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Impact of the pneumococcal conjugate vaccine on nasopharyngeal pneumococcal carriage in feverish infants in Mohamed VI teaching hospital of Marrakesh

THESIS

PRESENTED AND DEFENDED PUBLICLY ON **December 24th, 2019**

BY

Miss. Amal HABCHANE

Born on October 27th, 1991 in Tahannaout

TO OBTAIN A MEDICAL DOCTORATE

KEYWORDS

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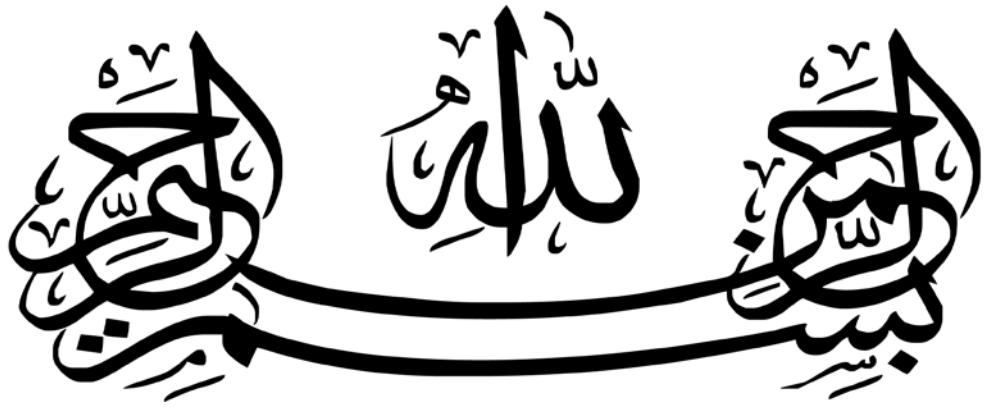

"رب أوزعني أن أشكر نعمتك التي
أنعمت عليَ وعلى والديَ وأن أعمل
صالحا ترضاه وأصلح لي في ذريتي إني
تبت إليك وإني من المسلمين"

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AS A MEMBER OF THE MEDICAL PROFESSION:

I SOLEMNLY PLEDGE to dedicate my life to the service of humanity;

THE HEALTH AND WELL-BEING OF MY PATIENT will be my first consideration;

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I WILL NOT PERMIT considerations of age, disease or disability, creed, ethnic origin, gender, nationality, political affiliation, race, sexual orientation, social standing, or any other factor to intervene between my duty and my patient;

I WILL RESPECT the secrets that are confided in me, even after the patient has died;

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I WILL FOSTER the honor and noble traditions of the medical profession;

I WILL GIVE to my teachers, colleagues, and students the respect and gratitude that is their due;

I WILL SHARE my medical knowledge for the benefit of the patient and the advancement of healthcare;

I WILL ATTEND TO my own health, well-being, and abilities in order to provide care of the highest standard;

I WILL NOT USE my medical knowledge to violate human rights and civil liberties, even under threat;

I MAKE THESE PROMISES solemnly, freely, and upon my honor.

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LISTE ARRÉTÉÉ LE 24/09/2019

DEDICATIONS

First praise is to ALLAH, the Almighty and the Greatest of all. I would like to thank ALLAH for giving me the opportunity, determination, and strength to complete this thesis.

I dedicate this Thesis:

To my dear father El Houssaine HABCHANE

Thank you for believing in me and supporting me endlessly. Without what you have done and still do for me, none of who I am today would have existed. I hope you are proud of me, and I will be working hard to always make you so.

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You are my love and escape. None of what I do would ever allow me to repay all that you have sacrificed for me, but I will always do my best to make you proud.

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ABBREVIATIONS

List of Abbreviations

S.p	: *Streptococcus pneumoniae*
NP	: Nasopharynx/ Nasopharyngeal
WHO	: World Health Organization
PCV	: Pneumococcal Conjugate Vaccine
CDC	: Centers for Disease Control and Prevention
BHI	: Brain and Heart infusion
CAN	: Colistin and Nalidixic Acid
CO2	: Carbon Dioxide
TSB	: Trypticase-Soy Broth
OXA1	: 1 µg oxacillin disk
EUCAST	: European Committee on Antimicrobial Susceptibility Testing
RR	: Rate Ratio
VS	: Vaccine Serotype
VE	: Vaccine Efficacy
OR	: Odds Ratio
CI	: Confidence Interval
NVS	: non-vaccine serotypes
PCR	: Polymerase Chain Reaction
PRSP	: Pneumococcus with Reduced Susceptibility to Penicillin
PSP	: Penicillin-Susceptible Pneumococcus
HIV	: Human Immunodeficiency Virus
PspA/C	: Pneumococcal surface proteins A/C
IPD	: Invasive pneumococcal disease
Non-IPD	: Non-Invasive pneumococcal disease
Cbp A	: choline-binding protein A
MIC	: Minimum Inhibitory Concentration
PBPs	: Penicillin-Binding Proteins

QRDR : Quinolone Resistance Determination Region

PPSV : Pneumococcal Polysaccharide Vaccine

USA : United States of America

Gavi : Global alliance for vaccines and immunization

cpsA : Capsular Polysaccharide Synthesis A

DI : Diversity Index

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INTRODUCTION

Streptococcus pneumoniae (*S.p*) is a commensal bacterium that colonizes the human superior airways, especially the nasopharynx (NP), during the early months of life. It is responsible for high rates of morbidity and mortality among children, by being the first cause of invasive bacterial infections in children aged three months to two years (pneumonia, bacteremia, meningitis, arthritis, and mastoiditis) and the second cause of acute otitis media (1). Pneumococcal infections are always preceded by a generally asymptomatic *S.pneumoniae* nasopharyngeal carriage that reaches its highest peak during early childhood. *S. p's* spread is conditioned by the virulence of the strain, which is related to both the bacterial capsule and the immunity status of the carrier.

In 2005, the World Health Organization (WHO) estimated that pneumococcal infections caused the death of 1.6 million people worldwide, of which, 700000 to 1 million were children younger than five years of age (2). Thus, these infections are a major pediatric health problem; through both the severity of their invasive forms (meningitis, bacteremia) and the elevated frequency of the non-invasive forms. Moreover, the prevalence of antibiotic-resistant pneumococcal strains has been steadily increasing in recent years, making therapeutic strategies more complicated. Therefore, vaccination remains the best preventive mean.

A study had been conducted before the implementation of the Pneumococcal Conjugate Vaccine (PCV) in the region of Marrakesh, in order to assess the nasopharyngeal pneumococcal carriage rate in children less than two years old, the strains' distribution and their adaptation to the commercialized vaccines. This study reported an overall nasopharyngeal carriage rate of 45.8% and the most carried strains were 19F, 6, 14, 23, 18 and 9A (3).

The implementation of the PCVs in the national immunization programs worldwide had a positive impact on reducing the nasopharyngeal carriage of vaccine serotypes as well as their transmission to the non-vaccinated individuals; this indirect effect is called the "herd effect". Thanks to this effect, the prevalence of invasive pneumococcal infections caused by these vaccine strains also decreased. In spite of this vaccine serotype reduction, there has been an increase in the rate of non-vaccine serotypes. This serotype replacement might be responsible

for more invasive pneumococcal infections and more antibiotic resistance. Thus, long term surveillance is needed.

Due to the observed pneumococcal invasive infections and the increase in the rate of drug-resistant strains, the Moroccan Health Ministry has expended the immunization against *S.p* in children less than 2 years old via its national immunization program. PCV13 was the first to be introduced in October 2010 in a 2+1 schedule. Then it has been replaced by PCV10 in July 2012 in the same schedule (4). Ever since this introduction, no surveillance or evaluation study of the vaccine effects has been conducted.

This study is a prospective cross-sectional study concerning feverish infants seen at the Pediatric Emergency Department of the Mother-Child hospital in Mohammed VI teaching hospital of Marrakesh. The infants were sampled by nasopharyngeal swabbing, over a period of 3 months (February to April 2017).

The main aim of this study is to provide epidemiological monitoring after the implementation of the PCV in the Moroccan immunization program and to assess its impact on the nasopharyngeal pneumococcal carriage in the region of Marrakesh. The specific goals are:

- To determine the overall *S. pneumoniae* nasopharyngeal carriage rate in feverish infants.
- To analyze the pneumococcal nasopharyngeal carriage risk factors in the target population (age, gender, mode of daycare, number of siblings…)
- To serotype the isolated pneumococcal strains in order to evaluate the impact of the PCV and detect the emerging serotypes.
- To detect the *S. pneumoniae* strains with reduced susceptibility to penicillin by using 1 µg oxacillin disks.
- To assess the correlation between serotypes and susceptibility to penicillin

PATIENTS & METHODS

I. Study characteristics

1. Study type

This is a prospective cross-sectional study, which lasted 3 months: February to April 2017.

2. Target population

Feverish infants aged 2 to 18 months, seen at the Pediatric Emergency Department of the Mother-Child hospital in Mohammed VI teaching hospital of Marrakesh.

3. Study location

The samples' collection took place in the Pediatric Emergency Department of the Mother-Child hospital in Mohammed VI teaching hospital of Marrakesh.

Swabs' bacteriological analysis, strains' identification and penicillin susceptibility tests were performed in the Microbiology laboratory of Ar-Razi hospital in Mohammed VI teaching hospital of Marrakesh.

Isolated strains' serogrouping was carried out in the Microbiology laboratory of the Avicenna Military Hospital.

The typing by PCR and swelling of the capsule reaction were done in collaboration with the Microbiology laboratory of Ibn-Rushd teaching hospital, Casablanca.

4. Inclusion criteria

The infants who had been included in this study had to:

- Be aged 2 to 18 months.
- Be feverish at, at least, 38°C.

5. Exclusion criteria

The infants who had taken antibiotics in the 7 days preceding the sampling were excluded from this study.

II. Work methodology

1. Data collection

1.1. Questionnaire

Epidemiological and clinical data were collected using a questionnaire (Annex) that focused on:

- Socio-demographic data: gender, mode of daycare, age, number of siblings.
- Antecedents: Number of received PCV doses, taking antibiotic treatment.
- Clinical features: Fever

1.2. Sampling

The sampling was performed by nasopharyngeal swabbing, using sterile swabs. The latter were introduced perpendicularly to the face, at the level of the middle nasal concha, until resistance was perceived (Figure 1). Then these simple and non-traumatic samples were rapidly carried to the microbiology laboratory of Ar-Razi hospital.

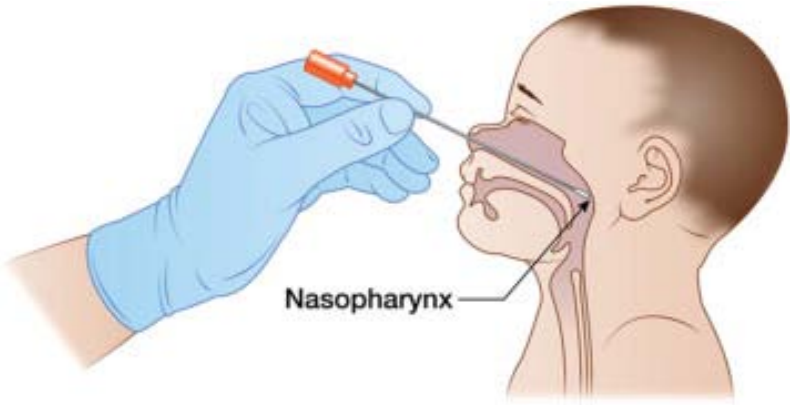

Figure 1: Nasopharyngeal swabbing technique in infants (5)

2. Microbiological analysis

S. pneumoniae identification was executed following the Centers for Disease Control and Prevention (CDC) recommendations (6).

2.1. Culturing

The collected swabs were put in a Brain and Heart infusion broth (BHI); a nutrient-rich liquid growth medium (Figure 2). Then they were sowed on a pneumococcus selective medium (Columbia Agar + CAN (Colistin and Nalidixic Acid) + 5% of blood)). Thereafter, they were incubated in a stove at 37°C, under 5% of CO2, during 24 to 48 hours.

Figure 2: Brain and Heart Infusion broth (7)

2.2. Strain identification

The pneumococcal strains were identified based on cultural, morphologic, biochemical and antigenic characteristics (hemolysis, optochin sensitivity, agglutination test).

a. Morphology

On Gram's stain, pneumococci are Gram-positive cocci. They appear to be encapsulated lanceolate 8-shaped or candle-flame-shaped diplococci (Figure 3).

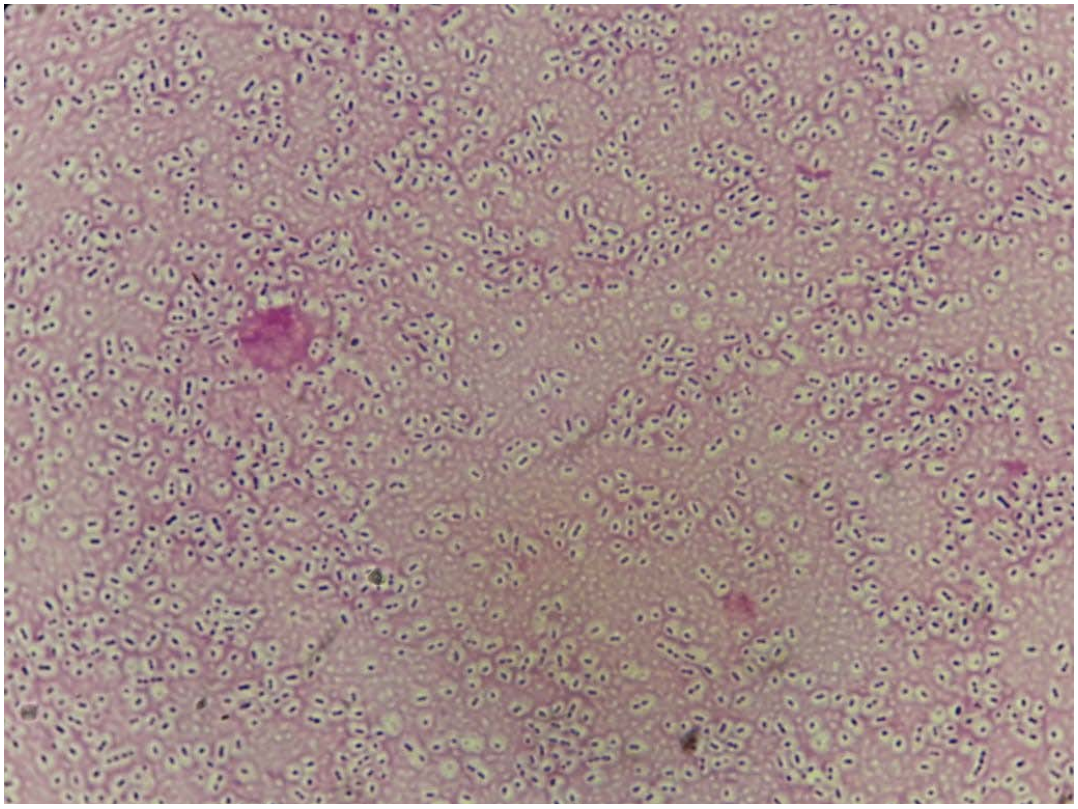

Figure 3: Encapsulated Gram-positive diplococci

b. Search for hemolysis

Hemolysin expression is favored by a CO2-rich or even an anaerobic incubation atmosphere. *S. pneumoniae* is generally characterized by alpha-type hemolysis. This type of hemolysis is recognized by the visualization of a greenish halo that surrounds the pneumococcal colonies (Figure 4). The *S.p* colonies were identified based on that aspect.

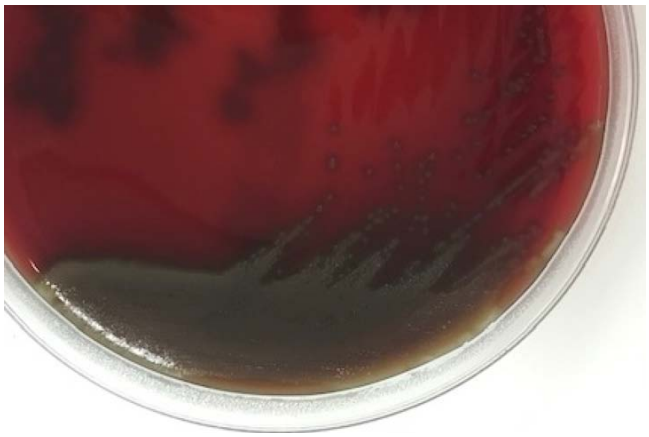

Figure 4: *Streptococcus pneumoniae* colonies surrounded by a greenish halo on blood agar, reflecting the alpha-type hemolysis

c. Optochin sensitivity test

S. pneumoniae is the only *streptococcus* that is sensitive to optochin, thus, the optochin sensitivity test is an essential criterion for pneumococcal identification.

The interpretation is done by measuring the diameter of the inhibition zone around the optochin disk. When the diameter is equal to or more than 14 mm, the strain is identified as pneumococcus (Figure 5). As for the strains with a diameter of less than 14 mm, further testing should be done for the identification of *S. pneumoniae*.

Figure 5:*S. pneumoniae* with optochin sensitivity

d. Agglutination test

S.p identification can also be done by detecting capsular antigens using latex particles sensitized with specific antibodies. When the matching antigen exists, the latex particles agglutinate heavily, while they remain in homogeneous suspension when it doesn't.

a) b)

Figure 6: a) Pastorex™ meningitidis (Bio-Rad) *Streptococcus pneumoniae* identification reagent, b) Positive agglutination

2.3. Storage

Every strain confirmed to be a *Streptococcus pneumoniae* was stored for further tests. The storage was done from pure and fresh *S.p* colonies into cryo-tubes containing preservation media (TSB: Trypticase-Soy Broth) plus 15% to 20% of glycerol. The tubes were stored at -80°C.

2.4. Pneumococcal penicillin susceptibility

Research for strains with reduced penicillin susceptibility was performed using 1 µg oxacillin (OXA1) disks, following EUCAST (European Committee on Antimicrobial Susceptibility Testing) recommendations (8).

A strain is said to be susceptible to penicillin G, and therefore to beta-lactams if the diameter of the inhibition zone around the OXA1 disk is more than or equal to 20 mm (Figure 7). While it is considered to be with reduced susceptibility to penicillin G, and thus to beta-lactams if this diameter is less than 20 mm.

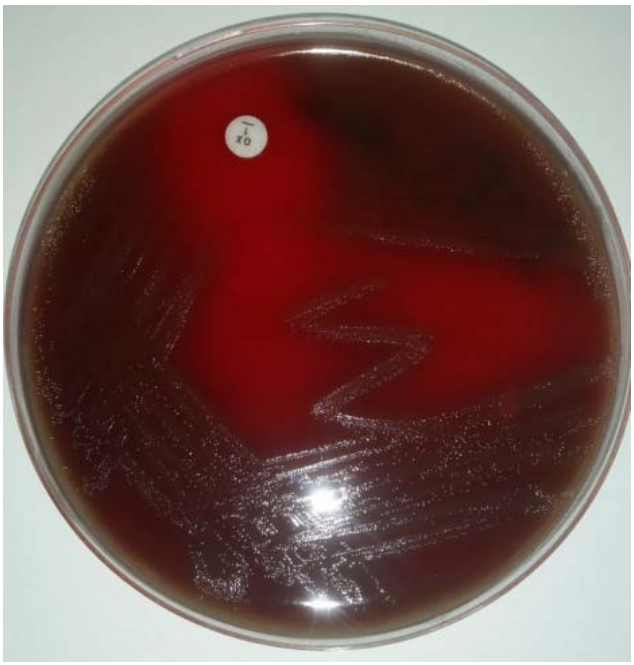

Figure 7 : penicillin susceptible pneumococcal strain.

2.5. Serogrouping by agglutination

Although serotyping and serogrouping of the pneumococcal isolates in patient specimens are not recommended on day-to-day practice, they become necessary in epidemiological studies aiming to monitor vaccine impact.

Strain serogrouping was performed using latex agglutination method, which is based on antigen-antibody reactions.

S. pneumoniae strain serogrouping was executed using Statens Serum Institut antiserums (from ImmuLexTM Pneumotest, Copenhagen, Denmark).

The type or group identification was first done via a test using the nine polyvalent antiserums from A to I until the acquisition of positive agglutination. After that, the strain was tested against antiserums from P to T until the visualization of agglutination. The type or group was read on the double-entry chessboard that comes with the kit.

The results were interpreted with the naked eye. The reaction is positive when large agglutinates appear in 5 seconds or less (Figure 8).

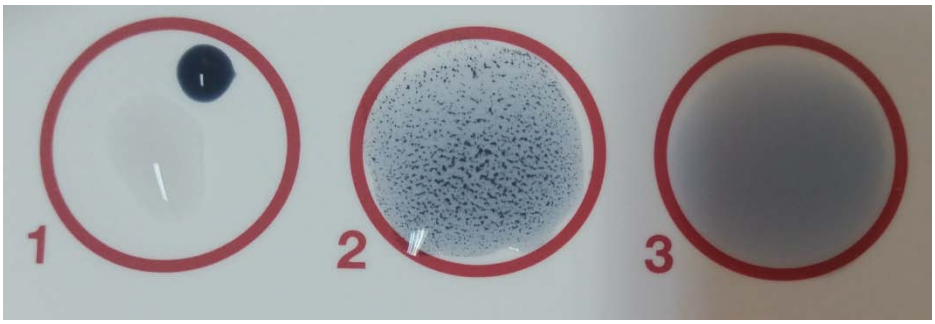

Figure 8: 1) A drop of the reagent + a drop of PBS (Phosphate Buffered Saline), 2) Positive agglutination aspect, 3) Negative agglutination aspect

2.6. Serotyping by molecular biology

Serotyping using molecular biology was executed in cooperation with the Microbiology Laboratory of Ibn-Rushd teaching hospital of Casablanca, according to the protocol and recommendations of pneumococcus molecular typing published by the CDC (9).

2.7. Serotyping using the capsular swelling reaction

This technique was used for serotyping strains belonging to serogroups 6 and 9.

The Quellung reaction, first described by Neufeld (10), consists of a change in the refractive index of the pneumococcal capsule, due to an interaction of this capsule with a type-specific antibody. This change makes the capsule swollen and clearly visible.

The antisera that were used in this reaction were serotype-specific antisera from Staten Serum Institut (Copenhagen, Denmark) (Table I).

Table I: Specific antisera tested for serogroups 6 and 9

Serogroups	Tested specific antisera
6	6A - 6B
9	9N/V - 9V

The results were examined under a phase-contrast microscope. The reaction is positive when a bright and clearly visible halo surrounding the bacterium capsule is visualized (Figure 9).

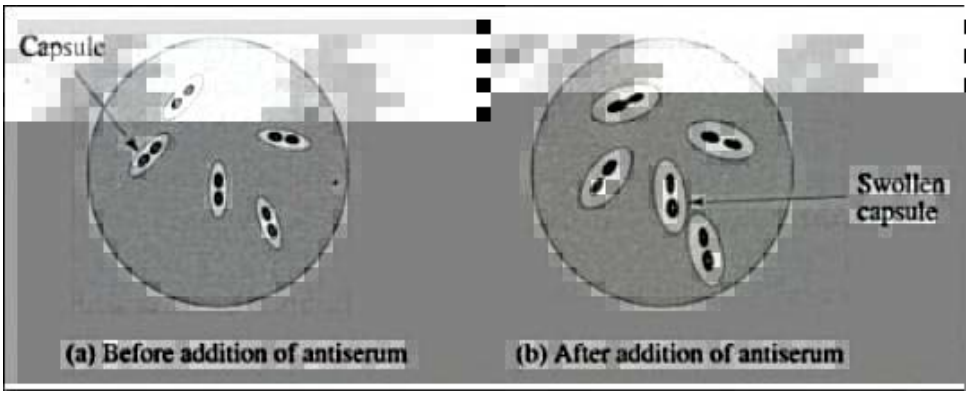

Figure 9: Schematic representation of the Quellung reaction results (11)

3. Statistical analyses

Raw data exploitation was performed using the Microsoft Excel 2007 program. Statistical analyses were carried out using the SPSS 20.0 software.

Simpson's diversity index was calculated in order to evaluate the change in the serotype diversity in the bacterial population, before and after the implementation of the vaccine.

The Chi-square test was used to compare the serotype distribution before and after the introduction of the PCV.

The Rate Ratio (RR) of the vaccine serotype (VS) carriage is the ratio of the VS prevalence in vaccinated children to the VS prevalence in non-vaccinated children (Table II).

$$RR = (a/N1) / (c/N2)$$

The PCV10 efficacy against the vaccine serotype carriage was calculated using the following equation:

$$VE = (1 - RR) \times 100$$

VE: Vaccine Efficacy.

Table II: Rate Ratio calculation parameters

	S. pneumoniae carriers		Total
	Vaccine serotypes	Non-vaccine serotypes	
Vaccinated children	a	B	N1
Non-vaccinated children	c	C	N2

a: Number of vaccinated children that are vaccine serotype carriers

b: Number of vaccinated children that are non-vaccine serotype carriers

c: Number of non-vaccinated children that are vaccine serotype carriers

d: Number of non-vaccinated children that are non-vaccine serotype carriers

N1 = a + b

N2 = c + d

RESULTS

I. Nasopharyngeal *Streptococcus pneumoniae* carriage prevalence in feverish infants in the region of Marrakesh

A total of 183 swabs were collected from feverish infants seen at the Emergency Department of Mohammed VI teaching hospital. Out of which, 125 strains were isolated, giving an overall nasopharyngeal pneumococcal carriage rate of 68.3% (Figure 10).

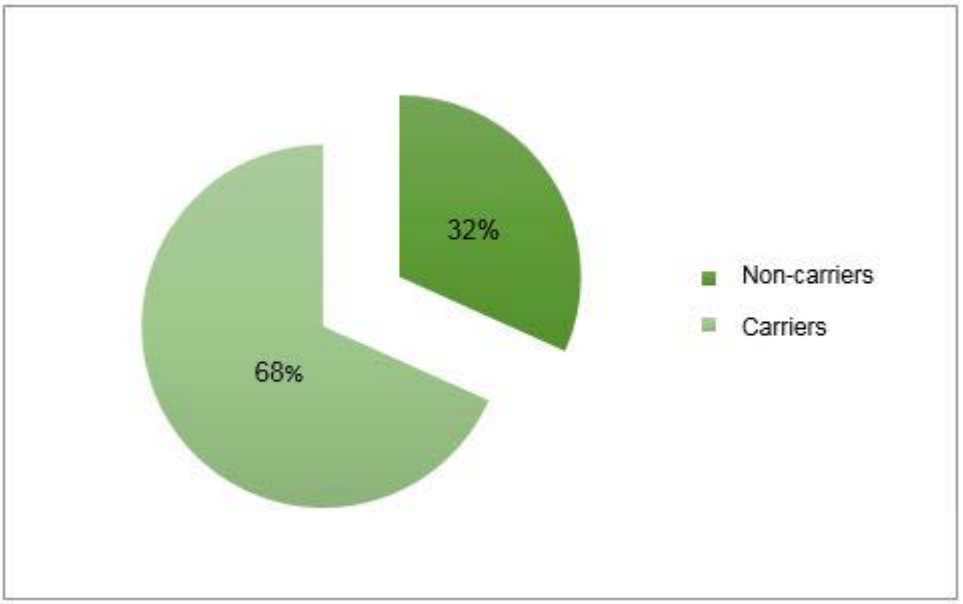

Figure 10: Overall prevalence of *Streptococcus pneumoniae* nasopharyngeal carriage in sampled infants

II. Characteristics of *Streptococcus pneumoniae* carriers

The average age of the feverish infants colonized by *S. pneumoniae* was 10.22 months (±5.04 months), with extremes ranging from 2 to 20 months. The sex ratio was 1.03. And the mean temperature detected in these infants was 38.48°C (±3.61°C).

Concerning immunization coverage, 84% of the carriers had received at least one dose of the vaccine, 14.4% had no information about their vaccination history, while only two infants were unvaccinated.

The main characteristics of the carrier infants are shown in Table III.

Table III: Characteristics of *S. pneumoniae* feverish carrier infants in the Marrakesh region

	n (%)
Characteristics:	
*Mean Age	10.22 ± 5.04
*Mean Temperature	38.48 ± 3.61
*Sexe ratio	Male : 63 (50.4%), female : 62 (49.6%)
*Daycare mode : Home	125 (100%)
PCV10 vaccination :	
*0 dose (unvaccinated)	2 (1.6%)
*1 dose	12 (9.6%)
*2 doses	60 (48%)
*3 doses	33 (26.4%)
*No information	18 (14.4%)
Colonization:	
*All serotypes combined	122
*PCV10 serotypes	8 (6.6%)
*Non-vaccine serotypes	89 (77.6%)

III. Univariate analysis of nasopharyngeal pneumococcal carriage risk factors in febrile infants in Marrakesh

The comparison of the risk factors associated with nasopharyngeal pneumococcal carriage between the carriers and non-carriers showed (Figure 11):

- No difference between the two groups concerning the number of siblings
- A different distribution according to age and immunization status: 66.2% of the carrier infants were aged 2 to 11 months and 68.86% had an incomplete vaccination schedule (The third dose was not yet received).

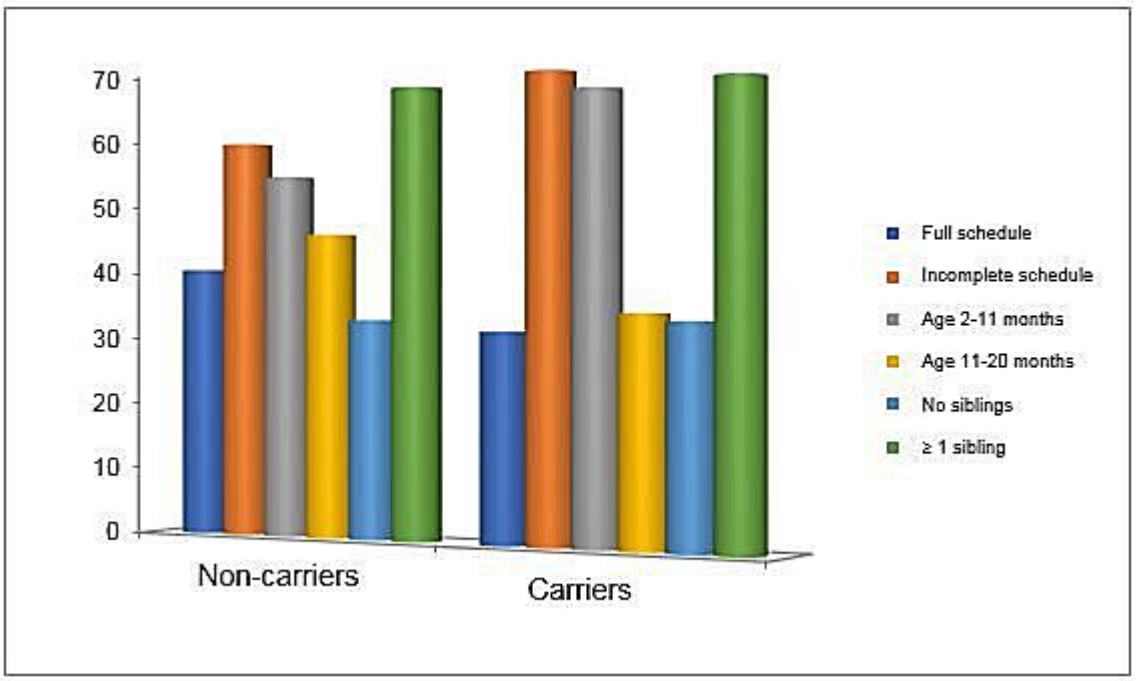

Figure 11: Comparison of risk factors associated with NP *S. pneumoniae* carriage between the feverish carrier and non-carrier infants in Marrakesh

According to the statistical analysis of these factors, only the association between the immunization status and NP pneumococcal carriage was statistically significant (OR = 1.50). Therefore, febrile infants with incomplete vaccination schedule had a higher risk of being colonized by *S.p* than those fully vaccinated (2 + 1) (Table IV).

This analysis did not show any significant difference between the two groups (colonized and non-colonized infants) concerning other factors (age, gender, number of siblings more than 1 and antibiotic treatment) (p = 0.05).

Given that all the sampled infants had a household daycare mode, daycare mode was not considered as a risk factor in this study.

Table IV: Pneumococcal carriage risk factors univariate analysis results in febrile infants in Marrakesh

Carriage risk factors	*S. pneumoniae carriage*	*p*	OR	CI 95 %
Siblings ≥ 1 (n= 123)	84 (68.3%)	0.996	0.998	0.514-1.938
Antibiotic treatment (n= 71)	48 (67.6%)	0.820	0.928	0.485-1.774
PCV10 doses: Incomplete schedule (n= 101)	73 (72.3%)	0.263	1.50	0.736-3.062
Male (n= 95)	63 (66.3%)	0.358	0.746	0.398-1.396
Age : 2 to 11 months (n= 113)	82 (72.6%)	0.130	0.611	0.322-1.159

OR: Odds Ratio **CI: Confidence Interval**

IV. Impact of immunization status on serotype carriage

Statistical analysis of the impact of the number of administrated vaccine doses on the vaccine and non-vaccine serotype carriage showed a highly significant difference ($p<0.001$) between the different immunization statuses. In fact, the decrease of vaccine serotype carriage was more important in infants who received the third PCV dose (Figure 12).

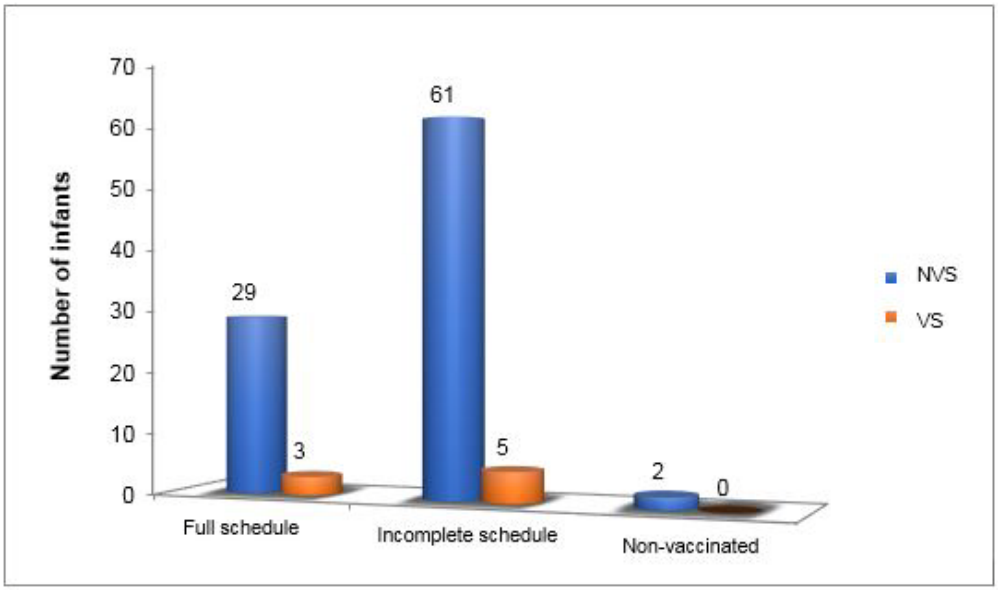

Figure 12: Impact of immunization status on vaccine (VS) and non-vaccine serotype (NVS) carriage

V. Distribution of isolated *Streptococcus pneumoniae* serotypes in sampled infants

1. Vaccine serotype distribution

The vaccine serotypes that were carried by the sampled infants accounted for a low percentage of 6.6%. The isolated serotypes were 19F (2 cases), 1 (2 cases) and one case for each of the following serotypes: 14, 23F, 6B and 9V (Figure 13).

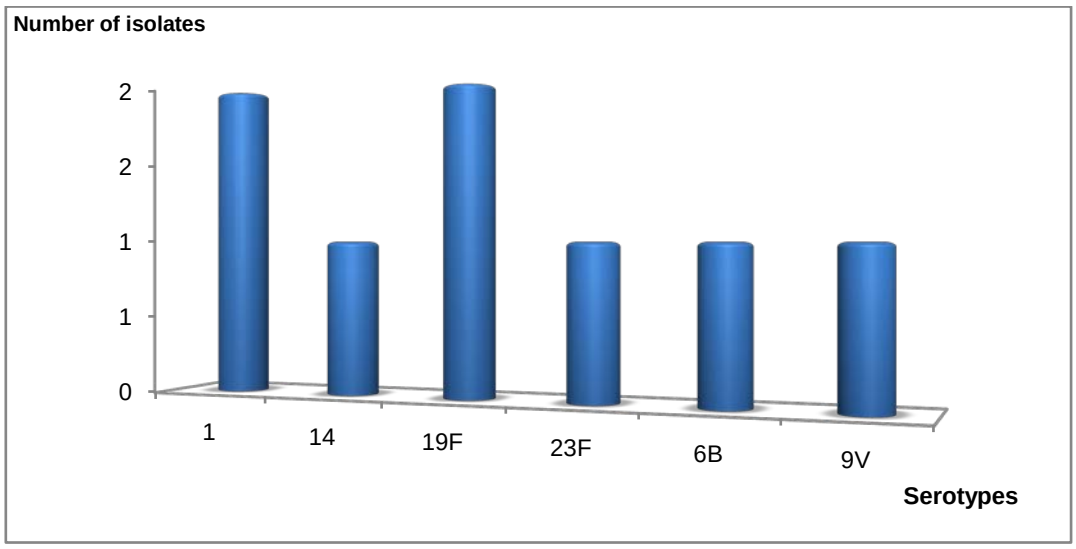

Figure 13: Distribution of isolated vaccine serotypes in feverish infants in the region of Marrakesh

2. Non-vaccine serotype distribution

This study concluded to an increase in the rate of the non-vaccine serotype carriage in the enrolled infants. This illustrates the serotype replacement phenomenon; vaccine serotypes have been replaced by non-vaccine serotypes. The isolated non-vaccine serotypes were: 6A (6.4%), 15A/15F (5.6%) and 3.2% for each of serotypes 20, 15B/C, 23B and 13 (Figure 14).

For non-typable and non-vaccine strains, PCR is needed to define their serotypes.

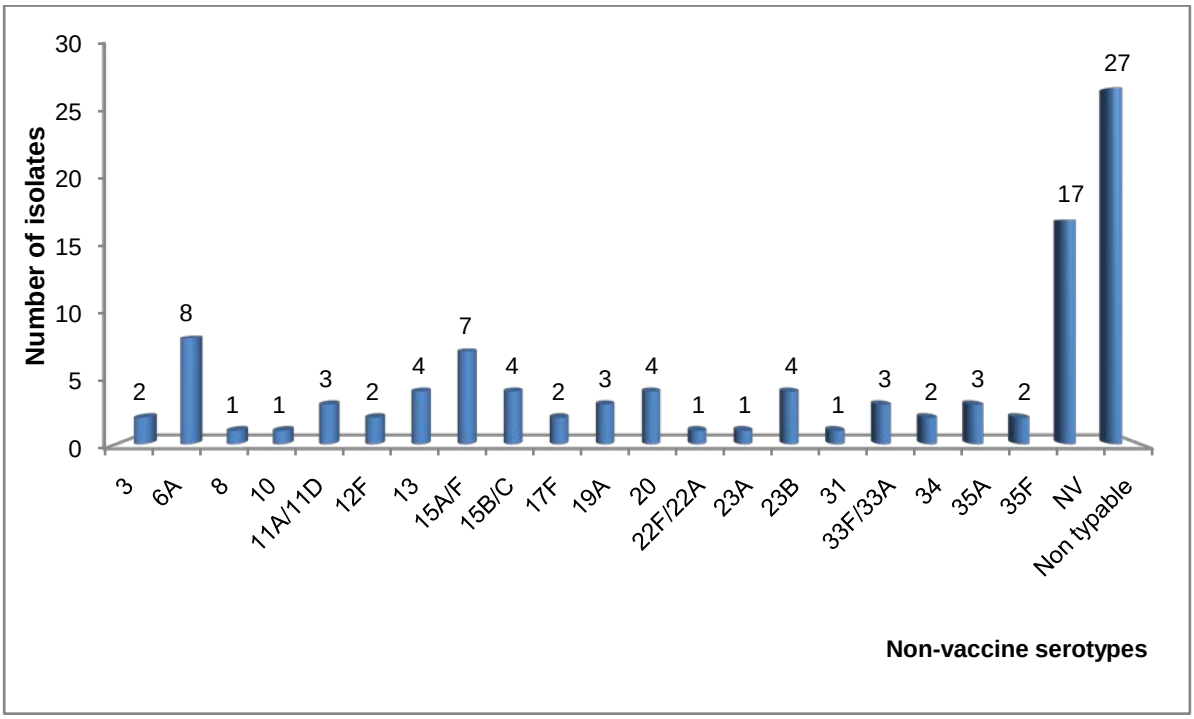

Figure 14: Distribution of isolated non-vaccine serotypes in feverish infants in the region of Marrakesh

***NV: Non-vaccine serotypes other than serotypes 3, 6A, 8, 10, 11A/11D, 12F, 13, 15A/15F, 15B/15C, 17F, 19A, 20, 22F/22A, 23A, 23B, 31, 33F/33A, 34, 35A and 35F. *Non-typable: Strains which hadn't agglutinated with any antiserum.**

VI. Effect of PCV10's introduction on the diversity of carried serotypes

Simpson's index of diversity was calculated before and after the implementation of the PCV10 in order to measure the serotype diversity in carriage. This index was significantly higher ($p<0.001$) in the post-vaccination period (Table V), reflecting a great serotype diversity compared to the pre-vaccination period.

Table V: Simpson's index of diversity in the pre and post-vaccination periods

	Before vaccination*	After vaccination	*p*
Simpson's index of diversity	0,83	0,92	< 0,001

*The index was calculated based on the results of the pneumococcal carriage study conducted in Marrakesh before the introduction of the vaccine (3).

VII. Effect of PCV10's introduction on vaccine serotypes' carriage

There was a clear change in the vaccine and non-vaccine serotype distribution after the implementation of the PCV. Before the introduction of the vaccine, the vaccine and non-vaccine serotype carriage rates were 53.9% and 34.2%, respectively. While in the post-vaccine period, the vaccine serotype proportion has significantly decreased ($p<0.001$) to 6.6% and the non-vaccine serotype percentage has increased to 77.6% (Table VI).

The calculated vaccine serotype Rate Ratio (RR (VS)) was less than 1 (0.117), which confirms the contribution of the vaccine in reducing the vaccine serotype carriage rate, with vaccine efficacy (VE) reaching 88.23% (Table VI).

Table VI: Distribution of vaccine and non-vaccine serotypes before and after PCV's introduction in Marrakesh

Population	Pneumococcal carriage		Total	P	RR (VS)	VE
	VS	NVS				
Vaccinated	8 (6,6%)	95 (77,6%)	103	< 0,001	0,117	88,23%
Unvaccinated	99 (53,9%)	51 (34,2%)	150			

VIII. Penicillin susceptibility of *S. pneumoniae* serotypes isolated in carriage in feverish infants in Marrakesh

1. Prevalence of *S. pneumoniae* serotypes with reduced susceptibility to penicillin

According to the penicillin susceptibility screening test that was systematically performed, 33.6% of the *S.p* serotypes isolated in carriage in the sampled infants were pneumococci with reduced susceptibility to penicillin (PRSP) (Figure 15).

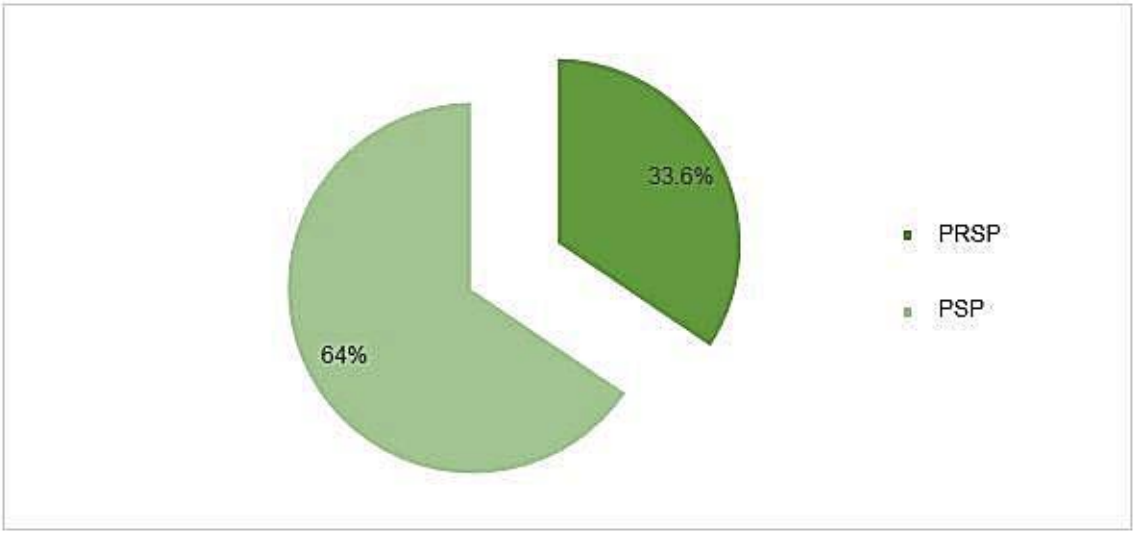

Figure 15: Prevalence of Pneumococci with reduced susceptibility to penicillin (PRSP) and penicillin-susceptible pneumococci (PSP) carried by feverish infants in the region of Marrakech.

2. Distribution of isolated serotypes according to their penicillin susceptibility profile

The study of the serotypes according to their penicillin susceptibility profile revealed the following results:

- Only two of the 8 isolated vaccine serotypes were PRSP, the serotypes in question were serotypes 14 and 6B (Figure 16).

- As for non-vaccine serotypes, 38 out of 102 strains were PRSP (which accounts for 82.6% of the overall PRSP serotypes), non-typable and penicillin-resistant strains were very common in this study (21 resistant strains out of 27 non-typable strains). (Figure 17).

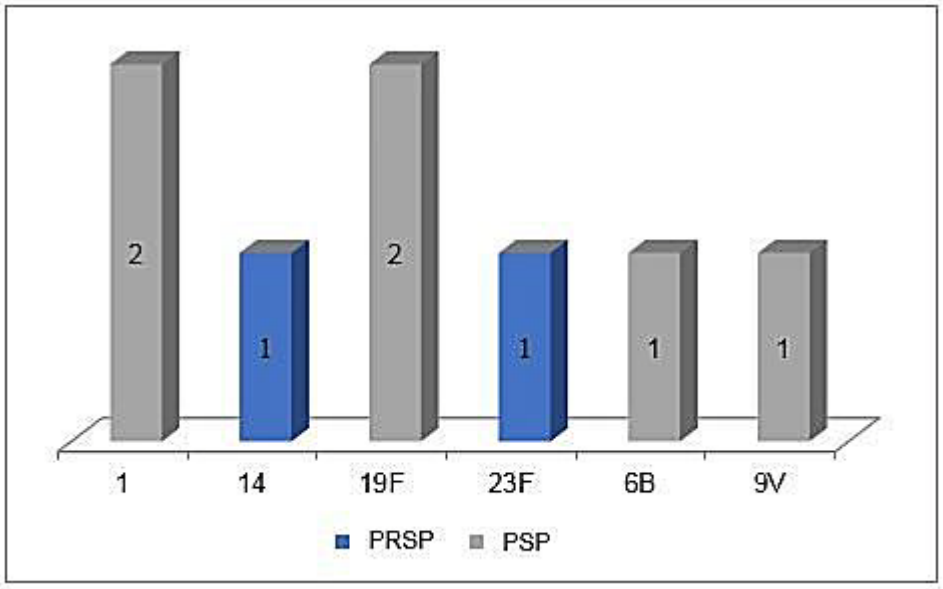

Figure 16: Distribution of vaccine serotypes according to their penicillin susceptibility (PRSP: Pneumococcus with reduced susceptibility to penicillin
- PSP: Penicillin susceptible pneumococcus)

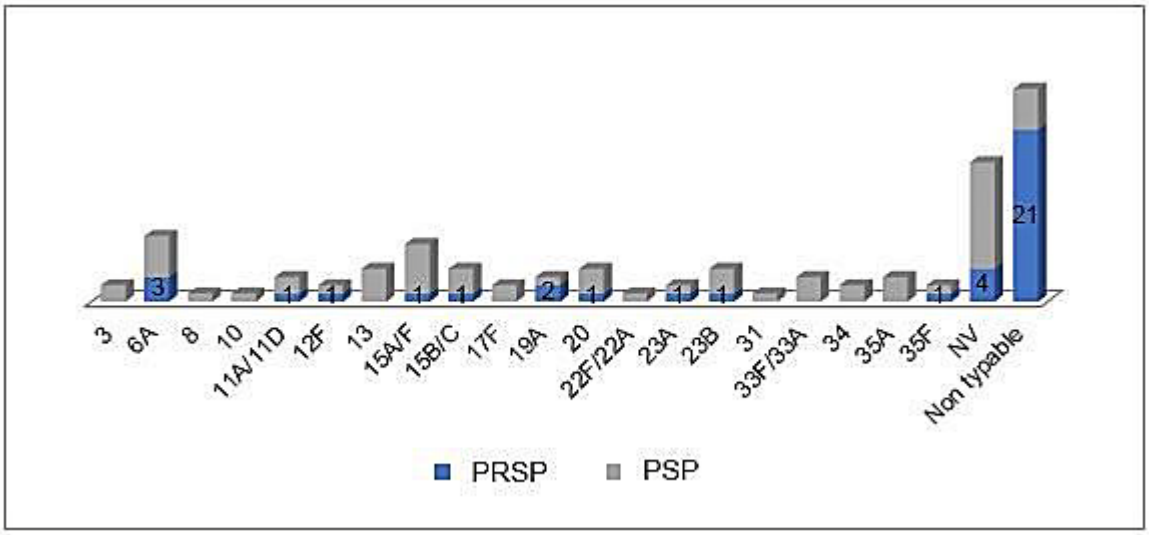

Figure 17: Distribution of non-vaccine serotypes according to their penicillin susceptibility (PRSP: Pneumococcus with reduced susceptibility to penicillin – PSP: Penicillin susceptible pneumococcus)

DISCUSSION

I. Generalities

1. History

Streptococcus pneumoniae was isolated for the first time in 1881, simultaneously by two microbiologists: George M. Sternberg in the United States and Louis Pasteur in France. They both independently described roughly lancet-shaped pairs of cocci in human saliva after injecting it into rabbits (12).

By 1886, this microorganism was being referred to as Pneumococcus by Fraenkel, because of its tendency to cause pulmonary disease. Then it was renamed Diplococcus pneumoniae in 1920, before being finally given its present name -*Streptococcus pneumoniae* - in 1974, primarily on the basis of its characteristic growth as chains of cocci in liquid media (12).

2. Taxonomy

Streptococcus pneumoniae (commonly known as Pneumococcus) belongs to the *Streptococcaceae* family, genus *Streptococcus*. This genus includes over 40 species grouped into six major groups: The *Pyogenic* group, the *Anginosus* group, the *Mitis* group, the *Salivarius* group, the *Bovis* group, and the *Mutans* group (13). According to the Lancefield Classification, *S.p* belongs to non-groupable species (14).

3. Epidemiology

3.1. Reservoir and transmission

S. pneumoniae is a commensal bacterium of the human upper airways, more precisely, the nasopharynx. (15).

Pneumococci are transmitted from human to human through respiratory droplets (16). The bacteria enter the nasal cavity, attach to the nasopharyngeal epithelial cells and might then either remain as a colonizer or spread to other organs, such as the middle ear, sinuses or down to the lungs via bronchi. Thus, it can potentially cross the mucosal barrier to enter the bloodstream, from which it can cross the blood-brain barrier and cause meningitis (17). (Figure18).

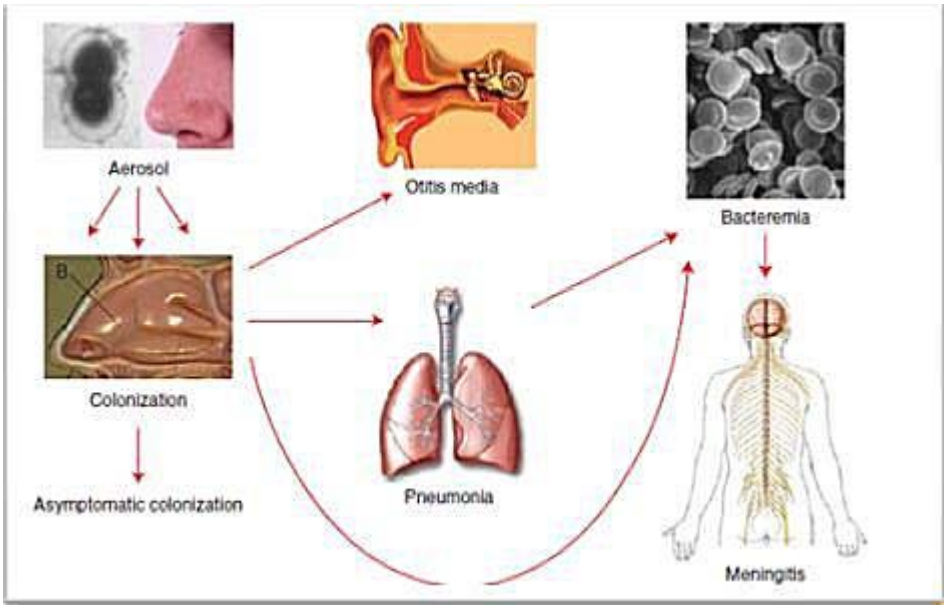

Figure 18: *Streptococcus pneumoniae*'s different ways of progression in the human body (17).

3.2. Pneumococcal carriage risk factors

Many previous studies have concluded to the presence of several factors according to which the rate of pneumococcal nasopharyngeal carriage varies. These factors include age less than two years, for that's when the registered rates were higher (18). Siblings ≥ 1, smoking environment, breastfeeding for less than two months and poor socio-economic conditions are also associated with pneumococcal colonization (3) as well as attending daycare centers, where promiscuity favors the transmission of the bacterium (19), and the winter season (20). Viral respiratory infections, especially with syncytial and influenza viruses increase the risk of both pneumococcal carriage and infections (18). A Finnish study has also reported the association of

the dietary factor which includes high consumption of sweet pastries and jam with an increased risk of *S.p* colonization (21).

3.3. Epidemiological aspects

Streptococcus pneumoniae infections are a major source of morbidity and mortality worldwide.

The World Health Organization estimates that invasive pneumococcal infections are responsible for the death of almost 500 000 children aged less than five years old, most of which are in developing countries (22). Moreover, hospitalizations for pneumococcal pneumonia (23), as well as medical management of acute otitis media (24), represent a considerable economic burden, especially in pediatrics. It should be noted that invasive pneumococcal infections are also common in the elderly and patients with underlying immunosuppressive diseases such as HIV infection and chronic liver disease (25).

The rate of pneumococcal infections and the deaths caused by them varies according to the socio-economic status of each country; it is higher in developing countries with major mortality prevalence in Sub-Saharan Africa and Southern Asia (61% of overall mortality) (26). Furthermore, the timing of disease onset in children also differs between low-income and high-income countries. In developing countries, most pneumococcal infections and deaths among children <5 years of age occur in the first year of life, with a peak in disease incidence before 6 months of age; in developed countries, these infections peak closer to 12 months of age with about half of the episodes occurring by 18 months (27). (Figure19).

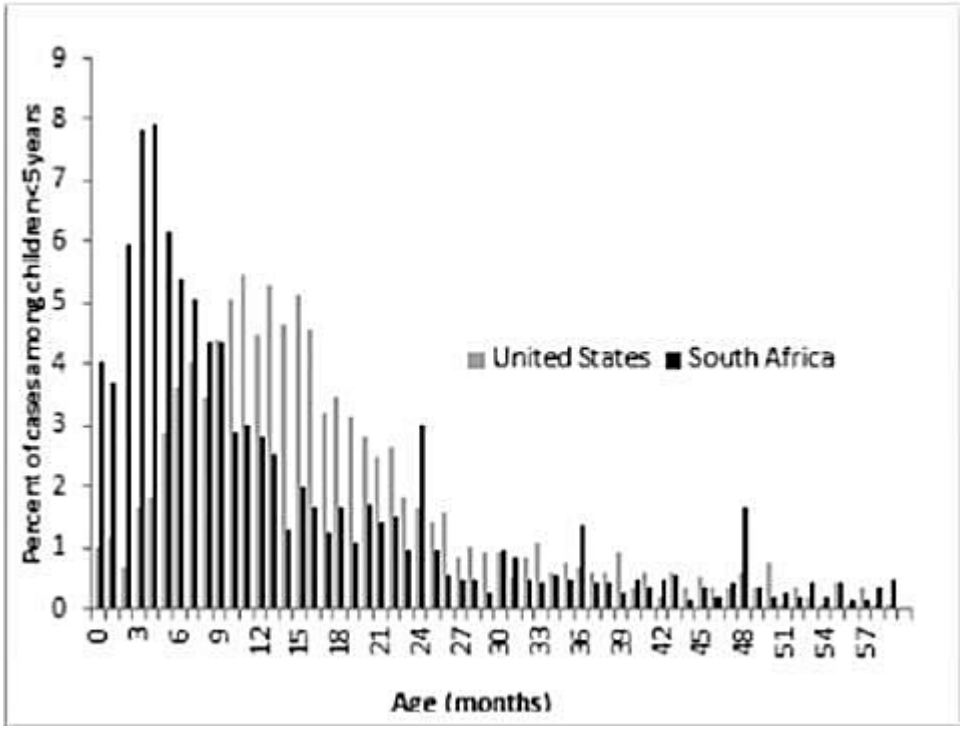

Figure 19: Distribution of cases of invasive pneumococcal disease for children <5 years, by months of age for children in a developing country (South Africa) and in an industrialized country (United States) (27).

In Morocco, the implementation of PCV13 in 2010 then PCV10 in 2012 significantly reduced the incidence of invasive pneumococcal infections in infants < 2 years of age, from 34.6 to 13.5 per 100 000 inhabitants, respectively before and after the vaccine introduction (28).

4. Microbiological aspects

4.1. Morphology and structure

On Gram's stain, pneumococci have a characteristic morphology: they are Gram-positive, lancet-shaped, encapsulated (Bright halo surrounding the bacteria) and 8-shaped or "candle-flame-shaped" diplococci (15). (Figure 20). They can also grow in chains (29).

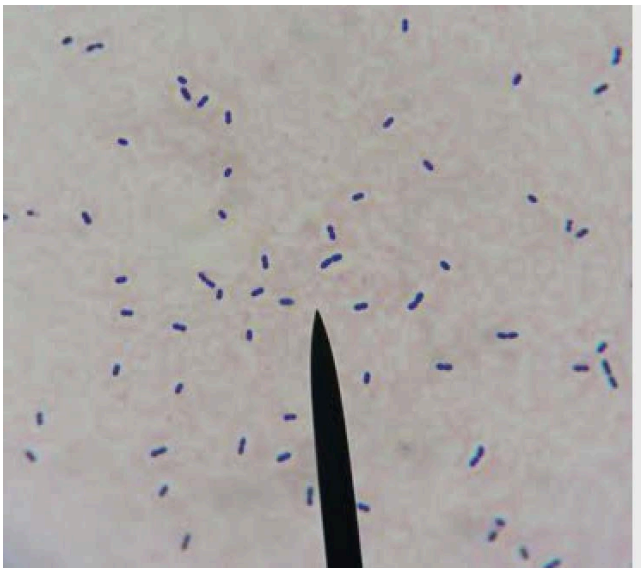

Figure 20: *Streptococcus pneumoniae* visualized as encapsulated Gram-positive diplococcic

4.2. Growth characteristics

a. Growth media

As a demanding bacterium, *S. pneumoniae* requires growth factors for its culture. Therefore, Soy-Trypticase and Columbia agar with 5% sheep blood are commonly used for culturing pneumococci. Chocolate blood agar to which a vitamin complex has been added is also a favorable medium for pneumococcal growth (15). Mueller Hinton agar with blood is used for antibiotic susceptibility testing (15). In liquid media, *S.p* can grow in Brain and Heart infusion broths (BHI).

b. Growth conditions

Pneumococci are anaerobic or facultative aerobic bacteria. The optimal conditions for their growth are a carbon-dioxide-enriched atmosphere (5 to 10%) or even an anaerobic atmosphere, a temperature ranging from 35 to 37°C and a pH=7.8 (6.5 – 8.3) (30), for an incubation period of 24 to 48 hours.

c. Colonies' aspect

S. pneumoniae colonies usually measure 0.5 to 1.5 mm and are surrounded by greenish halos showing incomplete hemolysis and transformation of hemoglobin into biliverdin (Alpha-type hemolysis). They are opaque or grayish, convex and with regular edges. Another feature characterizing *S. pneumoniae* colonies is the central umbilicus-like depression that is caused by the pneumococcal autolysin (Figure 21). Serotype 3 colonies often have a mucoid aspect, due to the excessive development of the capsule (Figure 22). (15).

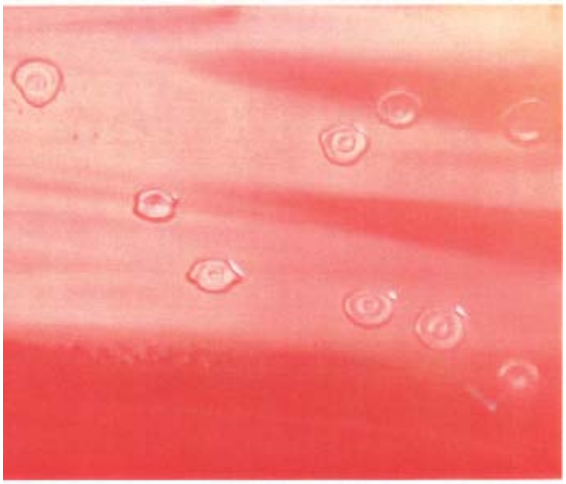

Figure 21 : *Streptococcus pneumoniae* colonies : note the central depression (29).

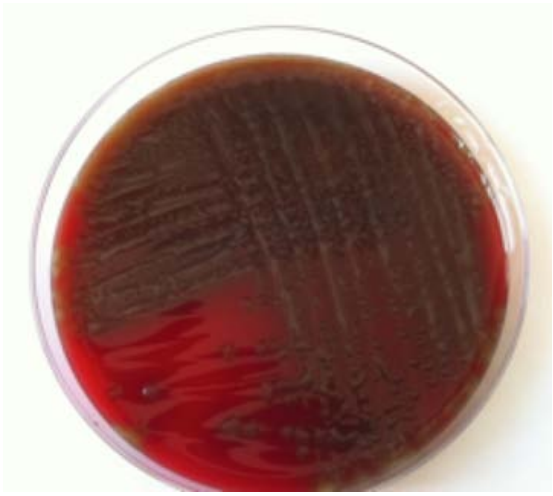

Figure 22: *Streptococcus pneumoniae* colonies with a mucoid aspect

4.3. Biochemical characteristics

Pneumococci, as *Streptococcaceae* family members, produce lactic acid by glucose fermentation (Homofermentative). They are catalase-negative, oxidase-negative and anaerobic-aero tolerant (15).

4.4. Antigenic characteristics

One of the major factors of pneumococcal virulence is the capsule. It is made up of polysaccharide macromolecules, and its antigenic structure allows pneumococcal strains serotyping (Lund's Danish Classification). Currently, over 90 serotypes have been described (31) (Table VII)

These serotypes have different propensities concerning pathogenicity and antibiotic resistance (32).

S.p possesses antigens other than its polysaccharide capsular antigens: The species-specific substance (C) which is a polysaccharide consisting of teichoic acid, the R antigen that is a protein and usually is masked by the capsular antigens, and the M antigen which is a protein type-specific antigen (30).

Table VII: Lund's Danish Classification of *Streptococcus pneumoniae* (31)

1, 2, 3, 4, 5, 6A, 6B, 7F, 7A, 7B, 7C, 8, 9A, 9L, 9N, 9V, 10F, 10A, 10B, 10C, 11F, 11A, 11B, 11C, 11D, 12F, 12A, 12B, 13, 14, 15F, 15A, 15B, 15C, 16F, 16A, 17F, 17A, 18F, 18A, 18B, 18C, 19F, 19A, 19B, 19C, 20, 21, 22F, 22A, 23F, 23A, 23B, 24F, 24A, 24B, 25F, 25A, 27, 28F, 28A, 29, 31, 32F, 32A, 33F, 33A, 33B, 33C, 33D, 34, 35F, 35A, 35B, 35C, 36, 37, 38, 39, 40, 41F, 41A, 42, 43, 44, 45, 46, 47F, 47A, 48

5. Pathogenicity

5.1. Virulence factors

S. pneumoniae has many virulence factors. They can be on the surface of the intact bacterium (Capsule, PspA…) or be expressed after its destruction or lysis (pneumolysin…). These factors are responsible for inflammatory reactions that can sometimes be very deleterious for the host via complement activation (33).

a. Capsule

The pneumococcal capsule is the first discovered and the most important virulence factor. It is the outermost element of the bacterium. In vivo, it allows the growth of pneumococci and considerably hinders phagocytosis by acting as a physical barrier and preventing phagocyte receptors from being in contact with complement components C3b that have eventually attached to the bacterial wall. The capsule is also able to electrostatically repulse the phagocytes that are negatively charged, like the capsular polysaccharides. And it protects the surface proteins from circulating antibodies.

The strains' virulence and invasiveness vary depending on the serotype, in other words, depending on the amount of produced capsule and its composition (33).

b. Pneumolysin

Pneumolysin, a thiol-activated toxin, is located in the cytoplasm. It is released in the outside under the action of LytA, a bacterial autolysin.

Pneumolysin has a cytotoxic activity. It inhibits the beating of the cilia involved in the mucociliary clearance of the bronchi and destroys the bronchial epithelium. It is responsible for a decrease in the bactericidal activity of monocytes and neutrophils. It also causes the inhibition of lymphocyte proliferation and reduction of antibody synthesis (33).

There is very little difference in the sequence of pneumolysin from one serotype to another which could be useful for the development of a pneumococcal protein vaccine (34).

c. Other virulence factors

c.1. Pneumococcal surface proteins (Psp A and C)

They facilitate pneumococcal systemic invasion by inhibiting the alternative complement pathway. Moreover, PspA, unlike PspC, is able to attach to lactoferrin, a human iron-sequestering glycoprotein, and thus provides enough iron for bacterial growth (33).

c.2. Other factors expressed after bacterial lysis

Wall components, especially teichoic and lipoteichoic acids and phosphorylcholine, can also be responsible for triggering inflammatory reactions (33).

c.3. Pili

Studies on the role of pili in the pathogenesis of pneumococcal infections are still few to date. However, in vitro, it has been shown that pili are involved in the process of pneumococcal adhesion to the pulmonary epithelial cells (35), as well as in the invasion and colonization (36).

5.2. Infections caused by Streptococcus pneumoniae

Although pneumococcal carriage is asymptomatic, it can lead to respiratory or even systemic infections (37). These infections can be invasive (Invasive pneumococcal disease; IPD) or non-invasive (non-IPD). The development of the pneumococcal disease is conditioned by many factors, the most important of which are the strain's virulence, the immunity status, especially the humoral immunity, and the presence of respiratory viral infections (16).

a. Non-invasive pneumococcal diseases

They are mucosal infections of the respiratory epithelium that are spread by contiguity, such as acute otitis media, sinusitis and pneumonia (16).

In fact, these infections are favored by surface proteins such as choline-binding protein A (Cbp A) and neuraminidase Nan A which cause a decrease in mucus viscosity and favor bacterial adhesion. During intercurrent infections by respiratory viruses, this mechanism is amplified under the effect of neuraminidase of viral origin (1).

b. Invasive pneumococcal diseases

IPD is defined as an infection confirmed by the isolation of *S. pneumoniae* from a normally sterile site (such as blood, cerebrospinal fluid, joint fluid, etc.). The most important infections are meningitis and bacteremia. These infections are frequent in young children, the elderly and patients with underlying diseases including HIV infection, sickle cell disease, terminal renal failure, etc. (38).

Bacteremia is caused by the bacteria crossing the respiratory mucosa which is facilitated by the epithelial destruction caused by pneumolysin (33) and surface proteins (1). The survival of the bacteria in the bloodstream is possible mostly thanks to the capsule alongside with other factors (16). Then pneumococci can stick to the cerebral capillary endothelium, cross the blood-brain barrier and cause meningitis (38).

6. Microbiological diagnosis

S. pneumoniae microbiological diagnosis is based on (15):

- Direct examination of biological fluids (Cerebrospinal fluid, blood, puncture fluids, sputum, pulmonary specimens) or pus (Otitis).
- Culture in blood supplemented media in order to search for alpha-type hemolysis.
- Gram Stain
- Species-specific tests:
  - Optochin sensitivity
  - Bile solubility
  - Agglutination test: Agglutination with latex particles sensitized with anti-capsular antibodies can confirm pneumococcal identification.
- In case of doubt, molecular biology, via PCR, can quickly confirm the identification of the strain (15).

7. Antibiotic resistance

7.1. Natural resistance

Pneumococci, like all the Gram-positive cocci, are naturally resistant to mecillinam, aztreonam, quinolones (except for anti-pneumococcal fluoroquinolones: levofloxacin and moxifloxacin) and colistin. Like all *streptococci*, they naturally have a low resistance to aminoglycosides (39).

They are also naturally susceptible to several antibiotic families such as beta-lactams (apart from mecillinam and aztreonam) (38), macrolides, Cotrimoxazole andfluoroquinolones with anti-Gram-positive activity (levofloxacin, moxifloxacin) (40).

7.2. Acquired resistance

a. Beta-lactams resistance

Pneumococci with reduced susceptibility to penicillin (PRSP) are defined as pneumococci possessing minimum inhibitory concentrations (MIC) of penicillin ≥ 0.1 mg / L. The first pneumococcal isolates with increased MICs of penicillin G were described in Australia in 1967. The mechanism of resistance is based on modifications of beta-lactam targets: penicillin-binding proteins (PBPs) (41).

Figure 23 represents MIC determination using antibiotic strips.

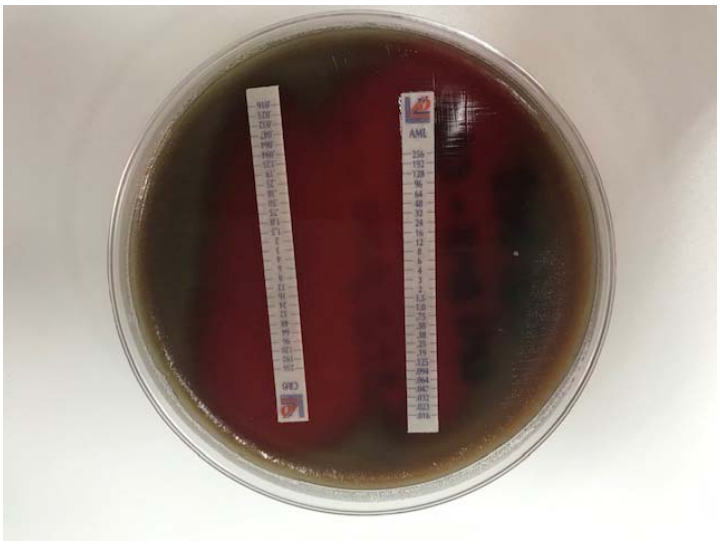

Figure 23: MIC determination using amoxicillin and ceftriaxone strips.

b. Fluoroquinolone resistance

Resistance to fluoroquinolones is due either to mutations of one of the two fluoroquinolone targets, gyrase and topoisomerase IV or to an increase in active efflux. Mutations usually occur in a region called QRDR (Quinolone Resistance Determination Region) (41).

c. Macrolide resistance

Three mechanisms are responsible for acquired macrolide resistance in *S. pneumoniae* (41):

- Target modification by methylation or ribosomal mutation.
- Efflux
- Enzymatic modification

8. Prophylaxis

Vaccination is the most effective way of preventing infections due to many pneumococcal serotypes. Currently, there are two types of pneumococcal vaccines: Pneumococcal polysaccharide vaccine (PPSV) and pneumococcal conjugate vaccine (PCV).

8.1. Pneumococcal polysaccharide vaccine

It contains capsular polysaccharide antigens of 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) (42).

One dose of PPSV is recommended to be administered to all seniors over the age of 65 years. It is also recommended that people aged 5 to 64 years with an underlying disease increasing the risk of developing an IPD, such as chronic heart disease, sickle cell disease, or HIV infection; receive PPSV doses with a maximum dose number of 3 throughout the lifetime and with at least a 5-year interval between 2 doses (42).

This vaccine has a moderate impact on the incidence of IPD and has no effect on the carriage. However, it did not generate any immune response in children less than 2 years old, who have the highest risk of developing IPDs (42).

8.2. Pneumococcal conjugate vaccine

In order to induce an immune response in children less than 2 years old, the polysaccharide conjugate vaccine was developed by linking the polysaccharide antigens to a carrier protein (diphtheria toxin). The first developed PCV was the heptavalent pneumococcal conjugate vaccine (PCV7) which contains the antigens of 7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F), and was first implemented in the USA in 2000 (42). Then two other conjugate vaccines were developed to cover more serotypes and thus be able to prevent more pneumococcal infections: PCV10 (4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, and 7F) (43) and PCV13 (4, 6B, 9 V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A, and 19A) (42).

PCV dosing schedules might differ from one country to another (42) (Figure 24). The vaccine manufacturers recommend 3 primary doses (the first dose can be given starting at 6 weeks of age) with an interval of at least 4 weeks between doses, plus a booster at least 6 months after the third dose (3p+1 schedule). It can also be administered according to a schedule consisting of 2 primary doses given 2 months apart, starting at the age of 2 months, followed by a booster at least 6 months after the second dose (2p+1 schedule) (44). However, some countries use a 3p+0 schedule, which consists of 3 primary doses without a booster (45).

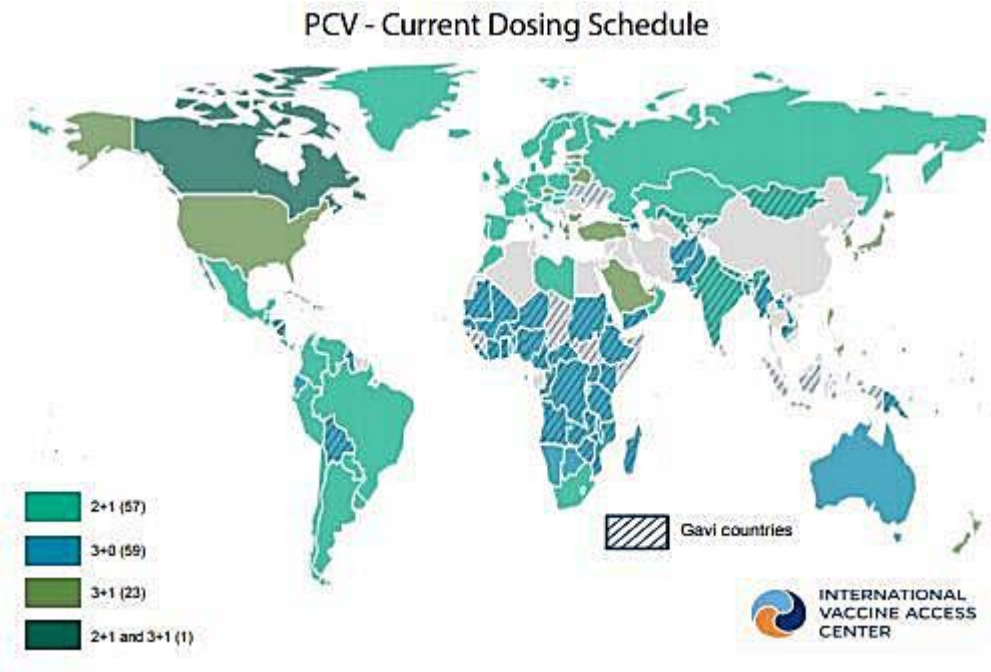

Figure 24: PCV current dosing schedules worldwide (June 2018) (45)

Gavi: Global alliance for vaccines and immunization.

II. Discussion of results

Pneumococcal infections represent a major cause of mortality and morbidity, especially in the pediatric population. Aiming to prevent these infections, the Moroccan Health Ministry introduced the pneumococcal conjugate vaccine in its national immunization program in 2010.

Based on literature reviewing, this study is, to our knowledge, the first national epidemiological monitoring study of the pneumococcal nasopharyngeal carriage after the introduction of the PCV in Morocco. A study conducted in Casablanca in 2015 concerned invasive pneumococcal infections (28).

This study aims to assess the impact of the PCV on the pneumococcal nasopharyngeal colonization in the region of Marrakech and to determine its prevalence and the risk factors associated with it. It also targets describing the distribution of serotypes and their antibiotic-susceptibility profiles.

1. Nasopharyngeal *Streptococcus pneumoniae* carriage prevalence in feverish infants in the region of Marrakesh

In this study, the overall pneumococcal nasopharyngeal carriage rate in the sampled infants was 68.3%. It is higher than the carriage rate before the introduction of the vaccine (45.8%) that was reported by a study conducted in the region of Marrakech which concerned healthy children aged less than 2 years in 2010 (3).

This prevalence is also greater than those reported by several internationally conducted studies which range from 5.5% to 56.4% (Table VIII). However, it is lower than the carriage rate detected in healthy children in certain countries, especially in Africa, such as The Gambia (46) and Mozambique (47) (84.3% and 84.5%, respectively).

Table VIII: Rates of nasopharyngeal carriage of *Streptococcus pneumoniae* in children at the international level

Study	Year	Carriage Rate
Hong-Kong (48)	2016	5,5%
Turkey (49)	2017	14%
Iran (50)	2015	18%
Japan (51)	2015	24,8%
United Kingdom (52)	2015	30%
Italy (53)	2013	32,9%
South Korea (54)	2016	36,4%
Colombia (55)	2013	44,2%
Brazil (56)	2016	48,8%
France (19)	2015	56,1%
Gambia (46)	2015	84,3%
Mozambique (47)	2018	84,5%

Comparing carriage rates between the said studies is difficult due to a number of factors including sample size, duration of study, age limit of enrolled children and their clinical features.

2. Risk factors associated with pneumococcal nasopharyngeal carriage

In this survey, we made a comparison of the number of *S.p* carriers in populations exposed and not exposed to certain risk factors. In fact, *S.p* NP colonization varies according to:

2.1. Age

Nasopharyngeal colonization begins during the early months of life, and peaks before 2 years of age (57).

In this study, the average age of the carriers was 10.22 months (± 5.04 months), and the predominant age group was 2 to 11 months. Nevertheless, age was not significantly associated with the pneumococcal carriage after the univariate analysis of the risk factors (OR= 0.611).

2.2. Immunization status

84% of infants colonized with *S.p* received at least one dose of the vaccine, which is almost similar to the proportion found in Germany in infants <2 years of age with invasive pneumococcal infections; in fact, in 2014-2015, 83.8% of these infants received at least one dose of the PCV at the time of the infection (58).

The colonization rate is significantly associated with the vaccination status (OR=1.50, CI=95%), as the majority of carrier infants had an incomplete vaccination schedule (72.3%). This result is in agreement with a Cypriot study which showed an increase in the rate of pneumococcal nasopharyngeal carriage (OR = 1.64) in children with an incomplete vaccination profile, compared to those who completed their pneumococcal immunization with PCV7 (59).

In addition, we found that pneumococcal carriage was higher (48%) in children who received 2 doses of the vaccine than in those who received a complete 3 dose schedule (2 primary doses and a booster); These results are comparable to those reported in the literature by several studies, particularly by randomized clinical trials that compared the effect of a booster dose, administered during the second year of life after two primary doses to the one containing only 3 primary doses on the nasopharyngeal carriage of the pneumococcus. In fact, the 2 + 1

schedule was more efficient (60,61). This is because the booster dose causes an increase in the concentration of antibodies rather than a primary series, despite the fact that the children are less protected during the interval between the primary doses and the booster compared to those who have completed 3 primary doses (62).

2.3. Other risk factors

In opposition to previous data (3,19,63,64), we found that having siblings ≥ 1 (OR = 0.998) is not a risk factor of *S. pneumoniae* carriage. The same data associate this carriage with daycare center attendance, but the daycare mode was not considered as a risk factor in our study since all the enrolled infants had a household daycare mode.

3. Distribution of isolated *Streptococcus pneumoniae* serotypes in sampled infants

One of the most important goals of this study was to elucidate the impact of the PCV on the pneumococcal NP carriage since this carriage represents an obligatory step for a pneumococcal disease to develop, whether it was invasive or not. This was possible through the study of isolated serotypes' distribution.

A total of 122 serotypes were isolated, of which 8 were PCV10 serotypes and 89 were non-vaccine serotypes (6.6% and 77.6%, respectively), which reflects the heterogeneity and diversity of isolated strains.

3.1. Vaccine serotypes

The proportion of vaccine serotypes was low (6.6%) with a predominance of serotypes 19F and 1 (2 cases each). Despite this low proportion, serotype 19 still retains its position as the lead vaccine serotype in carriage, as reported in several studies (3,65,66).

This study has highlighted the positive impact of PCV10 on nasopharyngeal colonization of *S.p*. Nasopharyngeal carriage of vaccine serotypes decreased from 53.9% to 6.6% ($p < 0.001$), which is consistent with studies that have previously explored this effect in different countries. A reduction of more than 90% ($p < 0.0001$) of vaccine serotypes was observed in Brazil (56). In Kenya as well, the rate of vaccine serotypes decreased from 34% to 13% for PCV10 (67). For PCV13, the proportion of vaccine serotypes was reduced from 21.4% to 3.5% ($p < 0.001$) in France (19).

The administration of PCVs decreases the carriage of vaccine serotypes, their transmission and the development of the infections they cause, not only in vaccinated children but also in non-vaccinated individuals living around these children. It's called indirect or group protection (herd immunization) (68). This effect has been extensively documented for PCV7 (68) through several studies; as in the United States (69) or The Gambia (70). However, data on PCV10 and PCV13 are still emerging (61,71). A Kenyan study has shown a reduction of two-thirds in the nasopharyngeal carriage of *S.p* in children less than 5 years of age as well as in adults after PCV10 administration (67). In Massachusetts, the USA, more than 50% reduction of the carriage of PCV13 vaccine serotypes was observed in unvaccinated children when PCV13 vaccination coverage reached 75% or more (72).

3.2. Non-vaccine serotypes

A raise in non-vaccine serotypes was noted after the introduction of the vaccine in this study, as their rate was 83.6%. Many studies have reported the same finding, which is known as serotype replacement (52,53,73-75). This phenomenon could explain the increase in the rate of *S.p* carriage after the introduction of PCV compared to the one found before its introduction in the Marrakech region.

Serotypes 6A and 15A / F represented a high rate (6.4% and 5.6% respectively) according to our findings. This is comparable with the results reported by a Kenyan study which showed a predominance of serotypes 6A and 15A during the post-PCV10 introduction era (67). The

prevalence of serotype 6A was also high in Brazil (56). The high prevalence of serotype 6A should be noted; actually, PCV10 immunogenicity data provided prior to its authorization suggested that it could provide cross-protection against serotypes related to vaccine serotypes, including serotypes 6A and 19A (76,77). A Finnish study had clearly elucidated this effect by highlighting a reduction in invasive pneumococcal infections caused by these two serotypes (78). This was not the case in our study. It should also be noted that the increase in the proportion of serotype 15A / F reported in this study is of significant value; in fact, according to data from literature reviewing, this serotype is known to be a frequent colonizer of vaccinated children and may be responsible for acute otitis media (79,80).

On the other hand, non-typable pneumococcal strains, which require PCR identification, also had a considerable proportion (22.13%). These data are consistent with those reported in The Gambia and South Korea (46,81). These non-typable strains are often non-encapsulated or have a disrupted and non-functional cpsA (capsular polysaccharide synthesis A) gene. Hence the need for molecular biology to identify these strains (82).

4. Effect of PCV10's introduction on the diversity of carried serotypes

By calculating Simpson's index, this study has shown the existence of high serotype diversity in carriage after the introduction of the PCV. In fact, about 20 non-vaccine serotypes were detected, with a DI = 0.92 during the post-vaccination period versus a DI = 0.83 during the pre-vaccination period ($p = 0.001$). A Swedish study had concluded to a similar result in 2016 (ID = 0.93 versus ID = 0.88 respectively ($P < 0.001$)) (74). In addition, the vaccine efficacy was very high against vaccine serotypes' carriage (VE = 88.23%), joining Brazil which had an almost similar efficacy rate (90%) (56).

5. Penicillin susceptibility of isolated *S. pneumoniae* serotypes

Concerning penicillin susceptibility, 33.6% of the strains were PRSP, and there was no significant difference between the pre-vaccine period (34.7%) (3) and the post-vaccination period. In fact, the vaccine had a reducing effect on the rate of penicillin-resistant vaccine serotypes; only two serotypes (14 and 23F) of the 8 isolated vaccine-serotypes were of diminished susceptibility to penicillin. While non-vaccine serotypes, as well as their resistance to antibiotics, emerged (82.6% of resistant serotypes), which represents a source of concern. The same effect was observed in Portugal following the introduction of the PCV7; a decline in penicillin-resistant vaccine serotypes at the expense of the emergence of resistant non-vaccine serotypes (83). The same result was found in South Korea with PCV13 (54), while in Turkey, there was a decrease in both nasopharyngeal pneumococcal carriage rate and PRSP strains' proportion after the introduction of PCV13 (49). This difference in results may be due to the difference in antibiotic consumption between countries. As for Morocco, unfortunately, no study has been published on the consumption of antibiotics.

CONCLUSION

Through this first national study on epidemiological monitoring of the pneumococcal nasopharyngeal carriage, conducted in febrile infants after the implementation of PCV10, we managed to describe the prevalence of the said carriage and its risk factors in the Marrakesh region, to serotype the isolated strains and to determine their antibiotic susceptibility profile. Moreover, we were able to elucidate the impact of the said vaccine on the *S.p* NP colonization.

In fact, the overall colonization prevalence was 68.3% and was significantly related to the immunization status. The rate of vaccine serotypes was reduced by 88.3% while non-vaccine serotypes increased. Thus, the effectiveness of PCV10 was well proven, simulating those of industrialized countries. The high rate of serotypes 15A / F and 6A should be emphasized. In addition, the prevalence of PRSP strains and non-vaccine serotypes is worrying and calls for cautious and more restrictive use of antibiotics in children.

Continuous epidemiological monitoring of pneumococci is essential for appropriate use of pneumococcal vaccines, and for evaluation of the impact of PCVs on pneumococcal infections and carriage. This assessment could also provide more data to encourage countries that have not yet introduced the PCV into their national immunization programs to do so.

ABSTRACTS

Résumé

Le portage rhinopharyngé du Streptococcus pneumoniae constitue l'étape clé précédant les infections pneumococciques qui sont une cause majeure de mortalité infantile. Le ministère de la santé marocain a introduit le vaccin antipneumococcique conjugué (PCV) dans son programme d'immunisation national depuis 2010.

L'objectif de cette étude est d'assurer un suivi épidémiologique après l'introduction du PCV10, et d'en évaluer l'impact sur le portage rhinopharyngé du pneumocoque, ainsi que la détermination de la prévalence de ce portage et ses facteurs favorisants, la distribution des sérotypes et le profil de sensibilité aux pénicillines chez les nourrissons fébriles au niveau de la région de Marrakech.

Un total de 183 prélèvements rhinopharyngés de nourrissons fébriles consultant aux urgences pédiatriques du CHU Mohamed VI, âgés de 2 à 18 mois, ont été recueillis, entre Février et Avril 2017. Leurs statuts vaccinaux et les facteurs de risques possibles ont été enregistrés. Les isolats ont été sérotypés et testés pour la sensibilité à la pénicilline.

Le taux du portage rhinopharyngé du pneumocoque était de 68.3%. 84% des nourrissons ont reçu au moins une dose du PCV10. Seul le statut vaccinal représentait un facteur favorisant cette colonisation après l'analyse univariée des facteurs de risque. Les sérotypes vaccinaux représentaient 6,6%, avec une prédominance des sérotype 19F et 1 (2 cas chacun). Le pourcentage des sérotypes non vaccinaux était 83,60%, les plus prédominant étaient les sérotypes 6A (6,4%) et 15A/F (5,6 %), avec une abondance des souches non typables (22,13%). Le taux des sérotypes du pneumocoque de sensibilité diminuée aux pénicillines était de 33,6% dont 82,6% étaient non vaccinaux. L'efficacité du vaccin a été estimée à 88,33%, et l'indice de Simpson de la diversité a augmenté durant la période post-vaccinale par rapport à la période pré-vaccinale (0,92 et 0,83 respectivement).

Le PCV10 a réussi à réduire le taux du portage des sérotype vaccinaux. Pourtant, l'augmentation des sérotypes non-vaccinaux ainsi que leur résistance aux pénicillines est inquiétante et impose une surveillance épidémiologique continue du portage et des infections pneumococciques.

Mots clés : Portage rhinopharyngé, pneumocoque, nourrissons fébriles, PCV10, sérotypes vaccinaux, sérotypes non vaccinaux.

Abstract

The nasopharyngeal (NP) carriage of Streptococcus pneumoniae is the key to the development of pneumococcal infections that are a major cause of pediatric mortality. The Moroccan Health Ministry included the pneumococcal conjugate vaccine (PCV) in its national program of immunization in 2010.

The aim of this study is to provide epidemiological monitoring after the implementation of PCV10 and to assess its impact on the pneumococcal nasopharyngeal carriage. Moreover, it aims to determine the prevalence of the said carriage, its risk factors, and the serotypes' prevalence and penicillin-susceptibility profile in feverish infants in the Marrakesh region.

183 nasopharyngeal swabs from feverish infants seen at the Pediatric Emergency Department of Mohamed VI teaching hospital of Marrakesh, aged between 2 and 18 months, were received from February to April 2017. The infants' vaccination status and the potential risk factors were registered. The isolated strains were serotyped and tested for penicillin-susceptibility.

The overall pneumococcal carriage rate was 68.3%. 84% of the infants had received at least one PCV10 dose. After the univariate analysis, vaccination status was the only risk factor associated with this carriage. The vaccine serotype carriage proportion was 6.6% and the most prevalent serotypes were 19F and 1 (2 cases each). The non-vaccine strains' colonization rate was 83.6%; serotypes 6A and 15A/F were the most predominant (6.4% and 5.6 % respectively). The non-typable strains were frequent (22.13%) in this survey. The rate of penicillin resistance among *S. pneumoniae* isolates was 33.6%, of which, 82.6% were non-vaccine serotypes. The vaccine effectiveness was estimated to be 88.33%. Simpson's index of diversity was significantly higher after than before vaccine introduction (0.92 and 0.83 respectively).

The PCV10 was successful at reducing the vaccine serotype carriage rate. However, the increase of non-vaccine strains' carriage, as well as their penicillin-resistance, is to worry about and imposes continuous epidemiological monitoring of pneumococcal carriage and infections.

Keywords: Nasopharyngeal carriage, pneumococcus, feverish infants, PCV10, vaccine serotypes, non-vaccine serotypes.

ملخص

يعتبر نقل بكتيريا المكورات الرئوية على مستوى البلعوم الأنفي مرحلة ضرورية لتكون التعفنات بسبب هذه البكتيريا . و تعد هذه التعفنات سببا رئيسيا لوفيات الأطفال . أدمجت وزارة الصحة المغربية اللقاح المقترن ضد المكورات الرئوية 10 (PCV10) ضمن برنامجها الوطني للتلقيح سنة 2012.

تهدف هذه الدراسة إلى تقديم متابعة إبيديميولوجية بعد تعميم هذا اللقاح وتقييم فعاليته تجاه النقل البلعوم-أنفي للمكورات الرئوية. كما تروم تحديد نسبة هذا النقل، والعوامل المؤثرة فيه، بالإضافة إلى دراسة توزيع الأنماط المصلية و حساسية المكورات الرئوية المعزولة عند الرضع المصابين بالحمى للبنسيلين في جهة مراكش.

تم جمع 183 عينة بلعوم-أنفية من رضع مصابين بالحمى، أثناء زيارتهم مستعجلات الأطفال بالمستشفى الجامعي محمد السادس بمراكش، والمتراوحة أعمارهم بين شهرين وثمانية عشر شهرا، في الفترة المتراوحة ما بين 2 فبرايرو 15 يونيو 2017 . كما تم تسجيل العوامل التي من الممكن أن تؤثر على هذا النقل وحالة التلقيح لكل رضيع. السلالات المعزولة تم تحديد نمطها المصلي واختبار حساسيتها للبنسلين.

قدرت نسبة النقل البلعوم-أنفي للمكورات الرئوية بهذه الدراسة ب 68.3% .حالة التلقيح كانت العامل المؤثر الوحيد في هذا النقل. الأنماط المصلية المضمنة في اللقاح التي تم رصدها بلغت نسبتها 6.6%، و قد كان النمطان 19F و1 الأكثر سيادة (حالتان لكل نمط). في حين أن نسبة الأنماط المصلية غير المضمن ة في اللقاح بلغت 83.60%، مع هيمنة النمطين 6A (6.4 بالمائة) و 15A/F (5.6 بالمائة) . نسبة السلالات غير القابل ة للتنميط كانت عالية (22.13%). معدل سلالات المكورات الرئوية ذات حساسية منخفضة للبنسلين هو 33.6%، من بين هذه السلالات 82.3 % كانت ذات أنماط مصلية غير مضم ة في اللقاح. قدرت نجاعة اللقاح ب 88.3% مع ارتفاع مؤشر سيمبسون للتنوع بعد فترة التلقيح مقارنة مع قبله (0.92 و 0.83).

لقد تمكن اللقاح المقترن ضد المكورات الرئوية (PCV10) 10 من خفض معدل النقل البلعوم-أنفي للأنماط المصلية المضمنة في اللقاح، إلا أن ارتفاع نسبة الأنماط المصلية غير المضمن ة ومقاومتها للبنسلين يدعو للقلق، ويجعل من التتبع الإبيديميولوجي لنقل المكورات الرئوية والتعفنات الناتجة عنها ضروريا.

كلمات المفتاح : النقل البلعوم-أنفي، المكورات الرئوية, PCV10, الرضع المصابون بالحمى، الأنماط المصلية اللقاحية، الأنماط المصلية غير اللقاحية.

ANNEX

Questionnaire

Order number:…

I. Socio-demographic data:

1) Patient Identity :

Patient Identifier (PI) :

……

Name :

……

……

Gender:

☐ F
☐ M

Age: ……

Birth Date: ……../……/…….

2) Siblings :

☐ None
☐ Siblings ≥ 1

3) Daycare Mode:

☐ Home
☐ Daycare center

II. Antecedents:

1) PCV doses :

☐ 0 dose
☐ 1 dose
☐ 2 doses
☐ 3 doses
☐ No information

2) Antibiotic treatment:

- ☐ Taken within the past 7 days
- ☐ Taken over a week ago but within the past 3 months
- ☐ Taken over 3 months ago
- ☐ No information

III. Clinical features:

Temperature: ……...

IV. Microbiological data:

1) Carriage:
   - ☐ Positive
   - ☐ Negative
2) Serotype:
   - ☐ PCV 10 vaccine serotype
     - ☐ 4
     - ☐ 6B
     - ☐ 9V
     - ☐ 14
     - ☐ 18C
     - ☐ 19F
     - ☐ 23F
     - ☐ 1
     - ☐ 5
     - ☐ 7F
   - ☐ Non-vaccine serotype:
     - ☐ Typable
       - Serotype: …….
     - ☐ Non-typable

3) Penicillin susceptibility:

- ☐ Penicillin susceptible pneumococcus
- ☐ Pneumococcus with reduced susceptibility to penicillin

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قسم الطبيب

أقْسِم بِالله العَظِيم

أن أراقبَ الله في مِهْنَتِي.

وأن أصُونَ حياة الإنسان في كآفّةِ أطوَارهَا في كل الظروف والأحَوال باذلة وسْعِي في انقاذها مِن الهَلاكِ والمرَضِ والألَم والقَلَق.

وأن أحفَظَ لِلنَاسِ كرَامَتهُم، وأسْتر عَوْرَتهُم، وأكتمَ سِرَّهُمْ.

وأن أكونَ عَلى الدوَام من وسائِل رحمة الله، باذلة رِعَايَتي الطبية للقريب والبعيد، للصالح والطالح، والصديق والعدو.

وأن أثابر على طلب العلم، وأسَخِّرَه لِنَفْعِ الإنْسَان لا لأذَاه.

وأن أُوَقّرَ مَن عَلَّمَني، وأُعَلّمَ مَن يَصْغرَني، وأكون أخـًا لِكُلِّ زَميلٍ في المِهنَةِ الطِّبِّيَة مُتعَاونِينَ عَلى البرِّ والتقوى.

وأن تكون حياتي مِصْدَاق إيمَاني في سِرّي وَعَلانيَتي، نَقِيَّة مِمّا يُشينهَا تجَاهَ الله وَرَسُولِهِ وَالمؤمِنين.

والله على ما أقول شهيد

كليـــــــة الـطـب
و الصيدلة - مراكش
FACULTÉ DE MÉDECINE
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جامعة القاضي عياض
UNIVERSITÉ CADI AYYAD

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تأثير اللقاح المقترن ضد بكتيريا المكورات الرئوية على الحمل البلعوم-أنفي لهذه البكتيريا عند الرضع المصابين بالحمى على مستوى المركز الاستشفائي الجامعي محمد السادس بمراكش

الأطروحة

قدمت ونوقشت علانية يوم **24 / 12 /2019**

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27 أكتوبر1991 بتحناوت

لنيل شهادة الدكتوراه في الطب

الكلمات المفتاح:

النقل البلعوم-أنفي - المكورات الرئوية - **PCV10** - الرضع المصابون بالحمى - الأنماط المصلية اللقاحية - الأنماط المصلية غير اللقاحية.

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