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Comparison of early effects of *S. pneumoniae* vaccination policies on nasopharyngeal carriage in a Palestinian population

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Dedication

To my almighty God, who gave me strength and knowledge through my life.

To Dr. Kifaya the great teacher and the exceptional human.

To my father who inspired me to be strong despite all the obstacles.

To my mother for her understanding and overwhelming support.

To my Wife and Son for their eternal love.

I dedicate this work…

Mahmoud Ahmad Mohammad Ramlawi

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed ……..

Mahmoud Ahmad Mohammad Ramlawi

Date: 21.04.2019

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Abstract

Background:

Streptococcus pneumoniae can asymptomatically colonize the nasopharynx and cause a various range of illnesses. Pneumococcal conjugate vaccines (PCVs) are at present used in different countries. The aim of the study is to determine the effect of different vaccination policies PCV7/13 to that PCV10 on the carriage rates and comparing the impact of different vaccination policies in East Jerusalem and West Bank region.

Methods:

Five cross-sectional surveillances *of S. pneumoniae* were carried out in East Jerusalem and Palestinian authority (PA), where two Palestinian populations with different vaccination policies were screened, with an annual average of 348 and 616 children., respectively, were performed during 2009-2016. Nasopharyngeal swabs and data were collected from children less than 5 years old visiting primary care physicians who visited any of three private pediatric clinics in Bethlehem, Nablus, and Ramallah in PA. In East-Jerusalem (EJ), PCV7 was implemented in 2009 and replaced by PCV13 in late 2010, while in Palestine (PA), PCV10 was implemented in 2011.

Swabs were streaked and incubated overnight at 35°C in 5% CO_2 enriched air to detect the presence of *S. pneumoniae*. Presumed colonies of *S. pneumoniae* were identified by morphological characteristics, such as α hemolysis and optochin susceptibility. *S. pneumoniae* serogroup was determined by the latex agglutination test and *S. pneumoniae* serotype was determined and confirmed using PCR and gel-electrophoresis.

Results:

A total of 4686 children were screened in EJ (n=1615) and PA (n=3070), the overall rate of *S. pneumoniae* carriage did not change significantly during the 5 first years of the study, in either population. The pediatric subjects from EJ were determined to carry *S. pneumoniae* during the 5 years study, 2009, 2010, 2011, 2014, and 2016 as 28.9%, 29.3%, 26.9%, 30.7% and 16.9%, respectively. In addition 35.9%, 33.6%, 28.8%, 28.6% and 32.9% of the pediatric subjects from PA were shown to carry *S. pneumoniae in 2009-2016*, respectively. By year 2016, *S. pneumoni-*

ae carriage was reduced significantly in EJ from ~29% on average to ~17%, following seven years application of PCV7/13. In PA, where follow-up included only 5 years after PCV10 application, *S. pneumoniae* carriage remained ~30%. Interestingly, VT7 strains gradually decreased following PCV implementation. Following vaccine implementation, during the study period, there was a significant decrease in carriage of *S .pneumoniae* in the EJ between 2009 and 2016 (*P*=0.001). No significant variation was seen in the overall carriage of *S. pneumoniae* between 2009 to 2016 in PA (*P*=0.065). PCV10 was introduced to PA late in 2011, but *S. pneumoniae* carriage was approximately (160/566) 28% in2011, prior to vaccine introduction, and (216/656), 32.9% in 2016, five years following vaccine implementation.

In PA region, PA VT13-10 strains declined from 18.87% in 2011 to 9.78% in 2014, but re-emerged to 18.06% in 2016.

In both EJ and PA, a significant increase of non-VT isolates was observed between 2009 and 2016 following vaccine implementation (*P*<0.0001 in both regions). Between 2009 and 2016, in EJ and PA, there was a decrease in PNSSP prevalence following vaccine implementation (p=0.2251 and p=0.1864, respectively).

S. pneumoniae carriage among the parents was relatively rare, with 3.3% of parents detected as nasopharyngeal carriers in both regions throughout the five study years.

Conclusions: Following PCV implementation, a decrease in the prevalence of VT strains was observed in EJ, and PA.

Keywords: Streptococcus pneumoniae, pneumococcal conjugate vaccine, vaccine-type strain, non-vaccine type strains, 7-valent PCV (PCV7),

مقارنة التأثيرات المبكرة لسياسات التطعيم ضد الالتهاب الرئوي على النقل البلعومي لدى السكان الفلسطينيين.

اعداد:محمود احمد محمد رملاوي

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الملخص:

خلفية الدراسة: بامكان المكورات العقدية الرئوية السيطرة على البلعوم الأنفي بدون أعراض وتسبب مجموعة متنوعة من الأمراض حيث حاليا تستخدم لقاحات المكورات الرئوية (PCVs) في بلدان مختلفة. تهدف الدراسة إلى تحديد تأثير سياسات التطعيم المختلفة PCV7/13 وكذلك PCV10 على معدلات الاصابة ومقارنة تأثير سياسات التطعيم المختلفة في القدس الشرقية والضفة الغربية.

منهجية البحث:تم إجراء خمسة دراسات مسحيه للالتهاب الرئوي في القدس الشرقية والضفة الغربية خلال 2009–2016، حيث تم فحص قسمين من السكان الفلسطينيين مع سياسات التطعيم المختلفة ، بمتوسط سنوي348 و 616 طفلا، على التوالي. حيث تم جمع مسحات البلعوم الأنفي وبيانات من أطفال تقل أعمارهم عن 5.5 سنة يزورون أطباء الرعاية الأولية من عيادات طب الأطفال الموجوده بالمناطق التالية بيت لحم ونابلس ورام الله في الضفة الغربية. تم الاقرار بتنفيذ PCV7 في القدس الشرقية في عام 2009، وحل محله PCV13 اواخر عام 2010 ، بينما في الضفة الغربية تم تنفيذ PCV10 في عام 2011.

تم زراعة المسحات ووضعها في حاضنه تحمل عند 35 درجة مئوية من الهواء المخصب من ثاني أكسيد الكربون طوال الليل للكشف عن وجود المكورات الرئوية. تم التعرف على المستعمرات المفترضة للالتهاب الرئوي بالخصائص المورفولوجية ، مثل انحلال الدم ألفا وحساسية الاوبتشين(optochin susceptibility).

تم تحديد المكورات الرئوية عن طريق اختبار latex agglutination وتم تحديد المصل المكورات الرئوية وتأكيدها باستخدام PCR والهلام الكهربائي(gel-electrophoresis).

النتائج:تم فحص مجموعه 4686 طفلاً في القدس الشرقية (عدد= 1615) والضفة الغربية (عدد = 3070) ولم يتغير المعدل الإجمالي للنقل العقدية الرئوية بشكل ملحوظ خلال السنوات الخمس الأولى من الدراسة ، في أي من السكان. تم تحديد اصابات الأطفال في القدس الشرقية الذين يحملون المكورات الرئوية خلال دراسة السنوات الخمس (2009 ، 2010 ، 2011 ، 2014 ، و 2016) بنسبة 28.9 ٪ ، 29.3 ٪ ، 26.9 ٪ ، 30.7 ٪ و 16.9 ٪ ، على التوالي. بالإضافة إلى ذلك تبين أن 35.9٪ ، 33.6٪ ، 28.8٪ ، 28.6٪ و 32.9٪ من الأطفال المصابين بالضفة الغربية يحملون التهاب رئوي على التوالي. بحلول عام 2016 ، انخفض معدل نقل الرئوية بشكل كبير في القدس الشرقية من 29٪ في المتوسط إلى 17٪ تقريبًا، بعد تطبيق PCV7/13 لمدة سبع سنوات. اما في الضفة الغربية تشمل المتابعة بعد 5 سنوات فقط من تطبيقPCV10 ، بقيت المكورات الرئوية ما يقارب 30 ٪. ومن المثير للاهتمام ،ان سلالات نوع اللقاح(VT7) انخفضت تدريجيا بعد تنفيذ اللقاح(PCV).

خلال فترة الدراسة ، كان هناك انخفاض ملحوظ في نقل البكتيريا الرئوية في القدس الشرقية بين عامي 2009 و 2016. لم يلاحظ أي اختلاف كبير في النقل الكلي للالتهاب الرئوي بين عامي 2009 و 2016 في الضفة الغربية. تم إدخال PCV10 إلى الضفة الغربية في أواخر عام 2011 ، ولكن نقل الرئوية كان حوالي (160/566) 28 ٪ في عام 2011 قبل إدخال اللقاح ، و (216/656) 32.9 ٪ في عام 2016 ، بعد خمس سنوات من تنفيذ اللقاح في منطقة الضفة الغربية ، انخفضت سلالات VT13-10 أيضًا من 18.87٪ في عام 2011 إلى 9.78٪ في عام 2014 ، ولكن عادت إلى 18.06٪ في عام 2016. في

كل من القدس الشرقية والضفة الغربية، لوحظت زيادة كبيرة في سلالات غير نوع اللقاح NVT بين عامي 2009 و2016 بعد تنفيذ اللقاح في كلتا المنطقتين. بين عامي 2009 و2016، في القدس الشرقية والضفة الغربية.كان هناك انخفاض غير مهم من الناحية الإحصائية في انتشار المكورات الرئوية المقاومة للبنسلين بعد تنفيذ اللقاح. كان نقل الالتهاب الرئوي بين الوالدين نادرًا نسبيًا، حيث تم اكتشاف 3.3٪ من الآباء على أنهم حاملات البلعوم الأنفي في كلتا المنطقتين خلال سنوات الدراسة الخمس.

الاستنتاجات: بعد تنفيذ لقاح المكورات الرئوية، لوحظ انخفاض في انتشار سلالات من نوع اللقاح في القدس الشرقية، الضفة الغربية.

الكلمات المفتاحية: المكورات العقدية الرئوية (*Streptococcus pneumoniae*)، لقاح المكورات الرئوية(pneumococcal conjugate vaccine PCV)، سلالة من نوع اللقاح (VT) ، سلالات من النوع غير اللقاح (NVT).

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List of abbreviations

Abbreviation	Term
*S.*pneumoniae	Streptococcus *p*neumoniae
IPD	Invasive Pneumococcal Disease
PspA	pneumococcal surface protein A
PspC	pneumococcal surface protein C
PsaA	pneumococcal surface adhesion A
PCV	pneumococcal conjugate vaccine
PPSV23	Pneumococcal Polysaccharide Vaccine23
NIP	National Immunization Plan
pIPD	PediatricInvasive Pneumococcal Disease
PBP	penicillin-binding proteins
HIV	Human immunodeficiency virus
PCR	Polymerase chain reaction
AR	Antibiotic Resistance
NT	Non-Typeable
PA	West Bank
MOH	Ministry of Health
EJ	East Jerusalem
CLSI	Clinical and Laboratory Standards Institute
OR	Odds ratio
IRB	institutional review board
PSSP	penicillin-susceptible S. pneumoniae
PRSP	penicillin- resistantS. pneumoniae
PNSSP	Penicillin Non-Susceptible Streptococcus Pneumoniae
WHO	World Health Organization
AOM	acute otitis media
BHI	brain heart infusion

Chapter One

Background

Pneumococcal infection caused by *Streptococcus pneumoniae*, cause high rates of morbidity and mortality. The main cause of deaths preventable by vaccination in children under age five worldwide (Brugger, Hathaway, and Mühlemann 2009). The World Health Organization (WHO) estimates that more than one million children die of pneumococcal disease in developing countries every year (WHO 2007). Community acquired pneumonia accounts for many of the severe diplococcus disease burden worldwide, although the danger of severe morbidity and death is highest for invasive pneumococcal disease (IPD) like bacteremia pneumonia, meningitis and sepsis(O'Brien et al. 2009). The presence of *S .pneumoniae* in the nasopharynx of healthy children is indicative of the strains circulating and causing infections in the community, and has often been reported as being a precursor to waves of invasive disease and a major factor in the spread of infection (Cardozo et al. 2007; Van Kerschaver et al. 2004)

Based on the structure of the capsular polysaccharides was split *S. pneumoniae* to more than 90 distinct serotypes, which are considered to be the major virulence factors (Sjostrom et al. 2007). Only some of the serotypes cause disease and, of those that do, some have a greater capacity to invade and cause bacteremia. Others are more frequently associated with respiratory tract disease without a bacteremia, while still others remain limited to the nasopharynx, bacteria lacking a polysaccharide capsule rarely cause invasive disease (Melin et al. 2010).

Prevention of infections caused by *S. pneumoniae* and their spread in young children is such an important goal of effective vaccination that new vaccines have been developed to achieve this. In 2007, the WHO recommended that pneumococcal conjugate vaccine 7 (PCV7), which contains the polysaccharides of the serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, be used in national immunization programs (NIP) (WHO 2007). Two more PCVs were recently introduced: PCV10, which contains the polysaccharides of the serotypes 1, 5, and 7F in addition to the serotypes of PCV7, and PCV13, which contains the polysaccharides of the serotypes 3, 6A, and 19A in addition to the serotypes of PCV10 (Thomas et al. 2007). PCVs of higher valence provide broader serotype coverage (Harboe et al. 2008; Kaltoft et al. 2008). The use of PCVs in young children

creates herd immunity by reducing transmission and, thus, circulation of the bacterial serotypes opposed by the vaccine, leading to a decrease in invasive pneumococcal disease (IPD) in the population (Aristegui et al. 2007).

Treatment of pneumococcal infections is becoming more difficult owing to the emergence of antibiotic resistant pneumococci. Studies on serotype distribution and their antibiotic sensitivities are necessary for planning rational national strategies for preventing and treating IPD (Tsai et al. 2006). Surveillance of pneumococcal diseases according to bacterial serotypes is essential, to learn about the current serotype distribution and to observe the efficacy of PCVs by following the dynamics of bacterial serotypes in the population, following the introduction of vaccination. Several studies have reported regional and temporal changes in the distribution of bacterial serotypes after the application of PCVs (Feikin and Klugman 2002).

Kattan and colleagues (Kattan et al. 2011) described the distribution of invasive *S. pneumoniae* from two Palestinian hospitals where bacteria of serotypes 6, 14, 1, and 9V predominated. The serotypes within serogroup 6 were not determined, however, it is important to identify the serotypes within serogroups such as serogroup 6 as serotype 6B polysaccharide is in PCV7 while serotypes 6A and 6C are not.

Following an introductory trial of the PCV7 vaccine in a pilot study in the Hebron, Bethlehem, Jericho and Tubas districts in September 2010, this study was done to determine the rate of nasopharyngeal pneumococcal infection in healthy children, identify the serotypes of the circulating strains of *S. pneumoniae* and determine their antibiotic susceptibilities.

1.1Problem statement

S. pneumoniae is an important cause of primary septicemia in children in the developing world. All disease states can lead to migration of S. *pneumoniae* from the site of infection into the bloodstream, resulting in pneumococcal sepsis.

1.2 Study justification

There are at least 40 serogroups of S. *pneumoniae* which are potentially pathogenic and possibly all are, but only a relatively small number of these groups accounts for the majority of IPD in children. There are geographic and age-related differences in the incidence of S. *pneumoniae* serotypes, and certain S. *pneumoniae* serotypes are recovered from IPD more frequently than others and have higher disease prevalence.

1.3 Study hypotheses

1) There are specific definable pathogen, host and population dependent factors that induce the evolution and spread of AR pathogens, and can explain the different Antibiotic Resistance (AR) patterns observed in our region.

2) Advocating judicious antibiotic practices result in reduction of antibiotic use by the population.

1.4 Study Goal

To show the effects of the pneumococcal conjugate vaccine on pneumococcal carriage among children and to compare the effects of the different PCV vaccination policies among Palestinians living in Jerusalem and the West Bank.

1.5 Study objectives

1. To report the effect of PCV13 and PCV10 which has been introduced gradually in Palestine by identifying the serotypes.

2. To determine the role of vaccination policies in effecting AR pattern

3. To determine the role of antibiotic use patterns in inducing AR and its spread in the community.

4. To identify the non vaccine type (nVT) strains

Chapter two:
Literature review

2.1 *S. pneumoniae* carriage and burden of disease

Pneumonia is a common disease affecting children, especially in developing countries (Izadnegahdar et al. 2013). *Streptococcus pneumoniae (S. pneumoniae)* is the predominant bacteria responsible for community acquired pneumonia (T.J. and M.B. 2005).

S. pneumonia was known in 1881 by Sternberg(Sternberg and Flaumenhajtt 1993). The pneumococcus could be a lancet formed gram positive diplococcus characterized by a surrounding polysaccharide capsule. Since it's alpha hemolytic, bile soluble and optochin sensitive, it can be known by standard microbiological tests, using morphological characteristics and biochemical tests (Desai et al. 2015). Based on the structure of the capsule, the pneumococci will be subclassified and more than 90 antigenically totally different serotypes (subtypes) have been recorded. Some non encapsulated variants of pneumococci exist, additionally called non typeable pneumococci (van der Poll and Opal 2009).

Streptococcus pneumoniae is a common human pathogen that causes high morbidity and mortality rates(Nasereddin et al. 2013). Epidemiological studies show that colonization of the neonatal respiratory tract with *S. pneumoniae*, *Haemophilus influenzae*, or *Moraxella catarrhalis* significantly increases the risk of asthma in the first 5 years of life as well as *Haemophilus influenzae*, or *Moraxella catarrhalis* (Rhedin 2017). Yearly, pneumococcal disease results in ≈ 600,000 deaths among children <5 years of age globally, with the majority occurring in Africa(Kassebaum et al. 2014).

In 2000, out of a total 10,600,000 deaths worldwidly, pneumococcal disease caused 826,000 deaths of children under the age of 5 years(O'Brien et al. 2009). Out of 90 *Streptococcus pneumoniae* serotypes that have been identified(Calix et al. 2012), approximately 20 are responsible for the majority of invasive pneumococcal disease (IPD) (Rudan et al. 2013).

S. pneumoniae infection is a common cause of acute otitis media, as well as more life threatening illnesses such as pneumonia, meningitis, and septicemia (O'Brien et al. 2009). Individuals who is Immunodeficient or those with anatomical asplenia, particularly sickle cell disease, are more susceptible to developing invasive pneumococcal disease (IPD) than the general population. Other risk factors include low socioeconomic status, upper respiratory and influenza infections, smoking, old age, and heart, liver and lung comorbidities (Butler and Schuchat 1999).

Nasopharyngeal *S. pneumoniae* carriage is the source for horizontal transmission of the pathogen between individuals and is the endogenous source for infection. Colonization of *S. pneumoniae* is generally asymptomatic and is a part of the nasopharyngeal flora established in individuals, but may sometimes develop into disease (Brugger, Hathaway, and Mühlemann 2009). *S. pneumoniae* is commonly carried by children under 5 years of age (Brugger, Hathaway, and Mühlemann 2009).

Risk factors correlated with previous antibiotic use, presence of young siblings high carriage rates include, age, and day care center attendance (Regev- Yochay, Keller, et al. 2004). In this study, *S. pneumoniae* was carried by only 4% of the adults, compared with 53% of children in the same community. Young age, day care center attendance, having young siblings, and no antibiotic use during the month before screening were associated with the high carriage rate among children, whereas the only risk factor associated with carriage among adults was the presence of a respiratory infection on the screening day. The trans oral nasopharyngeal and oropharyngeal swabs increase carriage detection in the adult population. The Frequency of carriage in children can be under the influence of environmental factors such as seasonal factors and crowded or closed settings (Rioux et al. 2011). Such as in the winter season, pneumococcal carriage and disease trends be likely to be higher and is in part due to the winter viral respiratory infection (Zhou et al. 2012). Nasopharyngeal colonization by *S. pneumoniae* typically persists for several months after the first exposure, but with age, acquisition of new serotypes results in shorter durations of carriage. Rarely, a colonization event results in invasion to other sites, such as the ears, sinuses, lungs or blood. Children under two years of age and adults older than 65 years of age are the most susceptible populations for pneumococcal disease. Because of that, the risk to human health posed by *S. p*neumoniae underscores the necessity and urgency of studying the bacteria.

2.2 The Pneumococcal Capsule

Pneumococci have many virulence factors, including capsular polysaccharides, pneumolysin, pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), and pneumococcal surface adhesin A (PsaA). Capsular polysaccharides are considered to be the most important virulence factor as they surround pneumococci and protected them from phagocytes. Currently, 94 capsular serotypes, including the recently reported serotypes 6C, 6D, 11E, and 20A/20B, have been reported (J. Y. Song, Nahm, and Moseley 2013) and at least 23 serotypes commonly cause invasive pneumococcal disease (IPD)(J. Y. Song, Nahm, and Moseley 2013). *Streptococcus pneumoniae* can asymptomatically colonize the nasopharynx and cause a various rang of illnesses.

This clinical spectrum from Colonization to invasive pneumococcal disease (IPD) appears to depend on the pneumococcal capsular serotype rather than the genetic background. Serotypes are serologically distinguishable strains belonging to the pneumococcal bacteria based on the structure of the capsular polysaccharides (J. Y. Song, Nahm, and Moseley 2013).

Types of *S. p*neumoniae that are antigenically associated are labeled with the same numerical value and assigned different letters, i.e. same serogroup, but different serotype (van Dam, Fleer, and Snippe 1990). These specific serotypes have been targeted for vaccine coverage(van Dam, Fleer, and Snippe 1990).

Pneumococcal capsules play the most important role in pathogenesis, and they have a great implication in vaccine development. Of note, serotype distributions of pneumococcal capsules vary geographically and temporally in terms of nasopharyngeal carriage, pathogenicity and clinical presentation (Hausdorff, Siber, and Paradiso 2001). The capsular polysaccharide structure plays a role in evading the host's immune system, including defense mechanisms such as phagocytosis, and persisting in carriage Serotype that produce heavily encapsulated polysaccharides avoid being phagocytosed, continue colonization within the host and persist in prevalence (Brandes et al. 2009).

The determination of pneumococcal serotypes is key to assess the effects of PCVs, including decreases in PCV serotypes and potential non PCV serotype replacement following the use of the vaccine over time. With PCVs being increasingly introduced into the routine infant immunization programs of several low and middle income countries serotype specific pneumococcal surveillance is key to assess the impact of the vaccine in diverse settings.

2.3 Pneumococcal Vaccines

Pneumococcal Polysaccharide Vaccine 23 (PPSV23)

In 1983, a 23-valent PPV (PPV23) was approved and developed a capsular polysaccharide vaccine targeting the 23 common serotypes (PPSV23) including serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 20, 22F, 23F, and 33F (Örtqvist, Hedlund, and Kalin 2005), which intensifying serotype coverage to more than 85% of the organisms causing IPD at the time (Rastogi et al. 2011). Covalent coupling of the polysaccharide to a protein carrier effectively converts the T-cell-independent Type 2 polysaccharide into a T-cell-dependent antigen; carrier proteins include a non toxic mutant of diphtheria toxin (CRM197), as well as other proteins such as protein D of non typeable *Haemophilus influenza* (Black et al. 2000). PPSV23 elicits a T cell independent immune response which makes it ineffective in children younger than 2 years of age (Bridy-Pappas et al. 2005),which make it not licensed for the them, while it was good for immune competent adult patients in the United States.

2.4 Pneumococcal Conjugate Vaccine (PCV)

The poor immunogenicity of T-cell-independent PPSV23 in infants led to development of the pneumococcal conjugate vaccine (PCV).

The introduction of pneumococcal conjugate vaccines (PCVs) into routine infant immunization programs worldwide has led to significant decreases in overall incidence of invasive pneumococcal disease (IPD), both in children and in adults (Esposito and Principi 2015).Unlikely, PPSV23, pneumococcal conjugate vaccines offer mucosal immunity, additionally acting upon cavum carriage acquisition, and thereby providing an indirect impact additionally to an on the spot community impact.

In 2000, the first 7-valent PCV (PCV7) was licensed in the United States (US) in and introduced to Korea in 2003. This PCV included capsular polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, representing approximately 80%-90% of IPD in children (Abu Seir et al. 2018).

While PCV7 was introduced to the United States in 2000, the vaccine was available in Israel for individual purchase since 2007 and implemented through the National Immunization Plan (NIP) in 2009. However, the protective coverage of PCV7 was reduced, due to the serotype shifts resulting from vaccine pressure, and PCV7 did not include serotypes 1, 3, and 5, which are common in Europe, Asia, and Africa (Hausdorff, Siber, and Paradiso 2001).Therefore, a 13-valent PCV (PCV13), which included serotypes 1, 3, 5, 6A, 7F, and 19A, was introduced for children in 2010 and later licensed for adults in 2012.

Since 2010, two improved pneumococcal conjugate vaccines, PCV13 and PCV10 became available to many countries. In Portugal, although available only in the private market, the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in 2001 led to significant changes in the serotypes responsible for pediatric IPD (pIPD) (Aguiar et al. 2008). Among the most relevant changes was the rise of non PCV7 serotypes 1, 7F and 19A as major causes of pIPD. During this period uptake peaked at 75% around 2008. In mid 2009, PCV10 became available, including PCV7 serotypes and additionally serotypes 1, 5 and 7F. Soon after this, in early 2010, PCV13 was introduced in Portugal, further including serotypes 3, 6A and 19A, and became the leading vaccine used in Portugal.

PCV13 was licensed and introduced in 2010 in the United States and in Israel a few months later (October 2010). In addition to the seven *S. pneumoniae* serotypes already found in PCV7, PCV13 includes six additional highly invasive serotypes: 1, 3, 5, 6A, 7F, and 19A. The serotypes found in PCV13 target 80%-90% of the pneumococcal capsular serotypes that were responsible for pneumococcal disease in the USA in the pre PCV era (Daniels, Rogers, and Shelton 2016). PCV10 contains serotypes found in PCV7 with three additional serotypes: 1, 5, and 7F. In a custom randomized trial conducted in Brazil, the effectiveness of PCV10 was found to be 83.8% against vaccinated serotypes and 77.9% against vaccine related serotypes(de Moraes et al. 2014). While they suggested that cross-reactivity for 19A high (82%) and low to 6A (15%), most studies, as well as a meta-analysis, report the opposite (Isturiz et al. 2017).

PCV 10 were used in Bangladesh, Belgium, Brazil, Chile, Colombia, Fiji, Finland, Georgia, Iceland, Nepal, Netherlands, Paraguay, and Peru. And PCV13 was used in Argentina, Australia, Canada, Costa Rica, Czech Republic, Denmark, France, Greece, Guatemala, Hungary, Ireland, Israel, Italy, Japan, Mexico, New Zealand, Nicaragua, Norway, Poland, Portugal, Russian Federation, Switzerland, Turkey, United Kingdom, United States, and Uruguay. Both PCV10 and PCV 13 were used in Austria, Estonia, Germany, Republic of Korea, Philippines, Slovakia, Slovenia, Sweden, and the Palestinian Authority.

PCV10 and PCV13 are the vaccines currently used in countries employing a pneumococcal conjugate vaccine. PCV10 consists of carrier protein from conserved lipoproteins of non type-able *Haemophilus influenzae*, while in PCV7 and PCV13 the carrier protein of a non-toxic mutant is the diphtheria toxin (Earnshaw et al. 2012). In an 8 years case control study conducted in Canada, protection against any IPD was 72% for PCV10 and 66% for PCV13 (where PCV effectiveness is calculated as one minus the odds ratio). Both vaccines were highly effective in conferring protection against serotypes targeted by each respective vaccine. Moreover, both vaccines displayed high levels of cross protection against IPD caused by serotype 19A, 71% by PCV10 and 74% by PCV13 (Cané, Hamelin, and Isturiz 2015). A study conducted in Finland in 2009-2010 showed that PCV10 confers herd immunity and displays cross protection against serotypes 19A and 6A in children less than 1.5 years of age (Jokinen et al. 2015).

In vaccine eligible children, significant reduction in PCV10 related IPD including 6A and 19A,suggesting that PCV10 may provide cross protection against vaccine related serotypes and consist with the study that done in Brazil that reported the significant effectiveness of PCV10 against serotype 19A (de Moraes et al. 2014).

PCV10 is cheaper; compared to PCV13, PCV13 covers 3 additional serotypes (3, 19A and 6A), which are a significant cause of IPD and has been suggested to provide higher protection against acute otitis media and IPD that may be caused by *Haemophilus influenzae* (Daana et al. 2015; Wu et al. 2015). In contrast, serotype 19A IPD prevalence was found to increase following PCV10 implementation in Columbia (Isturiz et al. 2017). Additionally, PCV13 was found to be more cost effective when compared to PCV10 by protecting against more IPD cases and deaths, even though PCV10 is cheaper (Ordóñez and Orozco 2015). A meta-analysis using published data from countries were PCV7, PCV10, and PCV13 were introduced, suggested that PCV7 and PCV10 do not appear to display herd protection against serotype 19A, while PCV13 was shown

to reduce IPD caused by serotype 19A and displayed herd protection against serotype 19A (Isturiz et al. 2017).A meta-analysis of 63 studies conducted between January 2006 through January 2014 concluded that both PCV10 and PCV13 are beneficial alternatives to PCV7, and the most optimal choice of vaccine for any country is dependent on several factors that vary by location, such as serotype replacement, serotype cross-protection, herd effects, cost-effectiveness, and the epidemiological distribution of serotypes present in the population (Wu et al. 2015).In 2017, 141 countries are known to have introduced or planned to introduce the pneumococcal conjugate vaccine. PCV13 is in use in 101 countries, PCV10 is in use in 33 countries, and 8 countries are using both PCV10 and PCV13 (Satzke et al. 2019).

2.5Cinical Presentations

Different clinical presentations of pneumococcal diseases are known due to different pneumococcal serotypes. Globally, serotypes 3, 6A, 6B, 9V, 14, 19A, 19F, and 23F were reported to cause acute otitis media (AOM) in young children (J. Y. Song, Nahm, and Moseley 2013). Serotype 19A pneumococcal acute otitis media increased predominantly after the introduction of PCV7 (Mahjoub-messai et al. 2009). Particularly, serotypes 3, 11A, and non typeable (NT) pneumococci are associated with acute conjunctivitis (Shouval et al. 2006).Concerning IPD, serotypes 1, 5, and 7F usually affect healthy young adults with a low mortality rate, while serotypes with low or intermediate invasive potential are more possible to affect the elderly with underlying comorbidities (Shouval et al. 2006)compared the incidence and serotype distribution of IPD among children less than 18 yr of age between the early (2001-2006) and late (2007- 2010) periods of the PCV7 era in the US. Overall IPD incidence rate was 7.5 cases per 100,000 population and was not statistically different from the observed incidence in 2001 to 2006 ($P > 0.05$). The proportion of bacteremia pneumonia among all IPD cases was almost 3-fold greater in 2009 to 2010 ($P < 0.01$). PCV7 serotypes represented for 7%, whereas the 13-valent pneumococcal conjugate vaccine serotypes accounted for 77% of all cases between 2007 and 2009. IPD due to serotypes 19A and 7F increased, and 19A and 7F were isolated in 41% and 20% of all IPD cases in the same period, respectively. However, serotypes causing IPD have changed pointedly in the last decade.

According to a meta-analysis (J. Y. Song, Nahm, and Moseley 2013), pneumococcal serotype 14 was the most prevalent etiologic agent of pneumococcal CAP, followed by serotypes 1 and 5, in Latin America and the Caribbean, which included the post-PCV7 periods, serotype 1 was predominant, followed by serotypes 19A and 3. The prevalence of serotype 14 was decreased to around 5%.

Serotypes 1, 3, 7F, 14, and 19A are known to be associated with empyema complications after pneumococcal infection (Krallis 2006). Also, According to surveillance in Ugandan children aged less than five years, the most common serotype was 6A/6B (40%), followed by 22A, 23F, 14, and 19A (Kisakye et al. 2009). Previously, serotypes 1, 3, and 5 were rarely reported in cases of meningitis (Kisakye et al. 2009).

Continual surveillance is important to determine the impact of 13 valent pneumococcal conjugate vaccine, as well as track potential changes in disease incidence and character due to non 13 valent pneumococcal conjugate vaccine serotype.

2.6 Serotype Replacement

Following widespread introduction of PCV7, the pneumococcal population has changed. Non vaccine serotypes (NVTs) have increased among asymptomatic carriers in a process called "serotype replacement(Biology 1997), and to a lesser extent, NVTs have also increased as causes of invasive disease. While the reported magnitude of this increase in disease among NVTs has been relatively modest in most countries, such changes have the potential to reduce the overall public health benefit of the vaccine. Consequentially, non-vaccine type strains take over the vacant niche and are the major colonizers in that niche, a phenomenon known as sero-type replacement (Huang et al. 2005).

Preceding to PCV7 implementation, seven serotypes caused the majority of invasive disease. Following PCV7 introduction, there was an increase in the non-VT-7 serotypes in the nasopharynx of individuals. Yet, the risk for IPD declined. PCV7 strains were nearly eliminated and only a minimal increase in non-VT strains was observed (Feikin et al. 2013).

Following nasopharyngeal serotype replacement, a lag was observed in the increase of IPD caused by non-VT serotypes, and varies in different populations and different geographical locations(Weinberger, Malley, and Lipsitch 2011). The six serotypes included in PCV13 (and not PCV7) were chosen, due to their known high virulence, it was therefore not surprising that they were among the most common emerging non-VT IPD causing serotypes replacing VT7 serotypes. Particularly, serotype 19A emerged dramatically in the United States and raised concern(Singleton et al. 2007). While serotype replacement is a universal phenomenon, which serotype among the non-VT serotypes emerge and replace the decreasing VT serotypes differs in different geographic locations. For example, a study in the United States reported that several year following PCV7 implementation, serotypes 19A, 6A, 15B/C, 35B, and 11A were observed to emerge and serotype 19A was particularly a high IPD causing serotype(Huang et al. 2005). While a study in Portugal reported that serotypes 1, 3, 7F, and 19A are the most common causing IPD serotypes following PCV7 implementation (Aguiar et al. 2008). Following PCV7 introduction, a universal increase in non-VT 13-7 serotypes was observed with some variation, but these serotypes were generally invasive. Following the introduction of PCV13, non-VT13 serotypes may emerge in the nasopharynx of individuals and follow the same trend that was observed after the introduction of PCV7(Weinberger, Malley, and Lipsitch 2011). There is strong evidence that colonization with NVTs increases in vaccinated populations. Randomized trials involve vaccination as the cause of these increases, and observational sign is reliable with theoretical estimates that both larger reductions in VT carriage and larger increases in NVT carriage must occur after mass vaccination.

2.7Antibiotic Resistance

In contrast to the capsular serotype, the genotype is more closely associated with antibiotic resistance. The capacity of S. pneumoniae to undergo horizontal gene transfer leads to its genetic diversity and aids the organism adapt to environmental changes, including antibiotic pressure. Theoretically, the genotype should primarily correlate with antibiotic resistance.

In a study done by Song et al., 2013 (J. Y. Song, Nahm, and Moseley 2013), he presented that Isolates belonging to serotypes 3 and 18C that were 100% sensitive to penicillin were significantly less competent than isolates belonging to serotypes 6B, 14, 19F, 9V, and 23F,

which were frequently resistant to penicillin. Intriguingly, the capsule (cps) locus of S. pneumoniae is flanked by the pbp2x and pbp1a genes, which code for penicillin-binding proteins (PBPs) (Thompson and Lipsitch 2004). PBPs are enzymes involved in cell wall synthesis and are targets for β-lactam antibiotics. Under the natural selection imposed by host immunity and antibiotics, the recombination events would involve PBP genes, as well as the cps operon, and would change both the serotype and the resistance profile of the strain.

Globally, the prevalence of antibiotic resistant S. pneumoniae has increased and related to the spread of pediatric pneumococcal serotypes (6A, 6B, 9V, 14, 15A, 19F, 19A, and 23F). After the introduction of PCV7, the prevalence of PCV7 serotypes and serotype 6A were decreased and subsequently replaced by non-PCV7 serotypes, including serotype 19A. Although Spain23F-ST81 and Spain6B-ST90 were well-established multidrug-resistant clones in the 1980s and 1990s, their prevalence decreased after the introduction of PCV7 (Ardanuy et al. 2009). Serotype 19A is the most prominent serotype worldwide, CC320/271 strains with serotype 19A are multidrug resistant and prevalent worldwide, particularly in North America and many Asian countries(J. Y. Song, Nahm, and Moseley 2013)both clinically important and multidrug-resistant (non-susceptible to ≥ 3 antibiotic classes) in the era of PCV7. More than 30% of serotype 19A isolates were multidrug resistant (Hulten et al. 2013).Among isolates with serotype 19A, 86.0% and 79.8% showed erythromycin resistance and MDR, respectively(J. Song et al. 2004).

With dual macrolide resistance mechanisms (ermB and mefA), they showed resistance to erythromycin, tetracycline, clindamycin, cefuroxime, and trimethoprim/ sulfamethoxazole (J. Song et al. 2004). Erythromycin resistance was highly correlated with azithromycin and clarithromycin resistance(Shin et al. 2011). Although the resistance rate for penicillin is quite low (minimum inhibitory concentration [MIC] ≥ 8 μg/mL, < 5%). Several clones of multidrug-resistant serotype 6C pneumococci emerged in the US and Europe, and a multidrug resistant 6D clone (ST282) has been identified in Korea (117- 119, 138, 139).The relations between the pneumococcal serotype and genotype were addressed with regard to antibiotic resistance.

Knowledge of the serotype distribution is important for conjugate vaccine usage, but the sero epidemiological data is insufficient in many countries. A nationwide sero surveillance system is vital to start.

2.8Culturing and molecular techniques

Culture remains the gold standard for the identification of the organism while the Quellung reaction remains the gold standard for serotype determination from isolates. Nonetheless, culture, is highly specific but has low sensitivity, requires long incubation periods and is not commonly available in many low-income countries (Slotved et al. 2004). In addition, antibiotic therapy prior to specimen collection or suboptimal culturing conditions may reduce the yield of cultures (Pai, Gertz, and Beall 2006). Polymerase chain reaction (PCR)-based methods targeting pneumococcal specific genes, such as lytA, have resulted in improved and timely diagnosis of pneumococcal diseases (Yu et al. 2005). The method involves streamlined DNA template preparation and agarose gel electrophoresis to analyze the amplification products. Capsular types identified by the PCR method invariably produced results concordant with the conventional serotyping technique. Even when the method presented does not fully type an isolate, the PCR data can guide the experimenter when using immunological serotyping. Multiplex PCR for the analysis of pneumococci provides an accurate, expeditious, and cost-effective way of reducing the number of strains that have to be serotyped by conventional immunological techniques (Satzke et al. 2014).Such methods can be easily implemented where molecular diagnostic capacity exists and could become an alternative diagnostic tool in settings where culture capacity is lacking or suboptimal. Nonetheless, the use of molecular diagnostic techniques for the evaluation of the impact of PCVs against IPD has been documented (Earnshaw et al. 2012).

Chapter Three:
Methodology

3.1 Study design, sample source and study population

The study is a repeated cross sectional surveillance of *S. pneumonia* carriage that was performed in 2016, to assess and isolate pneumococci from nasopharyngeal samples collected from Palestinian children who are living under two different policies for vaccination in East Jerusalem (EJ) and West Bank (PA), and then compare it with the previous 4 cross sectional surveys that done during the months May through July of 2009, 2010, 2011 and 2014 from the two Palestinian populations.

The results that we got here are compared to two previous studies done by Daana et al., 2015 and Abu Seir et al., 2018 to show the effects of PCVs (7, 10, 13) on streptococcal pneumonia nasopharyngeal carriage in studied population.

S. *Pneumoniae* carriage among Palestinian children (<5 years) is performed among two proximate Palestinian populations, living under two distinct health authorities, with different PCV vaccination policies (PCV7, PCV10 and PCV13). Areas of the Palestinian Authority has its own health ministry and began implementing PCV10 in late 2011, while East Jerusalem is governed by the Israeli Ministry of Health, and therefore implemented PCV7 in 2009 and PCV13 in 2010. This study provided a unique opportunity to study over-all and indirect effects of PCV7, PCV10 and PCV13 vaccination, in two closely related Palestinian populations governed by two distinct health authorities.

In this way, the study is designed to evaluate the effect of two different pneumococcal conjugate vaccine policies (PCV7/13 vs. PCV10) by comparing EJ and PA.It was thus possible to conduct an observational study that simulated some what a theoretical desired clinical trial measuring vaccine effectiveness, where PCV13 was introduced to one population, but not yet to the other. Here, the effects of PCVs on Streptococcus pneumoniae carriage, distribution of serotypes and antibiotic resistance patterns are reported**.** Children under 5.5 years of age residing in the West Bank under the PA who visited any three, private pediatric clinics in Bethlehem, Nablus, and

Ramallah were recruited by physicians conditional upon parents informed and signed consent. Samples were then transported to Al-Quds University Laboratory.

3.2 Data Collection

Each study participant filled a questionnaire by parents who were questioned to obtain medical information and demographics of the parent and child including, age, gender, day care attendance the life style and daily habits, environmental exposure, health and family history and socioeconomic information such as parent's profession, smoking habits, breastfeeding history, and type of pets owned. Medical information included chronic history of illnesses or recurrent illnesses, antibiotic use during the recent period, immunizations, hospitalizations during the past 6 months, and current medical status.

3.3 *S. pneumoniae* Screening and Laboratory

A nasopharyngeal swab, using rayon-tipped aluminum shaft swab placed in Amies transport media (Copan innovation, Brescia, Italy) was collected. Samples were transported within 24 hours. Swabs were streaked on tryptic soy agar plates supplemented with Sheep-blood and 5micrograms/ml gentamycin (Hy-labs) and were incubated overnight at 35°C in 5% CO_2 enriched air to detect the presence of *S. pneumoniae*. Presumed colonies of *S. pneumoniae* were further tested and identified by morphological characteristics, such as α hemolysis and optochin susceptibility. Other types of streptococcus are not susceptible to optochin. Once confirmed as *S. pneumoniae*, the sample was grown on tryptic soy broth agar (TSBA) blood plates (Hy-labs) overnight under the same conditions. *S. pneumoniae* samples were swabbed from TSBA and inserted in brain heart infusion (BHI) with 30% glycerol and was frozen at -80°C preserve for further characterization.

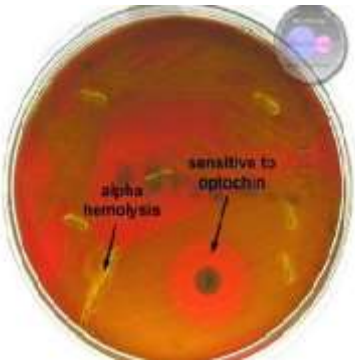


Figure 3A: Isolates from Early Years of Collection

During the study (2016), we characterized all of the *S. pneumoniae* samples that were collected from EJ, and PA.

3.4 Antibiotic Susceptibility Testing

Swabs were streaked on tryptic soy agar plates supplemented with Sheep-blood and 5 ug/ml gentamicin and were incubated overnight at 35◦C in 5% CO2-enriched air. *S. pneumoniae* were identified using morphological characteristics, alpha hemolysis and optochin susceptibility. Episolemeter test (E-test) strips for penicillin and ceftriaxone susceptibility were used following Clinical and Laboratory Standards Institute (CLSI) guidelines. Strains with MIC ≤ 0.06 g/ml were defined as penicillin-susceptible *S. pneumoniae* (PSSP), strains with MIC > 0.06 g/ml as penicillin non susceptible *S. Pneumonia* (PNSSP) and strains with MIC ≥ 2 _g/ml as penicillin resistant S. pneumoniae (PRSP). Antibiotic susceptibility to erythromycin, clindamycin, trimethoprim/sulfamethoxazole, ofloxacin and vancomycin was determined by disk-diffusion. Multidrug resistance (MDR) was defined as at least non susceptibility to penicillin and erythromycin resistance.

Defining multidrug resistance as non susceptibility at minimum to penicillin with a MIC greater than 0.06 mg/L and erythromycin with a MIC greater than 0.5 mg/L. Essentially growth inhibition of antibiotic susceptibilities via disk diffusion was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines as can be seen in **Table 3.1.**

Disk/ E-test strip	S (mm)	I (mm)	R (mm)	MIC (S)	MIC (I)	MIC (R)
Vancomycin	≥17	--	--	≤1	--	--
Trim/Sulfamethoxazole	≥19	16-18	≤15	≤0.5	1-2	≥4
Levofloxacin	≥17	14-16	≤13	≤2	4	≥8
Ofloxacin	≥16	13-15	≤12	≤2	4	≥8
Erythromycin	≥21	16-20	≤15	≤1	3	≥4
Clindamycin	≥19	16-18	≤15	≤0.25	--	≥1
Penicillin				≤0.06	0.12-1	≥2
Ceftriaxone				≤1	2	≥4

Table 3.1: Standard sensitivities to antibiotics according to the CLSI

3.5 Serotype Determination

The serogroup of the isolate was determined by latex agglutination test, bacteria colonies were grown overnight on TSBA blood plates 35°C in 5% CO_2 . Serogroup and serotype were determined by the latex agglutination test and the Quellung reaction (StatenSerum Institute, Copenhagen, Denmark).

The kit consists of 14 pneumococcal polyclonal pool antisera, (pools P to T and A to I). 10 µl of cell line was applied to disposable latex agglutination cards mixed with a pneumococcal sample retrieved from the TSBA blood plate with 1.50 µl of one pool antiserum. The solution was stirred and the card was rocked for 5-10 s. Agglutination signified a positive reaction, and was read by the naked eye. Agglutination occurs if the antiserum is homologous to the capsule of the pneumococcal culture. Specific combinations of antisera yield certain serogroups within 20 s. Pneumococcal samples were classified as omni negative when it does not react with the antisera. This method is sensitive and less expensive since it may be used as a screening tool to quickly identify the serotype, which subsequently can be confirmed by Quellung reaction**.**

Please see **Table 3.2** for combinations of antisera pools used and the serotypes resulting from these combinations.

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

Table 3.2: Distribution of the chessboard system of pneumococcal strains according to their serotypes using Pneumotest-Latex

3.6 Freezing of *S. Pneumoniae* Isolates

A pure fresh isolates of *S. pneumoniae* were streaked on blood agar to be inoculated, then, in bacterial preserver vials containing 25% glycerol at -80 °C until needed.

The freezing solution was prepared as the following: 37.8 gram of brain heart infusion broth (BHI) were weighed and suspended in a graduated cylinder with ddH2O till 750mL. The solution was mixed well to become homogenous and 250ml of pure glycerol were added. Then, the mixture was mixed well and autoclaved. After autoclaving, each 1 ml of the prepared freezing mixture was aliquoted in a screw capped sterile eppendorf tubes and freezed at -30 °C to be used for the collected isolates.

3.7 DNA Extraction and Quantification

Genomic DNA was extracted from overnight fresh cultures either by boiling method as described below and/or using the DNA extraction Kit "Nucleospin, Macherey-Nagel, Germany" (NucleoSpin® 2017).

For the extraction by boiling, a loopful suspension of overnight grown cultures on blood agar plates was prepared in 1.5 ml microcentrifuge tube containing a prepared lysis solution of 95 ul of sterile nuclease free distilled water, one microliter of lysostaphin (1 mg/mL), one microliter of lysosyme (1mg/mL),one microliter of 1M Tris and 2 ul 0.5M EDTA. The suspension was first incubated at room temperature for 5 min. then, incubated at 37 °C for 30 min and then heated in dry bath incubation at 95 °C for 10 min. the suspension was centrifuge at 10,000 rpm for 5 min; the supernatant was collected in a new clean collection microcentrifuge tube and used as DNA template.

Regarding the extraction by the Nucleospin Kit, it was done according to the manufacturer's instructions with some modifications Briefly,2-3 pure colonies were suspended in 180 ul T1 buffer and 25 ul proteinase K solution. Samples were completely covered with this lysis solution and colonies were lysed by vigorous vortex yielding to a turbid mixture. T1 buffer and proteinase K mixture are stable for 10-15 min before addition of samples because proteinase K tends to self-digest in T1 buffer without substrate. Thus, it was pre-mixed directly before use only. However,

it is important to add proteinase K to digest proteins and remove contamination from preparation of nucleic acid. Then, the mixture was incubated in a shaking incubator overnight at 56 °C until complete lysis is obtained. After lysis 200 ul of B3 buffer were added to each sample, vortexed vigorously and incubated at 70 °C for 15 min. Then, after incubation, samples were vortexed vigorously and adjusted for the DNA binding step. Then, 210 ul absolute ethanol (96-100%) were added to each sample, vortexed vigorously, and loaded to the NucleoSpin® Tissue column silica membrane with the collection tube, provided by the Kit. Then, samples were centrifuged for 1 min at 11,000 x g and first wash was done by adding 500 ul BW buffer and centrifuged for 1 min at 11,000 x g. After centrifugation, the flow through solution was discarded and columns were placed into new collection tubes. Second wash was done by adding 600 ul B5 buffer to the column and centrifugation for 1 min at 11,000 x g. Then, the flow through solution was discarded and the silica membrane columns were allowed to dry by centrifuging the columns with the collection tubes for 1 min at 11,000 x g. Residual ethanol is removed during this step.

The final step, the elution step was modified to yield to high pure and high concentration DNA. Half volume of elution buffer (50 ul) was added to the column and incubated for 10 min at room temperature before centrifugation. The elution buffer was pre-heated at 60°C to dissolve the pellet well. After centrifugation, the second half of the elution buffer (50 ul) was added to the column and incubated, also for 10 min at room temperature, then centrifuged again. By this, about 85-100% of bound nucleic acid is eluted in the standard elution volume and at a high concentration.

The extracted DNA by the two methods was stored at -30 °C for the molecular analysis.

The concentration of DNA were measured by a Nanodrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific Inc, Waltham MA, USA). The instrument was adjust using 1 ul distilled water as a blank for the boiling method and the Kit elution buffer as a blank for ncleo-spin Kit extraction method. The protein/DNA ratio (260/280 nm) was measured to estimate the purity of DNA samples. Whereas, samples with purity less than 1.2 were not used and re-extracted again.

The boiling method was faster and simpler, but the DNA extraction by Kit had shown higher purity and better stability of the DNA upon storage for further use.

The DNA concentration used for molecular typing of all samples ranged between 10-200 ng/ul

However, the volume of template DNA was increased for samples with low concentration and the highly concentrated DNA were diluted with nuclease free distilled water for better yield and purity. Figure3.1 (A) shows a summarized table taken randomly selected extracted samples that were used as template DNA for the Serotyping. Figure3.1 shows a representative graph for extracted DNA sample measured by the Nanodrop.

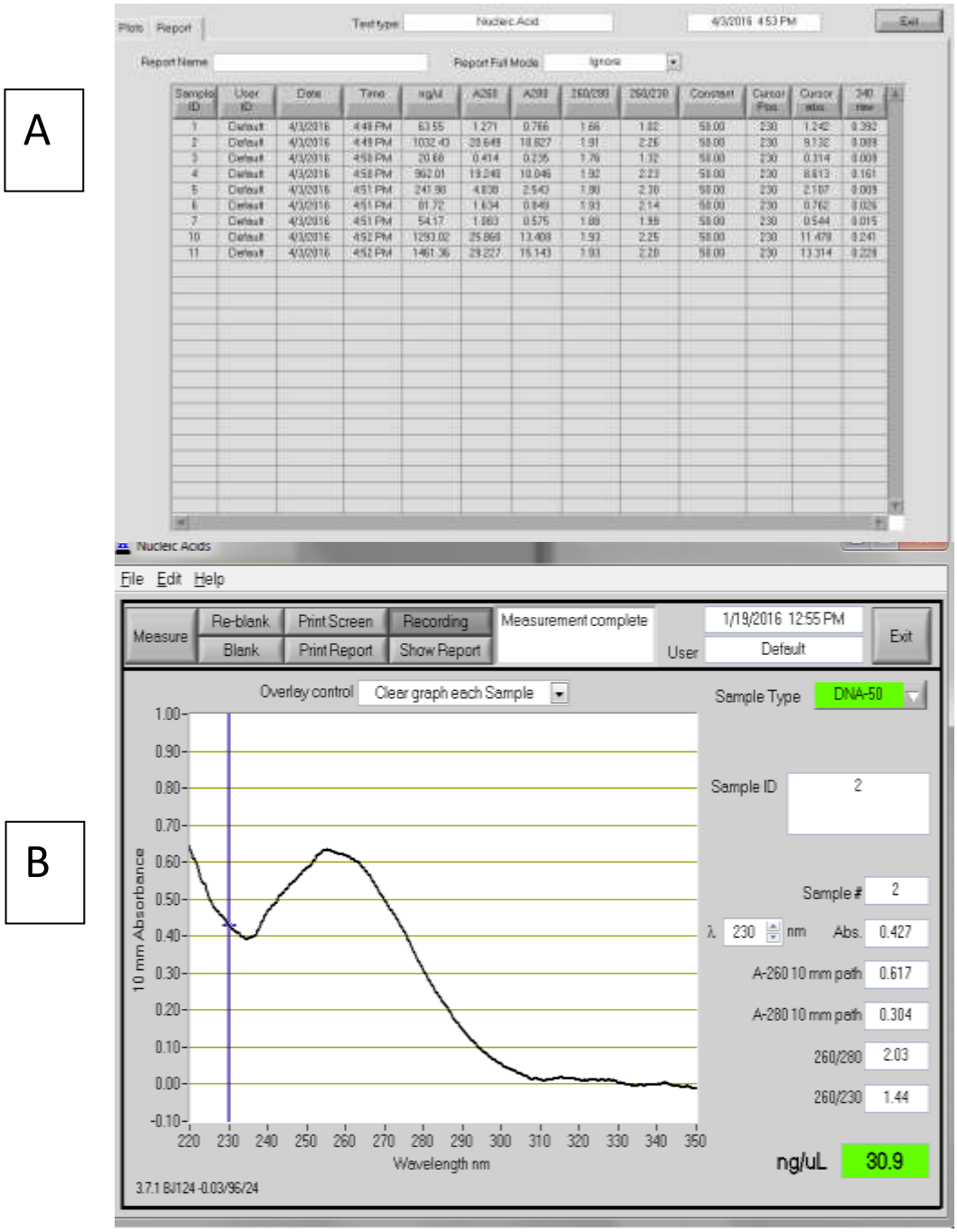


Figure 3.1: A: a table taken as snapshot from the (Nanodrop 1000 spectrophotometer) for some representative randomly selected extracted samples that were used as template DNA for the serotyping. B: a graph of representative extracted DNA sample as examined on the Nanodrop 1000 spectrophotmeter.

3.8 Molecular typing of serotype-specific genes encoding the capsule locus in *S. pneumonia:*

Following latex agglutination and determination of the serogroup, *S. pneumoniae* serotype was determined and confirmed through PCR and identified through gel electrophoresis. Based on the results of serotyping as serogroups that were positive by agglutination, the specific serotype of that serogroup were determined by PCR Multiplex PCR based approach for deducing serotypes done as described by (Feikin et al. 2013). The serotypes included (1, 2, 3, 4, 5, 8, 13,14, 20, 21, 31, 34, 37, 38, 39, 40, 44, 46, 6A, 6B, 7A, 7B, 7C, 7F, 9A, 9N, 9L, 9V,10A, 10F, 11A, 11D, 11F, 12A, 12B, 12F, 15A, 15B, 15C, 15F, 16A, 16F, 17F,18A, 18B, 18C, 18F, 19A, 19F, 19C, 22F, 22A, 23A, 23B, 23F, 25F, 28A, 24F, 33F,33A, 35B, 35F, 35A, 35C, and 47F). Table 3.3 lists the primers used in the single or multiplex PCR for the serotyping.

The PCR mix contained 10 µl ddH2O, 12.5 µl Ready Mix (PCR Master Mix by ABgene), 1.25 µl of the forward primer and 1.25 µl of the reverse primer per sample. or, PCR-ready product™ from syntezza (Pilishvili et al. 2010).In the case where two pairs of primers were used, then ddH2O was adjusted according to 7.5 µl to maintain a total of 25 µl PCR mix per PCR test tube.

The reaction conditions consist of 35 cycles at 95°C for 30 s, 54°C or 62°C for 1 min and 30s and 72°C for 1 min, followed by 10 min at 72°C. The reaction differed for serogroup 6 in that the reaction conditions consist of 35 cycles at 94°C for 30 s, 62°C for 1 min and 72°C for 1 min per kilobase, followed by 10 min at 72°C. PCR reactions were carried as described by (Feikin et al. 2013). In all amplification reactions, negative controls (mix without DNA) and positive controls (from pervious of PCR‘s in this study).

3.9 Agarose gel electrophoresis:

Agarose gel was cast and run in 2.0% agarose gels (FMC BioProducts, Rockland, ME,USA) run at 120 V in 1 X Tris–acetate–EDTA buffer, containing ethidium bromide (0.5g/mL) for visualization by UV light using a gel documentation system (the Bio-Imaging systems Mini Lumitransilluminator). The 100 bp or 1Kb molecular weight standard ladders (Thermo Scientific GeneRuler) was used as molecular weight standards for each gel.

Table 3.3: List of primers sequences used for pneumococcal serotype deduction by PCR and target amplicons.

Serotype	Primer Sequence (5'-3')	Base Pair Length	anneal-ing Tem	Multiplex Re-action
1	1-f: CTC TAT AGA ATG GAG TAT ATA AAC TAT GGT TA 1-r: CCA AAG AAA ATA CTA ACA TTA TCA CAA TAT TGG C	280	49	Reaction 1
9N/L	9N/L-f: GAA CTG AAT AAG TCA GAT TTA ATC AGC 9N/L-r: ACC AAG ATC TGA CGG GCT AAT CAA T	516	49	Reaction 1
7F	7F-f: CCT ACG GGA GGA TAT AAA ATT ATT TTT GAG 7F-r: CAA ATA CAC CAC TAT AGG CTG TTG AGA CTA AC	826	49	Reaction 1
10F/C	10F/C-f: GGA GTT TAT CGG TAG TGC TCA TTT TAG CA 10F/C-r: CTA ACA AAT TCG CAA CAC GAG GCA ACA	248	55	Reaction 2
6C	6C-f: CAT TTT AGT GAA GTT GGC GGT GGA GTT 6C-r: AGC TTC GAA GCC CAT ACT CTT CAA TTA	727	55	Reaction 2
4	4-f: CTG TTA CTT GTT CTG GAC TCT CGA TAA TTG G 4-r: GCC CAC TCC TGT TAA AAT CCT ACC CGC ATT G	430	55	Reaction 2
2	2-f: TAT CCC AGT TCA ATA TTT CTC CAC TAC ACC 2-r: ACA CAA AAT ATA GGC AGA GAG AGA CTA CT	290	53	Reaction 3
7F/A	7F/A-f: TCC AAA CTA TTA CAG TGG GAA TTA CGG	599	53	Reaction 3

	7F/A-r: ATA GGA ATT GAG ATT GCC AAA GCG AC			
9V/A	9V/A-f: GGG TTC AAA G TC AGA CAG TG A ATC TTA A 9V/A-r: CCA TGA ATG A AA TCA ACA TT G TCA GTA GC	816	53	Reaction 3
10A	10A-f: GGT GTA GAT TTA CCA TTA GTG TCG GCA GAC 10A-r: GAA TTT CTT CTT TAA GAT TCG GAT ATT TCT C	628	51	Reaction 4
21	21-f: CTA TGG TTA TTT CAA CTC AAT CGT CAC C 21-r: GGC AAA CTC AGA CAT AGT ATA GCA TAG	192	51	Reaction 4
39	39-f: TCA TTG TAT TAA CCC TAT GCT TTA TTG GTG 39-r: GAG TAT CTC CAT TGT ATT GAA ATC TAC CAA	98	51	Reaction 4
23A	23A-f: TAT TCT AGC AAG TGA CGA AGA TGC G 23A-r: CCA ACA TGC TTA AAA ACG CTG CTT TAC	722	54	Reaction 5
23B	23B-f: CCA CAA TTA G CG CTA TAT TCA TTC AAT CG 23B-r : GTC CAC GCT GAA TAA AAT GAA GCT CCG	199	54	Reaction 5
23F	23F-f: GTA ACA GTT GCT GTA GAG GGA ATT GGC TTT TC 23F-r: CAC AAC ACC TAA CAC TCG ATG GCT ATA TGA TTC	384	54	Reaction 5
19A	19A-f: GAG AGA TTC ATA ATC TTG CAC TTA GCC A 19A-r: CAT AAT AGC TAC AAA TGA CTC ATC GCC	566	52	Reaction 6
24	24-f: GCT CCC TGC TAT TGT AAT CTT TAA AGA G 24-r: GTG TCT TTT ATT GAC TTT ATC ATA GGT CGG	99	52	Reaction 6
35A/C	35A/C-f: ATT ACG ACT CCT TAT GTG ACG CGC ATA 35A/C-r: CCA ATC CCA AGA TAT ATG CAA CTA GGT T	280	52	Reaction 6
12F	12F-f: GCA ACA AAC GGC GTG AAA GTA GTT G 12F-r: CAA GAT GAA TAT CAC TAC CAA TAA CAA AAC	376	49	Reaction 7
31	31-f: GGA AGT TTT CAA GGA TAT GAT AGT GGT GGT GC 31-r: CCG AAT AAT ATA TTC AAT ATA TTC CTA CTC	701	49	Reaction 7
38/25A/F	38/25-f: CGT TCT TTT ATC TCA CTG TAT AGT ATC TTT ATG 38-/25r: ATG TTT GAA TTA AAG CTA ACG TAA CAA TCC	574	49	Reaction 7
15A/F	15A/F-f: ATT AGT ACA GCT GCT GGA ATA TCT CTT C 15A/15F-r: GAT CTA GTG AAC GTA CTA TTC CAA AC	434	50	Reaction 8
33F	33F-f: GAA GGC AAT CAA TGT GAT TGT GTC GCG	338	50	Reaction 8

	33F-r: CTT CAA AAT GAA GAT TAT AGT ACC CTT CTA C			
35F/47F	35F/47F-f: GAA CAT AGT CGC TAT TGT ATT TTA TTT AAA GCA A 35F/47F-r: GAC TAG GAG CAT TAT TCC TAG AGC GAG TAA ACC	517	50	Reaction 8
34	34-f: GCT TTT GTA AGA GGA GAT TAT TTT CAC CCA AC 34-r: CAA TCC GAC TAA GTC TTC AGT AAA AAA CTT TAC	408	52	Reaction 9
35B	35B-f: GAT AAG TCT GTT GTG GAG ACT TAA AAA GAA TG 35B-r: CTT TCC AGA TAA TTA CAG GTA TTC CTG AAG CAA G	677	52	Reaction 9
14	14-f: GAA ATG TTA CTT GGC GCA GGT GTC AGA ATT 14-r: GCC AAT ACT TCT TAG TCT CTC AGA TGA AT	189	52	Reaction 9
11A/D	11A/D-f: GGA CAT GTT CAG GTG ATT TCC CAA TAT AGT G 11A/D-r: GAT TAT GAG TGT AAT TTA TTC CAA CTT CTC CC	463	53	Reaction 10
3	3-f: ATG GTG TGA TTT CTC CTA GAT TGG AAA GTA G 3-r: CTT CTC CAA TTG CTT ACC AAG TGC AAT AAC G	371	53	Reaction 10
13	13-f: TAC TAA GGT AAT CTC TGG AAA TCG AAA GG 13-r: CTC ATG CAT TTT ATT AAC CG C TTT TTG TTC	655	53	Reaction 10
5	5-f: ATA CCT ACA CAA CTT CTG ATT ATG CCT TTG TG 5-r: GCT CGA TAA ACA TAA TCA ATA TTT GAA AAA GTA TG	362	49	Reaction 11
6A/B/C	6A/B/C-f: AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG 6A/B/C-r: TTA GCG GAG ATA ATT TAA AAT GAT GAC TA	250	49	Reaction 11
9V	9V-f: CTT CGT TAG TTA AAA TTC TAA ATT TTT CTA AG 9V-r: GTC CCA ATA CCA GTC CTT GCA ACA CAA G	753	49	Reaction 11
15B/C	15B/C-f: TTG GAA TTT TTT AAT TAG TGG CTT ACC TA 15B/C-r: CAT CCG CTT ATT AAT TGA AGT AAT CTG AAC C	496	49	Reaction 11
6A/B	6A/B-f: AAT TTG TAT TTT ATT CAT GCC TAT ATC TGG 6A/B-r: TTA GCG GAG ATA ATT TAA AAT GAT GAC TA	250	49	Reaction 12
18	18-f: CTT AAT AGC TCT CAT TAT TCT TTT TTT AAG CC 18-r: TTA TCT GTA AAC CAT ATC AGC ATC TGA AAC	573	49	Reaction 12
16F	16F-f: GAA TTT TTC AGG CGT GGG TGT TAA AAG 16F-r: CAG CAT ATA GCA CCG CTA AGC AAA TA	717	53	Reaction 13

6B	**6B-f: CGA CGT AAC AAA GAA CTA GGT GCt GAA AC** **6B-r: AAG TAT ATA ACC ACG CTG TAA AAC TCT GAC**	**220**	**53**	**Reaction 13**
18C	**18C-f: GCA TCt GTA CAG TGT GCT AAT TGG ATT GAA G** **18C-r: CTT TAA CAT CTG ACT TTT TCT GTT CCC AAC**	**354**	**53**	**Reaction 13**
7C/B	**7C/B-f: CTA TCT CAG TCA TCT ATT GTT AAA GTT TAC GAC GGG A** **7C/B-r: GAA CAT AGA TGT TGA GAC ATC TTT TGT AAT TTC**	**260**	**52**	**Reaction 14**
35F	**35F-f: GAA CAT AGT CGC TAT TGT ATT TTA TTT AAA GCA A** **35F-r: GAC TAG GAG CAT TAT TCC TAG AGC GAG TAA ACC**	**517**	**52**	**Reaction 14**
17F	**17F-f: TTC GTG ATG ATA ATT CCA ATG ATC AAA CAA GAG** **17F-r: GAT GTA ACA AAT TTG TAG CGA CTA AGG TCT GC**	**693**	**52**	**Reaction 14**
8	**8-f: GAT GCC ATG AAT CAA GCA GTG GCT ATA AAT C** **8-r: ATC CTC GTG TAT AAT TTC AGG TAT GCC ACC**	**294**	**55**	**Reaction 15**
22F	**22F-f: GAG TAT AGC CAG ATT ATG GCA GTT TTA TTG TC** **22F-r: CTC CAG CAC TTG CGC TGG AAA CAA CAG ACA AC**	**643**	**55**	**Reaction 15**
19F	**19F-f: GTT AAG ATT GCT GAT CGA TTA ATT GAT ATC C** **19F-r: GTA ATA TGT CTT TAG GGC GTT TAT GGC GAT AG**	**304**	**50**	**Reaction 16**
20	**20-f: GAG CAA GAG TTT TTC ACC TGA CAG CGA GAA G** **20-r: CTA AAT TCC TGT AAT TTA GCT AAA ACT CTT ATC**	**514**	**50**	**Reaction 16**

3.10 Ethical Considerations:

The IRB approvals were giving by local Research Ethical Committee at Al-Quds University for this study. Written informed consent was given by a parent for each participating child before recruitment. Also a permission from the Palestinian Ministry of Health (MOH) was obtained to review the clinical reports of patients and to collect the demographic data of patients.

3.11 Statistical Analysis:

Each isolate was coded, and data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics for all samples were presented as frequencies and percentages.

Chapter Four

Results

4.1 Study Design and study Population:

Palestinian population under two health policies; East Jerusalem (EJ) vs Palestinian authority (PA), two closely related Palestinian populations governed by two distinct health authorities, in five repeated cross-sectional surveillances during 2009 and 2016.

A total of 4,696 children under 5.5 years of age from both EJ and PA, joined the study during the years 2009, 2010, 2011, 2014, and 2016, with an annual average of 348 and 616 children, respectively. In EJ, children (n=1,740), full data were available for 1616, under Israeli Health policy who visited specific pediatric clinics were recruited. In the West Bank, children (n=3,080) under the Palestinian Authority who visited any of three, large private pediatric clinics in Bethlehem, Nablus, and Ramallah were recruited. Repeated cross-sectional surveillances were carried out in EJ and PA during summer season. The pediatric subjects from EJ were determined to carry *S. pneumoniae* during the 5 years study, 2009, 2010, 2011, 2014, and 2016 as (100/345,28.9%), (91/311, 29.3%), (87/324,26.9%), (88/287,30.7%), and (59/348,16.9%), respectively. In addition, (223/620, 35.9%), (200/595, 33.6%), (160/566,28.8%), (184/643,28.6%), and (216/656,32.9%) of the pediatric subjects from PA were shown to carry *S. pneumoniae*, respectively.

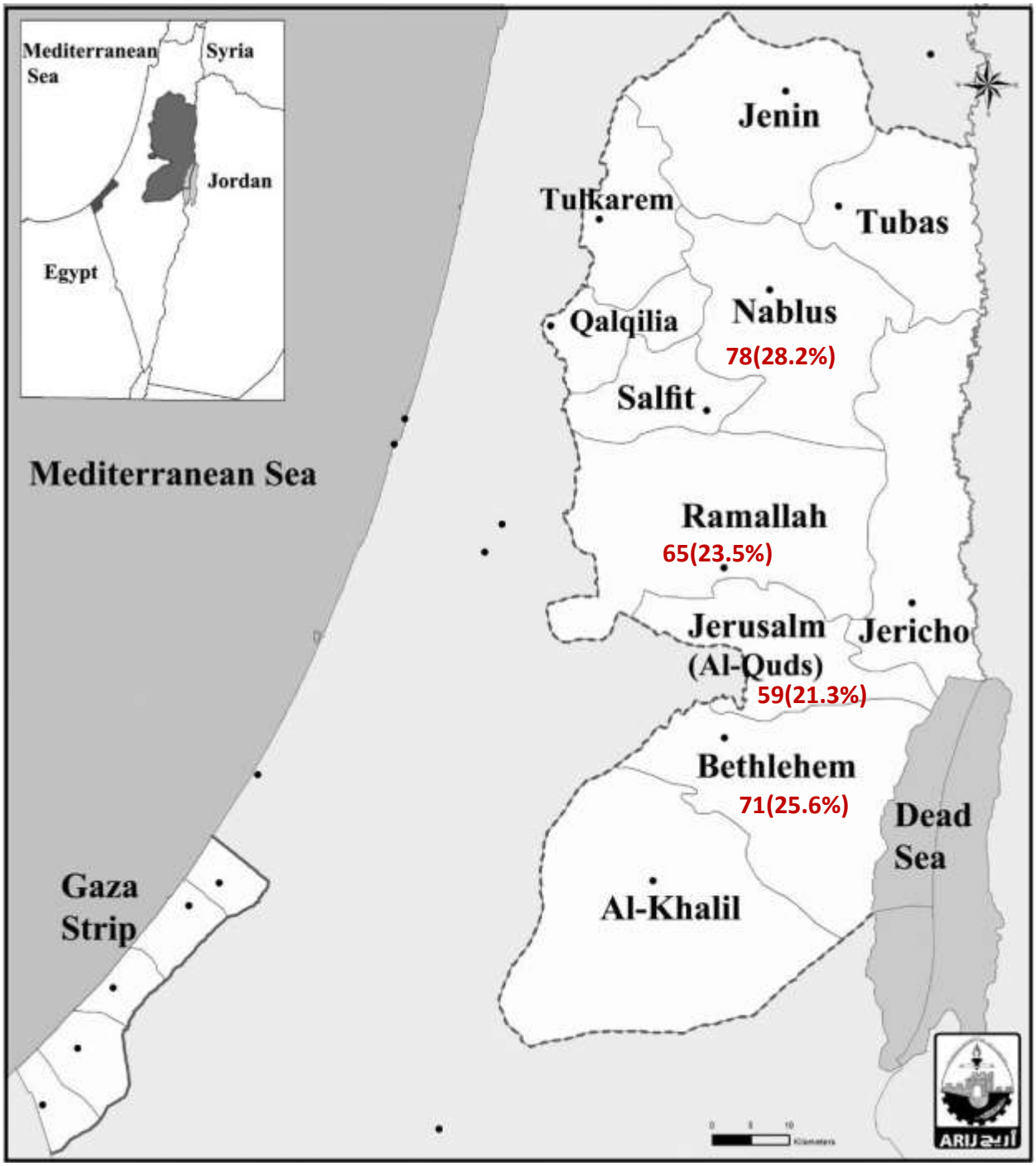


Figure4.1: A map of Jerusalem (Al-Quds) and the West Bank showing the distribution of *S. pneumoniae among cities in 2016 which are included in this study (Sampling area).*

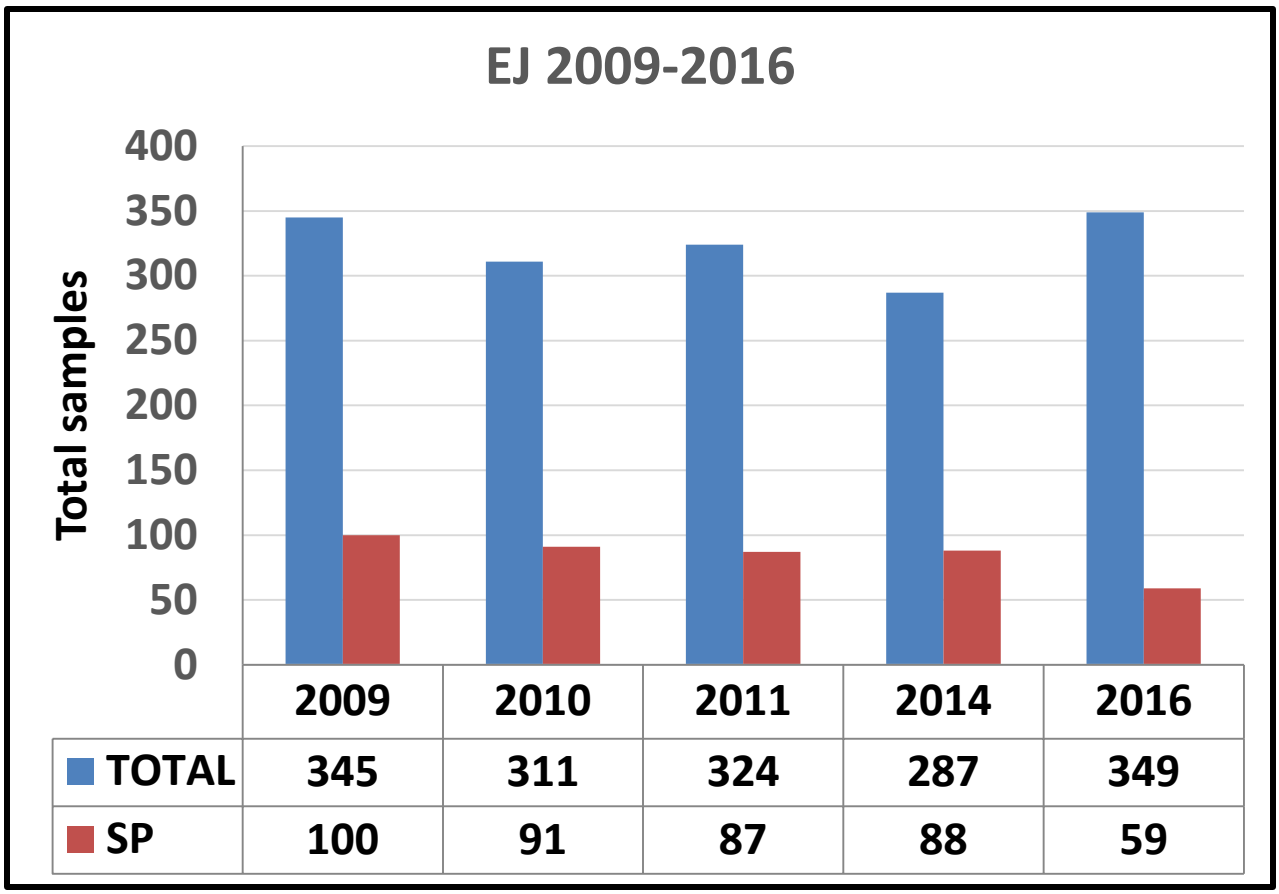
Full demographic data for each isolate including: the hospitalization period, age group, patient type, gender, place of residence and breastfed.

Place of residence for all included patients was documented from questionnaire. The most frequent regions of the collected isolates were from Nablus (78, 28.2%) followed by the Bethlehem (71, 25.6%), Ramallah (65, 23.5%) and East Jerusalem (59, 21.3) Table 4.1

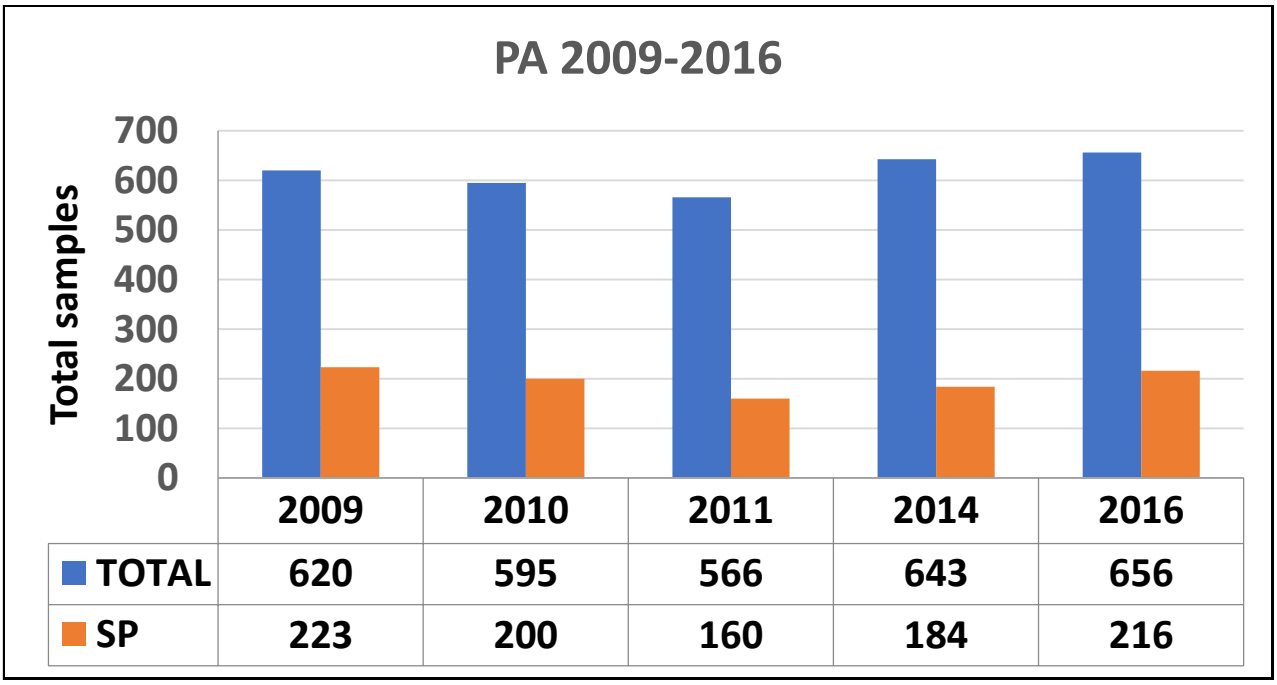
Region 2016	**Frequency (SP)**	**Percent %**
East Jerusalem (EJ)	**59**	**21.3**
Nablus (N)	**78**	**28.2**
Ramallah (R)	**65**	**23.5**
Bethlehem (B)	**71**	**25.6**
Total	**277**	**100.0**

Table 4.1: Number of Patients and their place of residence in this study.

As shown in **figure 4.2A&B** show, there was no significant change in the prevalence of carriage of pneumococci in any of the examined regions. The only significant change was observed in EJ in 2016, when pneumococcal carriage decreased significantly as compared to all previous years, and this is the most important limitation to change some stereotypes that not found in this region

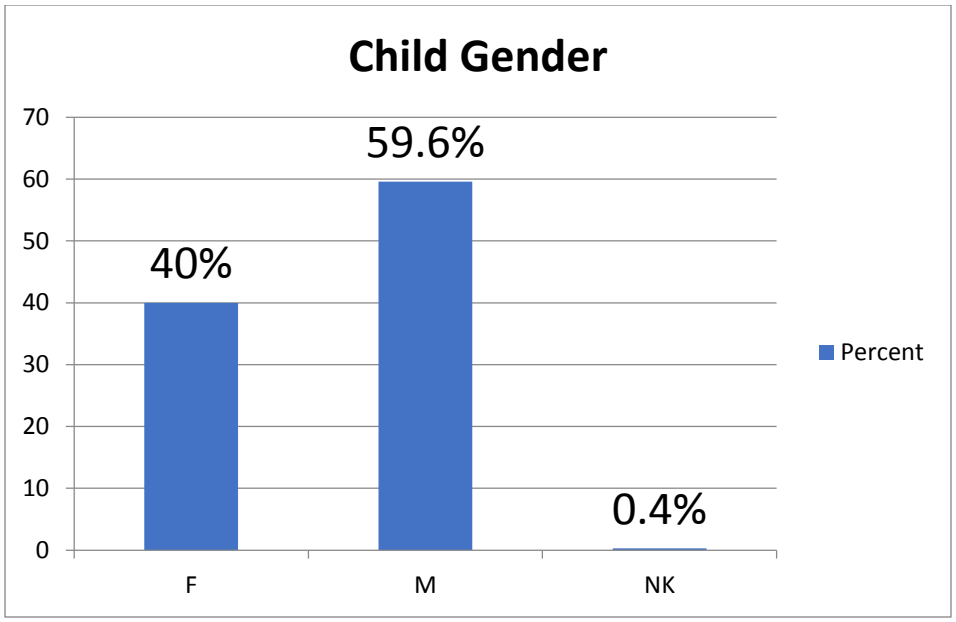


Fiqure 4.2A: Total samples and % of SP carriage in EJ



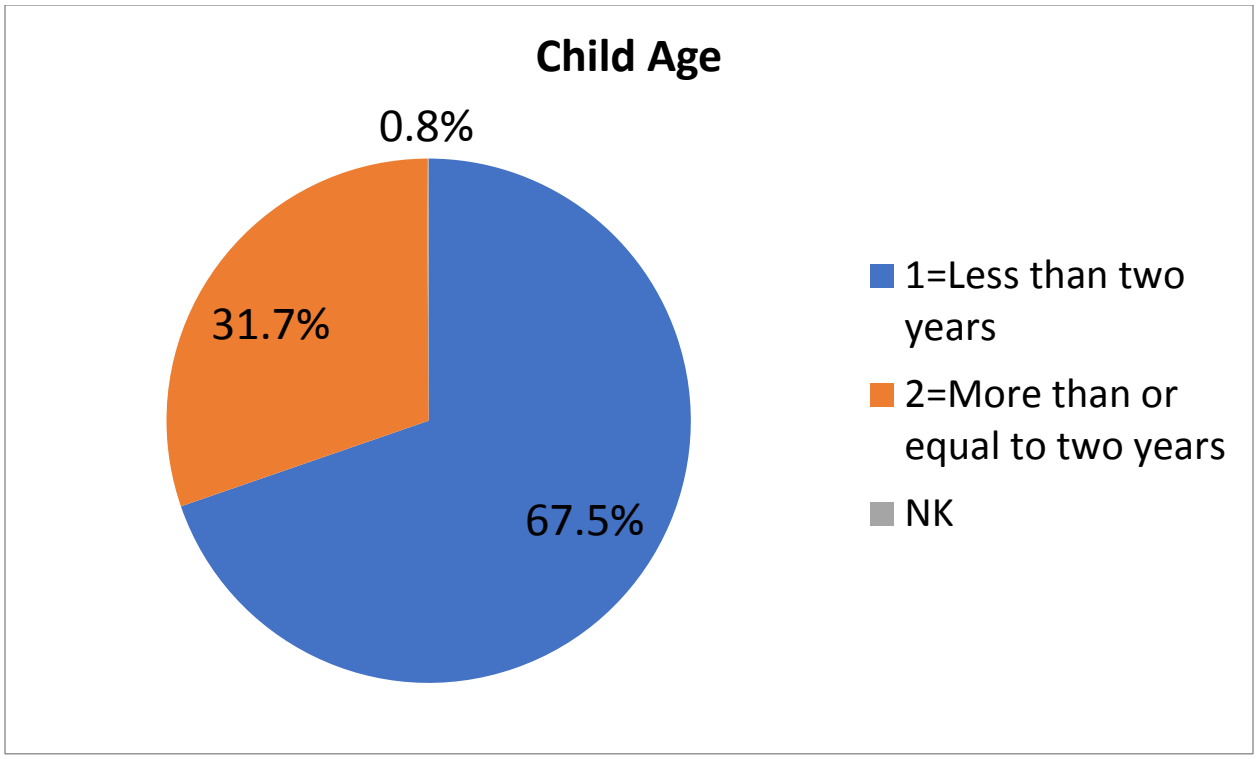
Fiqure 4.2B: Total samples and % of SP carriage in PA

A detailed description of the study population of the first four surveys (2009–2014) was previously reported(Abu Seir et al. 2018; Daana et al. 2015) Here, we present an additional surveillance that took place in 2016 in which 349 children from EJ and 656 children from PA were screened Figure 4.1. Population characteristics in this surveillance are slightly similar to those in the 4 initial surveillances, with aproportion of males (59.6% and 40% in PA and EJ), However, gender of 6(0.4%) of the samples were not found **figure4.2**.



Fiqure 4.3A: *S.pneumoniae* distribution according to gender in both EJ and PA.

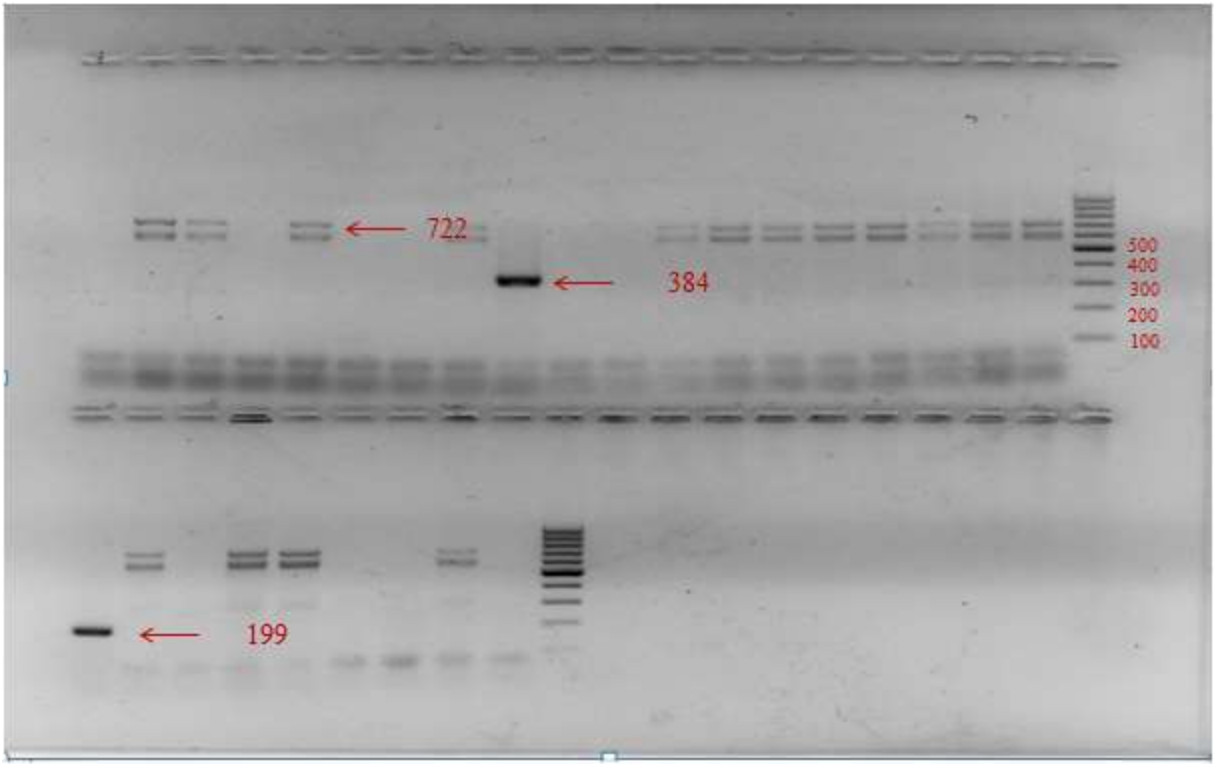
According to age, figure 4.3B shows the age distribution. Age was categorized to two age groups, as the following: 1; from 0-24 months, 2; more than 2 years. Approximately, 67.5% of the children between 0 and 24 months and almost 31.7% of the children more than 2 years. Accordingly, 73.1% (158/216) and 47.5% (28/59), of the children under age of 2 years old in PA and EJ respectively. Regarding breast fed, Nearly similar in both regions, where 94.9% and 97.2% in EJ and PA respectively. About 36, 16.7% of children from PA were hospitalized, and only four 6.8% children from EJ.



Fiqure 4.3B: *S.pneumoniae* distribution according to Age in both EJ and PA.

4.2 Detection of S. *pneumoniae* by Optochin susceptibility and Serotypes.

All of the S. pneumoniae samples were identified using morphological characteristics, alpha hemolysis and optochin susceptibility. E-test strips for penicillin and ceftriaxone susceptibility and the serogroup of the isolate is determined by latex agglutination test after that we confirmed the serotypes using PCR. Fiqure4.4 is a gel electrophoresis for detection of the 23A/B/F serotype.



Fiqure 4.4: A representative agarose gel electrophoresis for the 23A/B/F serotypes detection . M:100 bp DNA molecular weight marker. Band size 23A=722, 23B=199 and 23F=384

4.3 Vaccination uptake history and during the study period

The 7-valent PCV (PCV7) was available in private markets in EJ in 2009, but the vaccine was not implemented in the National Immunization Plan (NIP) during the first study year 2009. In 2009, less than 3% of the children received PCV7and <2% in PA received at least one dose of PCV7. After PCV7 was added to the NIP in EJ in 2010, the percentage of children in EJ receiving at least one dose of the vaccine increased to 74.9% and at least two doses of the vaccine to 57.8%. In late 2011, PCV10 was officially given in PA, and 13.1% children were estimated to be vaccinated that year (Daana et al., 2015), while it was 15 (4.1%) in 2010.Prior to PCV10 implementation in PA, in 2010 only 4.1% of children under the age of 2 received at least one dose of PCV7 (Daana et al., 2015). This proportion increased to 85.5% in PA three years after implementing PCV10 in the vaccination program compared to 92% of children in EJ.

In 2016 study, (28/29) 96.6% and (148/158) 93.7% of the children who had SP, which were less than two years old, were immunized with one or two dose in EJ and PA respectively. However, (30/30, 100%) and (52/58, 89.7%) were detected for children who were above two years old in both regions EJ and PA respectively. Interestingly, in 2016 study, 92.6% (200/216) and 98.3% (58/59) of children were vaccinated in both regions, PA and EJ., respectively. Table 4.2

Age of child	Region	Not vaccinated N(%)	Vaccinated N(%)	Total
< 2 years old	EJ	1	28	29
		%3.4	%96.6	%100.0
	PA	10	148	158
		%6.3	%93.7	%100.0
	Total	11	176	187
		%5.9	%94.1	%100.0
> 2 years old	EJ	0	30	30
		%0.0	%100.0	%100.0
	PA	6	52	58
		%10.3	%89.7	%100.0
	Total	6	82	88
		%6.8	%93.2	%100.0
Total	EJ	1	58	59
		%1.7	%98.3	%100.0
	PA	16	200	216
		%7.4	%92.6	%100.0
	Total	17	258	277
		%6.1	%93.1	%100.0

Table 4.2: Distribution of age groups of patients were that vaccinated from during study.

4.4*S. pneumoniae* Carriage

Carriage was detected in 425/1616 (26.3%) children in EJ and 983/3080(31.9%) in PA during the five-year study period. Similarly to previous study (Daana et al. 2015) findings where the carriage was detected in 279 (28.5%) children in EJ and 583(32.9%) in PA during the three-year study period.

Following vaccine implementation, during the study period, there was a significant decrease in carriage of *S. pneumoniae* in the EJ between 2009 and 2016 (p=0.001).

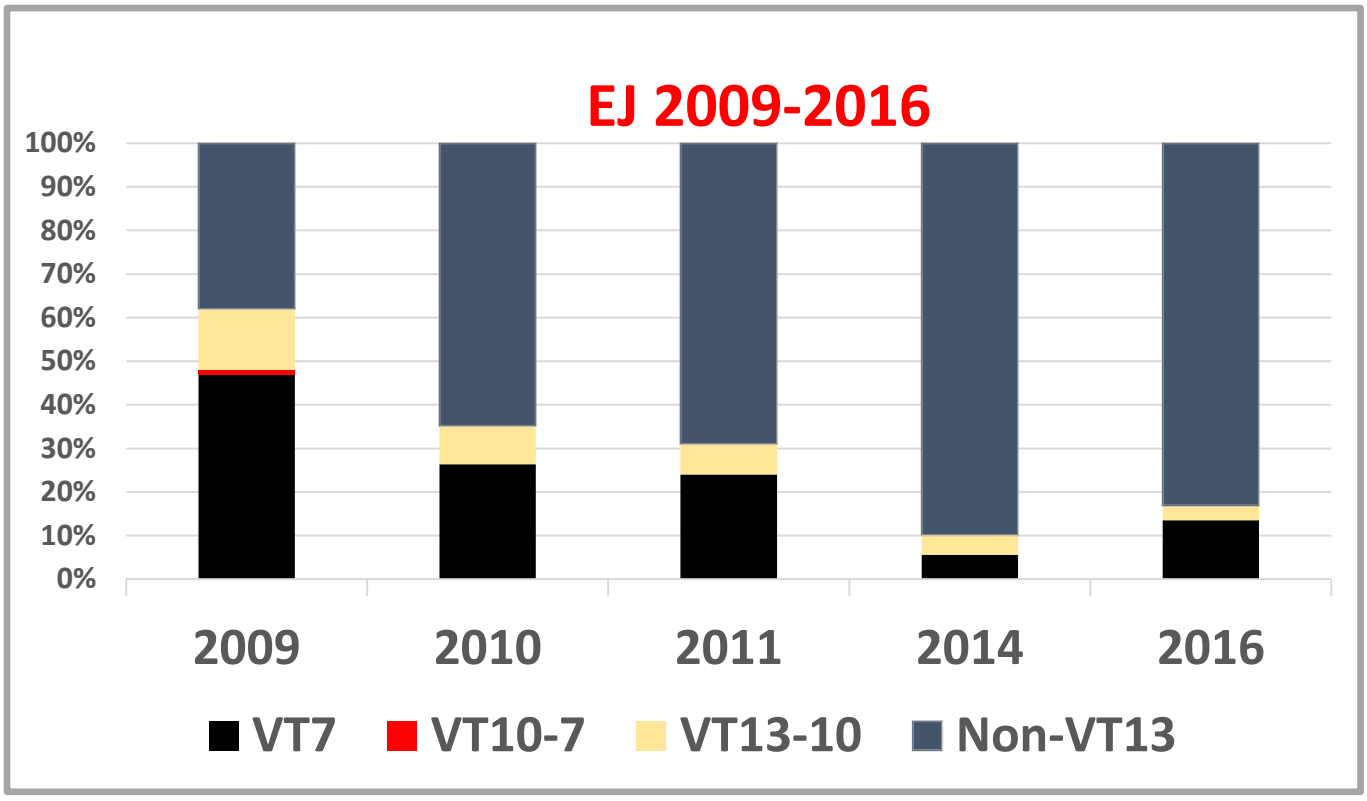


Figure 4.5A. Distribution of VT isolates and non-VT (NVT) isolates of *S. pneumoniae* in children from EJ.Bars represent *S. pneumoniae* carriage as a distribution of VT7 isolates, VT7-10 isolates, VT13-7 isolates, and non-VT-13 isolates of all isolates in each year.

In the PA region, no significant variation was seen in the overall carriage of *S. pneumonia* between 2009 to 2016 (p=0.065). PCV10 was introduced to PA late in 2011, but *S. pneumonia* carriage was approximately (160/566) 28% in2011, prior to vaccine introduction, and (216/656), 32.9% in 2016, five years following vaccine implementation. **Figure 4.5B**.

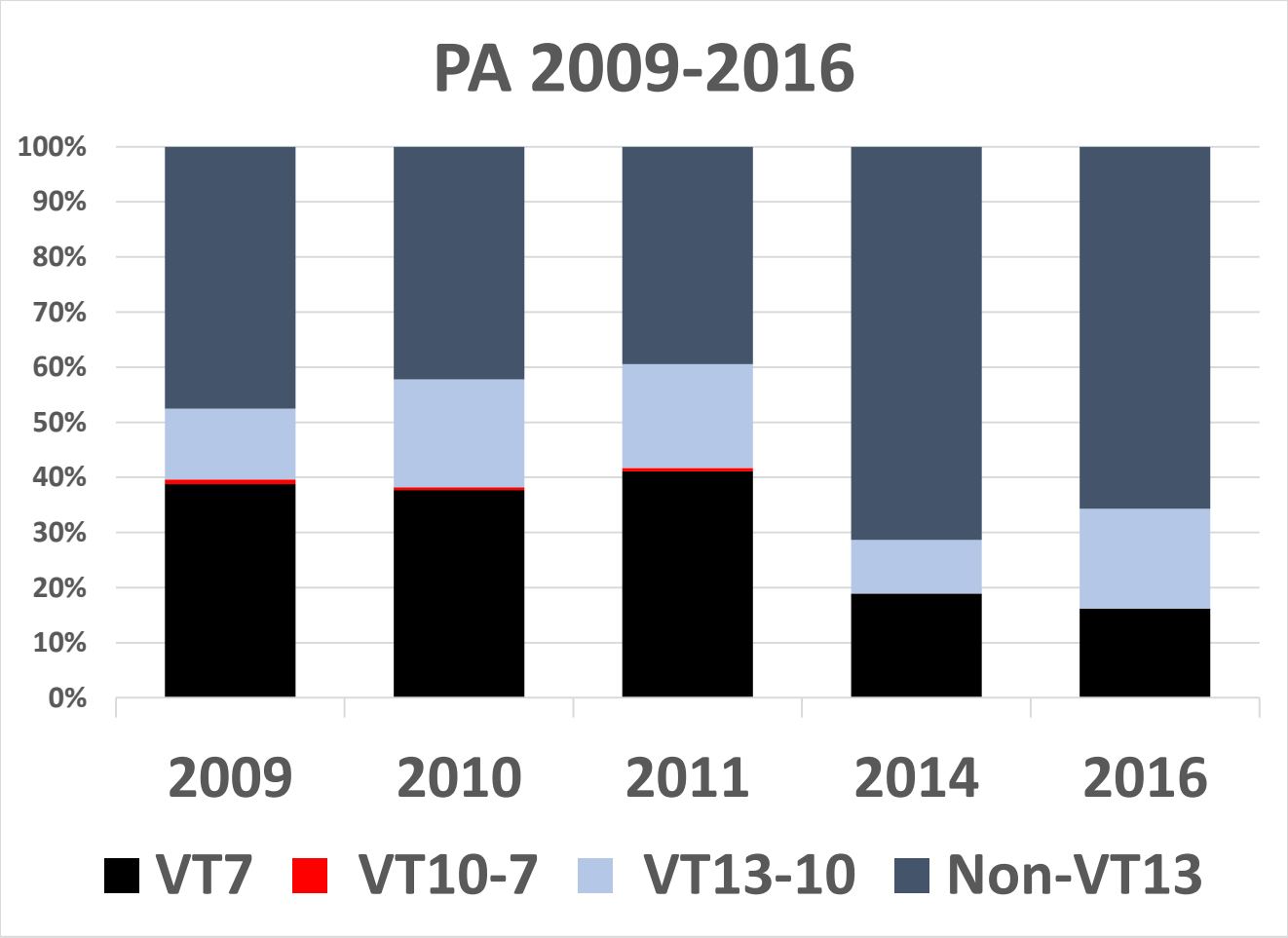


Figure 4.5B. Distribution of VT isolates and non-VT (NVT) isolates of *S. pneumoniae* in children from PA.Bars represent *S. pneumoniae* carriage as a distribution of VT7 isolates, VT7-10 isolates, VT13-7 isolates, and non-VT-13 isolates of all isolates in each year.

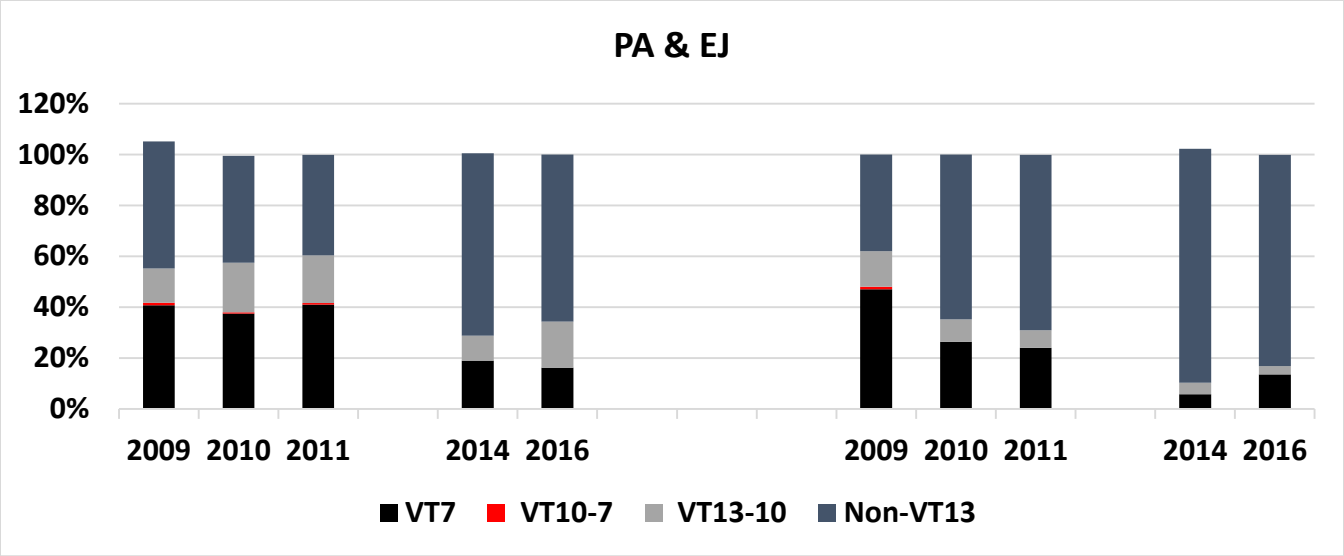
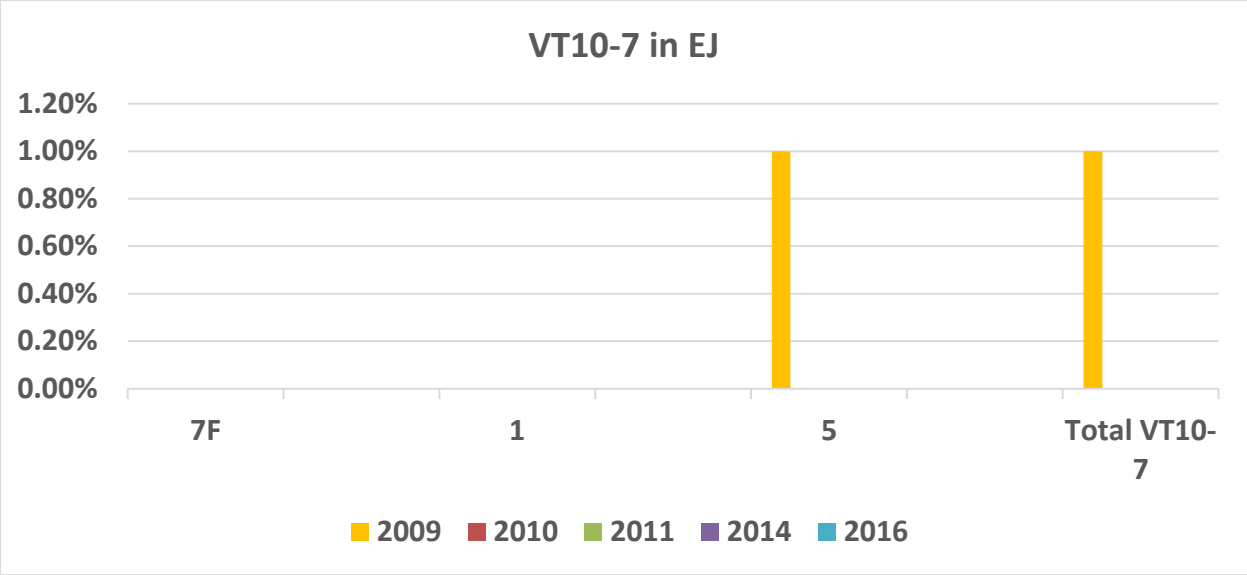


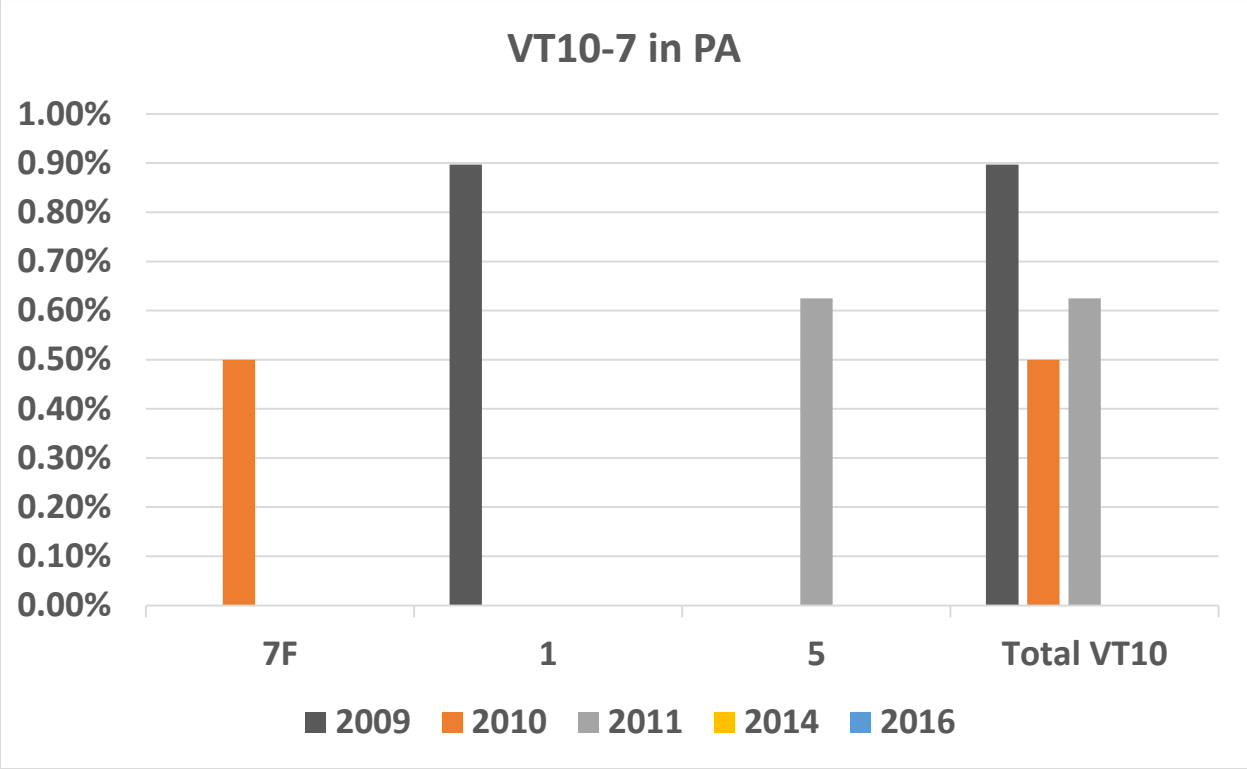
Figure4.6: In EJ and PA, the effect of the vaccination was seen in the distribution of *S. pneumoniae* strains during the study period.

4.5 Serotype Distribution

Prior to the introduction of PCV7 in EJ and PCV10 in PA, all 13 serotypes present in the vaccine, vaccine-type (VT) strains, represented 60% of all *S. pneumoniae* isolates **(see in appendex 6.1)**. Since the additional 3 serotypes belonging to PCV10: 1, 5, 7F were not detected in the study population between 2009 and 2016 just one case in 2009 in EJ and three cases in PA,one case in 2009, 2010 and 2011 in PA., respectively, VT7 and VT13-10 (VT13-10 isolates include serotypes 3, 6A, and 19A) SP strains were used to compare the effects of the two vaccines in EJ and PA.



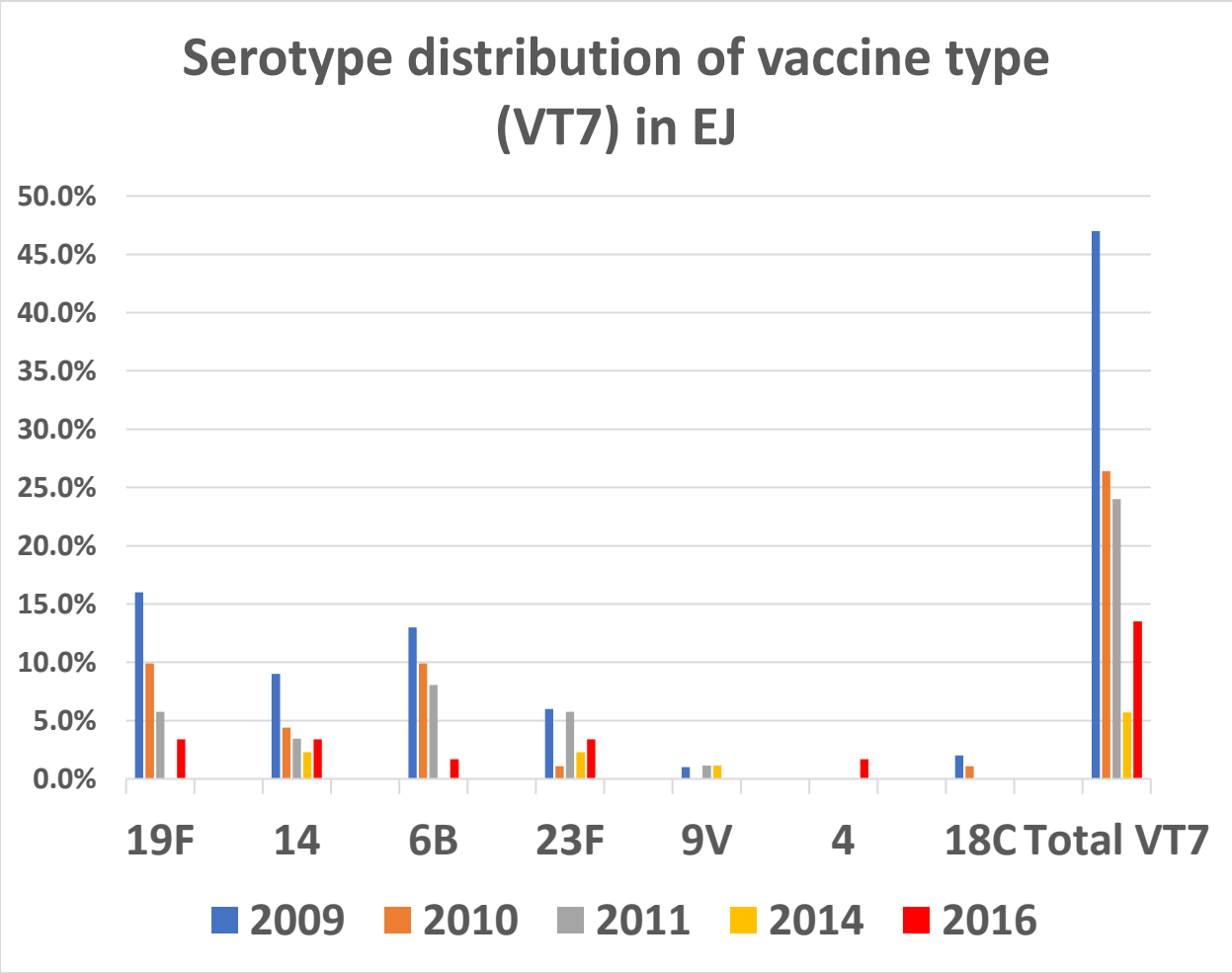
Fiqure 4.7A:Serotype distribution of vaccine type (VT10-7) in EJ



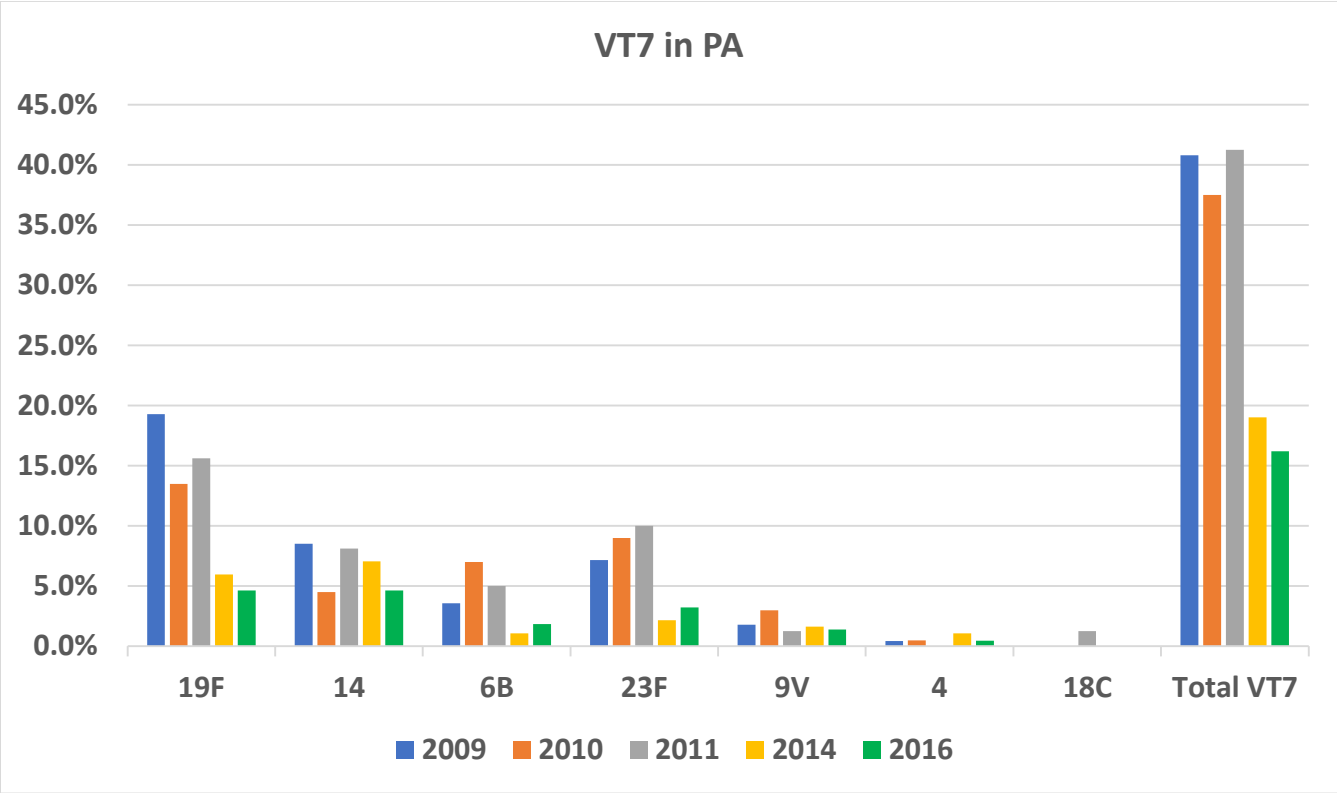
Fiqure 4.7B: Serotype distribution of vaccine type (VT10-7) in PA

In EJ, VT7 strains decreased significantly since the introduction of PCV7 and continued to decrease through 2016 ($p<0.0001$). VT7 strains in PA followed same trend as in EJ since the introduction of PCV10 in late 2011.

In EJ, the prevalence of VT7 strains was 47.00% in 2009. Following PCV7 implementation, the prevalence declined to approximately 26.4% by 2010 and 24.00% by 2011. Following PCV13 implementation, the prevalence of VT7 strains further decreased to 5.7% by 2014. However, in 2016 an increase in the prevalence of VT7 strains was observed to reach (13.5%) figure 4.8B.



Fiqure 4.8A: Serotype distribution of vaccine type (VT7) in EJ

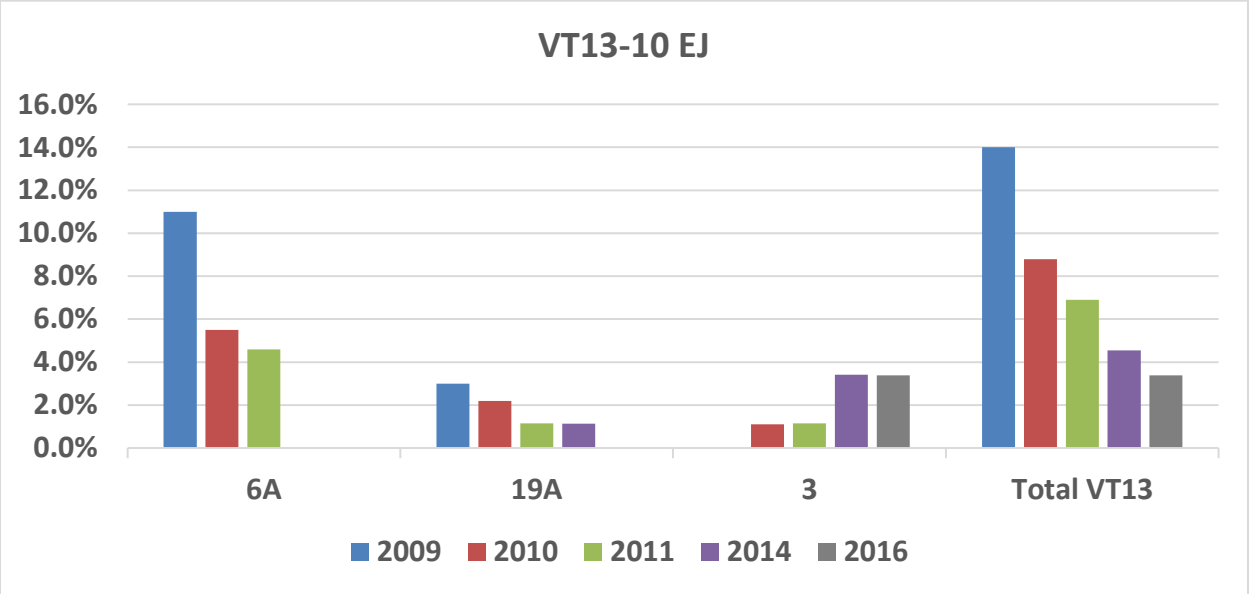


Fiqure 4.8B: Serotype distribution of vaccine type (VT7) in PA

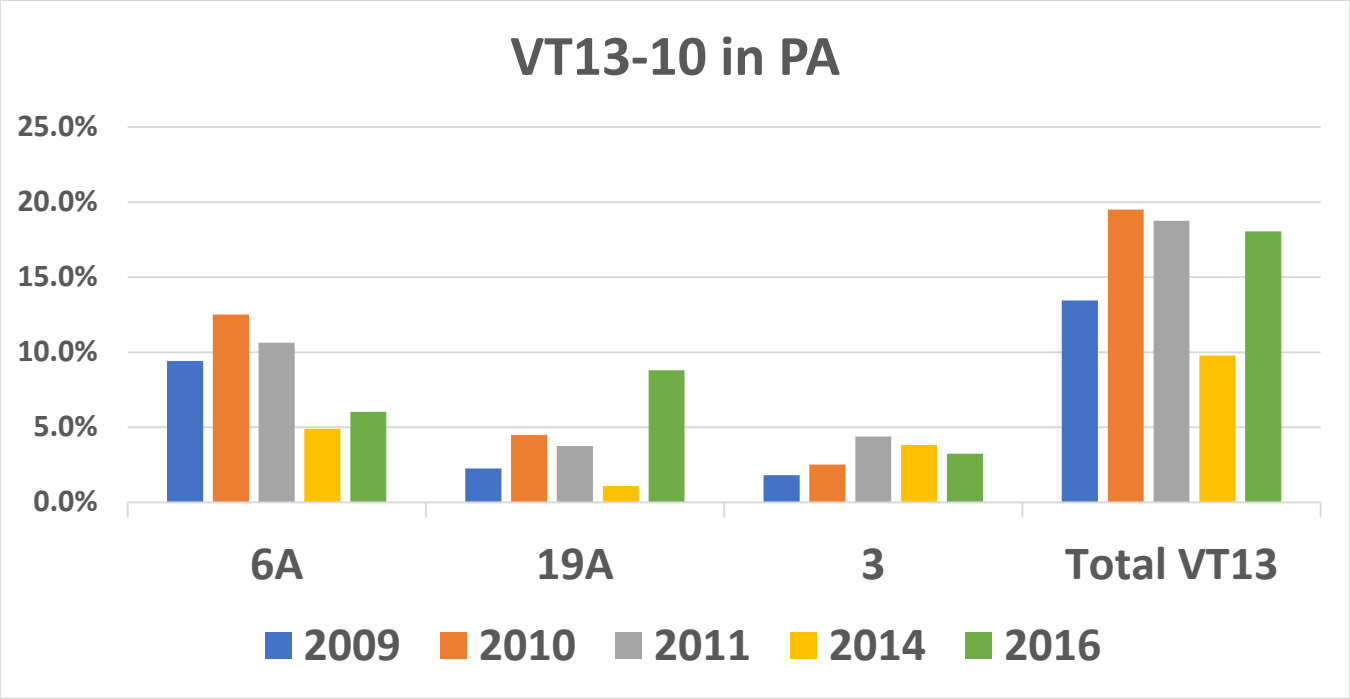
In both EJ and PA there is an overall decrease in all VT7 strains between 2009 and 2016, **(Figure 4.8A and 4.8B)** primarily serotypes 19F, 14, 6B, and 23F.in EJ, by 2016 serotypes 6B and 19A were eliminated, serotype 19F re-emerged following elimination in 2014. Five years following PCV10 implementation, by 2016, the prevalence of serotype 19A and 6A increased significantly (p=0.0150 and p=0.0213, respectively)

In PA region, PA VT13-10 strains declined as well from 18.87% in 2011 to 9.78% in 2014, but re-emerged to 18.06% in 2016. There was no observable effect of PCV10 implementation on the prevalence of VT13-10 isolates during the study period **(**p=0.9772**)**. If we assess the reduction in

VT10 strains 2 to 3 years following the introduction of either PCV7 or PCV10, i.e. in 2011 for EJ, and in 2014 for PA, a reduction of approximately 20% was seen. Moreover, in EJ and PA there was a statistically significant increase in non-vaccine type (non-VT or NVT) strains by 2016 **(**both p<0.0001**) Fiqure 4.9A&B.**

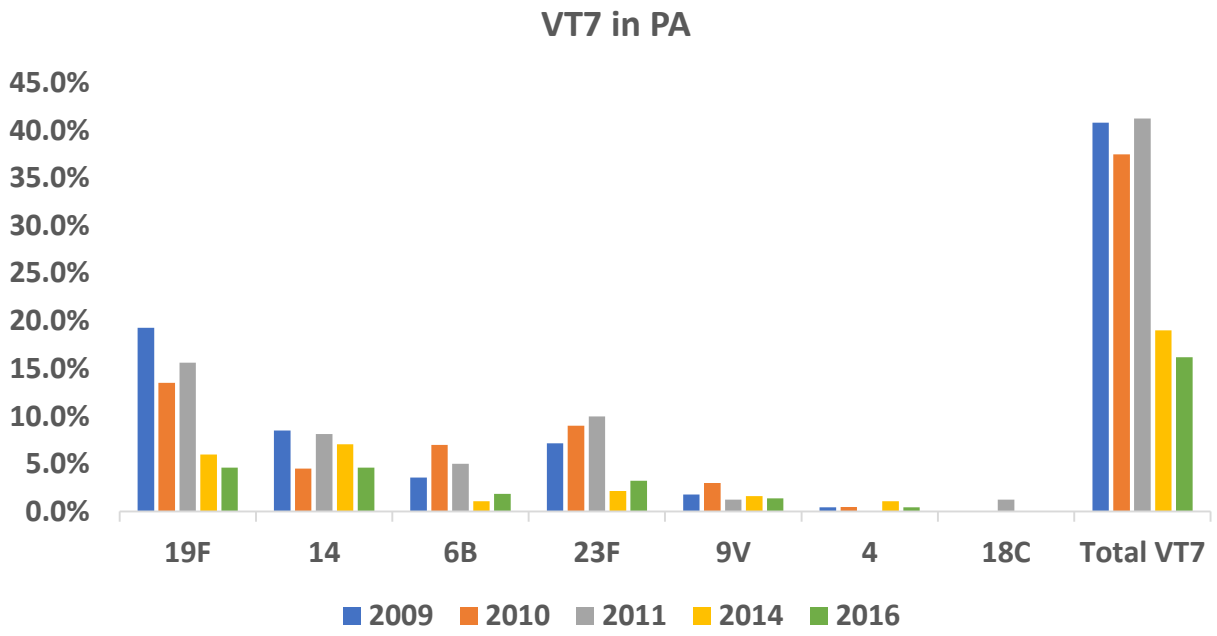


Fiqure 4.9A: VT13-10 strains in EJ decreased significantly (p=0.0327) from 2009 (14.00%) to 2014 (6.9%), followed by decrease in 2016 (3.4%).



Fiqure 4.9B: PA VT13-10 strains declined as well from 18.87% in 2011 to 9.78% in 2014, but re-emerged to 18.06% in 2016.

Following PCV10 implementation in PA, VT7 strains decreased significantly from 41.25% in 2011 to 16.20% in 2016 ($p<0.0001$) Fiqure4.9.



Fiqure 4.10: VT7 in PA strains decreased significantly from 41.25% in 2011 to 16.20% in 2016 ($p<0.0001$).

Between 2009 and 2016, the prevalence of VT7 isolates and VT13-10 isolates decreased significantly ($p<0.0001$ and $p=0.0327$, respectively) in EJ **(Figures 4.8A&4.9A)**. In PA, the prevalence of VT7 isolates decreased significantly during the study period ($p<0.0001$)**(Fiqure 4.8B),** while there was no significant change in the prevalence of VT13-10 isolates **(Figure 4.9B)**.

In both EJ and PA, a significant increase of non-VT isolates was observed between 2009 and 2016 following vaccine implementation ($p<0.0001$ in both regions).

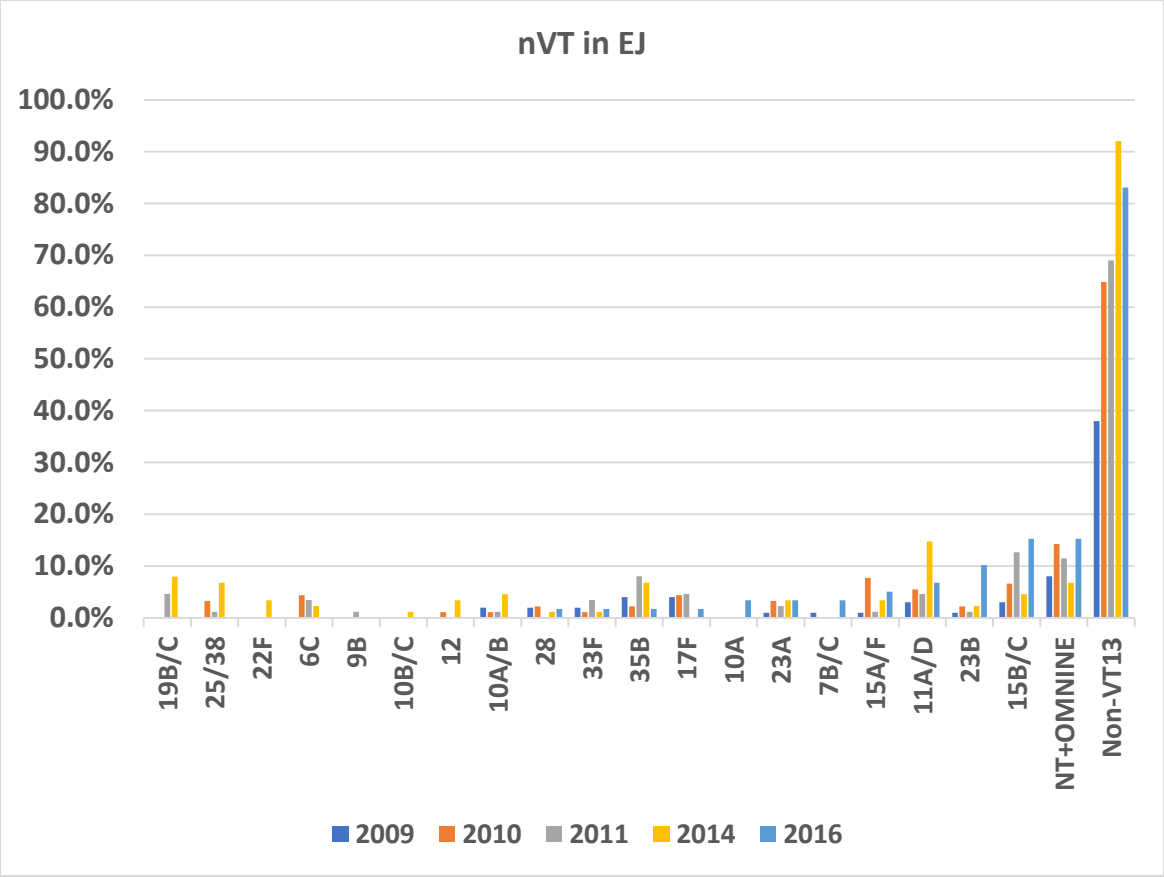


Figure 4.11A: Serotype Distribution of non VT Serotypes in EJ in each year.

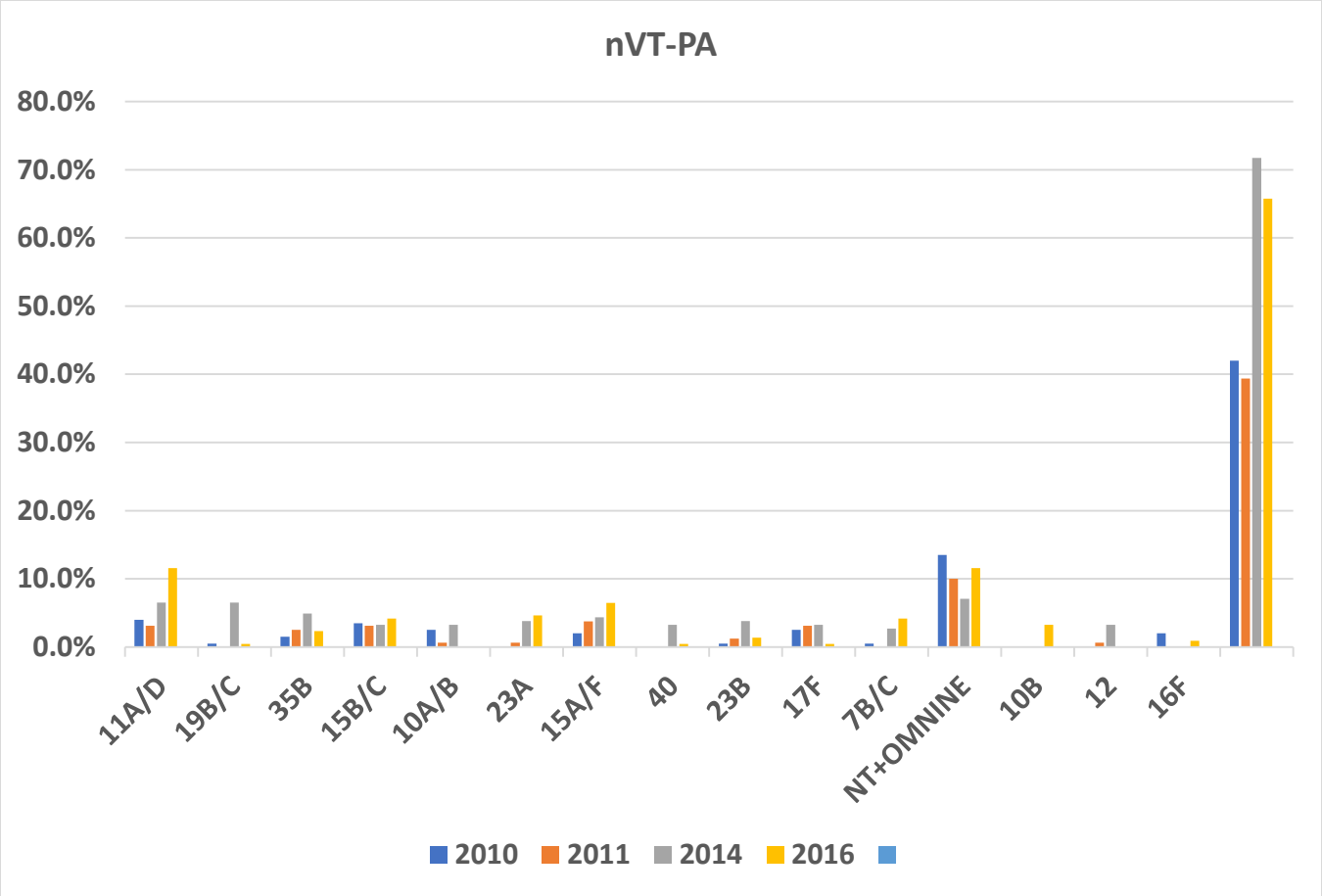


Figure 4.11B: Serotype Distribution of non VT Serotypes in PA in each year.

NonVT13 serotypes gradually replaced VT13 serotypes; therefore, overall carriage of *S. pnemonia* did not change significantly in both regions. The proportion of non-VT13 strains increased from 38.0% of all isolates in pre-PCV surveillance in 2009, to 83% five years after the introduction of PCV13 in EJ ($p<0.001$). As for PA, the proportion of non-VT13 serotypes increased from 39.4% in the pre-PCV period (2011) to 65.7% in 2016 ($p<0.001$). **Table 4.3A&B** presents the most common non-VT13 serotypes in both regions and their proportion among all isolates during the four surveillance periods, as well as p-values of the change pre to post-vaccine periods. By 2016, In both EJ and PA, the Five most common non-VT-13 serotypes are 11A/D(63.45), 15B/C (62.8%) ,35B(38.44%) ,15A/F(36.7%) and 19B/C(20.03%).

nVT -EJ(total %)	2009 (%)	2010 (%)	2011 (%)	2014 (%)	2016 (%)
19B/C	0.00	0.00	4.60	7.95	0.00
25/38	0.00	3.30	1.15	6.82	0.00
22F	0.00	0.00	0.00	3.41	0.00
6C	0.00	4.40	3.45	2.27	0.00
12	0.00	1.10	0.00	3.41	0.00
10A/B	2.00	1.10	1.15	4.55	0.00
33F	2.00	1.10	3.45	1.14	1.69
35B	4.00	2.20	8.05	6.82	1.69
17F	4.00	4.40	4.60	0.00	1.69
10A	0.00	0.00	0.00	0.00	3.39
23A	1.00	3.30	2.30	3.41	3.39
7B/C	1.00	0.00	0.00	0.00	3.39
15A/F	1.00	7.69	1.15	3.41	5.08
11A/D	3.00	5.49	4.60	14.77	6.78
23B	1.00	2.20	1.15	2.27	10.17
15B/C	3.00	6.59	12.64	4.55	15.25
NT+OMNINE	8.00	14.29	11.49	6.82	15.25
Non-VT13	38.00	64.84	68.97	92.05	83.05

Table 4.3A: The proportion of non-VT13 strains increased from 38.0% of all isolates in pre-PCV surveillance in 2009, to 83% five years after the introduction of PCV13 in EJ

nVT -PA(total %)	2009 (%)	2010 (%)	2011 (%)	2014 (%)	2016 (%)
11A/D	3.59	4	3.13	6.52	11.57
19B/C	0	0.5	0	6.52	0.46
35B	4.48	1.5	2.5	4.89	2.31
15B/C	6.73	3.5	3.13	3.26	4.17
10A/B	1.79	2.5	0.63	3.26	0
23A	0.9	0	0.63	3.8	4.63
15A/F	1.79	2	3.75	4.35	6.48
40	0	0	0	3.26	0.46
23B	0.9	0.5	1.25	3.8	1.39
17F	3.14	2.5	3.13	3.26	0.46
7B/C	0	0.5	0	2.72	4.17
NT+OMNINE	8.52	13.5	10	7.07	11.57
10B	0	0	0	0	3.24
12	0	0	0.63	3.26	0
16F	3.14	2	0	0	0.93
Total nvt13 sero-types	50.22	42	39.38	71.74	65.74

Table 4.3B: The proportion of non-VT13 serotypes increased from 39.4% in the pre-PCV period (2011) to 65.7% in 2016.

4.6 Parental carriage

S. pneumoniae carriage among the parents was relatively rare, with 3.3% (n = 157/4696) of parents detected as nasopharyngeal carriers in both regions throughout the five study years. In 2016, *S. pneumoniae* carriage among the parents was 1.4% (n = 18) of parents seen in both EJ (n=1) and PA (n=17). Three of parent strains were belonged to VT7 and two strains were belonged to VT13 only in PA. Parental strains that belonged to non-VT13 serotypes detected in 13 strains in EJ and PA, respectively. All of the 18 parents who were carriers, had a child also carrier of the same serotype. Yet, only eight of those parent-child carrier pairs had an identical serotype on screening. The small sample size of parental strains did not allow us to assess PCV effect on parental carriage or strain distribution.

While in Abu Seir and colleagues, the Overall, 18.3% (n = 23) of parent strains belonged to VT10 serotypes in both regions. Serotypes 14, 19F and 23F constituted the majority of VT10 serotypes (73.9%, n = 17). Parental strains that belonged to non-VT13 serotypes constituted 67.5% (n = 27) and 72.1% (n = 62) in EJ and PA, respectively.

Sixty percent of the parents who were carriers, had a child who was also a pneumococcal carrier, yet, only 42.7% of those parent-child carrier pairs had an identical serotype on screening. In both regions, once PCV was implemented, none of the VT13 carrier parents had a child who carried a VT13 strain.

	EJ	***PA***
	n(%)	n(%)
Parent carriage	49/1616	108/3080
Strains available for serotyping	41	103
VT7	7/41	18/103
VT10	7/41	16/103
VT13	13/41	26/103
Non-VT13	28/41	84/103
Child also carrier of the same sero-type	13/41	27/103

Table4.4: pre-vaccine period in EJ included data form 2009 and PA from 2009-2011. post vaccine period in EJ included data from 2010-2016 and in PA from 2014-2016

4.7Antibiotic use and its resistance

In EJ, 97/100 samples were tested for penicillin non-susceptible *S. pneumoniae* (PNSSP) and penicillin resistant *S. pneumoniae* (PRSP) in 2009, and 90/91 in 2010. In 2011 87/87, 2014 88/88, and in 2016 59/59 samples were tested. Of the samples collected in PA, 192/221 were tested in 2009, 197/198 in 2010, 160/160 in 2011, 183/184 in 2014, and 218/218 in 2016 were tested for PNSSP and PRSP. All samples were present as no samples were missing in examining multi-drug resistant (MDR) isolates in EJ and PA. Between 2009 and 2016, in EJ and PA, there was a non-statistically significant decrease in PNSSP prevalence following vaccine implementation **(**p=0.2251 and p=0.1864, respectively**)**. In EJ, there was a nearly significant overall decrease in PRSP (p=0.0561) and a statistically significant decrease in MDR (p=0.0005) isolates between 2009 and 2016.

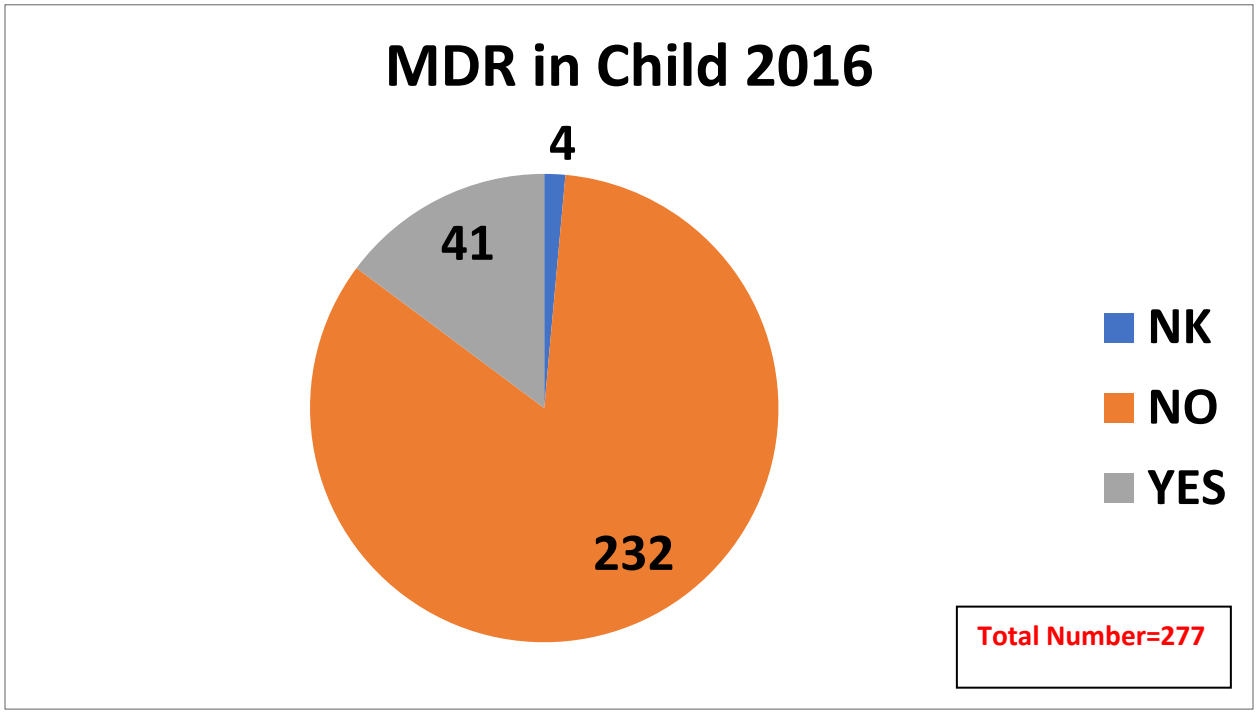


Figure 4.12: Prevalence of isolates number among antibiotic resistant strains.

Antibiotic use was reported in about half the children during the three months preceding to screening in both districts. About7% of antibiotics were not prescribed by primary care physicians in both districts as noted from the medical file, but were informed to have been used by the parent and were habitually bought over the counter. In 2016 surveillance, out of 277 *S.pneumoniae*, 60.6% were did not take any antibiotic, followed by 30.35 carried one antibiotic and 7.2% took two and 0.7% were taken there and more from both regions **(P=0.00).**

Number of antibiotic use in 2016			**0**	**1**	**2**	**3+**	**NK**	
Region	**EJ**	**N**	**41**	**13**	**4**	**1**	**0**	**59**
		%	**69.5%**	**22.0%**	**6.8%**	**1.7%**	**0.0%**	**100.0%**
	PA	**N**	**127**	**71**	**16**	**1**	**1**	**216**
		%	**58.8%**	**32.9%**	**7.4%**	**.5%**	**.5%**	**100.0%**
Total		**N**	**168**	**84**	**20**	**2**	**3**	**277**
		%	**60.6%**	**30.3%**	**7.2%**	**.7%**	**1.1%**	**100.0%**

Table4.5: Number of antibiotic use in 2016 in two region.

Of all *S. pneumoniae* in 2016, 277 isolates (98.6%) were tested for antibiotic susceptibility. With 14.8% (41 strains) were MDR in both EJ and PA. PNSSP prevalence with 56% in both EJ and PA, and 19.5% were PRSP in both regions. Of all *S. pneumoniae*, 829 isolates (96.3%) were tested for antibiotic susceptibility. PNSSP prevalence was extremely high in the first study year, with 83% in both EJ and PA pre-vaccine introduction**.** This is in Daana work(Daana et al. 2015)

Chapter Five:
Discussion, Conclusions, Limitations and Recommendations

This Comparison of PCVs study was conducted to investigate the impact of vaccine of *S. pneumoniae* among Palestinians children. The findings of this study provide an estimate for the prevalence of several of pneumococcal serotypes that have not been previously considered. This chapter highlights the major findings and the recommendations and limitations of the study.

5.1 Discussion

Vaccination and the type of vaccine is an important issue for decreasing the prevalence of diseases caused by S. pneumonia. In this study, we conducted an additional surveillance that was done in 2016, following the introduction of PCV10 in 2011 and to the previously unvaccinated populations.

Thus we evaluated PCVs on pneumococcal carriage among children and their parents and the effects of the different vaccination programs.

In our study, we have shown the effects of the pneumococcal conjugate vaccine on pneumococcal carriage among children, as well as the effects of the examined vaccination programs on serotype distribution in two closely related populations living in EJ, and PA throughout 2009-2016. Here, we conducted an additional surveillance in 2016, following the introduction of PCV10 and PCV7/13 to the previously unvaccinated population and to the population that were vaccinated.

The main effect observed in these two regions was that following the introduction of the pneumococcal conjugate vaccines, the prevalence of VT serotypes reduced until eventually they were nearly eliminated. However, this decrease did not result in an overall decrease in the carriage of *S. pneumoniae* since serotype replacement followed in the two regions, both in the Palestinian Authority (PA) as well as the low socio-economic level populations from East Jerusalem (EJ) regardless of whether PCV10 or PCV7/13 was introduced.

5.2 S. pneumoniae Carriage

For the prevalence of carriage of pneumococci, the only significant change was observed in EJ in 2016, when pneumococcal carriage decreased significantly as compared to all previous years 2009, 2010, 2011 and 2014. The decline in *S. pneumoniae* carriage in EJ goes with the findings of an active surveillance study done in EJ in 2009, which compared *S. pneumoniae* carriage in a middle-class EJ population and a low socio-economic level EJ population. Both PCV7 and PCV13 uptake were high, reaching~90% by July 2012 and December 2013, respectively. That study determined that all-pneumococcal carriage rates significantly declined by approximately 10%, from 54.3% in the early-PCV7 period, to 49.1% in the PCV13 impact period with a significant decline detected in the EJ population, and a mild decline perceived in the EJ population following PCV7 and later PCV13 implementation (Ben-Shimol et al. 2016). This difference in carriage was attributed to overcrowding and large families, similar to the situation observed in EJ. Unlike EJ, in PA, a significant variation was not observed in overall pneumococcal carriage between 2009 and 2016 (~32% on average), and this is probably due to the difference in timing of corresponding vaccine implementations in EJ and PA. The trend in the prevalence of *S. pneumoniae* carriage in PA is predicted to follow the trend observed in EJ 7 years after vaccine implementation due to similar background factors shared in EJ and PA. Therefore, a longer follow-up study is recommended.

The higher effectiveness and cost-effectiveness of PCV13 compared to PCV10 was reported in few studies, one study reported 97% effectiveness against VT-IPD for PCV10, but 86% effectiveness for PCV13 (Deceuninck et al. 2015).

while other studies (Domingues et al. 2014), showed no differences between the two vaccines. So there is disagreement over the advantage of PCV13 compared to PCV10, especially regarding the effect on 19A, that arise following PCV10 in some studies but decrease in other. This may be due to differences in the study design, different outcomes assessed (carriage,IPD, etc.), or different vaccine coverage. However, this may be due to differences in the circulating clones in the different geographic regions.

The explanation for the relatively stable prevalence of *S. pneumoniae* carriage despite the introduction of an effective vaccine is that while VT strains are affected by vaccination, they are replaced by non-VT strains. This phenomenon of serotype replacement has been pronounced in other geographical areas and has been discussed (Flasche et al. 2011).

By the end of our study period in 2016 and after PCV7/13 had been implemented in EJ and PCV10 in PA, similar non-VT serotypes emerged in two regions, including serotypes 10, 11A/D, 15A/F, 15B/C, 23A, 23B, and 7B/C.

5.3 PCV Impact on Serotype Distribution

A trend was observed in EJ where VT7 strains declined from 47% of all serotypes in 2009, prior to vaccine implementation, to 5.0% of all serotypes in 2014, post vaccine implementation. By 2016, we observed a slight non-significant increase in the prevalence of VT7 strains; by that point they account for 8.0% of all isolates in EJ. Additionally, a similar trend was observed in PA, where PCV10 was implemented where the prevalence of VT7 strains was approximately 40.8% prior to vaccine administration and decreased to 16.2% by 2016, 5 years after vaccine implementation.

According to previous studies, serotypes 1, 4, 5, 7F, 8, 12F, 14, 18C, and 19A are prospective to cause IPD. Although serotypes 1 and 19A are the predominant causes of IPD, serotype 14 still one of the most common etiologic agents of non bacteremic pneumonia in adults, even after PCV7 introduction. Serotypes 1, 3, and 19A pneumococci are likely to cause empyema and hemolytic uremic syndrome (J. Y. Song, Nahm, and Moseley 2013)**.**

The observed increase in the prevalence of VT7 strains in EJ during the last year of this study was surprising. This increase can be mainly attributed to the reappearance of serotype 19F, although it is covered by the 3 PCVs, and its prevalence in the population was expected to further decrease with ongoing vaccination. Supporting to our findings, a similar observation was reported in Taiwan, where serotype 19F persisted in the population several years following PCV13 implementation (Cho et al. 2017). The persistence of serotype 19F may be due to its invasiveness, higher colonization rate, and advantages conferred by its antibiotic resistance properties.

Over the course of this study, serotypes 1, 5, and 7F were not detected in the populations in EJ, or PA. This is not surprising, since these serotypes are rarely carried, highly virulent and associated with epidemics.

When comparing the impact of PCV10 with that of PCV13, the most interesting finding is the impact on the serotypes covered by one vaccine, but not the other, (i.e., serotypes 3, 6A, and 19A which are referred in our study as VT13-10 strains). In EJ, a significant decline was observed in the prevalence of VT13-10 between 2009 and 2010, following PCV7 implementation, and may also be attributed to the decrease observed in serotype 6A. Following implementation of PCV13 in EJ, where the proportion of detected VT13-10 fell to 4.55% by 2014 from 8.79% in 2010. However, the prevalence of VT13-10 increased in EJ to 3.39% by 2016. This slight decrease is likely due to a decrease in the prevalence of serotype 3. In PA where PCV10 was introduced, the proportion of VT13-10 strains in the pre-PCV10 era, consisted of 18.75% in 2011, similar to vaccine rates in 2016. This similarity due to the increase in the prevalence of serotype 19A.

In that study (Ben-Shimol et al. 2016), a respective declines for PCV7, 6A and additional PCV13 serotypes carriage rates were 76%, 90% and 66%. In contrast, non-PCV13 serotypes carriage rates increased significantly throughout the study, Interestingly, the comparison of the impact of the different PCVs on serotypes covered by one vaccine, but not the other (serotypes 3, 6A and 19A). Serotype 6A has been repeatedly reported to decrease following PCV7 or PCV10 implementation (J. Y. Song, Nahm, and Moseley 2013).

This is due to cross-protection by 6B in PCV7/PCV10. Serotype 19A, which is not included in either PCV7or PCV10, could similarly be cross-protected by 19F which is included in both these vaccines. However, only modest protection has been suggested (Hausdorff, Hoet, and Schuerman 2010). In EJ, following PCV10 implementation, the prevalence of VT13-10 strains decreased to 10.33% in 2014. This surprising effect was due to a significant decrease in both serotypes 6A and 19A, within 3 years of PCV implementation. This decrease could potentially be explained by cross-reaction of the PCV10 effect on 6B and 6A and similarly on 19F and 19A. While cross-reaction between 6A and 6B has been repeatedly reported (Whitney et al. 2006), there is much debate about the effect of PCV10 on 19A. (Prymula et al. 2006).

Unlike PCV10, the use of PCV7 has been repeatedly shown to be followed by increases in 19A and no cross-reactivity is assumed for this vaccine (Moore et al. 2008).

PCV7 introduction in many countries, led to emergence of serotype 19A both in carriage and in IPD (Isturiz et al. 2017),that did not observe emergence of 19A in EJ after PCV7 and before PCV13 was implemented. Similarly a nationwide IPD surveillance study in Israel did not report 19Aincrease following PCV7 introduction among children or adults (Regev-Yochay et al. 2017).

A plausible explanation could be the rapid transition from PCV7 to PCV13 within less than two years in Israel, or a different clonal background distribution. In contrast to PCV7, the impact of PCV10 on serotype 19A is much more debated. Several studies reported PCV10 effectiveness against serotype 19A (Wagenvoort et al. 2016).

Invasive Pneumococcal Disease 3 Years after Introduction of 10-Valent Pneumococcal Conjugate Vaccine, the Netherlands .Emerging infectious diseases. while other studies reported emergence of serotype 19A following PCV10 introduction (Huang et al. 2009). The changing epidemiology of invasive pneumococcal disease at a tertiary children's hospital through the 7-valent pneumococcal conjugate vaccine era: a case for continuous surveillance (Isaacman, McIntosh, and Reinert 2010).

Interestingly, in PA, the decrease in VT13-10 strains was temporally transient and likely due to changes after PCV10 implementation. By 2016, the prevalence of VT13-10 strains in that region had increased to 18.06%, and this increase was mainly due to an increase in serotype 19A. Our results were consistent with other studies that have compared the effects of PCV7, PCV10, and PCV13. In our study, PCV10 does not appear to be as effective as PCV13 in targeting serotype 19A, suggesting that PCV10 does not provide direct immunity against serotype 19A (Isturiz et al. 2017; Wysocki et al. 2009). These results emphasize the need to continue and monitor the changing epidemiology and serotype distribution of *S. pneumoniae* following different vaccination policies. However, it is important to note that these are only short-term (three years) observations, and longer follow-up is mandatory to determine the long-term effect of PCV10 on serotype 19A. Serotype 3 is one of the three additional serotypes not included in PCV10, is unique in many aspects, heavily encapsulated with a mucoid phenotype, highly resistant to phagocytosis. While some have suggested that despite this it is not invasive (Brueggemann et al. 2004).
Many studies from different geographical regions have reported ineffectiveness of PCV13 against serotype 3 (Naucler et al. n.d.). We show a nearly significant increase in serotype 3

following PCV13 implementation in EJ (p = 0.05) and no change after PCV10 implementation in PA. Whether serotype 3 will eventually decrease in a longer follow-up is yet to be observed.

Serotype 6C, a non-VT serotype, is biochemically different from serotype 6A and 6B, but serologically similar to serotype 6A (Park et al. 2007). Serotype 6C was reported to increase in prevalence in carriage and in IPD cases following PCV7 implementation in the United States (Millar et al. 2010). Cross protection between serotype 6A in PCV13 and 6C has been reported (Cooper et al. 2011).

In EJ, following PCV7 implementation, the prevalence of serotype 6C increased in 2010 (4.4%), while following PCV13 implementation, the prevalence of serotype 6C decreased and was eliminated by 2016 (0.0%), similar to the trend reported in the United States. Unlike EJ, in PA, the prevalence of serotype 6C increased following PCV10 implementation. This was in line with our expectations based on the fact that cross protection against serotype 6C was not seen for PCV10 in a cross-sectional study conducted in Brazil in 2010 and 2013 (M.-C. C. de Brandileone et al. 2016).

Serotype replacement is a common phenomenon in which non-VT serotypes emerge and replace VT serotypes with geographic variability(Weinberger, Malley, and Lipsitch 2011).

The emerging serotypes we observed following the two different vaccination policies were nearly similar. In 2016 surveillance, In EJ, the most notable emerging non-VT serotypes were 15B/C and 23B and in PA they were 11A/D, 15B/C, 23A, 15A/F, 7B/C and 10B. while in 2014, In EJ, the most notable emerging non-VT serotypes were 11A/D, 19B/C, 25/38, 40, 21, 9N/L and 12F and in PA they were 19B/C, 40, 23A and 7B/C(Abu Seir et al. 2018). A study in Massachusetts, USA reported that several years following PCV7 implementation, serotypes 19A, 6A, 15B/C, 35B,and 11A emerged (Huang et al. 2009).

In Northern Japan, serotypes 15A, 23A, 11A, 10A and 35B accounted for the majority of non-VT13 serotypes after the introduction of PCV13 (Kawaguchiya et al. 2016)Serotype 6C was shown to decline after PCV13 introduction but not PCV7 or PCV10 (M. C. de C. Brandileone et al. 2016).Interestingly, we observed emergence of 6C following PCV7 implementation, but a decrease after PCV13 in EJ, but increased after PCV10 implementation in PA.

5.4 Antibiotic Resistance

Since antibiotic resistance was relatively common among VT strains, and most of the observed antibiotic-resistant strains were VT strains, it was important to note that more than 60% did not use antibiotics. In 2009-2011 study (Daana et al. 2015), antibiotic resistance in the pre-vaccine period was wide spread in both EJ and PA, similar to observed rates in EJ populations (Greenberg et al. 2008). but much higher than reported in USA, Europe or Israeli Jewish populations (Huang et al. 2009), where approximately 10% PRSP and 35% PNSSP have been reported. This may be expected considering the high antibiotic use reported in our population. In 2016, PNSSP prevalence with 56% in both EJ and PA, and 19.5% were PRSP in both regions. With 14.8% (41 strains) were MDR in both EJ and PA. Since most antibiotic resistant strains in the pre-PCV period belonged to VT serotypes, it was not surprising that the introduction of PCV7/PCV13 and PCV10 resulted in decline in resistant-VT strains. Nevertheless, this increase in antibiotic resistance may serve as a window of opportunity to enhance careful antibiotic use that may be required together with PCV pressure to produce along-lasting reduction in antibiotic resistant pneumococci. The prevalence of antibiotic-resistant *S. pneumoniae* has increased worldwide and might be related to the spread of pediatric pneumococcal serotypes (6A, 6B, 9V, 14, 15A, 19F, 19A, and 23F). After the introduction of PCV7, the prevalence of PCV7 serotypes and serotype 6A were reduced and subsequently replaced by non-PCV7 serotypes, including serotype 19A. Although Spain 23F-ST81 and Spain6B-ST90 were well-established multidrug-resistant clones in the 1980s and 1990s, their prevalence decreased after the introduction of PCV7.

Parental nasopharyngeal pneumococcal carriage was rare in our population, this could be due to our determination of the carriage via nasopharyngeal swabbing, while recent reports suggested higher yield in adults when swabbing both pharynx and nasopharynx or adding salivary testing (Trzciński et al. 2013).

In 2016, only 45.3% of those parent child co-carrier pairs had an identical serotype, While only 42.7% of those parent child co-carrier pairs had an identical serotype (Rania et al., 2018).

Similar dis concordance was previously reported among adults in Israel, where intra-familial transmission could not be demonstrated (Regev- Yochay, Raz, et al. 2004).

Children were shown to carry pneumococcal strains for months, while adults typically carry pneumococci for only very short durations (Shak, Vidal, and Klugman 2013),Yet, this does not essentially explain the relative dis-concordance of serotypes between children and their parents. The difference between serotypes carried by children's and their parents' could be due to the difference of the direct PCV impact and the indirect (herd effect) impact, on their parents, particularly the potential time lag of the effects.

5.5 Study Limitations

It is important to note that this study was an observational study and not a controlled intervention trial. So the following points we considered as limitation to our study**. First:** the policies of vaccination were introduced at different times, PCV7/13 in 2009/2010 in EJ, andPCV10 in 2011 in PA. Those differences between the private clinics of PA and EJ could be an additional concern, although the socioeconomic status of the people who visit those private clinics is similar to that of the population served in EJ. **Second,** while the two compared populations are closely related, they differed in several variables that were adjusted. For our study did not consider possible mixing or cross-contamination between EJ and PA due to the mixing of the populations of those regions, but such mixing is most likely limited due to existing barriers. **Third,** the overall carriage in the children was relatively low, probably due to the 'off season' periods we chose, i.e. spring and summer, when carriage of pneumococci is lower. This was intentional in order to overcome seasonal variability, but limited the power to detect some differences.

5.6 Conclusion

In conclusion, following the introduction of PCV, the overall prevalence of carriage did not change significantly, but the prevalence of vaccine-type serotypes decreased and those serotypes were nearly eliminated, regardless of which PCV was used. Despite the short follow-up interval after implementation of PCV13 in EJ, a dramatic decrease in the VT13 serotypes (including serotypes 6A and 19A but not serotype 3) was observed in EJ. While implementation of PCV10 in PA shows a dramatic increase in VT13 serotypes in PA. Replacement by non-VT13 was also

observed in both populations regardless of the vaccination used. Longer follow up is needed to compare the long-term effects.

5.7 Recommendations

Our study highlights the need for large population-based reconciliation studies. Further studies should be conducted on the PCV. Moreover, in addition to NVT, the evaluation of PCVs lead to decreased deaths of children. This study is short follow-up interval that only reports the short-term impact of the vaccines, so to assess the long-term differences between the vaccines, longer follow-up is needed. Educational and awareness programs are recommended for both physicians and medical laboratories for appropriate and reliable diagnosis and appropriate antibiotic treatment decisions. This can help reduce the development of more complex antibiotics

Chapter Six

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Chapter Seven:

Appendices

Table 6.1: Frequency of VT Serotypes in PA

Serotype	PA					
	2009	2010	2011	2014	2016	p-trend
	sp (n=223)	sp (n=200)	sp (n=160)	sp (n=184)	sp (n=216)	
	N %	N %	N %	N %	N %	2009-2014
19F	43	27	25	11	10	P = 0.000
	19.28%	13.50%	15.63%	5.98%	4.63%	
14	19	9	13	13	10	P = 0.291
	8.52%	4.50%	8.13%	7.07%	4.63%	
6B	8	14	8	2	4	P = 0.030
	3.59%	7.00%	5.00%	1.09%	1.85%	
23F	16	18	16	4	7	P = 0.007
	7.17%	9.00%	10.00%	2.17%	3.24%	
9V	4	6	2	3	3	P = 0.473
	1.79%	3.00%	1.25%	1.63%	1.39%	
4	1	1	0	2	1	P = 0.726
	0.45%	0.50%	0.00%	1.09%	0.46%	
18C	0	0	2	0	0	P = 0.977
	0.00%	0.00%	1.25%	0.00%	0.00%	
Total VT7	**91**	**75**	**66**	**35**	**35**	**P = 0.000**
	40.81%	37.50%	41.25%	19.02%	16.20%	
7F	0	1	0	0	0	P = 0.511
	0.00%	0.50%	0.00%	0.00%	0.00%	
1	2	0	0	0	0	ND
	0.90%	0.00%	0.00%	0.00%	0.00%	
5	0	0	1	0	0	P = 0.983
	0.00%	0.00%	0.63%	0.00%	0.00%	
Total VT10	**2**	**1**	**1**	**0**	**0**	**P = 0.098**
	0.90%	0.50%	0.63%	0.00%	0.00%	
6A	21	25	17	9	13	P = 0.024
	9.42%	12.50%	10.63%	4.89%	6.02%	
19A	5	9	6	2	19	
	2.24%	4.50%	3.75%	1.09%	8.80%	
3	4	5	7	7	7	P = 0.262

	1.79%	2.50%	4.38%	3.80%	3.24%	
Total VT13	**30**	**39**	**30**	**18**	**39**	**P = 0.917**
	13.45%	19.50%	18.75%	9.78%	18.06%	
All VT	123	115	97	53	74	P = 0.000

Table 6.2: Frequency of non-VT (NVT) Serotypes in PA

	Non-VT13 SEROTYPES PA					
	2009	**2010**	**2011**	**2014**	**2016**	**p-trend**
	sp (n=223)	**sp (n=200)**	**sp (n=160)**	**sp (n=184)**	**sp (n=216)**	
	N %	**N %**	**N %**	**N %**	**N %**	**2009-2014**
11A/D	8	8	5	12	25	P = 0.000
	3.59%	4.00%	3.13%	6.52%	11.57%	
19B/C	0	1	0	12	1	P = 0.014
	0.00%	0.50%	0.00%	6.52%	0.46%	
25/38	0	2	1	4	0	P = 0.569
	0.00%	1.00%	0.63%	2.17%	0.00%	
35B	10	3	4	9	5	P = 0.706
	4.48%	1.50%	2.50%	4.89%	2.31%	
15B/C	15	7	5	6	9	P = 0.210
	6.73%	3.50%	3.13%	3.26%	4.17%	
10A/B	4	5	1	6	0	P = 0.266
	1.79%	2.50%	0.63%	3.26%	0.00%	
12F	0	0	1	6	0	P = 0.110
22F	0	1	1	2	2	P = 0.150
	0.00%	0.50%	0.63%	1.09%	0.93%	
23A	2	0	1	7	10	P = 0.000
	0.90%	0.00%	0.63%	3.80%	4.63%	
15A/F	4	4	6	8	14	P = 0.004
	1.79%	2.00%	3.75%	4.35%	6.48%	
40	0	0	0	6	1	P =

						0.035
	0.00%	0.00%	0.00%	3.26%	0.46%	
21	2	1	0	1	2	P = 0.959
	0.90%	0.50%	0.00%	0.54%	0.93%	
9N/L	1	3	1	1	4	P = 0.354
	0.45%	1.50%	0.63%	0.54%	1.85%	
6C	1	3	2	0	5	P = 0.273
	0.45%	1.50%	1.25%	0.00%	2.31%	
23B	2	1	2	7	3	P = 0.136
	0.90%	0.50%	1.25%	3.80%	1.39%	
17F	7	5	5	6	1	P = 0.150
	3.14%	2.50%	3.13%	3.26%	0.46%	
7B/C	0	1	0	5	9	P = 0.000
	0.00%	0.50%	0.00%	2.72%	4.17%	
8	0	2	0	1	0	P = 0.722
	0.00%	1.00%	0.00%	0.54%	0.00%	
9A	1	0	0	2	0	P = 0.971
	0.45%	0.00%	0.00%	1.09%	0.00%	
9B	0	0	1	0	0	P = 0.983
	0.00%	0.00%	0.63%	0.00%	0.00%	
NT+OMNINE	19	27	16	13	25	P = 0.940
	8.52%	13.50%	10.00%	7.07%	11.57%	
10A	0	0	0	0	1	P = 0.168
	0.00%	0.00%	0.00%	0.00%	0.46%	
10B	0	0	0	0	7	P = 0.000
	0.00%	0.00%	0.00%	0.00%	3.24%	
10B/C	0	0	0	1	0	P = 0.485
	0.00%	0.00%	0.00%	0.54%	0.00%	
10F/C	2	1	0	0	0	P = 0.054
	0.90%	0.50%	0.00%	0.00%	0.00%	

12	0	0	1	6	0	P = 0.110
	0.00%	0.00%	0.63%	3.26%	0.00%	
13	0	0	0	0	4	P = 0.006
	0.00%	0.00%	0.00%	0.00%	1.85%	
13/47	3	3	2	4	0	P = 0.362
	1.35%	1.50%	1.25%	2.17%	0.00%	
16F	7	4	0	0	2	P = 0.010
	3.14%	2.00%	0.00%	0.00%	0.93%	
18A/B/F	1	1	1	1	0	P = 0.524
	0.45%	0.50%	0.63%	0.54%	0.00%	
24	3	1	0	0	2	P = 0.434
	1.35%	0.50%	0.00%	0.00%	0.93%	
28	4	0	2	2	2	P = 0.715
	1.79%	0.00%	1.25%	1.09%	0.93%	
29	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
31	2	0	2	0	3	P = 0.569
	0.90%	0.00%	1.25%	0.00%	1.39%	
32	1	0	0	0	0	ND
	0.45%	0.00%	0.00%	0.00%	0.00%	
33A/B/C	1	0	0	1	0	P = 0.652
	0.45%	0.00%	0.00%	0.54%	0.00%	
33F	2	0	1	1	3	P = 0.409
	0.90%	0.00%	0.63%	0.54%	1.39%	
34	1	0	2	0	0	P = 0.454
	0.45%	0.00%	1.25%	0.00%	0.00%	
35AC/42	0	0	0	0	2	P = 0.051
	0.00%	0.00%	0.00%	0.00%	0.93%	
35F47F	0	0	1	2	0	P = 0.412
	0.00%	0.00%	0.63%	1.09%	0.00%	
36/37	2	0	0	1	0	P =

						0.254
	0.90%	0.00%	0.00%	0.54%	0.00%	
39	0	0	0	1	0	ND
	0.00%	0.00%	0.00%	0.54%	0.00%	
42	4	0	0	0	0	ND
	1.79%	0.00%	0.00%	0.00%	0.00%	
43/44/46	1	0	0	1	0	P = 0.652
	0.45%	0.00%	0.00%	0.54%	0.00%	
7	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
7A	1	0	0	0	0	ND
	0.45%	0.00%	0.00%	0.00%	0.00%	
22A	1	0	0	0	0	ND
	0.45%	0.00%	0.00%	0.00%	0.00%	
17A	0	0	0	3	0	P = 0.226
	0.00%	0.00%	0.00%	1.63%	0.00%	
Total non-VT13 SEROTYPES	**112**	**84**	**63**	**132**	**142**	
	50.22%	**42.00%**	**39.38%**	**71.74%**	**65.74%**	**P = 0.000**

Table 6.3: Frequency of VT Serotypes in EJ

	East Jerusalem					
	2009	**2010**	**2011**	**2014**	**2016**	**p-trend**
	sp (n=100)	**sp (n=91)**	**sp (n=87)**	**sp (n=88)**	**sp (n=59)**	
	N %	**N %**	**N %**	**N %**	**N %**	**2009-2016**
19F	16	9	5	0	2	P = 0.000
	16%	9.89%	5.75%	0.00%	3.39%	
14	9	4	3	2	2	P = 0.045
	9%	4.40%	3.45%	2.27%	3.39%	
6B	13	9	7	0	1	P = 0.000
	13%	9.89%	8.05%	0.00%	1.69%	
23F	6	1	5	2	2	P = 0.480
	6%	1.10%	5.75%	2.27%	3.39%	
9V	1	0	1	1	0	P = 0.866
	1%	0.00%	1.15%	1.14%	0.00%	
4	0	0	0	0	1	P = 0.108
	0	0.00%	0.00%	0.00%	1.69%	
18C	2	1	0	0	0	P = 0.063
	2%	1.10%	0.00%	0.00%	0.00%	
Total VT7	**47**	**24**	**21**	**5**	**8**	**P = 0.000**
	47%	**26.40%**	**24%**	**5.70%**	**13.50%**	
7F	0	0	0	0	0	ND
	0	0.00%	0.00%	0.00%	0.00%	
1	0	0	0	0	0	ND
	0	0.00%	0.00%	0.00%	0.00%	
5	1	0	0	0	0	ND
	1%	0.00%	0.00%	0.00%	0.00%	
Total VT10	**1**	**0**	**0**	**0**	**0**	**ND**
	1%	0.00%	0.00%	0.00%	0.00%	
6A	11	5	4	0	0	P = 0.000
	11%	5.49%	4.60%	0.00%	0.00%	
19A	3	2	1	1	0	P = 0.119
	3%	2.20%	1.15%	1.14%	0.00%	
3	0	1	1	3	2	P = 0.040
	0	1.10%	1.15%	3.41%	3.39%	
Total VT13	**14**	**8**	**6**	**4**	**2**	**P = 0.006**
	14.00%	8.79%	6.90%	4.55%	3.39%	
All VT	62	32	27	9	10	P = 0.000

Table 6.4: Frequency of non-VT (NVT) Serotypes in EJ

Serotype	Non-VT13 SEROTYPES EJ					
	2009	2010	2011	2014	2016	p-trend
	sp (n=100)	sp (n=91)	sp (n=87)	sp (n=88)	sp (n=59)	
	N %	N %	N %	N %	N %	2009-2014
11A/D	3	5	4	13	4	P = 0.027
	3.00%	5.49%	4.60%	14.77%	6.78%	
19B/C	0	0	4	7	0	P = 0.040
	0.00%	0.00%	4.60%	7.95%	0.00%	
25/38	0	3	1	6	0	P = 0.243
	0.00%	3.30%	1.15%	6.82%	0.00%	
35B	4	2	7	6	1	P = 0.738
	4.00%	2.20%	8.05%	6.82%	1.69%	
15B/C	3	6	11	4	9	P = 0.028
	3.00%	6.59%	12.64%	4.55%	15.25%	
10A/B	2	1	1	4	0	P = 0.876
	2.00%	1.10%	1.15%	4.55%	0.00%	
12F	0	1	0	3	0	P = 0.305
	0.00%	1.10%	0.00%	3.41%	0.00%	
22F	0	0	0	3	0	P = 0.128
	0.00%	0.00%	0.00%	3.41%	0.00%	
23A	1	3	2	3	2	P = 0.349
	1.00%	3.30%	2.30%	3.41%	3.39%	
15A/F	1	7	1	3	3	P = 0.565
	1.00%	7.69%	1.15%	3.41%	5.08%	
40	0	0	0	2	1	P = 0.052
	0.00%	0.00%	0.00%	2.27%	1.69%	
21	0	0	0	2	0	P = 0.214
	0.00%	0.00%	0.00%	2.27%	0.00%	
9N/L	0	0	2	2	1	P = 0.101
	0.00%	0.00%	2.30%	2.27%	1.69%	
6C	0	4	3	2	0	P = 0.961
	0.00%	4.40%	3.45%	2.27%	0.00%	

23B	1	2	1	2	6	P = 0.008
	1.00%	2.20%	1.15%	2.27%	10.17%	
17F	4	4	4	0	1	P = 0.128
	4.00%	4.40%	4.60%	0.00%	1.69%	
7B/C	1	0	0	0	2	P = 0.272
	1.00%	0.00%	0.00%	0.00%	3.39%	
8	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
9A	0	0	0	1	0	ND
R	0.00%	0.00%	0.00%	1.14%	0.00%	
9B	0	0	1	0	0	ND
	0.00%	0.00%	1.15%	0.00%	0.00%	
NT+OMNINE	8	13	10	6	9	P = 0.632
	8.00%	14.29%	11.49%	6.82%	15.25%	
10A	0	0	0	0	2	P = 0.023
	0.00%	0.00%	0.00%	0.00%	3.39%	
10B	0	0	0	0	1	P = 0.108
	0.00%	0.00%	0.00%	0.00%	1.69%	
10B/C	0	0	0	1	0	ND
	0.00%	0.00%	0.00%	1.14%	0.00%	
10F/C	0	0	0	1	0	ND
	0.00%	0.00%	0.00%	1.14%	0.00%	
12	0	1	0	3	0	P = 0.031
	0.00%	1.10%	0.00%	3.41%	0.00%	
13	0	0	0	0	1	P = 0.108
	0.00%	0.00%	0.00%	0.00%	1.69%	
13/47	1	1	1	1	0	P = 0.660
	1.00%	1.10%	1.15%	1.14%	0.00%	
16F	1	1	0	1	1	P = 0.769
	1.00%	1.10%	0.00%	1.14%	1.69%	
18A/B/F	0	0	1	0	1	P = 0.214
	0.00%	0.00%	1.15%	0.00%	1.69%	
24	1	0	1	2	0	P = 0.769
	1.00%	0.00%	1.15%	2.27%	0.00%	
28	2	2	0	1	1	P = 0.942
	2.00%	2.20%	0.00%	1.14%	1.69%	
29	1	0	0	1	0	P = 0.756
	1.00%	0.00%	0.00%	1.14%	0.00%	

31	0	0	1	0	0	P = 0.884
	0.00%	0.00%	1.15%	0.00%	0.00%	
32	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
33A/B/C	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
33F	2	1	3	1	1	P = 0.917
	2.00%	1.10%	3.45%	1.14%	1.69%	
34	1	1	0	1	0	P = 0.554
	1.00%	1.10%	0.00%	1.14%	0.00%	
35AC/42	0	0	0	0	1	P = 0.108
	0.00%	0.00%	0.00%	0.00%	1.69%	
35F47F	0	0	0	1	0	P = 0.381
	0.00%	0.00%	0.00%	1.14%	0.00%	
36/37	0	0	0	0	1	P = 0.108
	0.00%	0.00%	0.00%	0.00%	1.69%	
39	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
42	0	2	0	0	0	P = 0.408
	0.00%	2.20%	0.00%	0.00%	0.00%	
43/44/46	0	0	0	1	0	P = 0.381
	0.00%	0.00%	0.00%	1.14%	0.00%	
7	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
7A	0	0	0	0	0	ND
	0.00%	0.00%	0.00%	0.00%	0.00%	
22A	1	0	0	0	0	ND
	1.00%	0.00%	0.00%	0.00%	0.00%	
17A	0	0	1	0	0	P = 0.408
	0.00%	0.00%	1.15%	0.00%	0.00%	
Total non-VT13 SEROTYPES	**38**	**59**	**60**	**81**	**49**	
Non-VT13	**38.00%**	**64.84%**	**68.97%**	**92.05%**	**83.05%**	**P = 0.001**

Freezing Thawing Protocol for *S. pneumonia* isolates

1. Fresh grown colonies of *S. aureus* should be frozen in 25% glycerol/BHI at -80^{o}C. For short durations (up to 1 week freezing in -4^{o}c is adequate):
    a. Label the side of the eppendorf tube with the same code that was given to the patient in the original sampling laboratory and also use a new serial number for each patient and label the cap of the eppendorf
    b. Weigh **37.8** gram of brain heart infusion (BHI) and put in a graduated cylinder
    c. Add ddH2O till 750ml
    d. Stir on a stirrer till the solution is homogenous
    e. Add 250ml of pure glycerol and
    f. Mix well and autoclave
    g. Aliquote 1 ml of the freezing solution in a screw capped sterile eppendorf tubes
    h. Freeze to be used when the bacteria is available, then thaw
    i. Swab as much as possible of the pure fresh grown isolates and mix with the freezing solution, squeeze on the edges of the Eppendorf tube .
    j. Freeze at -80^{o}C. For short durations (up to 1 week freezing in -20^{o}C is adequate)
    k. Enter all patient data into your computerized system by taking the bar code of the label and then enter the serial number manually, box number, freezer number, shelf number, then all patients data and susceptibility results…(according to the built patient sheet)

Thawing Protocol for *S. pneumonia* isolates

1. Take out the required frozen samples from the -80^{o}C freezer according to the freezing numbers.
2. Open the box in the hood, open the Eppendorf carefully and don't cause contamination (perform it quickly otherwise put the samples on ice to prevent complete thawing)
3. Streak a bit of the frozen bacterial on blood agar (half a plate or use one plate for three samples).
4. Incubate at 37^{o}C for 24hrs to proceed in serotyping and PCRs
5. In case of contamination, try to get a pure culture by streaking a single colony on blood agar (you can use blood agar with gentamycin for *Streptococcus pneumoniae)*

Freeze Tube Protocol for *S. pneumonia* isolates

1. Add 37.8 gram of brain heart infusion (BHI).
2. Dissolve BHI with 1 letter of dH2O.
3. Add 333.33 ml glycerol to solution.
4. Put in autoclave.

- This protocol for 1 liter,1000 freeze tube approximately.
- If we need less than 1L can we prepare about 500 ml dH2O with 18.9 g of BHI and 166.66 ml glycerol.

Protocol for culturing *Streptococcus pneumonia*

1. Label all of the required plates and eppendorf tubes with the same original patient green code that was given in the original laboratory
2. Subculture colonies on 3 blood agar plates for **identification** and **susceptibility testing (AST)**
    a. **Two Blood agar** for antibiotic susceptibility testing as the following protocol:
        i. Put 1-2ml Brain Heart Infusion Broth in a clear tube
        ii. Take one small colony of *Streptococcus pneumonia*, dip in the tube and mix till you get the same turbidity as McFarland standard tube
        iii. Streak on two plates of blood agar and add the following antibiotics:
            1. Plate I: cefoxitin, erythromycin, clindamycin, gentamicin and TMP/SMX (Trimethoprim-sulfamethoxazole)
            2. Optochin disc diffusion and strips of penicillin and ceftriaxone
            3. Plate II tetracycline, rifampin, fusicid acid, ofloxacin and vanco-mycin.check??
    b. **Blood agarfor freezing**: to be frozen for research purposes
3. Incubate at 37^{o}C and 5% CO_2 for 18hrs
4. On the second day, report antibiotic susceptibility testing results
5. Freeze optochin sensitive fresh grown colonies of *Streptococcus pneumonia* according to freezing protocol in a screw capped 1.5ml eppendorf tube that is also labeled with the

same patient code and also given a serial number on the cap – fresh grown colonies on blood agar 25% glycerol/BHI at -80^{o}C.

6. Enter the patients data and AST results into the computerized research file

Protocol for invasive pneumococcal disease (IPD)

in Hospitals

1. Start from positive blood culture bottles – Following 24h incubation in 37^{o}C in 5% CO_2
2. Differentiate gram positive from gram negative by culturing the sample on blood agar, macconkey agar and on chocolate agar.
3. Incubate for 18 hrs at 37 ^{o}C
4. In case of alpha hemolysis and colony characteristics typical to *Streptococcus pneumoniae* then sub-culture as the following:
    a. Plate 1: on Blood agar (to be transported for research) and call the person in charge for this project
    b. Plate 2 &3: on blood agar for antibiotic susceptibility testing (AST) for optochin (ethylhydrocupreine hydrochloride) sensitivity and other Antibiotics susceptibility) including penicillin, ceftriaxone, erythromycin and clindamycin should be performed following CLSI guidelines. Other suggested antibiotics: tetracycline, TMP/SMX, ofloxacin and vancomycin.
5. label plate 1 of optochin susceptible isolates with a green barcode and label the patient data sheet with the same code
6. Incubate all plates at 37^{o}C for 18hrs and confirm your finding to the project assigned person.
7. Fill basic data collection of each case - see attached questionnaire
8. Report if *Step. pneumoniae* was within 72h of hospitalization or after 72h of hospitalization
9. Into your computer research file, fill in all the required data
10. Arrange for plate 1 to be transported at 4^{o}C during **8 hours** to the central laboratory in Ramallah
11. In the central laboratory in Ramallah, plates and their data sheets should handed to _____
12. Call the person in charge of receiving the samples to confirm proper transportation and handling

Data Form

ID of patient_____

Date of Birth_____ Date of SP isolation_____

Source: Blood / CSF / Other_____

Address_____ City_____

Date of Hospitalization_____ Date of Discharge_____

At discharge: Alive / Dead

Antibiotic Susceptibility:

	Pen	Cef	Ery	Clin	Tet	TMP/ SMX	Oflo	van
S								
I								
R								
MIC								

Additional data (from Medical file):

Major Diagnosis: Pneumonia / Meningitis / Bacteremia with no source / sepsis / Other_____

Comorbidities:

	Yes	No		Yes	No
Smoker			Immunosuppressed		
Alcohol Abuse			HIV		
Diabetes Mellitus			Hematologic Malignancy		
Ch. Lung Dis			Bone Marrow Transplant		
Congestive Heart Dis.			Past Neurosurg / CSF leak		
Ch. Liver Dis.			Cirrhosis		
IV drug abuse			Ch. Renal Failure		
Malignancy (non-hematologic)			Splenectomy / asplenic		

Protocol for *Streptococcus pneumonia* in the Central Laboratory in Ramallah

1. In the central laboratory, keep the plates at $4^{o}C$
2. Inform the assigned student **that Streptococcus pneumonia** samples arrived
3. Samples should be processed directly according to the below protocol and should be kept at $-80^{o}C$