagnostics, and prevention. *Frontiers in Immunology*, *9*(JUN). https://doi.org/10.3389/fimmu.2018.01366.

Burki, T. (2015). Yemen health situation "moving from a crisis to a disaster". *The Lancet*, *385*(9978), 1609. https://doi.org/10.1016/S0140-6736(15)60779-6.

Carvalho, M. D. G. S., Tondella, M. L., McCaustland, K., Weidlich, L., McGee, L., Mayer, L. W., … Sampson, J. S. (2007). Evaluation and Improvement of Real-Time PCR Assays Targeting lytA , ply , and psaA Genes for Detection of Pneumococcal DNA. *Journal of Clinical Microbiology*, *45*(8), 2460–2466. https://doi.org/10.1128/JCM.02498-06.

Carvalho, M. D. G. S., Pimenta, F. C., Jackson, D., Roundtree, A., Ahmad, Y., Millar, E. V., O'Brien, KL., Whitney, CG., Cohen, AL., Beall, BW. (2010). Revisiting Pneumococcal Carriage by Use of Broth Enrichment and PCR Techniques for Enhanced Detection of Carriage and Serotypes. *Journal of Clinical Microbiology*, *48*(5), 1611–1618. https://doi.org/10.1128/JCM.02243-09.

Cavalieri, S. J., Harbeck, R. J., McCarter, Y. S., Ortez, J. H., Rankin, I. D., Sautter, R. L., *et al*. (2005). Streptococcus pneumoniae. In M. B. Cole (Ed.), *Manual of antimicrobial susceptibility testing.* (pp. 133–140). Washington, DC, USA: Pan American Health Organization.

CDC. (2017). *Antibiotic Use in the United States, 2017: Progress and Opportunities. Atlanta, GA:US Department of Health and Human Services, CDC.* https://archive.cdc.gov/www_cdc_gov/antibiotic-use/stewardship-report/2017.html.

CDC. (2013). Progress in introduction of pneumococcal conjugate vaccine - worldwide, 2000-2012. *MMWR Morb Mortal Wkly Rep. 2013 Apr 26;62(16):308-11. PMID: 23615674; PMCID: PMC4604961.*

Ceyhan, M., Karadag-Oncel, E., Hascelik, G., Ustundag, G., Gurbuz, V., Samlioglu, P., *et al.* (2021). Nasopharyngeal carriage of Streptococcus pneumoniae in healthy children aged less than five years. *Vaccine*, *39*(15), 2041–2047. https://doi.org/10.1016/j.vaccine.2021.03.028.

Chang, B., Akeda, H., Nakamura, Y., Hamabata, H., Ameku, K., Toma, T., Miyagib, M,. Ohnishi, M. (2020). Impact of thirteen-valent pneumococcal conjugate vaccine on nasopharyngeal carriage in healthy children under 24 months in Okinawa, Japan. *Journal of Infection and Chemotherapy*, *26*(5), 465–470. https://doi.org/10.1016/j.jiac.2019.12.009.

Chao, Y., Bergenfelz, C., & Hakansson, A. P. (2019). Growing and Characterizing Biofilms Formed by Streptococcus pneumoniae. In F. Iovino (Ed.), *Streptococcus pneumoniae. Methods in Molecular Biology* (pp. 147–171). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-9199-0_13.

Chao, Y., Marks, L. R., Pettigrew, M. M., & Hakansson, A. P. (2015). Streptococcus pneumoniae biofilm formation and dispersion during colonization and disease. *Frontiers in Cellular and Infection Microbiology*, *4*(January), 1–16. https://doi.org/10.3389/fcimb.2014.00194.

Charalambous, B. M., Batt, S. L., Peek, A. C., Mwerinde, H., Sam, N., & Gillespie, S. H. (2003). Quantitative Validation of Media for Transportation and Storage of Streptococcus pneumoniae. *Journal of Clinical Microbiology*, *41*(12), 5551–5556. https://doi.org/10.1128/JCM.41.12.5551-5556.2003.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries* (2nd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9780511543470.

Chereau, F., Opatowski, L., Tourdjman, M., & Vong, S. (2017). Risk assessment for antibiotic resistance in South East Asia. *BMJ (Online)*, *358*, 2–8. https://doi.org/10.1136/bmj.j3393.

Chewapreecha, K. C. (2014). *Evolution of Streptococcus pneumoniae during carriage Functional and evolutionary analyses of pneumococcal genome variation*. (Doctoral dissertation) University of Cambridge.

Chi, Y.-C., Rahkola, J. T., Kendrick, A. A., Holliday, M. J., Paukovich, N., Roberts, T. S., Janoff, E. N., Eisenmesser, E. Z. (2017). Streptococcus pneumoniae IgA1 protease: A metalloprotease that can catalyze in a split manner in vitro. *Protein Science*, *26*(3), 600–610. https://doi.org/10.1002/pro.3110.

Chiu, F.-F., Tu, L.-L., Chen, W., Zhou, H., Liu, B.-S., Liu, S.-J., & Leng, C.-H. (2023). A broad-spectrum pneumococcal vaccine induces mucosal immunity and protects against lethal Streptococcus pneumoniae challenge. *Emerging Microbes & Infections*, *12*(2), 2272656. https://doi.org/10.1080/22221751.2023.2272656.

Chow, S. (2021). Pneumonia History. *News-Medical*. Retrieved 30 October 2021, from https://www.news-medical.net/health/Pneumonia-History.aspx.

CLSI. (2021). *Performance Standards for Antimicrobial Susceptibility Testing* (M 100 ed 31st). USA: Clinical and Laboratory Standards Institute.

Cohen, A., Troib, S., Dotan, S., Najmuldeen, H., Yesilkaya, H., Kushnir, T., *et al.* (2019). Streptococcus pneumoniae Cell Wall-Localized Trigger Factor Elicits a Protective Immune Response and Contributes to Bacterial Adhesion to the Host. *Scientific Reports*, *9*(1), 4295. https://doi.org/10.1038/s41598-019-40779-0.

Cowan, M. K., & Smith, H. (2018). Infectious Diseases Affecting the Respiratory System. In *Microbiology A System Approach* (5th ed., pp. 612–647). New York: McGraw-Hill Higher Education.

Cvitkovic Spik, V., Beovic, B., Pokorn, M., Drole Torkar, A., Vidmar, D., Papst, L., Seme, K., Kogoj, R., Müller Premru, M. (2013). Improvement of pneumococcal pneumonia diagnostics by the use of rt-PCR on plasma and respiratory samples. *Scandinavian Journal of Infectious Diseases*, *45*(10), 731–737. https://doi.org/10.3109/00365548.2013.804631.

Daana, M., Rahav, G., Hamdan, A., Thalji, A., Jaar, F., Abdeen, Z., *et al.* (2015). Measuring the effects of pneumococcal conjugate vaccine (PCV7) on Streptococcus pneumoniae carriage and antibiotic resistance: The Palestinian-Israeli Collaborative Research (PICR). *Vaccine*, *33*(8), 1021–1026. https://doi.org/10.1016/j.vaccine.2015.01.003.

Davis, K. M., Akinbi, H. T., Standish, A. J., & Weiser, J. N. (2008). Resistance to Mucosal Lysozyme Compensates for the Fitness Deficit of Peptidoglycan Modifications by Streptococcus pneumoniae. *PLoS Pathogens*, *4*(12), e1000241. https://doi.org/10.1371/journal.ppat.1000241.

Dayie, N. T. K. D., Tettey, E. Y., Newman, M. J., Bannerman, E., Donkor, E. S., Labi, A. K., & Slotved, H. C. (2019). Pneumococcal carriage among children under five in Accra, Ghana, five years after the introduction of pneumococcal conjugate vaccine. *BMC Pediatrics*, *19*(1), 316. https://doi.org/10.1186/s12887-019-1690-5.

De Angelis, G., Moschioni, M., Muzzi, A., Pezzicoli, A., Censini, S., Delany, I., *et al.* (2011). The Streptococcus pneumoniae Pilus-1 Displays a Biphasic Expression Pattern. *PLoS ONE*, *6*(6), e21269. https://doi.org/10.1371/journal.pone.0021269.

De La Campa, A. G., Balsalobre, L., Ardanuy, C., Fenoll, A., Pérez-Trallero, E., Liñares, J. (2004). Fluoroquinolone resistance in penicillin-resistant Streptococcus pneumoniae Clones, Spain. *Emerging Infectious Diseases*, *10*(10), 1751–1759. https://doi.org/10.3201/eid1010.040382.

De la Fuente-Núñez, C., Reffuveille, F., Fernández, L., & Hancock, R. E. W. (2013). Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies. *Current Opinion in Microbiology*, *16*(5), 580–589. https://doi.org/10.1016/j.mib.2013.06.013.

Dessing, M. C., Schouten, M., Draing, C., Levi, M., von Aulock, S., & van der Poll, T. (2008). Role played by Toll-like receptors 2 and 4 in lipoteichoic acid-induced lung inflammation and coagulation. *The Journal of Infectious Diseases*, *197*(2), 245–252. https://doi.org/10.1086/524873.

Dockrell, D. H., Whyte, M. K. B., & Mitchell, T. J. (2012). Pneumococcal pneumonia: mechanisms of infection and resolution. *Chest*, *142*(2), 482–491. https://doi.org/10.1378/chest.12-0210.

Domachowske, J. (2019). *Introduction to Clinical Infectious Diseases*. (J. Domachowske, Ed.). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-91080-2.

Domínguez, J., Andreo, F., Blanco, S., Ruiz-Manzano, J., Prat, C., Latorre, I., Galí, N., Rivelo, R., Matas, L., Ausina, V. (2006). Rapid detection of pneumococcal antigen in serum samples for diagnosing pneumococcal pneumonia. *Journal of Infection*, *53*(1), 21–24. https://doi.org/10.1016/j.jinf.2005.09.008.

Donlan, R. M. (2002). Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases*, *8*(9), 881–890. https://doi.org/10.3201/eid0809.020063.

Dowson, C. G., Barcus, V., King, S., Pickerill, P., Whatmore, A., & Yeo, M. (1997). Horizontal gene transfer and the evolution of resistance and virulence determinants in Streptococcus. *Journal of Applied Microbiology Symposium Supplement*, *83*, 42S-51S.

Dowson, C. G., Hutchison, A., Woodford, N., Johnson, A. P., George, R. C., & Spratt, B. G. (1990). Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of Streptococcus pneumoniae. *Proceedings of the National Academy of Sciences*, *87*(15), 5858–5862. https://doi.org/10.1073/pnas.87.15.5858.

Draz, I. H., Halawa, E. F., Wahby, G., Ismail, D. K., & Meligy, B. S. (2015). Pneumococcal infection among hospitalized Egyptian children. *Journal of the Egyptian Public Health Association*, *90*(2), 52–57. https://doi.org/10.1097/01.EPX.0000465234.31794.b1.

Du, S., Vilhena, C., King, S., Sahagún-Ruiz, A., Hammerschmidt, S., Skerka, C., & Zipfel, P. F. (2021). Molecular analyses identifies new domains and structural differences among Streptococcus pneumoniae immune evasion proteins PspC and Hic. *Scientific Reports*, *11*(1), 1–15. https://doi.org/10.1038/s41598-020-79362-3.

Dube, F. S., Kaba, M., Whittaker, E., Zar, H. J., & Nicol, M. P. (2013). Detection of Streptococcus pneumoniae from Different Types of Nasopharyngeal Swabs in Children. *PLoS ONE*, *8*(6), e68097. https://doi.org/10.1371/journal.pone.0068097.

Dzaraly, N. D., Muthanna, A. R., Mohd Desa, M. N., Taib, N. M., Masri, S. N., Rahman, N. I., Suhaili, Z., Tuan Soh, T.s., Abdullah, F. H. (2020). Pilus islets and the clonal spread of piliated Streptococcus pneumoniae: A review. *International Journal of Medical Microbiology*, *310*(7), 151449. https://doi.org/10.1016/j.ijmm.2020.151449.

Eichmann, K., & Krause, R. M. (2013). Fred Neufeld and pneumococcal serotypes: foundations for the discovery of the transforming principle. *Cellular and Molecular Life Sciences*, *70*(13), 2225–2236. https://doi.org/10.1007/s00018-013-1351-z.

Eijkelkamp, B. A., Morey, J. R., Neville, S. L., Tan, A., Pederick, V. G., Cole, N., *et al.* (2019). Dietary zinc and the control of Streptococcus pneumoniae infection. *PLoS Pathogens*, *15*(8), e1007957. https://doi.org/10.1371/journal.ppat.1007957.

El-Kholy, A., Badawy, M., Gad, M., & Soliman, M. (2020). Serotypes and antimicrobial susceptibility of nasopharyngeal isolates of streptococcus pneumoniae from children less than 5 years old in egypt. *Infection and Drug Resistance*, *13*, 3669–3677. https://doi.org/10.2147/IDR.S250315.

El-Nawawy, A. A., Hafez, S. F., Meheissen, M. A., Shahtout, N. M. A., & Mohammed, E. E. (2015). Nasopharyngeal carriage, capsular and molecular serotyping and antimicrobial susceptibility of Streptococcus pneumoniae among asymptomatic healthy children in Egypt. *Journal of Tropical Pediatrics*, *61*(6), 455–463. https://doi.org/10.1093/tropej/fmv060.

El-Beyrouty, C., Buckler, R., Mitchell, M., Phillips, S., & Groome, S. (2022). Pneumococcal vaccination—A literature review and practice guideline update. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *42*(9), 724–740. https://doi.org/10.1002/phar.2723.

El Moujaber, G., Osman, M., Rafei, R., Dabboussi, F., & Hamze, M. (2017). Molecular mechanisms and epidemiology of resistance in Streptococcus pneumoniae in the Middle East region. *Journal of Medical Microbiology*, *66*(7), 847–858. https://doi.org/10.1099/jmm.0.000503.

Eliopoulos, G. M. (2004). Quinolone resistance mechanisms in pneumococci. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *38 Suppl 4*(SUPPL. 4), S350-6. https://doi.org/10.1086/382692.

Emgård, M., Msuya, S. E., Nyombi, B. M., Mosha, D., Gonzales-Siles, L., Nordén, R., *et al.* (2019). Carriage of penicillin-non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. *International Journal of Infectious Diseases*, *81*, 156–166. https://doi.org/10.1016/j.ijid.2019.01.035.

English, B. K., & Gaur, A. H. (2010). The Use and Abuse of Antibiotics and the Development of Antibiotic Resistance. In *Finn, A., Curtis, N., Pollard, A. (eds) Hot Topics in Infection and Immunity in Children VI. Advances in Experimental Medicine and Biology* (Vol. 659, pp. 73–82). New York: Springer. https://doi.org/10.1007/978-1-4419-0981-7_6.

Esteghamati, A., Nazari-Alam, A., Badamchi, A., Faramarzi, M., Alipoor, M., Baradaran Moghaddam, A., *et al*. (2022). Determination of Streptococcus pneumonia Serotypes Isolated from Clinical Specimens: A Step Toward the Production of a Native Vaccine in Iran. *Archives of Clinical Infectious Diseases*, *16*(6), 1–7. https://doi.org/10.5812/archcid.112897.

Ezoji, K., Yaghoubi, M., Nojomi, M., Mahmoody, S., Zahraie, S. M., Moradi Lakeh, M., Tabatabaei, S.R., Karimi, A. (2019). Cost-effectiveness of introducing the pneumococcal conjugate vaccine for children under 5 years in the Islamic Republic of Iran. *Eastern Mediterranean Health Journal*, *25*(10), 686–697. https://doi.org/10.26719/emhj.19.039.

Fallah, F., Tabatabaei, S. R., Yousefi, M., Hashemi, A., Nazari-Alam, A., & Saadat, A. (2021). Association of Erythromycin Resistance with mefA and ermB Genes among Clinical Isolates of Streptococcus Pneumoniae in Tehran, Iran. *Mediterranean Journal of Infection Microbes and Antimicrobials*, *10*(March). https://doi.org/10.4274/mjima.galenos.2021.2020.19.

Fliegauf, M., Sonnen, A. F. P., Kremer, B., & Henneke, P. (2013). Mucociliary Clearance Defects in a Murine In Vitro Model of Pneumococcal Airway Infection. *PLoS ONE*, *8*(3), e59925. https://doi.org/10.1371/journal.pone.0059925.

Francis, J. P., Richmond, P. C., Michael, A., Siba, P. M., Jacoby, P., Hales, B. J., *et al.* (2016). A longitudinal study of natural antibody development to pneumococcal surface protein A families 1 and 2 in Papua New Guinean Highland children: a cohort study. *Pneumonia*, *8*(1), 1–7. https://doi.org/10.1186/s41479-016-0014-x.

Frayha, H. H., & Al Mazrou, Y. Y. (2005). Vaccination Against Invasive Pneumococcal Disease in Saudi Arabia: Where Do We Stand? *Annals of Saudi Medicine*, *25*(2), 90–93. https://doi.org/10.5144/0256-4947.2005.90.

Ganaie, F., Saad, J. S., McGee, L., van Tonder, A. J., Bentley, S. D., Lo, S. W., *et al.* (2020). A New Pneumococcal Capsule Type, 10D, is the 100th Serotype and Has a Large cps Fragment from an Oral Streptococcus. *MBio*, *11*(3). https://doi.org/10.1128/mBio.00937-20.

Gandhi G. (2015). Charting the evolution of approaches employed by the Global Alliance for Vaccines and Immunizations (GAVI) to address inequities in access to immunization: A systematic qualitative review of GAVI policies, strategies and resource allocation mechanisms through an equity lens (1999-2014). BMC Public Health, 15(1). https://doi.org/10.1186/s12889-015-2521-8.

Garber, K., Fox, C., Abdalla, M., Tatem, A., Qirbi, N., Lloyd-Braff, L., Al-Shabi, K., Ongwae, K., Dyson, M., Hassen, K. (2020). Estimating access to health care in Yemen, a complex humanitarian emergency setting: a descriptive applied geospatial analysis. *The Lancet Global Health*, *8*(11), e1435–e1443. https://doi.org/10.1016/S2214-109X(20)30359-4.

Gebre, T., Tadesse, M., Aragaw, D., Feye, D., Beyene, H., Seyoum, D., & Mekonnen, M. (2017). Nasopharyngeal Carriage and Antimicrobial Susceptibility Patterns of Streptococcus pneumoniae among Children under Five in Southwest Ethiopia. *Children*, *4*(4), 27. https://doi.org/10.3390/children4040027.

Ghahfarokhi, S. H., Mosadegh, M., Ahmadi, A., Pourmand, M. R., Azarsa, M., Rahbar, M., & Nikmanesh, B. (2020). Serotype distribution and antibiotic susceptibility of streptococcus pneumoniae isolates in Tehran, Iran: A surveillance study. *Infection and Drug Resistance*, *13*, 333–340. https://doi.org/10.2147/IDR.S234295.

Gharibani, K. M., Azami, A., Parvizi, M., Khademi, F., Mousavi, S. F., & Arzanlou, M. (2019). High frequency of macrolide-resistant Streptococcus pneumoniae colonization in respiratory tract of healthy children in Ardabil, Iran. *Tanaffos*, *18*(2), 118–125.

Gierke R., Wodi A. P., & Kobayashin M. (2021). Pneumococcal Disease. In *Epidemiology and Prevention of Vaccine-Preventable Diseases (The Pink Book: Course Textbook)* (14th ed., pp. 255–274). CDC. Retrieved from https://www.cdc.gov/vaccines/pubs/pinkbook/pneumo.html.

Gil, E., Noursadeghi, M., & Brown, J. S. (2022). Streptococcus pneumoniae interactions with the complement system. *Frontiers in Cellular and Infection Microbiology*, *12*(July), 929483. https://doi.org/10.3389/fcimb.2022.929483.

Ginsburg, I. (2002). Role of lipoteichoic acid in infection and inflammation. *The Lancet Infectious Diseases*, *2*(3), 171–179. https://doi.org/10.1016/S1473-3099(02)00226-8

Gordon, S. B., Kanyanda, S., Walsh, A. L., Goddard, K., Chaponda, M., Atkinson, V., *et al.* (2003). Poor Potential Coverage for 7-Valent Pneumococcal Conjugate Vaccine, Malawi. *Emerging Infectious Diseases*, *9*(6), 747–749. https://doi.org/10.3201/eid0906.030020.

Goyal, R., Singh, N., Kaur, M., & Talwar, V. (2007). Antimicrobial Resistance in Invasive and Colonising Streptococcus Pneumoniae in North India. *Indian Journal of Medical Microbiology*, *25*(3), 256–259. https://doi.org/10.1016/s0255-0857(21)02117-4.

Grimwood, K., Anderson, V. A., Bond, L., Catroppa, C., Hore, R. L., Keir, E. H., Nolan, T., Roberton, D. M. (1995). Adverse Outcomes of Bacterial Meningitis in School-Age Survivors. *Pediatrics*, *95*(5), 646–656. https://doi.org/10.1542/peds.95.5.646.

Gupte, S. (2010). *The Short Textbook of Medical Microbiology* (10th ed.). New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. https://doi.org/10.5005/jp/books/11044.

Guo, M.-Y., Shi, X.-H., Gao, W., Tian, J.-L., Yuan, L., Yang, J., *et al.* (2023). The dynamic change of serotype distribution and antimicrobial resistance of pneumococcal isolates since PCV13 administration and COVID-19 control in Urumqi, China. *Frontiers in Cellular and Infection Microbiology*, *13*(January), 1–9. https://doi.org/10.3389/fcimb.2023.1110652.

Gut, H., King, S. J., & Walsh, M. A. (2008). Structural and functional studies of Streptococcus pneumoniae neuraminidase B: An intramolecular trans-sialidase. *FEBS Letters*, *582*(23–24), 3348–3352. https://doi.org/10.1016/j.febslet.2008.08.026.

Habib, M., Porter, B. D., & Satzke, C. (2014). Capsular Serotyping of Streptococcus pneumoniae Using the Quellung Reaction. *Journal of Visualized Experiments*, (84), 1–4. https://doi.org/10.3791/51208.

Hadjipanayis, A., Efstathiou, E., Alexandrou, M., Panayiotou, L., Zachariadou, C., Petrou, P., & Papaevangelou, V. (2016). Nasopharyngeal pneumococcal carriage among healthy children in Cyprus post widespread simultaneous implementation of PCV10 and PCV13 vaccines. *PLoS ONE*, *11*(10), 1–15. https://doi.org/10.1371/journal.pone.0163269.

Halboup, A., Abdi, A., Ahmed, M., Al-Qadasi, F., & Othman, G. Q. (2020). Access to antibiotics without prescription in community pharmacies in Yemen during the political conflict. *Public Health*, *183*, 30–35. https://doi.org/10.1016/j.puhe.2020.03.003.

Hall, S. C., Smith, D. R., Dyavar, S. R., Wyatt, T. A., Samuelson, D. R., Bailey, K. L., & Knoell, D. L. (2021). Critical Role of Zinc Transporter (ZIP8) in Myeloid Innate Immune Cell Function and the Host Response against Bacterial Pneumonia. *The Journal of Immunology*, *207*(5), 1357–1370. https://doi.org/10.4049/jimmunol.2001395.

Hammitt, L. L., Murdoch, D. R., Scott, J. A. G., Driscoll, A., Karron, R. A., Levine, O. S., & O'Brien, K. L. (2012). Specimen Collection for the Diagnosis of Pediatric Pneumonia. *Clinical Infectious Diseases*, *54*(suppl_2), S132–S139. https://doi.org/10.1093/cid/cir1068.

Hanna-Wakim, R., Chehab, H., Mahfouz, I., Nassar, F., Baroud, M., Shehab, M., *et al.* (2012). Epidemiologic characteristics, serotypes, and antimicrobial susceptibilities of invasive Streptococcus pneumoniae isolates in a nationwide surveillance study in Lebanon. *Vaccine*, *30*(SUPPL. 6), G11–G17. https://doi.org/10.1016/j.vaccine.2012.07.020.

Harris, M., Clark, J., Coote, N., Fletcher, P., Harnden, A., McKean, M., & Thomson, A. (2011). British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. *Thorax*, *66*(Suppl 2), ii1–ii23. https://doi.org/10.1136/thoraxjnl-2011-200598.

Hasanuzzaman, M., Saha, S., Malaker, R., Rahman, H., Sajib, M. S. I., Das, R. C., *et al.* (2021). Comparison of Culture, Antigen Test, and Polymerase Chain Reaction for Pneumococcal Detection in Cerebrospinal Fluid of Children. *JID*, 224, S209–S217. https://doi.org/10.1093/infdis/jiab073.

Henriques-Normark, B., & Tuomanen, E. I. (2013). The Pneumococcus: Epidemiology, Microbiology, and Pathogenesis. *Cold Spring Harbor Perspectives in Medicine*, *3*(7), a010215–a010215. https://doi.org/10.1101/cshperspect.a010215.

Heß, N., Waldow, F., Kohler, T. P., Rohde, M., Kreikemeyer, B., Gómez-Mejia, A., *et al.* (2017). Lipoteichoic acid deficiency permits normal growth but impairs virulence of Streptococcus pneumonia. *Nature Communications*, *8*(1). https://doi.org/10.1038/s41467-017-01720-z.

Higgs, C., Kumar, L. S., Stevens, K., Strachan, J., Sherry, N. L., Horan, K., *et al.* (2023). Population structure, serotype distribution and antibiotic resistance of Streptococcus pneumoniae causing invasive disease in Victoria, Australia. *Microbial Genomics*, *9*(7), 1–14. https://doi.org/10.1099/mgen.0.001070.

Hill, H., Mitsi, E., Nikolaou, E., Blizard, A., Pojar, S., Howard, A., *et al*. (2023). A Randomized Controlled Clinical Trial of Nasal Immunization with Live Virulence Attenuated Streptococcus pneumoniae Strains Using Human Infection Challenge. *American Journal of Respiratory & Critical Care Medicine*, *208*(8), 868–878. https://doi.org/10.1164/rccm.202302-0222OC.

Hill, P. C., Yin, B. C., Akisanya, A., Sankareh, K., Lahai, G., Greenwood, B. M., & Adegbola, R. A. (2008). Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian infants: A longitudinal study. *Clinical Infectious Diseases*, *46*(6), 807–814. https://doi.org/10.1086/528688.

Hilleringmann, M., Kohler, S., Gámez, G., & Hammerschmidt, S. (2015). Pneumococcal Pili and Adhesins. In J. Brown, S. Hammerschmidt, & C. B. T.-S. P. Orihuela (Eds.), *Streptococcus Pneumoniae* (pp. 309–346). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-12-410530-0.00017-X.

Hirayama, S., Domon, H., Hiyoshi, T., Isono, T., Tamura, H., Sasagawa, K., Takizawa, F., Terao, Y. (2022). Triosephosphate isomerase of Streptococcus pneumoniae is released extracellularly by autolysis and binds to host plasminogen to promote its activation. *FEBS Open Bio*, *12*(6), 1206–1219. https://doi.org/10.1002/2211-5463.13396.

Hjálmarsdóttir, M., Haraldsson, G., Quirk, S. J., Haraldsson, Á., Erlendsdóttir, H., & Kristinsson, K. G. (2020). Reduction of antimicrobial resistant pneumococci seven years after introduction of pneumococcal vaccine in Iceland. *PLoS ONE*, *15*(3), 1–13. https://doi.org/10.1371/journal.pone.0230332.

Houri, H., Tabatabaei, S. R., Saee, Y., Fallah, F., Rahbar, M., & Karimi, A. (2017). Distribution of capsular types and drug resistance patterns of invasive pediatric Streptococcus pneumoniae isolates in Teheran, Iran. *International Journal of Infectious Diseases*, *57*, 21–26. https://doi.org/10.1016/j.ijid.2017.01.020.

Howe, J., & Wilson, T. (1972). Co-Trimoxazole-Resistant Pneumococci. *The Lancet*, *300*(7769), 184–185. https://doi.org/10.1016/S0140-6736(72)91354-2.

Hu, Y., Park, N., Seo, K. S., Park, J. Y., Somarathne, R. P., Olivier, A. K., Fitzkee N. C., Thornton, J. A. (2021). Pneumococcal surface adhesion A protein (PsaA) interacts with human Annexin A2 on airway epithelial cells. *Virulence*, *12*(1), 1841–1854. https://doi.org/10.1080/21505594.2021.1947176.

Ibrahim, D. D., Ousmane, S., Moumouni, K., & Mahamane, A. E. (2017). Measurement of pneumococcal IgG antibody, carriage and infection with Streptococcus pneumoniae in children under five years of age with acute respiratory infection in Niger. *Journal of Infection and Public Health*, *10*(5), 657–660. https://doi.org/10.1016/j.jiph.2017.05.007.

Infante, A. J., McCullers, J. A., & Orihuela, C. J. (2015). Mechanisms of Predisposition to Pneumonia. In O. C. Brown J, Hammerschmidt S (Ed.), *Streptococcus Pneumoniae* (pp. 363–382). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-12-410530-0.00019-3.

Iovino, F., Nannapaneni, P., Henriques-Normark, B., & Normark, S. (2020). The impact of the ancillary pilus-1 protein RrgA of Streptococcus pneumoniae on colonization and disease. *Molecular Microbiology*, *113*(3), 650–658. https://doi.org/10.1111/mmi.14451.

Iuchi, H., Ohori, J., Kyutoku, T., Ito, K., & Kurono, Y. (2019). Role of phosphorylcholine in Streptococcus pneumoniae and nontypeable Haemophilus influenzae adherence to epithelial cells. *Auris, Nasus, Larynx*, *46*(4), 513–519. https://doi.org/10.1016/j.anl.2018.11.003.

Jamsheer, A., Rafay, A. M., Daoud, Z., Morrissey, I., & Torumkuney, D. (2016). Results from the Survey of Antibiotic Resistance (SOAR) 2011–13 in the Gulf States. *Journal of Antimicrobial Chemotherapy*, *71*(suppl 1), i45–i61. https://doi.org/10.1093/jac/dkw064.

Jean, S. S., & Hsueh, P. R. (2011). High burden of antimicrobial resistance in Asia. *International Journal of Antimicrobial Agents*, *37*(4), 291–295. https://doi.org/10.1016/j.ijantimicag.2011.01.009.

Jhelum, H., Sori, H., & Sehgal, D. (2018). A novel extracellular vesicle-associated endodeoxyribonuclease helps Streptococcus pneumoniae evade neutrophil extracellular traps and is required for full virulence. *Scientific Reports*, *8*(1), 7985. https://doi.org/10.1038/s41598-018-25865-z.

Jroundi, I., Mahraoui, C., Benmessaoud, R., Moraleda, C., Munoz Almagro, C., Seffar, M., *et al.* (2017). Streptococcus pneumoniae carriage among healthy and sick pediatric patients before the generalized implementation of the 13-valent pneumococcal vaccine in Morocco from 2010 to 2011. *Journal of Infection and Public Health*, *10*(2), 165–170. https://doi.org/10.1016/j.jiph.2016.02.012.

Kabir, F., Muneer, S., Kalam, A., Sami, A., Qureshi, S., Hotwani, A., *et al*. (2017). Experience with the quantitative lytA gene real-time polymerase chain reaction for the detection of Streptococcus pneumoniae from pediatric whole blood in Pakistan. *Biomedical and Biotechnology Research Journal (BBRJ)*, *1*(1), 71. https://doi.org/10.4103/bbrj.bbrj_26_17.

Kaltoft, M. S., Skov Sørensen, U. B., Slotved, H.-C., & Konradsen, H. B. (2008). An easy method for detection of nasopharyngeal carriage of multiple Streptococcus pneumoniae serotypes. *Journal of Microbiological Methods*, *75*(3), 540–544. https://doi.org/10.1016/j.mimet.2008.08.010.

Karami, M., Hosseini, S. M., Hashemi, S. H., Ghiasvand, S., Zarei, O., Safari, N., Erfani, H., Alikhani, M. Y. (2019). Prevalence of nasopharyngeal carriage of Streptococcus pneumoniae in children 7 to 14 years in 2016: A survey before pneumococcal conjugate vaccine introduction in Iran. *Human Vaccines & Immunotherapeutics*, *15*(9), 2178–2182. https://doi.org/10.1080/21645515.2018.1539601.

Karimaei, S., Tohidinik, H. R., Afshar, D., Pourmand, M. R., Ghahfarokhi, S. H., Goodarzi, N. N., & Azarsa, M. (2021). Antimicrobial susceptibility pattern and serotype distribution of streptococcus pneumoniae in the middle east region: A systematic review and meta-analysis. *Acta Medica Iranica*, *59*(2), 64–78. https://doi.org/10.18502/acta.v59i2.5572.

Kauffmann, F., Lund E., Eddy B. E. (1960). Proposal for A change in the Nomenclature of Diplococcus pneumoniae and A comparison of the Danish and American Type Designations. *International Bulletin of Bacterioological Nomenclature and Taxonomy*, 10(1), 31-40.

Kawaguchiya, M., Urushibara, N., Aung, M. S., Ohashi, N., Takamatsu, R., Tsutida, S., Ito, M., Kobayashi, N. (2023). Spread of multidrug resistance in non-PCV13/PCV20 serotypes of Streptococcus pneumoniae: a cross-sectional study ten years after the introduction of pneumococcal conjugate vaccine in Japan. *Journal of Microbiology, Immunology and Infection*, 118159. https://doi.org/10.1016/j.jmii.2023.07.004.

Kearns, A. M., Graham, C., Burdess, D., Heatherington, J., & Freeman, R. (2002). Rapid Real-Time PCR for Determination of Penicillin Susceptibility in Pneumococcal Meningitis, Including Culture-Negative Cases. *Journal of Clinical Microbiology*, *40*(2), 682–684. https://doi.org/10.1128/JCM.40.2.682-684.2002.

Keller, L. E., Robinson, D. A., & McDaniel, L. S. (2016). Nonencapsulated Streptococcus pneumoniae: Emergence and Pathogenesis. *MBio*, *7*(2), e01792. https://doi.org/10.1128/mBio.01792-15.

Kietzman, C. C., Gao, G., Mann, B., Myers, L., & Tuomanen, E. I. (2016). Dynamic capsule restructuring by the main pneumococcal autolysin LytA in response to the epithelium. *Nature Communications*, *7*. https://doi.org/10.1038/ncomms10859.

Kim, G. L., Seon, S. H., & Rhee, D. K. (2017). Pneumonia and Streptococcus pneumoniae vaccine. *Archives of Pharmacal Research*, *40*(8), 885–893. https://doi.org/10.1007/s12272-017-0933-y.

Kim, G. R., Kim, E. Y., Kim, S. H., Lee, H. K., Lee, J., Shin, J. H., *et al*. (2023). Serotype Distribution and Antimicrobial Resistance of Streptococcus pneumoniae Causing Invasive Pneumococcal Disease in Korea Between 2017 and 2019 After Introduction of the 13-Valent Pneumococcal Conjugate Vaccine. *Annals of Laboratory Medicine*, *43*(1), 45–54. https://doi.org/10.3343/alm.2023.43.1.45.

King, S. J. (2010). Pneumococcal modification of host sugars: a major contributor to colonization of the human airway? *Molecular Oral Microbiology*, *25*(1), 15–24. https://doi.org/10.1111/j.2041-1014.2009.00564.x.

Koliou, M. G., Andreou, K., Lamnisos, D., Lavranos, G., Iakovides, P., Economou, C., & Soteriades, E. S. (2017). Serotypes and antimicrobial resistance of S. pneumoniae nasopharyngeal carriage in children from Cyprus: A country with relatively low coverage with the seven-valent pneumococcal conjugate vaccine. *Journal of Epidemiological Research*, *3*(2), 51. https://doi.org/10.5430/jer.v3n2p51.

Koliou, M. G., Andreou, K., Lamnisos, D., Lavranos, G., Iakovides, P., Economou, C., & Soteriades, E. S. (2018). Risk factors for carriage of Streptococcus pneumoniae in children. *BMC Pediatrics*, *18*(1), 144. https://doi.org/10.1186/s12887-018-1119-6.

Kono, M., Zafar, M. A., Zuniga, M., Roche, A. M., Hamaguchi, S., & Weiser, J. N. (2016). Single Cell Bottlenecks in the Pathogenesis of Streptococcus pneumoniae. *PLOS Pathogens*, *12*(10), e1005887. https://doi.org/10.1371/journal.ppat.1005887.

Ktari, S., Ben Ayed, N. E. H., Maalej, S., Mnif, B., Rhimi, F., & Hammami, A. (2021). Clinical optochin resistant Streptococcus pneumoniae and Streptococcus pseudopneumoniae strains in Tunisia. *Journal of Infection in Developing Countries*, *15*(5), 672–677. https://doi.org/10.3855/jidc.13106.

Ktari, S., Jmal, I., Mroua, M., Maalej, S., Ben Ayed, N. E. H., Mnif, B., Rhimi, F., Hammami, A. (2017). Serotype distribution and antibiotic susceptibility of Streptococcus pneumoniae strains in the south of Tunisia: A five-year study (2012–2016) of pediatric and adult populations. *International Journal of Infectious Diseases*, *65*, 110–115. https://doi.org/10.1016/j.ijid.2017.10.015.

Kuch, A., Gołębiewska, A., Waśko, I., Ronkiewicz, P., Markowska, M., Hryniewicz, W., & Skoczyńska, A. (2014). Usefulness of Pneumotest-Latex for direct serotyping of Streptococcus pneumoniae isolates in clinical samples. *Journal of Clinical Microbiology*, *52*(7), 2647–2649. https://doi.org/10.1128/JCM.00451-14.

Kumar, A., Faiq, M., Pareek, V., Kumari, C., Narayan, R., & Ghosh, S. (2019). *Discovery of the DNA Double-Helix vis-a-vis Publication Ethics in Modern Science: Could it Survive the Standard Editorial Scrutiny and Peer Review?* [Preprint]. ResearchGate. https://doi.org/10.13140/RG.2.2.20495.94887.

Kumar, S. (2016). Pneumococcus (Diplococcus pneumoniae: Streptococcus pneumoniae). In *Essentials of Microbiology* (1/e ed, pp. 183–187). New Delhi: JP Medical Ltd.

Laan, Z. (2020). *Humoral responses induced by an enzymatically active , whole-cell killed pneumococcal vaccine*. (Master's thesis, School of Biological Sciences) University of Adelaide. Retrieved from https://hdl.handle.net/2440/127329.

Lagousi, T., Basdeki, P., De Jonge, M. I., & Spoulou, V. (2020). Understanding host immune responses to pneumococcal proteins in the upper respiratory tract to develop serotype-independent pneumococcal vaccines. *Expert Review of Vaccines*, *19*(10), 959–972. https://doi.org/10.1080/14760584.2020.1843433.

Lan, Y., Liu, L., Hu, D., Ge, L., Xiang, X., Peng, M., *et al.* (2023). Limited protection of pneumococcal vaccines against emergent Streptococcus pneumoniae serotype 14/ST876 strains. *Infection*, (0123456789). https://doi.org/10.1007/s15010-023-02110-y.

Lee, N. Y., Song, J.-H., Kim, S., Peck, K. R., Ahn, K.-M., Lee, S.-I., *et al*. (2001). Carriage of Antibiotic-Resistant Pneumococci among Asian Children: A Multinational Surveillance by the Asian Network for Surveillance of Resistant Pathogens (ANSORP). *Clinical Infectious Diseases*, *32*(10), 1463–1469. https://doi.org/10.1086/320165.

Letsiou, E., Teixeira Alves, L. G., Felten, M., Mitchell, T. J., Müller-Redetzky, H. C., Dudek, S. M., & Witzenrath, M. (2021). Neutrophil-Derived Extracellular Vesicles Activate Platelets after Pneumolysin Exposure. *Cells*, *10*(12), 3581. https://doi.org/10.3390/cells10123581.

Leung, M. (2012). *Phenotypic and genotypic diversity in Streptococcus pneumoniae strains in Tanzania and the United Kingdom*. (Doctoral thesis). University College London). Retrieved from http://discovery.ucl.ac.uk/1359412/1/1359412_Leung_Thesis_Final.pdf.

Littorin, N. (2020). *Streptococcus pneumoniae infections before and after the introduction of a conjugated pneumococcal vaccine* (Doctoral thesis). Lund University. Retrieved from https://portal.research.lu.se/portal/files/79697940/e_spik_ex_Nils_L.pdf.

Iliyasu, G., Habib, A. G., & Aminu, M. B. (2015). Antimicrobial susceptibility pattern of invasive pneumococcal isolates in North West Nigeria. *Journal of Global Infectious Diseases*, *7*(2), 70–74. https://doi.org/10.4103/0974-777X.154440.

Lobb, B., Lee, M. C., McElheny, C. L., Doi, Y., Yahner, K., Hoberman, A., Martin, M.J., Hirota J. A., Doxey, A. C., Shaikh, N. (2023). Genomic classification and antimicrobial resistance profiling of Streptococcus pneumoniae and Haemophilus influenzae isolates associated with paediatric otitis media and upper respiratory infection. *BMC Infectious Diseases*, *23*(1), 596. https://doi.org/10.1186/s12879-023-08560-x.

Loose, M., Hudel, M., Zimmer, K.-P., Garcia, E., Hammerschmidt, S., Lucas, R., Chakraborty, R., Pillich, H. (2015). Pneumococcal Hydrogen Peroxide–Induced Stress Signaling Regulates Inflammatory Genes. *Journal of Infectious Diseases*, *211*(2), 306–316. https://doi.org/10.1093/infdis/jiu428.

Maestro, B., & Sanz, J. M. (2016). Choline Binding Proteins from Streptococcus pneumoniae: A Dual Role as Enzybiotics and Targets for the Design of New Antimicrobials. *Antibiotics*, *5*(2), 21. https://doi.org/10.3390/antibiotics5020021.

Manenzhe, R. I., Moodley, C., Abdulgader, S. M., Robberts, F. J. L., Zar, H. J., Nicol, M. P., & Dube, F. S. (2019). Nasopharyngeal carriage of antimicrobial-resistant pneumococci in an intensively sampled South African Birth Cohort. *Frontiers in Microbiology*, *10*(MAR), 1–10. https://doi.org/10.3389/fmicb.2019.00610.

Masomian, M., Ahmad, Z., Ti Gew, L., & Poh, C. L. (2020). Development of Next Generation Streptococcus pneumoniae Vaccines Conferring Broad Protection. *Vaccines*, *8*(1), 132. https://doi.org/10.3390/vaccines8010132.

Mathew, B. J., Gupta, P., Naaz, T., Rai, R., Gupta, S., Gupta, S., *et al*. (2023). Role of Streptococcus pneumoniae extracellular glycosidases in immune evasion. *Frontiers in Cellular and Infection Microbiology*, *13*(February), 1–8. https://doi.org/10.3389/fcimb.2023.1109449.

Mellroth, P., Daniels, R., Eberhardt, A., Rönnlund, D., Blom, H., Widengren, J., Normark, S., Henriques-Normark, B. (2012). LytA, major autolysin of Streptococcus pneumoniae, requires access to nascent peptidoglycan. *Journal of Biological Chemistry*, *287*(14), 11018–11029. https://doi.org/10.1074/jbc.M111.318584.

Méroc, E., Fletcher, M. A., Hanquet, G., Slack, M. P. E., Baay, M., Hayford, K., Gessner B. D., Grant, L. R. (2023). Systematic Literature Review of the Epidemiological Characteristics of Pneumococcal Disease Caused by the Additional Serotypes Covered by the 20-Valent Pneumococcal Conjugate Vaccine. *Microorganisms*, *11*(7), 1816. https://doi.org/10.3390/microorganisms11071816.

Michelow, I. C., Lozano, J., Olsen, K., Goto, C., Rollins, N. K., Ghaffar, F., *et al*. (2002). Diagnosis of Streptococcus pneumoniae Lower Respiratory Infection in Hospitalized Children by Culture, Polymerase Chain Reaction, Serological Testing, and Urinary Antigen Detection. *Clinical Infectious Diseases*, *34*(1), e1–e11. https://doi.org/10.1086/324358.

Miellet, W. R., van Veldhuizen, J., Litt, D., Mariman, R., Wijmenga-Monsuur, A. J., Badoux, P., *et al*. (2022). It Takes Two to Tango: Combining Conventional Culture With Molecular Diagnostics Enhances Accuracy of Streptococcus pneumoniae Detection and Pneumococcal Serogroup/Serotype Determination in Carriage. *Frontiers in Microbiology*, 13(April), 1–16. https://doi.org/10.3389/fmicb.2022.859736.

Miguélez, S. A., Guirao, G. Y., Ruíz, A. I. M., Sanchez-Solís, M., Lucas, M. D., González-Camacho, F., *et al*. (2021). Impact of pneumococcal vaccination in the nasopharyngeal carriage of streptococcus pneumoniae in healthy children of the murcia region in Spain. *Vaccines*, *9*(1), 1–13. https://doi.org/10.3390/vaccines9010014.

Mitchell, T. J., & Dalziel, C. E. (2014). The Biology of Pneumolysin. In *Sub-Cellular Biochemistry* (Vol. 80, pp. 145–160). https://doi.org/10.1007/978-94-017-8881-6_8.

Miyazaki, H., Shibuya, R., Chang, B., Inukai, T., Miyazaki, Y., Ubukata, K., Nakamura, S., Matsumoto, T. (2020). Genetic characteristics of piliated Streptococcus pneumoniae serotype 35B, increased after introduction of pneumococcal vaccines in Japan. *Journal of Infection and Chemotherapy*, *26*(11), 1198–1204. https://doi.org/10.1016/j.jiac.2020.06.016.

Mohanna, M. (2010). Self-medication with Antibiotic in Children in Sana'a City, Yemen. *Oman Medical Journal*, *25*(1), 41–43. https://doi.org/10.5001/omj.2010.10.

Mohanna, M. A. B., & Bahannan, A. A. (2016). Bacterial Profile And Antibiogram Of Otitis Media Among Children In Yemen. *Journal of Ayub Medical College, Abbottabad*, *28*(3), 480–483.

Mokaddas, E., & Albert, M. J. (2012). Impact of pneumococcal conjugate vaccines on burden of invasive pneumococcal disease and serotype distribution of Streptococcus pneumoniae isolates: An overview from Kuwait. *Vaccine*, 30, G37-G40. https://doi.org/10.1016/j.vaccine.2012.10.061.

Mokaddas, E. M., Rotimi, V. O., & Albert, M. J. (2007). Increasing Prevalence of Antimicrobial Resistance in Streptococcus pneumoniae in Kuwait: Implications for Therapy. *Microbial Drug Resistance*, *13*(4), 227–234. https://doi.org/10.1089/mdr.2007.774.

Mokaddas, E. M., Rotimi, V. O., & Albert, M. J. (2008). Implications of Streptococcus pneumoniae Penicillin Resistance and Serotype Distribution in Kuwait for Disease Treatment and Prevention. *Clinical and Vaccine Immunology*, *15*(2), 203–207. https://doi.org/10.1128/CVI.00277-07.

Mokaddas, E., & Albert, M. J. (2016). Serotype distribution and penicillin-non-susceptibility of Streptococcus pneumoniae causing invasive diseases in Kuwait: A 10-year study of impact of pneumococcal conjugate vaccines. *Expert Review of Vaccines*, *15*(10), 1337–1345. https://doi.org/10.1080/14760584.2016.1198698.

Mokaddas, E. M., Shibl, A. M., Elgouhary, A., & Elsobky, M. (2018). Effect of the introduction of pneumococcal conjugate vaccines on serotype prevalence in Kuwait and Saudi Arabia. *Vaccine*, *36*(43), 6442–6448. https://doi.org/10.1016/j.vaccine.2018.07.067.

Mokaddas, E., Syed, S., & Albert, M. J. (2021). The 13-valent pneumococcal conjugate vaccine (PCV13) does not appear to provide much protection on combined invasive disease due to the six PCV13 non-PCV7 serotypes 1, 3, 5, 6A, 7F, and 19A in Kuwait during 2010–2019. *Human Vaccines & Immunotherapeutics*, *17*(11), 4661–4666. https://doi.org/10.1080/21645515.2021.1968216.

Morens, D. M., Taubenberger, J. K., & Fauci, A. S. (2008). Predominant Role of Bacterial Pneumonia as a Cause of Death in Pandemic Influenza: Implications for Pandemic Influenza Preparedness. *The Journal of Infectious Diseases*, *198*(7), 962–970. https://doi.org/10.1086/591708.

Morpeth, S. (2015). *Understanding the dynamics and spread of pneumococcal infection from healthy carriage to pneumonia and invasive disease, in Kilifi, Kenya* (Doctoral thesis). The Open University. https://doi.org/https://doi.org/10.21954/ou.ro.0000efe8.

Mosser, J. F., Grant, L. R., Millar, E. V., Weatherholtz, R. C., Jackson, D. M., Beall, B., *et al*. (2014). Nasopharyngeal Carriage and Transmission of Streptococcus pneumoniae in American Indian Households after a Decade of Pneumococcal Conjugate Vaccine Use. *PLoS ONE*, *9*(1), e79578. https://doi.org/10.1371/journal.pone.0079578.

Moyo, S. J., Steinbakk, M., Aboud, S., Mkopi, N., Kasubi, M., Blomberg, B., *et al*. (2012). Penicillin resistance and serotype distribution of Streptococcus pneumoniae in nasopharyngeal carrier children under 5 years of age in Dar es Salaam, Tanzania. *Journal of Medical Microbiology*, *61*(PART7), 952–959. https://doi.org/10.1099/jmm.0.042598-0.

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., *et al*. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2021). Bacteriology. In *Medical microbiology E-book* (19th ed., pp. 203–207). USA: Elsevier Health Sciences.

Musher, D. M., Anderson, R., & Feldman, C. (2022). The remarkable history of pneumococcal vaccination: an ongoing challenge. *Pneumonia*, *14*(1), 5. https://doi.org/10.1186/s41479-022-00097-y.

Nasereddin, A., Shtayeh, I., Ramlawi, A., Salman, N., Salem, I., & Abdeen, Z. (2013). Streptococcus pneumoniae from Palestinian nasopharyngeal carriers: Serotype distribution and antimicrobial resistance. *PLoS ONE*, *8*(12). https://doi.org/10.1371/journal.pone.0082047.

Nature. (1947). Carl Friedländer (1847–87). *Nature*, *160*(4072), 668–668. https://doi.org/10.1038/160668b0.

Navne, J. E. (2014). *Epidemiology of Streptococcus pneumoniae in Greenland Colonization, Invasive Disease and Vaccine Impact* (Doctoral thesis). Faculty of Health Science, University of Copenhagen.

Neal, E. F. G., Chan, J., Nguyen, C. D., & Russell, F. M. (2022). Factors associated with pneumococcal nasopharyngeal carriage: A systematic review. *PLOS Global Public Health*, *2*(4), e0000327. https://doi.org/10.1371/journal.pgph.0000327.

Negash, A. A., Asrat, D., Abebe, W., Hailemariam, T., Gebre, M., Verhaegen, J., Aseffa, A,. Vaneechoutte, M. (2019). Pneumococcal Carriage, Serotype Distribution, and Risk Factors in Children With Community-Acquired Pneumonia, 5 Years After Introduction of the 10-Valent Pneumococcal Conjugate Vaccine in Ethiopia. *Open Forum Infectious Diseases*, *6*(6), 1–8. https://doi.org/10.1093/ofid/ofz259.

Negri, M. C., Morosini, M. I., Loza, E., & Baquero, F. (1994). In vitro selective antibiotic concentrations of beta-lactams for penicillin-resistant Streptococcus pneumoniae populations. *Antimicrobial Agents and Chemotherapy*, *38*(1), 122–125. https://doi.org/10.1128/AAC.38.1.122.

Nelson, A. L., Roche, A. M., Gould, J. M., Chim, K., Ratner, A. J., & Weiser, J. N. (2007). Capsule Enhances Pneumococcal Colonization by Limiting Mucus-Mediated Clearance. *Infection and Immunity*, *75*(1), 83–90. https://doi.org/10.1128/IAI.01475-06.

Neufeld, F. (1902). Ueber die Agglutination der Pneumokokken und über die Theorieen der Agglutination. *Zeitschrift Für Hygiene Und Infektionskrankheiten*, *40*(1), 54–72. https://doi.org/10.1007/BF02140530.

Nichols, T., & Freeman, R. (1980). A new selective medium for Streptococcus pneumoniae. *Journal of Clinical Pathology*, *33*(8), 770–773. https://doi.org/10.1136/jcp.33.8.770.

O'Brien, K. L., Bronsdon, M. A., Dagan, R., Yagupsky, P., Janco, J., Elliott, J., *et al*. (2001). Evaluation of a medium (STGG) for transport and optimal recovery of Streptococcus pneumoniae from nasopharyngeal secretions collected during field studies. *Journal of Clinical Microbiology*, *39*(3), 1021–1024. https://doi.org/10.1128/JCM.39.3.1021-1024.2001.

O'Brien, K. L., Wolfson, L. J., Watt, J. P., Henkle, E., Deloria-Knoll, M., McCall, *et al*. (2009). Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *The Lancet*, *374*(9693), 893–902. https://doi.org/10.1016/S0140-6736(09)61204-6.

Ojal, J., Goldblatt, D., Tigoi, C., & Scott, J. A. G. (2018). Effect of Maternally Derived Anti-protein and Anticapsular IgG Antibodies on the Rate of Acquisition of Nasopharyngeal Carriage of Pneumococcus in Newborns. *Clinical Infectious Diseases*, *66*(1), 121–130. https://doi.org/10.1093/cid/cix742.

Olarte, L., & Jackson, M. A. (2021). Streptococcus pneumoniae. *Pediatrics In Review*, *42*(7), 349–359. https://doi.org/10.1542/pir.2020-0062.

Oliver, M. B., Jones, C., Larson, T. R., Calix, J. J., Zartler, E. R., Yother, J., & Nahm, M. H. (2013). Streptococcus pneumoniae serotype 11D has a bispecific glycosyltransferase and expresses two different capsular polysaccharide repeating units. *Journal of Biological Chemistry*, *288*(30), 21945–21954. https://doi.org/10.1074/jbc.M113.488528.

Örtqvist, Å., Hedlund, J., & Kalin, M. (2005). Streptococcus pneumoniae : Epidemiology, Risk Factors, and Clinical Features. *Seminars in Respiratory and Critical Care Medicine*, *26*(06), 563–574. https://doi.org/10.1055/s-2005-925523.

Orubu, E. S. F., Al-Dheeb, N., Ching, C., Bu Jawdeh, S., Anderson, J., Sheikh, R., Hariri, F. Zaman, M. H. (2021). Assessing Antimicrobial Resistance, Utilization, and Stewardship in Yemen: An Exploratory Mixed-Methods Study. *Am. J. Trop. Med. Hyg*, *105*(5), 1404–1412. https://doi.org/10.4269/ajtmh.21-0101.

Otte, S., Haugan, M. S., Eriksen, H. B., Kerrn, M. B., & Elverdal, P. L. (2014). ImmuLex^{TM} S. pneumoniae Omni a new latex agglutination test for rapid detection of S. pneumoniae in blood cultures and plate cultures. In *9th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD-9), Hyderabad, India*.

Özdemir, H., Çiftçi, E., Durmaz, R., Güriz, H., Aysev, A. D., Karbuz, A., *et al*. (2014). Nasopharyngeal carriage of Streptococcus pneumoniae in healthy Turkish children after the addition of PCV7 to the national vaccine schedule. *European Journal of Pediatrics*, *173*(3), 313–320. https://doi.org/10.1007/s00431-013-2156-7.

Parija, S. C. (2012). Pneumococcus. In *Textbook of Microbiology & Immunology-E-book* (2ed ed., pp. 195–200). Haryana, India: Elsevier Health Sciences.

Parker, D., Soong, G., Planet, P., Brower, J., Ratner, A. J., & Prince, A. (2009). The NanA neuraminidase of Streptococcus pneumoniae is involved in biofilm formation. *Infection and Immunity*, *77*(9), 3722–3730. https://doi.org/10.1128/IAI.00228-09.

Pereira, J. M., Xu, S., Leong, J. M., & Sousa, S. (2022). The Yin and Yang of Pneumolysin During Pneumococcal Infection. *Frontiers in Immunology*, *13*(April), 1–21. https://doi.org/10.3389/fimmu.2022.878244.

Pericone, C. D., Overweg, K., Hermans, P. W. M., & Weiser, J. N. (2000). Inhibitory and Bactericidal Effects of Hydrogen Peroxide Production by Streptococcus pneumoniae on Other Inhabitants of the Upper Respiratory Tract. *Infection and Immunity*, *68*(7), 3990–3997. https://doi.org/10.1128/IAI.68.7.3990-3997.2000.

Pericone, C. D., Park, S., Imlay, J. A., & Weiser, J. N. (2003). Factors Contributing to Hydrogen Peroxide Resistance in Streptococcus pneumoniae Include Pyruvate Oxidase (SpxB) and Avoidance of the Toxic Effects of the Fenton Reaction. *Journal of Bacteriology*, *185*(23), 6815–6825. https://doi.org/10.1128/JB.185.23.6815-6825.2003.

Petousis-Harris, D. H., Turner, N., Heffernan H., & N. M. (2014). 2012 Antigen Review for the New Zealand National Immunisation Schedule: Pneumococcal. Retrieved from http://hdl.handle.net/2292/47713.

Pettigrew, M. M., Fennie, K. P., York, M. P., Daniels, J., & Ghaffar, F. (2006). Variation in the presence of neuraminidase genes among Streptococcus pneumoniae isolates with identical sequence types. *Infection and Immunity*, *74*(6), 3360–3365. https://doi.org/10.1128/IAI.01442-05.

Pinti, M., Appay, V., Campisi, J., Frasca, D., Fülöp, T., Sauce, D., Larbi, A., Weinberger, B., Cossarizza, A. (2016). Aging of the immune system: Focus on inflammation and vaccination. *European Journal of Immunology*, *46*(10), 2286–2301. https://doi.org/10.1002/eji.201546178.

Podolsky, S. H. (2005). The Changing Fate of Pneumonia as a Public Health Concern in 20th-Century America and Beyond. *American Journal of Public Health*, *95*(12), 2144–2154. https://doi.org/10.2105/AJPH.2004.048397.

Pommerville, J. C. (2018). *Fundamentals of microbiology, Part IV, Chapter 11* (11th ed.). Burlington: Jones & Bartlett Publishers.

Procop, G. W., Church, D. L., Hall, G. S., & Janda, W. M. (2017). *Koneman's color atlas and textbook of diagnostic microbiology*. (J. Joyce, Ed.) (17th ed.). USA: Wolters Kluwer.

Quintero Moreno, B., Araque, M., & Mendoza, E. (2017). Evaluation of Two Supplemented Culture Media for Long-Term, Room-Temperature Preservation of Streptococcus pneumoniae Strains. *BioMed Research International*, *2017*, 1–9. https://doi.org/10.1155/2017/1218798.

Rai, P., Parrish, M., Tay, I. J. J., Li, N., Ackerman, S., He, F., Kwang, J., Chow, V, T., Engelward, B. P. (2015). Streptococcus pneumoniae secretes hydrogen peroxide leading to DNA damage and apoptosis in lung cells. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), E3421–E3430. https://doi.org/10.1073/pnas.1424144112.

Rajam, G., Anderton, J. M., Carlone, G. M., Sampson, J. S., & Ades, E. W. (2008). Pneumococcal surface adhesin A (PsaA): A review. *Critical Reviews in Microbiology*, *34*(3–4), 131–142. https://doi.org/10.1080/10408410802275352.

Regev-Yochay, G., Abullaish, I., Malley, R., Shainberg, B., Varon, M., Roytman, Y., *et al*. (2012). Streptococcus pneumoniae Carriage in the Gaza Strip. *PLoS ONE*, *7*(4), e35061. https://doi.org/10.1371/journal.pone.0035061.

Reslan, L., Finianos, M., Bitar, I., Moumneh, M. B., Araj, G. F., Zaghlout, A., *et al*. (2021). The Emergence of Invasive Streptococcus pneumoniae Serotype 24F in Lebanon: Complete Genome Sequencing Reveals High Virulence and Antimicrobial Resistance Characteristics. *Frontiers in Microbiology*, *12*(February), 1–10. https://doi.org/10.3389/fmicb.2021.637813.

Reslan, L., Youssef, N., Boutros, C. F., Assaf-Casals, A., Fayad, D., Khafaja, S., *et al*. (2022). The impact of vaccination on the burden of invasive pneumococcal disease from a nationwide surveillance program in Lebanon: an unexpected increase in mortality driven by non-vaccine serotypes. *Expert Review of Vaccines*, *21*(12), 1905–1921. https://doi.org/10.1080/14760584.2022.2143349.

Ricketson, L. J., Lidder, R., Thorington, R., Martin, I., Vanderkooi, O. G., Sadarangani, M., & Kellner, J. D. (2021). PCR and Culture Analysis of Streptococcus pneumoniae Nasopharyngeal Carriage in Healthy Children. *Microorganisms*, *9*(10), 2116. https://doi.org/10.3390/microorganisms9102116.

Riedel S., Hobden J. A., Miller S., *et al*. (2019). Section III Bacteriology. In *Jawetz, Melnick & Adelberg's Medical Microbiology* (28th ed., pp. 164, 215–228). USA: McGraw-Hill Education.

Rijkers, G. T., Sanders, E. A. M., Breukels, M. A., & Zegers, B. J. M. (1998). Infant B cell responses to polysaccharide determinants. *Vaccine*, *16*(14–15), 1396–1400. https://doi.org/10.1016/S0264-410X(98)00098-X.

Ryan, K. J., Ahmad, N., Alspaugh, J. A., Drew, W. L., Lagunoff, M., Pottinger, P., Reller, L., Sterling L., & W. S. (2018). *Sherris Medical Microbiology*. (K. Ryan, Ed.) (17th ed., Vol. 1). New York: McGraw-Hill Companies.

Saadi, A. T., Garjees, N. A., & Rasool, A. H. (2017). Antibiogram profile of septic meningitis among children in Duhok, Iraq. *Saudi Medical Journal*, *38*(5), 517–520. https://doi.org/10.15537/smj.2017.5.19300.

Sadeq, H., Husain, E. H., Alkoot, A., Atyani, S., Al-fraij, A., Al-Daithan, A., AlSaleem, T., Taher, A., Alenezi, M. (2017). Childhood meningitis in Kuwait in the era of post pneumococcal conjugate vaccination: A multicenter study. *Journal of Infection and Public Health*, *10*(6), 766–769. https://doi.org/10.1016/j.jiph.2016.11.009.

Sader, H. S., & Pignatari, A. C. C. (1994). E Test: a novel technique for antimicrobial susceptibility testing. *Sao Paulo Medical Journal*, *112*(4), 635–638. https://doi.org/10.1590/S1516-31801994000400003.

Sallam, M. (2019). Trends in Antimicrobial Drug Resistance of Streptococcus pneumoniae Isolates at Jordan University Hospital (2000–2018). *Antibiotics*, *8*(2), 41. https://doi.org/10.3390/antibiotics8020041.

Salsabila, K., Paramaiswari, W. T., Amalia, H., Ruyani, A., Tafroji, W., Winarti, Y., Khoeri, M. M., Safari, D. (2022). Nasopharyngeal carriage rate, serotype distribution, and antimicrobial susceptibility profile of Streptococcus pneumoniae isolated from children under five years old in Kotabaru, South Kalimantan, Indonesia. *Journal of Microbiology, Immunology and Infection*, *55*(3), 482–488. https://doi.org/10.1016/j.jmii.2021.06.006.

Salvadori, G., Junges, R., Morrison, D. A., & Petersen, F. C. (2019). Competence in streptococcus pneumoniae and close commensal relatives: Mechanisms and implications. *Frontiers in Cellular and Infection Microbiology*, *9*(APR), 1–8. https://doi.org/10.3389/fcimb.2019.00094.

Sanaei, A., Abdinia, B., & Karimi, A. (2012). Nasopharyngeal Carrier Rate of Streptococcus pneumoniae in Children: Serotype Distribution and Antimicrobial Resistance. *Arch Iran Med, 15*(8), 500–503. PMID: 22827788.

Sanders, M. E., Norcross, E. W., Robertson, Z. M., Moore, Q. C., Fratkin, J., & Marquart, M. E. (2011). The Streptococcus pneumoniae Capsule Is Required for Full Virulence in Pneumococcal Endophthalmitis. *Investigative Ophthalmology & Visual Science*, *52*(2), 865–872. https://doi.org/10.1167/iovs.10-5513.

Sanz, J. C., Culebras, E., Ríos, E., Rodríguez-Avial, I., Wilhelmi, I., Ramos, B., Sanz, J. C., Picazo, J. J. (2010). Direct serogrouping of Streptococcus pneumoniae strains in clinical samples by use of a latex agglutination test. *Journal of Clinical Microbiology*, *48*(2), 593–595. https://doi.org/10.1128/JCM.01651-09.

Sapkota, M., & Knoell, D. L. (2018). Essential role of zinc and zinc transporters in myeloid cell function and host defense against infection. *Journal of Immunology Research*, 2018. https://doi.org/10.1155/2018/4315140.

Sastry, A. S., & Bhat, S. (2019). *Essentials of medical microbiology* (2nd ed.). JP Medical Ltd. New Delhi, India.

Satzke, C., Turner, P., Virolainen-Julkunen, A., Adrian, P. V., Antonio, M., Hare, K. M., *et al*. (2014). Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine*, *32*(1), 165–179. https://doi.org/10.1016/j.vaccine.2013.08.062.

Sayyahfar, S., Esteghamati, A., Fahimzad, S. A., Hajisadeghi-Isfahani, S., Nazari-Alam, A., & Azimi, L. (2021). Serotype Distribution of Streptococcus pneumoniae Carriage in Six-Month-Old Infants: A Cross-sectional Study During 2017-18, Tehran, Iran. *Archives of Pediatric Infectious Diseases*, *10*(1), 1–8. https://doi.org/10.5812/pedinfect.112705.

Schrag, S. J., Beall, B., & Dowell, S. (2001). *Resistant pneumococcal infections : the burden of disease and challenges in monitoring and controlling antimicrobial resistance*. (World Health Organization Communicable Diseases Cluster). World Health Organization. https://iris.who.int/handle/10665/66846.

Schroeder, M. R., & Stephens, D. S. (2016). Macrolide Resistance in Streptococcus pneumoniae. *Frontiers in Cellular and Infection Microbiology*, *6*(SEP), 98. https://doi.org/10.3389/fcimb.2016.00098.

Shadi, A. Z. (2019). Antibiotic resistance in Saudi Arabia and some Middle Eastern countries: Current status. *African Journal of Microbiology Research*, *13*(8), 151–157. https://doi.org/10.5897/AJMR2019.9048.

Shaper, M., Hollingshead, S. K., Benjamin, W. H., & Briles, D. E. (2004). PspA Protects Streptococcus pneumoniae from Killing by Apolactoferrin, and Antibody to PspA Enhances Killing of Pneumococci by Apolactoferrin. *Infection and Immunity*, *72*(9), 5031–5040. https://doi.org/10.1128/IAI.72.9.5031-5040.2004.

Sharew, B., Moges, F., Yismaw, G., Abebe, W., Fentaw, S., Vestrheim, D., & Tessema, B. (2021). Antimicrobial resistance profile and multidrug resistance patterns of Streptococcus pneumoniae isolates from patients suspected of pneumococcal infections in Ethiopia. *Annals of Clinical Microbiology and Antimicrobials*, *20*(1), 1–8. https://doi.org/10.1186/s12941-021-00432-z.

Shibl, A. M. (2008). Distribution of serotypes and antibiotic resistance of invasive pneumococcal disease isolates among children aged 5 years and under in Saudi Arabia (2000–2004). *Clinical Microbiology and Infection*, *14*(9), 876–879. https://doi.org/10.1111/j.1469-0691.2008.02058.x.

Shormin, M., Shamsuzzaman, S., Afroz, S., & Rashed, A. (2022). Antimicrobial Susceptibility Pattern of Streptococcus pneumoniae among Healthy Carrier Children under Five Years Old attended at Outpatient Department of Largest Teaching Hospital in Bangladesh. *Bangladesh Journal of Infectious Diseases*, *8*(1), 12–17. https://doi.org/10.3329/bjid.v8i1.57950.

Sia, S. B., Lagrada, M. L., Gayeta, J. M., Masim, M. A. L., Abad, J. P., Magbanua, M. A., & Ablola, F. B. (2021). Serotype distribution and antimicrobial resistance of Streptococcus pneumoniae in the Philippines, 2012–2018. *Western Pacific Surveillance and Response Journal*, *12*(4), 2012–2018. https://doi.org/10.5365/wpsar.2021.12.4.834.

Siberry, G., & Brahmadathan, K., Pandian, R., Lalitha, MK., Steinhoff MC, John TJ (2001). Comparison of different culture media and storage temperatures for the long-term preservation of Streptococcus pneumoniae in the tropics. *Bulletin of the World Health Organization*, 79(1):43-7. Epub 2003 Nov 5. PMID: 11217666; PMCID: PMC2566344.

Sigurdsson, S. (2018). *The impact of vaccination with conjugated pneumococcal vaccine on pneumococcal carriage and disease caused by pneumococci in Icelandic*. (Doctoral dissertation). Háskóli Íslands University of Iceland. Retrieved from https://hdl.handle.net/20.500.11815/1187.

Skinner, J. M., Indrawati, L., Cannon, J., Blue, J., Winters, M., MacNair, J., *et al*. (2011). Pre-clinical evaluation of a 15-valent pneumococcal conjugate vaccine (PCV15-CRM197) in an infant-rhesus monkey immunogenicity model. *Vaccine*, *29*(48), 8870–8876. https://doi.org/10.1016/j.vaccine.2011.09.078.

Skov Sørensen, U. B., Yao, K., Yang, Y., Tettelin, H., & Kilian, M. (2016). Capsular polysaccharide expression in commensal Streptococcus species: Genetic and antigenic similarities to Streptococcus pneumoniae. *MBio*, *7*(6), 1–17. https://doi.org/10.1128/mBio.01844-16.

Slotved, H. C., Kaltoft, M., Skovsted, I. C., Kerrn, M. B., & Espersen, F. (2004). Simple, Rapid Latex Agglutination Test for Serotyping of Pneumococci (Pneumotest-Latex). *Journal of Clinical Microbiology*, *42*(6), 2518–2522. https://doi.org/10.1128/JCM.42.6.2518-2522.2004.

Slotved, H. C., & Satzke, C. (2013). In vitro growth of pneumococcal isolates representing 23 different serotypes. *BMC Research Notes*, *6*(1), 208. https://doi.org/10.1186/1756-0500-6-208.

Smith-Vaughan, H., Crichton, F., Beissbarth, J., Morris, P. S., & Leach, A. J. (2008). Survival of pneumococcus on hands and fomites. *BMC Research Notes*, *1*(1), 112. https://doi.org/10.1186/1756-0500-1-112.

Sondag, J. E., Morgens, R. K., Hoppe, J. E., & Marr, J. J. (1977). Detection of pneumococci in respiratory secretions: clinical evaluation of gentamicin blood agar. *Journal of Clinical Microbiology*, *5*(4), 397–400.

Song, J. H., Jung, S. I., Ko, K. S., Kim, N. Y., Son, J. S., Chang, H. H., *et al*. (2004). High prevalence of antimicrobial resistance among clinical Streptococcus pneumoniae isolates in Asia (an ANSORP study). *Antimicrobial Agents and Chemotherapy*, *48*(6), 2101–2107. https://doi.org/10.1128/AAC.48.6.2101-2107.2004.

Sørensen, U. B. S., Henrichsen, J., Chen, H.-C., & Szu, S. C. (1990). Covalent linkage between the capsular polysaccharide and the cell wall peptidoglycan of Streptococcus pneumoniae revealed by immunochemical methods. *Microbial Pathogenesis*, *8*(5), 325–334. https://doi.org/10.1016/0882-4010(90)90091-4.

Sourav, S., Patricia, A., Sharma, S., Kanungo, R., Jayachandran, S., & Prashanth, K. (2010). Detection of pneumolysin and autolysin genes among antibiotic resistant Streptococcus pneumoniae in invasive infections. *Indian Journal of Medical Microbiology*, *28*(1), 34–39. https://doi.org/10.4103/0255-0857.58726.

Soysal, A., Karabağ-Yılmaz, E., Kepenekli, E., Karaaslan, A., Cagan, E., Atıcı, S., *et al*. (2016). The impact of a pneumococcal conjugate vaccination program on the nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among healthy children in Turkey. *Vaccine*, *34*(33), 3894–3900. https://doi.org/10.1016/j.vaccine.2016.05.043.

Srinivasan, V., du Plessis, M., Beall, B. W., & McGee, L. (2011). Quadriplex real-time polymerase chain reaction (lytA, mef, erm, pbp2bwt) for pneumococcal detection and assessment of antibiotic susceptibility. *Diagnostic Microbiology and Infectious Disease*, *71*(4), 453–456. https://doi.org/10.1016/j.diagmicrobio.2011.08.017.

Subramanian, K., Neill, D. R., Malak, H. A., Spelmink, L., Khandaker, S., Marchiori, D. L. G. *et al*. (2019, November 12). Pneumolysin binds to the mannose receptor C type 1 (MRC-1) leading to anti-inflammatory responses and enhanced pneumococcal survival. *Nature Microbiology*. Springer US. https://doi.org/10.1038/s41564-018-0280-x.

Suleiman, M., Ejembi, J., Giwa, F., Jimoh, O., Suleiman, A., & Olayinka, A. (2018). Serotype distribution pattern of Streptococcus Pneumoniae isolates from invasive infections at a university teaching hospital in Northern Nigeria. *Annals of Tropical Pathology*, *9*(2), 145. https://doi.org/10.4103/atp.atp_33_18.

Sutcliffe, C. G., Shet, A., Varghese, R., Veeraraghavan, B., Manoharan, A., Wahl, B., *et al*. (2019). Nasopharyngeal carriage of Streptococcus pneumoniae serotypes among children in India prior to the introduction of pneumococcal conjugate vaccines: a cross-sectional study. *BMC Infectious Diseases*, *19*(1), 605. https://doi.org/10.1186/s12879-019-4254-2.

Sütçü, M., Aktürk, H., Karagözlü, F., Somer, A., Gürler, N., & Salman, N. (2017). Empyema due to Streptococcus Pneumoniae Serotype 9V in a Child Immunized with 13-Valent Conjugated Pneumococcal Vaccine. *Balkan Medical Journal*, *34*(1), 74–77. https://doi.org/10.4274/balkanmedj.2015.0937.

Swarthout, T. D., Gori, A., Bar-Zeev, N., Kamng'ona, A. W., Mwalukomo, T. S., Bonomali, F., *et al*. (2020). Evaluation of Pneumococcal Serotyping of Nasopharyngeal-Carriage Isolates by Latex Agglutination, Whole-Genome Sequencing (PneumoCaT), and DNA Microarray in a High-Pneumococcal-Carriage-Prevalence Population in Malawi. *Journal of Clinical Microbiology*, *59*(1), 1–11. https://doi.org/10.1128/JCM.02103-20.

Swiatlo, E., & McDaniel, L. S. (2020). Mucosal Vaccines for Streptococcus pneumoniae. In 2 (Ed.), *Mucosal Vaccines* (pp. 597–609). Elsevier. https://doi.org/10.1016/B978-0-12-811924-2.00034-1.

Syed, S., Hakala, P., Singh, A. K., Lapatto, H. A. K., King, S. J., Meri, S., Jokiranta T. S., Haapasalo, K. (2019). Role of Pneumococcal NanA Neuraminidase Activity in Peripheral Blood. *Frontiers in Cellular and Infection Microbiology*, *9*(JUN), 218. https://doi.org/10.3389/fcimb.2019.00218.

Syrjänen, R. K., Kilpi, T. M., Kaijalainen, T. H., Herva, E. E., & Takala, A. K. (2001). Nasopharyngeal Carriage of Streptococcus pneumoniae in Finnish Children Younger Than 2 Years Old. *The Journal of Infectious Diseases*, *184*(4), 451–459. https://doi.org/10.1086/322048.

Tabatabaei, S. R, Shamshiri, A., Nasiri, M., Weinberger, D., Dadashi, M., & Karimi, A. (2019). Pneumococcal meningitis in Iran: a systematic review and meta–analysis. *Journal of Acute Disease*, *8*(3), 99. https://doi.org/10.4103/2221-6189.259108.

Tabatabaei, S. R, Karimi, A., Rahbar, M., Shirvani, F., Azimi, L., Shirdoost, M., & Fallah, F. (2021). Profile of streptococcus pneumoniae serotypes of children with invasive disease in Tehran, Iran. An implication for vaccine coverage. *Iranian Journal of Pediatrics*, *31*(2). https://doi.org/10.5812/ijp.106086.

Tabatabaei, S. R., Shamshiri, A., Azimi, L., Nazari-Alam, A., Karimi, A., Mirjavadi, S. A., & Tariverdi, M. (2022). Co-infection with dual Streptococcus pneumoniae serotypes as a cause of pediatric bacterial meningitis in Iran: a multi-center cross-sectional study. *BMC Infectious Diseases*, *22*(1), 1–5. https://doi.org/10.1186/s12879-022-07606-w.

Taha, A., & Ali, K. (2019). Streptococcus pneumonia isolated from the nasal carriage and its antibiotic susceptibility profiles in children. *Zanco Journal of Medical Sciences*, *23*(3), 315–321. https://doi.org/10.15218/zjms.2019.039.

Taj-Aldeen, S. J., & Shamseldin Elshafie, S. (2016). Emerging resistant serotypes of invasive Streptococcus pneumoniae. *Infection and Drug Resistance*, *Volume 9*, 153–160. https://doi.org/10.2147/IDR.S102410.

Takeuchi, N., Naito, S., Ohkusu, M., Abe, K., Shizuno, K., Takahashi, Y., *et al*. (2020). Epidemiology of hospitalized paediatric community-acquired pneumonia and bacterial pneumonia following the introduction of 13-valent pneumococcal conjugate vaccine in the national immunization programme in Japan. *Epidemiology and Infection*, *148*(e91), e91. https://doi.org/10.1017/S0950268820000813.

Talaro, K. P., & Chess, B. (2018). *Foundations in Microbiology* (10th ed.). New York: The McGraw-Hill Companies. 0-07-112275-3.

Talbird, S. E., Carrico, J., La, E. M., Carias, C., Marshall, G. S., Roberts, C. S., *et al*. (2022). Impact of Routine Childhood Immunization in Reducing Vaccine-Preventable Diseases in the United States. *Pediatrics*, *150*(3). https://doi.org/10.1542/peds.2021-056013.

Tariq, W. U. Z., Abou Hassanein, A., & Hashmey, R. H. (2016). Changes in susceptibility pattern of streptococcus pneumonia at Tawam Hospital in Al Ain, United Arab Emirates during (2004-2011). *Pakistan Armed Forces Medical Journal, 66*(1), 14-21.

Tavares, D. A., Handem, S., Carvalho, R. J., Paulo, A. C., de Lencastre, H., Hinds, J., & Sá-Leão, R. (2019). Identification of Streptococcus pneumoniae by a real-time PCR assay targeting SP2020. *Scientific Reports*, *9*(1), 3285. https://doi.org/10.1038/s41598-019-39791-1.

Thummeepak, R., Leerach, N., Kunthalert, D., Tangchaisuriya, U., Thanwisai, A., & Sitthisak, S. (2015). High prevalence of multi-drug resistant Streptococcus pneumoniae among healthy children in Thailand. *Journal of Infection and Public Health*, *8*(3), 274–281. https://doi.org/10.1016/j.jiph.2014.11.002.

Tiley, K. S., Ratcliffe, H., Voysey, M., Jefferies, K., Sinclair, G., Carr, M., *et al*. (2022). Nasopharyngeal Carriage of Pneumococcus in Children in England up to 10 Years After 13-Valent Pneumococcal Conjugate Vaccine Introduction: Persistence of Serotypes 3 and 19A and Emergence of 7C. *The Journal of Infectious Diseases*, 1–12. https://doi.org/10.1093/infdis/jiac376.

Tille P. (2014). PART III Bacteriology, SECTION 2; Catalase-Positive, Gram-Positive Cocci. In *Bailey & Scott's Diagnostic Microbiology* (13th ed., pp. 247–262). China: Mosby, Inc., an affiliate of Elsevier Inc. Retrieved from http://evolve.elsevier.com/Tille/micro/.

Torumkuney, D., Dolgum, S., van Hasselt, J., Abdullah, W., & Keles, N. (2022). Country data on AMR in Saudi Arabia in the context of community-acquired respiratory tract infections: links between antibiotic susceptibility, local and international antibiotic prescribing guidelines, access to medicine and clinical outcome. *Journal of Antimicrobial Chem*otherapy, 77 Suppl 1: i70–i76. https://doi.org/10.1093/jac/dkac219.

Torumkuney, D., Mokaddas, E., Jiman-Fatani, A., Ageel, A., Daoud, Z., Bouferraa, Y., Zerdan, M. B., Morrissey, I. (2020). Results from the Survey of Antibiotic Resistance (SOAR) 2015–17 in the Middle East (Kuwait, Lebanon and Saudi Arabia): data based on CLSI, EUCAST (dose-specific) and pharmacokinetic/pharmacodynamic (PK/PD) breakpoints. *Journal of Antimicrobial Chemotherapy*, *75*(Supplement_1), i60–i75. https://doi.org/10.1093/jac/dkaa084.

Troxler, L. J., Werren, J. P., Schaffner, T. O., Mostacci, N., Vermathen, P., Vermathen, M., *et al*. (2019). Carbon source regulates polysaccharide capsule biosynthesis in Streptococcus pneumoniae. *Journal of Biological Chemistry*, *294*(46), 17224–17238. https://doi.org/10.1074/jbc.RA119.010764.

Tvedskov, E. S. F., Hovmand, N., Benfield, T., & Tinggaard, M. (2022). Pneumococcal carriage among children in low and lower-middle-income countries: A systematic review. *International Journal of Infectious Diseases*, *115*, 1–7. https://doi.org/10.1016/j.ijid.2021.11.021.

Ueno, M., Ishii, Y., Tateda, K., Anahara, Y., Ebata, A., Iida, M., *et al*. (2013). Prevalence and risk factors of nasopharyngeal carriage of Streptococcus pneumoniae in healthy children in Japan. *Japanese Journal of Infectious Diseases*, *66*(1), 22–25. https://doi.org/10.7883/yoken.66.22.

UNICEF and WHO. (2006). *Pneumonia: the forgotten killer of children*. Retrieved from https://www.who.int/publications/i/item/9789280640489.

van der Poll, T., & Opal, S. M. (2009). Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *The Lancet*, *374*(9700), 1543–1556. https://doi.org/10.1016/S0140-6736(09)61114-4.

Van Dyke, M. K., Pirçon, J.-Y., Cohen, R., Madhi, S. A., Rosenblüt, A., Macias Parra, M., *et al*. (2017). Etiology of Acute Otitis Media in Children Less Than 5 Years of Age. *Pediatric Infectious Disease Journal*, *36*(3), 274–281. https://doi.org/10.1097/INF.0000000000001420.

Velusamy, S., Tran, T., Mongkolrattanothai, T., Walker, H., McGee, L., & Beall, B. (2020). Expanded sequential quadriplex real-time polymerase chain reaction (PCR) for identifying pneumococcal serotypes, penicillin susceptibility, and resistance markers. *Diagnostic Microbiology and Infectious Disease*, *97*(2), 115037. https://doi.org/10.1016/j.diagmicrobio.2020.115037.

Venkatesan, P., & Macfarlane, J. T. (1992). Role of pneumococcal antigen in the diagnosis of pneumococcal pneumonia. *Thorax*, *47*(5), 329–331. https://doi.org/10.1136/thx.47.5.329.

Verghese, V. P., Veeraraghavan, B., Jayaraman, R., Varghese, R., Neeravi, A., Jayaraman, Y., *et al*. (2017). Increasing incidence of penicillin- and cefotaxime-resistant Streptococcus pneumoniae causing meningitis in India: Time for revision of treatment guidelines? *Indian Journal of Medical Microbiology*, *35*(2), 228–236. https://doi.org/10.4103/ijmm.IJMM_17_124.

Vidanapathirana, G., Angulmaduwa, S., Munasinghe, T., Ekanayake, A., Kudagammana, T., Dissanayaka, N., & Liyanapathirana, V. (2020). Pneumococcal colonization among healthy and hospitalized vaccine-naive Sri Lankan children. *Vaccine*, *38*(46), 7308–7315. https://doi.org/10.1016/j.vaccine.2020.09.040.

Vögele, M., Bhaskara, R. M., Mulvihill, E., van Pee, K., Yildiz, Ö., Kühlbrandt, W., Müller, D. J., Hummer, G. (2019). Membrane perforation by the pore-forming toxin pneumolysin. *Proceedings of the National Academy of Sciences*, *116*(27), 13352–13357. https://doi.org/10.1073/pnas.1904304116.

Voss, S., Hallström, T., Saleh, M., Burchhardt, G., Pribyl, T., Singh, B., *et al*. (2013). The choline-binding Protein PspC of Streptococcus pneumoniae interacts with the C-terminal heparin-binding domain of vitronectin. *Journal of Biological Chemistry*, *288*(22), 15614–15627. https://doi.org/10.1074/jbc.M112.443507.

Wada, F. W., Tufa, E. G., Berheto, T. M., & Solomon, F. B. (2019). Nasopharyngeal carriage of Streptococcus pneumoniae and antimicrobial susceptibility pattern among school children in South Ethiopia: Post-vaccination era. *BMC Research Notes*, *12*(1), 1–6. https://doi.org/10.1186/s13104-019-4330-0.

Walekhwa, M., Muturi, M., Gunturu, R., Kenya, E., & Kabera, B. (2018). Streptococcus pneumoniae serotype epidemiology among PCV-10 vaccinated and unvaccinated children at Gertrude's Children's Hospital, Nairobi County: a cross-sectional study. *F1000Research*, *7*(0), 879. https://doi.org/10.12688/f1000research.14387.1.

Wangirapan, A., Ayuthaya, S. I. N., Katip, W., Kasatpibal, N., Mektrirat, R., Anukool, U., & Oberdorfer, P. (2020). Serotypes and Vaccine Coverage of Streptococcus Pneumoniae Colonization in the Nasopharynx of Thai Children in Congested Areas in Chiang Mai. *Pathogens*, *9*(12), 988. https://doi.org/10.3390/pathogens9120988.

Wani, J. H., Gilbert, J. V., Plaut, A. G., & Weiser, J. N. (1996). Identification, cloning, and sequencing of the immunoglobulin A1 protease gene of Streptococcus pneumoniae. *Infection and Immunity*, *64*(10), 3967–3974. https://doi.org/10.1128/iai.64.10.3967-3974.1996.

Warren, L. (2016). Gram-Positive Cocci. In *Review of medical microbiology and immunology* (14th ed., pp. 123–125). New York: McGraw-Hill Education.

Watson, D. A., Musher, D. M., Jacobson, J. W., & Verhoef, J. (1993). A Brief History of the Pneumococcus in Biomedical Research: A Panoply of Scientific Discovery. *Clinical Infectious Diseases*, *17*(5), 913–924. https://doi.org/10.1093/clinids/17.5.913.

Weinberger, D. M., Bruden, D. T., Grant, L. R., Lipsitch, M., O'Brien, K. L., Pelton, S. I., Sanders. E. A. M., Feikin, D. R. (2013). Using Pneumococcal Carriage Data to Monitor Postvaccination Changes in Invasive Disease. *American Journal of Epidemiology*, *178*(9), 1488–1495. https://doi.org/10.1093/aje/kwt156.

Weinberger, D. M., Trzciński, K., Lu, Y.-J., Bogaert, D., Brandes, A., Galagan, J., Anderson, P. W., Malley, R., Lipsitch, M. (2009). Pneumococcal Capsular Polysaccharide Structure Predicts Serotype Prevalence. *PLoS Pathogens*, *5*(6), e1000476. https://doi.org/10.1371/journal.ppat.1000476.

Weiser, J. N., Ferreira, D. M., & Paton, J. C. (2018). Streptococcus pneumoniae: transmission, colonization and invasion. *Nature Reviews Microbiology*, *16*(6), 355–367. https://doi.org/10.1038/s41579-018-0001-8.

Werren, J. P., Mostacci, N., Gjuroski, I., Holivololona, L., Troxler, L. J., Hathaway, L. J., Furrer, J., Hilty, M. (2023). Carbon Source-dependent Capsule Thickness Regulation in Streptococcus pneumoniae. *Frontiers in Cellular and Infection Microbiology*, *13*, 1279119. https://doi.org/10.3389/fcimb.2023.1279119.

Whitsett, J. A., & Alenghat, T. (2015). Respiratory epithelial cells orchestrate pulmonary innate immunity. *Nature Immunology*, *16*(1), 27–35. https://doi.org/10.1038/ni.3045

WHO. (2012). Pneumococcal vaccines WHO position paper – 2012 – Recommendations. *Vaccine*, *30*(32), 4717–4718. https://doi.org/10.1016/j.vaccine.2012.04.093.

WHO. (2013). *Introduction of Pneumococcal Vaccine PCV13, A Handbook for District and Health Facility Staff* (WHO/IVB/13). Geneva: The Department of Immunization, Vaccines and Biologicals Geneva, Switzerland. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/90380/WHO_IVB_13.10_eng.pdf.

WHO. (2017). *Meningitis in Yemen*. *Weekly Epidemiological Monitor,* Volume 10; Issue no 35, 27 August 2017. Retrieved from https://reliefweb.int/report/yemen/who-emro-weekly-epidemiological-monitor-volume-10-issue-35-27-august-2017.

WHO. (2018). *Pneumococcus.* In *Surveillance standards for vaccine-preventable diseases* (2ed ed., pp. 1–14). Geneva: World Health Organization. Retrieved from https://www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-pneumococcus.

WHO. (2022). *Immunization data*. World Health Organization Immunization Data portal. https://immunizationdata.who.int/listing.html?topic=coverage&location=emr. Published 2022. Accessed 18 Apr 2023.

WHO. (2022). *Pneumonia in children.* Retrieved from https://www.who.int/news-room/fact-sheets/detail/pneumonia#:~:text=Pneumonia.

Wouters, I., Van Heirstraeten, L., Desmet, S., Blaizot, S., Verhaegen, J., Goossens, H., *et al*. (2018). Nasopharyngeal s. pneumoniae carriage and density in Belgian infants after 9 years of pneumococcal conjugate vaccine programme. *Vaccine*, *36*(1), 15–22. https://doi.org/10.1016/j.vaccine.2017.11.052.

Wu, H., Moser, C., Wang, H.-Z., Høiby, N., & Song, Z.-J. (2015). Strategies for combating bacterial biofilm infections. *International Journal of Oral Science*, *7*(1), 1–7. https://doi.org/10.1038/ijos.2014.65.

Wyllie, A. L. (2016). *Molecular surveillance of pneumococcal carriage in all ages.* [Doctoral thesis) Utrecht University, New Zealand. https://dspace.library.uu.nl/handle/1874/341331.

Yang, Z., Bedugnis, A., Levinson, S., Dinubile, M., Stossel, T., Lu, Q., & Kobzik, L. (2019). Delayed Administration of Recombinant Plasma Gelsolin Improves Survival in a Murine Model of Penicillin-Susceptible and Penicillin-Resistant Pneumococcal Pneumonia. *The Journal of Infectious Diseases*, *220*(9), 1498–1502. https://doi.org/10.1093/infdis/jiz353.

Yang, Z., Chiou, T. T.-Y., Stossel, T. P., & Kobzik, L. (2015). Plasma gelsolin improves lung host defense against pneumonia by enhancing macrophage NOS3 function. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *309*(1), L11–L16. https://doi.org/10.1152/ajplung.00094.2015.

Yüksek, S. K., Tezer, H., Gülhan, B., Özkaya Parlakay, A., Güldemir, D., Coskun-Ari, F. F., … Baran Aksakal, F. N. (2020). Nasopharyngeal pneumococcal carriage in healthy Turkish children after 13-valent conjugated pneumococcal vaccine implementation in the national immunization program. *Journal of Infection and Public Health*, *13*(2), 266–274. doi: 10.1016/j.jiph.2019.10.009. Epub 2019 Dec 6. PMID: 31818710.

Zahlten, J., Herta, T., Kabus, C., Steinfeldt, M., Kershaw, O., García, P., *et al*. (2015). Role of pneumococcal autolysin for KLF4 expression and chemokine secretion in lung epithelium. *American Journal of Respiratory Cell and Molecular Biology*, *53*(4), 544–554. https://doi.org/10.1165/rcmb.2014-0024OC.

Zysk, G., Schneider-Wald, B. K., Hwang, J. H., Bejo, L., Kim, K. S., Mitchell, T. J., Hakenbeck, R., Heinz, H.-P. (2001). Pneumolysin is the main inducer of cytotoxicity to brain microvascular endothelial cells caused by Streptococcus pneumoniae. *Infection and Immunity*, *69*(2), 845–852. doi: 10.1128/IAI.69.2.845-852.2001. PMID: 11159977; PMCID: PMC97961.

Appendices

Current Infectious Disease Reports
https://doi.org/10.1007/s11908-023-00807-7

PEDIATRIC INFECTIOUS DISEASES (I BROOK, SECTION EDITOR)

Prevalence and Resistance Patterns of *Streptococcus pneumoniae* Recovered from Children in Western Asia

Yasser M. Matran[1,2] · Ahmed M. Al-Haddad[3] · Divakar Sharma[4] · Nitin Pal Kalia[5] · Sarika Sharma[6] · Manoj Kumar[7] · Sandeep Sharma[2]

Accepted: 2 June 2023

Abstract

Purpose of Review Despite the available pneumococcal conjugate vaccination (PCV), children in developing countries suffer significant morbidity and mortality from pneumococcal illness. This review outlines the pneumococcal carriage rate, common serotypes, antibiotic resistance, and PCV coverage among western Asian children.

Recent Findings The carriage rate and prevalence of PCV serotypes remain high among children in Western Asia. In recent times, the national immunization rates have increased, but there was a considerable rate of invasive pneumococcal diseases (IPD) strains and non-invasive pneumococcal diseases (NIPD) serotypes with high antimicrobial resistance rates and many communities in western Asian countries are experiencing the phenomenon of pneumococcal vaccine serotype replacement (PVSR).

Summary An accurate and updated disease surveillance in Western Asia is urgently needed to guide immunization efforts and protect children effectively. Increasing the PCV covering rate and antimicrobial stewardship is crucial for countries to implement strict systems and effectively minimize the high rate of resistance.

Keywords S. pneumoniae · Nasopharyngeal · Epidemiology · Western Asian Countries

✉ Divakar Sharma
divakarsharma88@gmail.com

✉ Sandeep Sharma
sandeep.23995@lpu.co.in

1 Department of Para-Clinic, Unit of Clinical Microbiology, Faculty of Medicine and Health Sciences, University of Aden, Aden, Yemen

2 Department of Medical Laboratory Science, Lovely Professional University, Phagwara, Punjab 144411, India

3 Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Hadhramout University, Al Mukalla, Yemen

4 Department of Microbiology, Lady Hardinge Medical College, New Delhi 110001, India

5 Department of Biological Sciences (Pharmacology & Toxicology), National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad, Telangana 500037, India

6 Department of Sponsored Research, Lovely Professional University, Phagwara, Punjab 144411, India

7 Research Department, Maternal and Child Health Program, Sidra Medicine, Doha, Qatar

Introduction

Pneumococcal carriage is prevalent in low- and lower-middle-income countries, particularly among children, and encompasses a broad range of serotypes [1]. According to the World Health Organization (WHO), *Streptococcus pneumoniae* is responsible for the mortality of over 300,000 children under the age of 5 globally on an annual basis. The majority of fatalities take place in developing nations [2]. The carriage of *S. pneumoniae* in the nasopharynx, even in the absence of symptoms, is a crucial factor in the transmission of this pathogen. It typically occurs prior to the onset of invasive pneumococcal diseases [3]. The pneumococcal carriage, antimicrobial resistance, and pneumococcal conjugate vaccination (PCV) coverage vary across regions, influenced by various factors, such as the socioeconomic status of the population, vaccination rate, and antibiotic misuse [4•]. The year 1977 was an important historical event, with the licensing of the first pneumococcal vaccine in the USA which was composed of 14 pneumococcal serotypes, and in 1983 had licensed a 23-valent polysaccharide vaccine, but this vaccine was not effective in protecting children; the suffering

Published online: 03 July 2023

JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Streptococcus pneumoniae among the children of Aden, Yemen: a cross-sectional report of post-pneumococcal conjugate vaccine

Yasser M Matran[1,2], Ahmed M Al-Haddad[3], Avleen Kour[2], Hussein Al-Shehabi[4], Sarika Sharma[5], Ashish Suttee[6], Sandeep Sharma[2]

[1] Department of Para-Clinic, Unit of Clinical Microbiology, Faculty of Medicine and Health Sciences, University of Aden, Yemen
[2] School of Allied Medical Sciences, Lovely Professional University, India
[3] Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Hadhramout University, Yemen
[4] Department of HIV and Other Retroviruses, Robert Koch Institute, Germany
[5] Department of Sponsored Research, Lovely Professional University, India
[6] Department of Pharmacognosy, Lovely Professional University, India

Abstract

Introduction: *Streptococcus pneumoniae* cause a significant global health challenge. We aimed to determine nasopharyngeal carriage, serotypes distribution, and antimicrobial profile of pneumococci among the children of Aden.

Methodology: A total of 385 children, aged 2-17 years, were included. Asymptomatic samples were randomly collected from children in selected schools and vaccination centers. Symptomatic samples were obtained from selected pediatric clinics. The nasopharyngeal swabs were tested for pneumococci using culture and real time polymerase chain reaction (RT-PCR). Serotyping was done with a pneumotest-latex kit and antimicrobial susceptibility was tested by disc diffusion and Epsilometer test.

Results: The total pneumococcal carriage was 44.4% and 57.1% by culture and RT-PCR, respectively. There was a statistically significant association between carriage rate and living in single room (OR = 7.9; $p = 0.00001$), sharing a sleeping space (OR = 15.1; $p = 0.00001$), and low monthly income (OR = 2.02; $p = 0.007$). The common serotypes were 19, 1, 4, 5, 2, and 23. The proportion of non-pneumococcal conjugate vaccine (non-PCV13) serotypes was 24%. Pneumococci were resistant to penicillin (96.5%), cefepime (15.8%), ceftriaxone (16.4%), and amoxicillin-clavulanate (0%). Erythromycin, azithromycin, and doxycycline had resistance rates of 48%, 31%, and 53.3%, respectively.

Conclusions: A high pneumococcal carriage rate was observed in Yemeni children, particularly in low-income households and shared living conditions. There was significant penicillin resistance at meningitis breakpoint. Furthermore, non-PCV13 serotypes were gradually replacing PCV13 serotypes. The findings underscore the urgent need for enhanced surveillance and stewardship to improve vaccination and antibiotic policies in Yemen.

Key words: pneumococci; Yemen; serotypes; children; vaccine; antimicrobial.

J Infect Dev Ctries 2024; 18(4):579-586. doi:10.3855/jidc.18935

(Received 22 July 2023 – Accepted 16 November 2023)

Introduction

Streptococcus pneumoniae (pneumococci) are upper airway commensals and have a complex relationship with their host [1,2]. Their carriage increases from 9% in infancy to 43% in childhood due to many factors [3,4]. Asymptomatic carriage in the nasopharynx is crucial for transmission and precedes invasive pneumococcal diseases [1,2,5]. In 2019, pneumococci were ranked as the fourth leading bacteria contributing to mortality from antimicrobial resistance (AMR) [6]. Studies indicate that implementing the pneumococcal conjugate vaccine (PCV13) in the Middle East can reduce pneumococcus mortality by approximately 38% [7]. There were considerable variations in the vaccination covering rates of PCV13 among the children of neighboring countries: 66.7% in Bahrain, 37.2% in Oman, and 75-78.3 in Qatar [8,9,10]. Additionally, recent evidence suggests that the PCV13 serotypes 1, 3, 5, 6A, 7F, and 19A did not provide effective protection against invasive pneumococcal disease (IPD) in Kuwait [11]. Although PCV saves lives and reduces the occurrence of AMR strains, it also leads to the emergence of non-PCV strains with AMR [12,13]. In Yemen, 44.3% of the children were reported to have respiratory diseases and it is a common illness [14]. The isolation rate of *Streptococcus pneumoniae* (*S. pneumoniae*) among Yemeni children with bacterial meningitis was 34.9% [15]. Furthermore, pneumococci

LOVELY PROFESSIONAL UNIVERSITY
Bridging the Distance in Education

NAAC GRADE A++

iNSERB DIA

Alert Diagnostic

G20 2023 INDIA

Azadi Ka Amrit Mahotsav

RTBS 2023

International Conference on Recent Trends in Biomedical Sciences (RTBS-2023)

Certificate of Achievement

This is to certify that Prof./Dr./Mr./Ms. ...Yasser Matran..... of ..Lovely Professional University.....has presented Oral/Poster entitled..Antimicrobial resistance trends among Pneumococcal Serotypes in Yemen.....In **International Conference on Recent Trends in Biomedical Sciences (RTBS-2023)** organized by Department of Medical Laboratory Sciences from 6th-7th October 2023 at Lovely Professional University, Punjab, India.

Sandeep Sharma

Prof (Dr.) Sandeep Sharma
Covenor/Organizing Secretary
RTBS2023

Monica Gulati

Prof (Dr.) Monica Gulati
Chairperson
RTBS-2023

ICMBSDG
Microbial Bioprospecting Towards Sustainable Development Goals

LOVELY PROFESSIONAL UNIVERSITY
Transforming Education Transforming India

NAAC GRADE A++

Certificate No. 298894

Certificate of Participation

This is to certify that **Prof./Dr./Mr./Ms. Yasser M. Matran** of **Lovely Professional University** has Participated/Presented a Poster Presentation entitled **Detection of Streptococcus pneumoniae carriage by real-time pcr** in International Conference on **"Microbial Bioprospecting Towards Sustainable Development Goals"** held on **24th- 25th November 2023** organized by Association of Microbiologist of India-LPU Unit and Society of Chemical and Synthetic Biology at Lovely Professional University, Punjab..

Date of Issue : 12-12-2023
Place : Phagwara (Punjab), India

Prepared by **(Administrative Officer-Records)**	**Dr. Arun Karnwal** **President** **AMI-LPU Unit**	**Dr. Karthik Loganathan** **President** **SCSB**	**Dr. Ashish Vyas** **Organizing Secretary** **AMI-LPU Unit**	**Dr. Neeta Raj Sharma** **Senior Dean** **SBEB, LPU**

DEPARTMENT OF BIOTECHNOLOGY
MINISTRY OF SCIENCE & TECHNOLOGY, GOVERNMENT OF INDIA