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The impact of pneumococcal conjugate vaccine on pneumococcal nasopharyngeal ecology in children 2 months through 5 years

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Thesis

THE IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINE ON PNEUMOCOCCAL NASOPHARYNGEAL ECOLOGY IN CHILDREN 2 MONTHS THROUGH 5 YEARS

by

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"We look to medical research to discover remedial measures to insure better health and more happiness for mankind."

Thomas Hunt Morgan

DEDICATION

I would like to dedicate this work to my parents Asya and Ahmad Anthony Khan, my sisters Adilah and Mareyah Khan, my grandparents Mohammed and Bibi Ameena Amin and Omar Farouk and Amina Thairun Khan, my aunt Akela Amin, my uncle John Amin and my pets, Zain and Zara Khan. These individuals taught me to live a life fueled by passion, purpose and authenticity. For that, I am forever grateful.

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THE IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINE ON PNEUMOCOCCAL NASOPHARYNGEAL ECOLOGY IN CHILDREN 2 MONTHS THROUGH 5 YEARS

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ABSTRACT

This study evaluates the ecology of *Streptococcus pneumoniae* (SP) nasopharynx (NP) colonization in response to the pneumococcal conjugate vaccines, specifically 7-Valent Pneumococcal Conjugate Vaccine (2000-2009), 13-Valent Pneumococcal Conjugate Vaccine (2010-2023) and 20-Valent Pneumococcal Conjugate Vaccine (2023-future date). It is anticipated that the replacement of PCV13 with PCV20, a pneumococcal conjugate vaccine with 7 additional polysaccharide conjugates to CRM197 will enhance the protection against non-vaccine serotypes which are in circulation in communities. The project will evaluate the dynamic changes in pneumococcal colonization over the 5-year time line from 2021-2026. Pneumococcal nasopharynx colonization is detected through nasopharyngeal culture and molecular techniques. The primary source of pneumococcal transmission occurs among the pediatric population and between children and adults. The impact of PCV7 and 13 on pneumococcal colonization over the prior 20 years created a herd effect that resulted in a reduction in pneumococcal disease in unimmunized children and adults. Studies of NP colonization has led to a deeper understanding of pneumococcal conjugate vaccine (PCV) effectiveness and the role of herd immunity in protecting the population, the emergence of replacement serotypes, the variation in invasive capability of each serotype and

evolution of antimicrobial resistance resulting from the evolving ecology. In this 5-year-study, researchers at the Pelton Lab in Boston Medical Center set out to understand the prevalence of NP carriage of 13vPnC serotypes, the 7 unique 20vPnC serotypes and NVST (non-vaccine serotypes) within the pediatric population prior to and subsequent to the introduction of PCV 20 (Fall 2023).

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13vPnC	13-valent Conjugate Vaccine
20vPnC	20-valent Conjugate Vaccine
BHI	Brain Heart Infusion
CAP	Community-acquired Pneumonia
IPD	Invasive Pneumococcal Disease
NP	Nasopharynx, Nasopharyngeal
PCR	Polymerase Chain Reaction
PBS	Phosphate-buffered Saline
PCV	Pneumococcal Conjugate Vaccine
PPV	Pneumococcal Polysaccharide Vaccine
RT-PCR	Real Time Polymerase Chain Reaction
SP	*Streptococcus pneumoniae*
STGG	Skim-milk, Tryptone, Glucose, Glycerin
TSB	Tryptic Soy Broth
NVST	Non-vaccine Serotype
NVT	Non-vaccine Serotype
VST	Vaccine Serotype
VT	Vaccine Serotype

IMPORTANCE OF THE PNEUMOCOCCAL CONJUGATE VACCINE

Background

Streptococcus pneumoniae (SP) is Gram-positive, lancet-shaped facultative anaerobic bacteria that is found in nature either in pairs or short distinct chains. Pneumococcal disease results from *Streptococcus pneumoniae* due to their unique structure to evade host defenses and cause a spectrum of clinical manifestation or syndromes. The polysaccharide capsule of this bacterium is the major virulence factor responsible for its pathogenesis. Currently there are over 100 pneumococcal serotypes, based on the capsular polysaccharide differences in composition, that have been identified (Dion & Ashurst, 2022). This study primarily focuses on the prevalence of pneumococcal carriage in young children in the era of pneumococcal vaccines (Dion & Ashurst, 2022). The pathogenesis of pneumococcal disease begins with nasopharyngeal colonization of SP in children. Invasion of this bacteria to the lungs leads to pneumonia; to the bloodstream leads to sepsis; to the brain leads to meningitis. SP invasion of the middle-ear leads to otitis media and sinusitis cases (NFID, 2022). Historically, SP was first identified and isolated in 1881 from a patient's saliva sample, who had a concurrent diagnosis of rabies (Dion & Ashurst, 2022). Just over a century later, the first polysaccharide vaccine targeting pneumococci was produced and subsequently introduced in the United States (Dion & Ashurst, 2022).

Community-acquired pneumonia (CAP) is a leading cause of both morbidity and mortality in the United States, with about 95% of pneumonia cases attributed to SP in the pre-antibiotic era and about 15% of pneumonia cases attributed to SP in the postantibiotic era (Dion & Ashurst, 2022). We now recognize that viral-bacterial interactions in the context of co-infections are an important part of the pathogenesis of pneumococcal disease. Recent studies demonstrate that respiratory viruses, such as Respiratory Syncytial Virus (RSV), are epidemiologically linked to pneumococcal pneumonia, invasive disease and otitis media (Nickbakhsh, 2019).

Much of recent research on SP colonization and evolution stems from the historical interest in pathogen-pathogen interaction in the context of co-infections, specifically the crossover of respiratory viruses, known as *viral interference.* Primary or first-stage viral infection in the respiratory tract can either decrease or increase infection upon exposure to a secondary or second-stage viral infection. Interestingly enough, the human respiratory system is a reservoir for exposed viruses that tend to coexist and/or combat over a period of time. Recent studies on the interactions between coexisting respiratory tract pathogens have identified previously overlooked mechanisms to evaluate overarching respiratory infections, such as the *non-incidental co-seasonality* of mutual increases in influenza and pneumococcal disease rates. It has been understood that primary infection by the influenza virus leads to decreased host immunity and increased susceptibility to secondary bacterial colonization (Nickbakhsh, 2019). In terms of background, SP disease is known to be more frequent in patients older than 65 years and younger than 2 years, or those who have underlying comorbid illnesses, such as: recurrent otitis media, recurrent pneumonia, recurrent sinusitis, immune deficiency, sickle cell disease and chronic lung disease, including but not limited to bronchopulmonary dysplasia, asthma, etc. (Pelton & Lapidot, 2021). As a result, it is of interest and importance to study this infection in pediatric patients between the ages of 2 months to 5 years from all demographics (except those that present with *study exclusion criteria* or are under Massachusetts Department of Children and Family Custody (challenge obtaining consent) and/or have a facial deformity that would hinder a nasal swab that are seen at Boston Medical Center that may present as healthy or otherwise sick, with flu-like, upper-respiratory infection (RTI) symptoms (*study inclusion criteria*). With the introduction of the first PCV over 2 decades ago, it is clear that invasive pneumococcal disease (IPD) has dramatically declined in both vaccinated and unvaccinated children. The decline in unimmunized children support the importance of herd immunity- when a large part of the community is protected by reducing circulation and transmission of the pathogen within the community (Pruthi, et al., 2022). Herd immunity or herd benefit emphasizes that there is a decrease in SP colonization in response to either vaccine or natural immunity following disease. In the case of PCV, there is specifically a reduction in circulation of vaccine serotypes. However, serotypes not present in the vaccines (or non-vaccine serotypes) are observed to replace those previously colonizing serotypes. This results in persistent colonization despite immunization, but with an altered distribution of serotypes. Serotypes, essential to the focus of this paper, are based on polysaccharide capsules found on SP that have a major role in virulence, interfering with host immune combat mechanisms.

The study of PCV and its relation to IPD has provided insight not only on herd immunity but on *invasive capacity* (incidence of event occurrence, in this case IPD, divided by colonization prevalence) which uniquely varies among the known 100 serotypes (Pelton, et al., 2019). Evidence from the aforementioned editorial estimated the difference in invasive capacity between serotypes having the highest (7F, 38, 19A, 3, 33F) and the lowest (6C, 35B, 21, 11A, 23B, and 23A) invasive capacity to be as much as 100-fold. In general, disease due to SP with lower invasive capacity were generally seen in children with pre-existing comorbidities, many with immune defects making the children more susceptible to SP of low invasiveness (Pelton, et al., 2019).

Pfizer's Pneumococcal 20-valent Conjugate Vaccine (20vPnC), currently licensed for adults ages 18 years or older, contains capsular polysaccharide conjugates for the following serotypes: 8, 10A, 11A, 12F, 15B, 22F, 33F, in addition to those serotypes found in Pneumococcal 13-valent Conjugate Vaccine (13vPnC) (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F). According to the CDC, 20vPnC is targeted for use in adults aged 65 years and older, in addition to adults aged 19-64 years with known and specific underlying medical conditions and preexisting risk factors, who have not yet received a pneumococcal conjugate vaccine.

Historically, the Pelton Lab has monitored childhood IPD in Massachusetts before the implementation of 13vPnC (2002-2009) and after the implementation of 13vPnC (2010-2021). Prior studies aimed to examine how the introduction of 13vPnC affected non-vaccine-serotype-colonization and morbidity and mortality rates in children. The same principle applies to the lab's current study, examining NP colonization in Massachusetts before the implementation of 20vPnC (2021-2023) and after the implementation of 20vPnC (2024-2026). Important to note is the enhanced molecular technology employed in the Pelton Lab at Boston Medical Center, which is designed to account for and detect low density colonization of SP in the nasopharynx. The enhanced detection techniques include: 4-hour pre-incubation, DNA extraction, identification of pneumococcal DNA by Real-Time Polymerase Chain Reaction (RT-PCR) followed by molecular serotyping as modified from the Centers for Disease Control and Prevention (CDC) protocol. The specific procedure includes harboring identification of SP by RT-PCR using specific primers that detect the *lytA* and *piaB* genes; samples confirmed to be positive for both genes are considered as SP-positive specimens and are further tested for molecular serotyping through a multiplex RT-PCR according to CDC published assay.

Objectives

The Pelton Lab and Pfizer Inc. have identified primary and secondary research objectives to guide the premise of the 20vPnC Surveillance Study, while also citing important literature to move forward with PCV development. *Primarily*, the Pelton Lab aims to understand the annual vaccine serotype (VST) prevalence of pneumococcal bacteria colonization for a period of 5 consecutive years (2021-2026) to understand vaccine impact before and after 20vPnC is introduced, among children between the ages of 2 months to less than 60 months who present as healthy or sick with an upper respiratory tract infection (URTI). *Secondarily,* the study aims to compare NP SP carriage of the seven 20vPnC-additional serotypes in immunized vs. non-immunized children 2 to <6 months of age receiving care at the Primary Care Center or included in the Nasopharyngeal Carriage Study Cohort in order to identify 20vPnC uptake necessary to create herd immunity. While published literature on IPD and PCV is quite extensive, this paper focuses on 3 main questions: *1) How much vaccination is necessary to allow* *herd immunity? 2) What are the implications of non-vaccine serotypes in the community? 3) What are the mechanisms that allow the conjugate vaccine to combat colonization?*

Invasive Pneumococcal Disease

In the context of invasive pneumococcal disease (IPD), pneumococcal colonization of the upper respiratory system is common and expected to be detected in about 20 to 60% of healthy children tested (Canada, 2021). The concern stems in the asymptomatic presentation of SP, with no observable signs for detection. In terms of transmission, SP can be spread through oral contact, in addition to oral secretions or respiratory-generated airborne particles. Provided that SP resides within saliva and/or respiratory secretions, an infected individual is capable of transmitting IPD to others. To further expand upon disease distribution, on both a domestic and global scale, it is worth highlighting that SP is a leading cause of both morbidity and mortality. Supporting the premise behind this Surveillance Study, the World Health Organization predicts that SP is among the leading top 2 causes of bacterial meningitis and globally around 500,000 deaths can be attributed to IPD in children between 2 months to 5 years annually (Canada, 2021). Bacteremia, or presence of bacteria in the bloodstream, is the most common presentation of IPD among the aforementioned age range. Bacteremia attributed to SP pneumonia is also the most common presentation of IPD among adults, especially among individuals aged 65 years and older (Canada, 2021). Specifically, IPD in the form of bacteremia, affects 17 out of 100,000 adults over the age of 18 in the United States, while IPD in the form of meningitis affects about 1.5 out of 100,000 adults annually (Canada, 2021). To understand the published literature on PCV and IPD and delve further into the research questions outlined, it is important to start at the molecular level to first understand how the NP samples are collected and then tested for SP culture.

RESEARCH METHODS

Sample Collection and Processing

Nasopharyngeal samples were collected from the population of interest via calcium alginate swabs, in which two swabs will be wrapped around each other, bent at approximately 30 degrees and simultaneously inserted into one naris. It was recommended to insert to collect the sample from the naris that may evidently contain secreted mucous and/or ensure that mucous is visualized on the swab. Upon collection, samples were placed in transport tubes containing a medium of skim-milk, tryptone, glucose and glycerin (STGG), transported to the Maxwell Finland Laboratory for Infectious Diseases, stored in designated freezer at -80°C and kept until processing stage. From the collected samples, SP isolates were purified, serotyped using an antibody bases assay called Quellung reaction and tested for antimicrobial susceptibility, while the frozen transport media, as mentioned above, underwent molecular processing to test for the molecular markers- autolysin (*lytA*) and permease gene of the pia ABC transporter (*piaB*) to confirm/deny evident colonization (Pelton & Lapidot, 2021).

To begin processing, the STGG tube was thawed to 4°C overnight. The next day, the liquid mixture was vortexed vigorously, in order to mix the STGG solution (used for storage) with a tryptic soy broth (TSB)/glycerol base (used for cryo-protection). Afterwards, 100 microliters of the solution were taken and added to 3 milliliters of Brain Heart Infusion (BHI) Broth to prepare the sample for growth. Then, the contents of the tube were incubated at 37°C in a candle jar, providing an adequate anaerobic environment to induce SP growth for 4 hours; 100 microliters of the above solution are plated on a blood agar plate infused with Gentamicin (purchased from Remel). Since SP is known to be Gram-positive and resistant to this aminoglycoside antibiotic, Gentamicin is expected to target and inhibit the growth of Gram-negative bacteria that may reside on the plate. Important to note is that presence of SP can be detected through the morphological characteristic of *alpha-hemolysis* on the blood agar-Gentamicin plates, where the SP colonies appear as dense, moist colonies with a distinct *dark zone* (CDC, 2016). Following alpha-hemolytic testing, we performed optochin sensitivity testing, which is a hallmark to confirm presence of SP. After culturing the dark alpha-hemolytic zone from the previous plate and streaking it on a new plate, an optochin (ethylhydrocuprein hydrochloride) disk is placed and observed in order to confirm/deny the specific presence of SP. Of importance, optochin is an antibiotic that interferes with ATPase and ATP production in the crucial electron transport chain of the bacteria. The outside of the disk represents the *inhibition zone*, showing that SP is sensitive to this antibiotic inhibiting its growth.

After SP has been confirmed, the serotyping process was carried out in order to identify which serotype(s) are prevalent in the specific NP samples. To accomplish this, on a clean slide, 10 microliters of the sample will be placed on the bacterial phosphate-buffered saline (PBS) suspension was placed and a loop full of the bacteria from the plate was mixed with buffer to make bacterial suspension. Afterwards, serotype-specific antibodies (raised in rabbits by the Staten Serum Institute) using pool, groups and factors were added in a stepwise manner to identify the specific serotype agglutination. Serotype pooling was both an effective and efficient way to narrow down the presence of specific serotypes in SP positive samples. Since there are 100 serotypes identified till date, a pool of 10 identified serotypes were applied at a time to a harbored sample. With physical evidence of *agglutination* or clumping of the SP antigen with the serotype-specific-rabbit-antibody, we were able to identify and test each specific serotype within that pool. If there was no physical evidence of agglutination, we proceeded to test the next pool, which contained serotype-specific antibodies for another 10 serotypes. This process was completed and repeated until all 100 serotypes had been tested for. This dictated the specific serotype *group* and *factor* that was evident in the original collected NP sample.

In summation, since this study is specific to 20vPnc, the nasopharyngeal nares *swab* first undergoes the Optochin Test to confirm/deny the presence of *Streptococcus pneumoniae*, then the Quellung Reaction Test where the serotype-specific antibodies bind to the capsule of the *Streptococcus Pneumoniae* (Pelton & Lapidot, 2021). Furthermore, DNA isolated from the nasopharyngeal nares swab sample first undergoes a polymerase chain reaction (PCR) assay using molecular markers *lytA* and *piaB* to confirm/deny the presence of *Streptococcus Pneumoniae*, then RT-PCR using primers for serotypes 10A, 11A, 12F, 15B, 22F, 33F to confirm/deny the presence of *Streptococcus Pneumoniae* and *VST serotypes* (Pelton & Lapidot, 2021). Ultimately, the microbiologic and molecular tests collectively provided confidence to identify SP colonization and serotype distribution, as devised by the Pelton Lab and CDC publications.

Figure 1. Nasopharyngeal Sample Collection and Processing Chart (Pelton Lab).

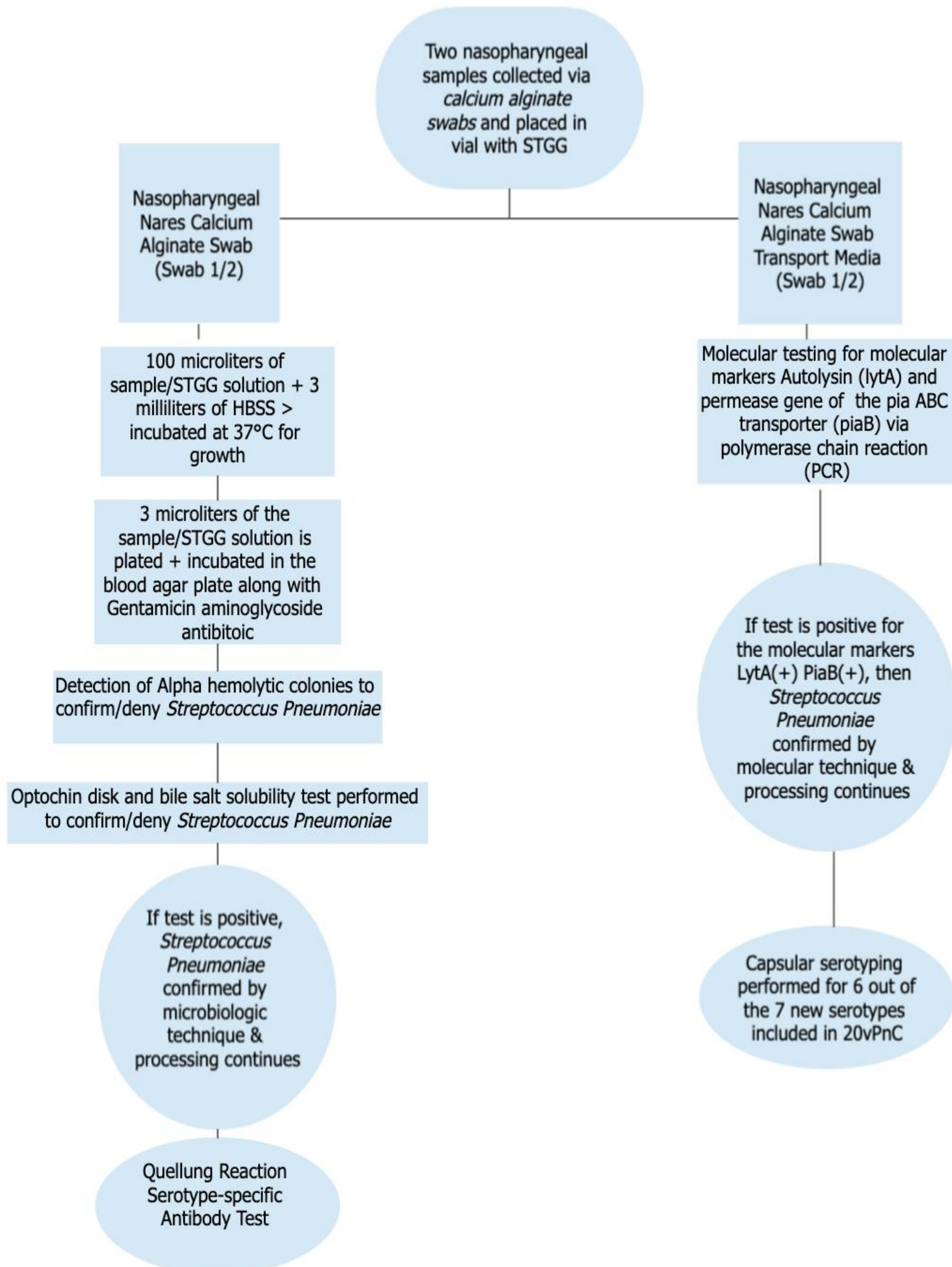


Illustration 1. Sample Processing Diagram (Adapted from the Pelton Lab)

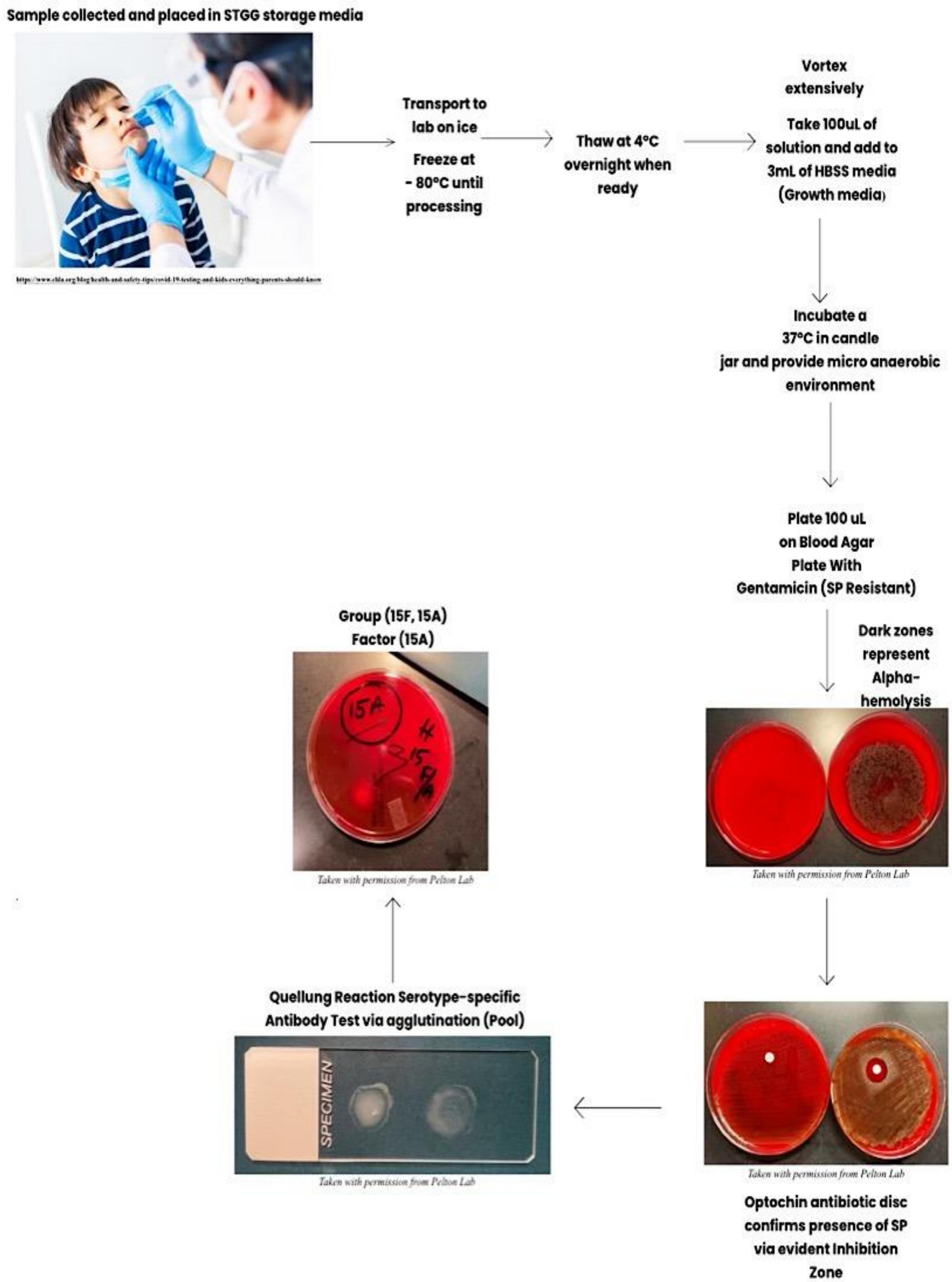
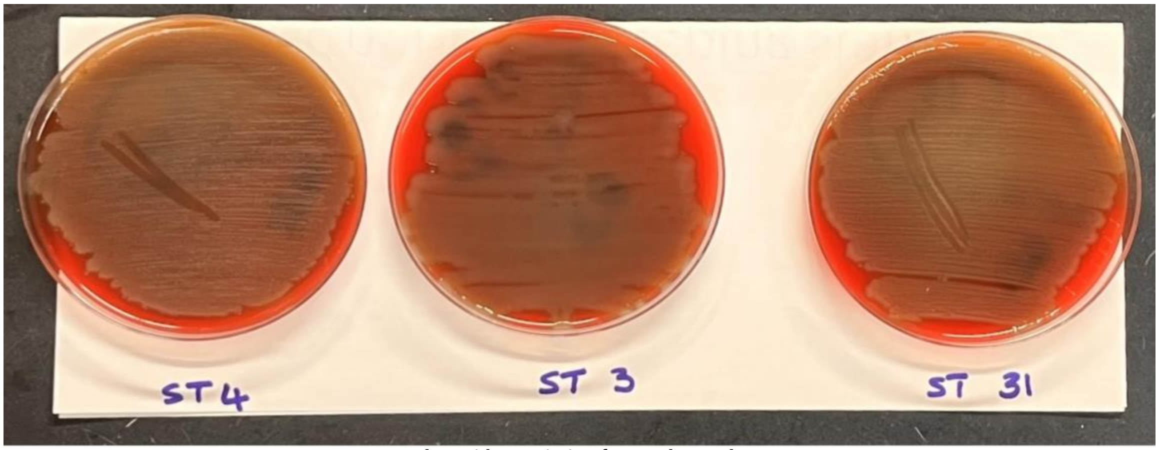


Figure 2. End Result of Specific Serotypes.



Taken with permission from Pelton Lab

A *secondary objective* of the NP Surveillance Study is to evaluate trends in antibiotic susceptibility among the pneumococci recovered from NP samples collected from children that are either healthy or diagnosed with URTI (Pelton & Lapidot, 2021). Only the samples that both demonstrate alpha hemolytic colonies on the Gentamicin aminoglycoside antibiotic plates and demonstrate an inhibition zone around the Optochin disk (sensitive to Optochin) to confirm *Streptococcus pneumoniae* presence will then undergo specific serotyping, followed by antibiotic susceptibility testing. This secondary objective leads to the *secondary endpoint* of determining the ratio of vaccine serotype (VST) and non-vaccine serotype (NVST) samples deemed resistant to antimicrobial treatment in the 2-year period before and 3-year period after 20vPnC introduction. Antimicrobial susceptibility testing will be performed using E-test strips, which has a gradient of antibiotic concentrations coated on the strip (bioMérieux, 2022). All bacterial stocks were stored in the freezing medium at -80°C for future applications. The antimicrobial susceptibility of the SP isolates will specifically be tested against

Amoxicillin, Ceftriaxone, Azithromycin, Benzylpenicillin and Trimethoprim. According to previous antimicrobial research, the antibiotics which are effective against *Streptococcus pneumoniae* include: Ceftriaxone (95% effective), high-dose Amoxicillin (74% effective), Clindamycin (85% effective), and standard-dose Amoxicillin (74% effective), which is why these antibiotics are specifically being tested as part of the NP Surveillance Study (Bell, 2013). Overall, this lends great insight to the process of bacterial evolution within a given population and the most up-to-date susceptibility patterns to guide antimicrobial treatment. In terms of the specific E-testing procedure, *Streptococcus pneumoniae* identified isolates will be grown overnight in trypticase soy broth and after that period of time, will then be diluted to appropriate turbidity. The identified isolates will then be plated on Mueller Hinton Agar plates (Thermoscientific-Remel) which contain a small percentage of sheep blood to induce growth. Afterwards, E test strips representing each of the aforementioned antibiotics will be placed on top of the plate, and plates will be incubated overnight at 37°C in a candle jar. Based on the amount of growth inhibition due to antibiotics, we determined the minimum inhibitory concentration (MIC) of a certain antibiotic needed to inhibit SP growth and correlated the serotype to antibiotic sensitivity. An antibiotic is deemed to be more effective should a lower concentration amount be able to prevent large growth of *Streptococcus pneumoniae*, evident through the lack of dark zones on the plate (see Figure 3). This information can then benefit both researchers and clinicians alike, in attempting to understand the most effective treatment that targets *Streptococcus pneumoniae* disease. The antibiotic sensitivity test aims to determine: *serotype differences in susceptibility to* *the aforementioned antibiotics, the difference in sensitivity between isolates from healthy versus sick children, and identify emerging SP serotypes able to resist antibiotic activity*. Ultimately, antibiotic sensitivity testing for specifically *Streptococcus pneumoniae* allows researchers to understand the most effective antibiotics and support clinician decisions for adequately managing the antibiotic selection course to improve a patient's long-term health.

Historically, the antibiotic susceptibility test produces the following results: *resistant, susceptible*, *intermediate* (Reller et. al, 2009). Resistant indicates that the bacteria are not affected or inhibited by the tested antibiotic. Susceptible indicates that the tested antibiotic inhibits the specific pathogen. Intermediate indicates that the bacteria falls into the range of susceptibility, otherwise viewed as a "buffer zone" in which a high concentration or even one that exceeds the concentration on the E-test strip may need to be incorporated into the therapy plan in order for the patient to overcome the bacterial infection. Over time, antibiotic resistance is known to develop through several mechanisms, including but not limited to: natural recombination and internal integration, external conjugation, transformation, transduction (Khan et. al, 2019). With antibiotic resistance, or the inherent ability of bacteria to translocate resistance genes that block the inhibitory effect of antibiotics prescribed for use within the human body, proving to be an ongoing trend, the Pelton Lab in collaboration with Pfizer continued the initiative to test isolated SP strains against the antibiotics currently used in clinical applications. The images on the next page represent antibiotic sensitivity testing as part of the ongoing 20vPnC Surveillance Study.

Figure 3. E-test Plates.

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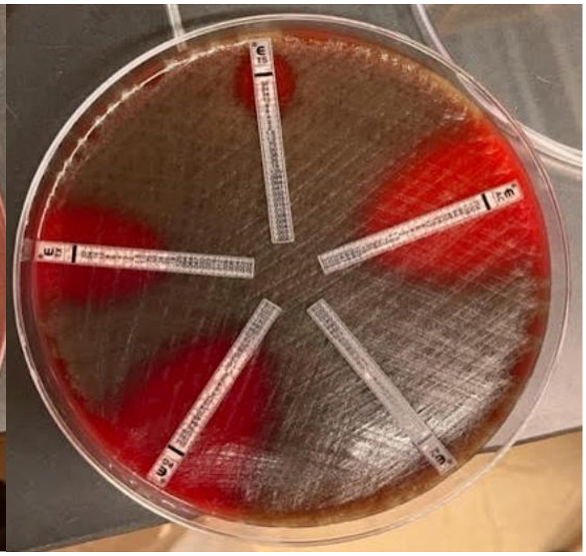

Taken with permission from Pelton Lab

Figure 4: Microscopic Image (Live and Gram-stained).

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In reference to Figure 3, the specific antibiotic is labeled under the letter 'E' on the upper portion of the test strip. For example, AC represents Amoxicillin; PG represents Benzylpenicillin; AZ represents Azithromycin; TX represents Ceftriaxone; TS represents Trimethoprim. According to the antibiotic sensitivity test in this figure, Azithromycin is the least effective as treatment against the *Streptococcus Pneumoniae* strain tested for this assay, as it requires greater than MIC 256 µg/ml to inhibit growth. In the case of Trimethoprim, is more effective than Azithromycin but less effective than the others, as MIC 6 µg/ml or more is required to inhibit growth. Ceftriaxone is more effective than Azithromycin and Trimethoprim as an antibiotic therapy as it requires MIC as low as 0.25 µg/ml to inhibit SP growth. Amoxicillin and Benzylpenicillin seem to be equally effective, give or take, requiring MIC approximately 0.94 µg/ml to inhibit SP growth, as tested for the particular isolate of SP. In reference to Figure 4, the microscopic image presented is both live and gram-stained. A gram stain is an efficient and effective laboratory test that can help to identify and differentiate the presence of bacteria based on chemical and/or physical properties of the cell wall composition. If bacteria appear in a crystal blue color, then they belong to a Gram positive group; if bacteria appear as red, then they belong to a Gram negative bacterial group. Since *Streptococcus pneumoniae* is gram-positive with a cell wall composed of a thick 90% peptidoglycan-like layer, we expect it to have a blue-to-purple hue under a gram stain, as shown (Cleveland Clinic, 2022). In terms of the actual Gram stain, we first applied a primary stain (Crystal violet), then an inorganic oxide (Iodine solution), followed by rapid de-colorization (alcohol) and then a counterstain (Safranin) to uncover select color and shape characteristics of SP. For example, cocci usually present in clusters or chains and because SP is Gram-positive, it will also present with a distinct hue (Cleveland Clinic, 2022). Now that the process of nasopharyngeal sample collection, processing and analysis has been elucidated, the purpose and principle of the Pelton Lab's NP Surveillance Study can be understood in detail. The Surveillance Study has been ongoing, with the same procedures outlined and applied for 7vPnC, 10vPnC, 13vPnC, 15vPnC and currently 20vPnC.

HERD IMMUNITY

How much vaccination is necessary to allow herd immunity?

As discussed previously, the pneumococcal conjugate vaccine promotes *herd protection* through the pediatric and consequently adult communities. Researchers as part of the Pneumonia Program set out to understand how herd immunity works, since vaccination of children was sufficient to reduce the transmission of vaccine serotypes to those who are unable to be vaccinated, including but not limited to children under the age for immunization, middle-aged adults with existing comorbidities, autoimmune diseases or older individuals (Klugman, 2014). The Pneumonia Program also explored the financial benefit PCV vaccination due to herd immunity, as many individuals who are unable to afford vaccination or who live in countries where vaccines are only available for children can still be protected against vaccine serotypes by immunizing children. Evidence of herd effect was reported first in high income countries, where disease declined in adults following introduction of PCV in the national childhood immunization program. This observation enabled researchers to understand herd immunity, which could be expected to work in developing countries, where vaccine serotype identification and accurate rates of

Invasive Pneumococcal Disease are not accounted for (Klugman, 2014). The Pneumonia Program has begun their research in Africa, where the introduction of 7vPnC has objectively reduced the spread of vaccine-associated serotypes over a measured course of time within the developing communities. For instance, researchers as part of this program decided to closely monitor 7vPnC introduction and administration over a 6-week, 14-week, and 9-month schedule in underdeveloped and underserved areas of South Africa (Klugman, 2014). As observed in high income countries, transmission of vaccine-specific serotypes was reduced in children, adults and elders including those who did who did not receive the vaccine. As an extension of this study, authors performed similar analysis with 10vPnC in a rural community in Kenya around 2011. The same result was demonstrated, indicating there was reduction in the nasopharyngeal colonization of vaccine serotypes in both immunized and non-immunized children, adults and elders in the community within a few years of introducing this vaccine to children. An interesting idea was then introduced, to test the effectiveness of herd immunity as attributed to 10vPnC in the area, where children between the ages of 2 months to 5 years were offered the vaccine. Researchers noticed that vaccine-specific serotypes were reduced by approximately 66% in children, adults and elders that were unimmunized (Klugmann, 2014). This observation provided evidence in that immunized children between 2 months to 5 years were key conveyers of vaccine-specific serotypes in the widespread community. Based on the data collected on 10vPnC in the rural community of Kenya, the Pneumonia Program highlights that the vaccine administration of about two-thirds (66%) of children between 2 months to 5 years induced and promoted herd immunity significantly in the community (Klugmann, 2014). This percentage may not apply to all developing countries, as the following factors differ: preexisting pneumococcal colonization density within the community, effect of preexisting viral and bacterial infections, such as HIV, socioeconomic status, and differences, if any, in the evolution of patient immunosuppression to the serotype-specific vaccines of 7vPnC, 10vPnC and 13vPnC (Klugmann, 2014).

In Boston, Massachusetts, the Pelton team estimated that vaccination of about 65-75% of children between 2 months to 5 years was required to induce and promote herd immunity to the serotypes of 13vPnC within the unimmunized children at Boston Medical Center. The NP Surveillance Study showcased a novel way that developing countries can test and understand induced herd immunity against Invasive Pneumococcal Disease as a result of preventative vaccination among children below 5 years of age. At Boston Medical Center, children between the ages of 2 months to 5 years of age were not only swabbed for SP cultures, but their medical history was reviewed for previous vaccinations, underlying and/or current medical conditions, and familial, cultural and social status. Parents were also asked questions in relation to vaccine hesitation, safety and efficacy. These factors are important when trying to determine the effectiveness of herd immunity when the 13vPnC vaccination program introduced 10vPnC in the community. As the Pneumonia Program suggests, developing countries can strongly benefit from the NP Surveillance Study by testing children between the ages of 2 months to 5 years, particularly children with upper-respiratory tract infection (URTI) symptoms and/or signs of pneumonia, simultaneously analyzing the patient's vaccination history. Since these patients would be categorized as "sick-URTI", this method would allow clinicians in developing countries to detect serotypes such as 1, 4, 5 and 7F, which are typically only found in non-healthy subjects (Kluggman, 2014). As Klugmann and members of the Pneumonia Program defined, herd immunity is the rate of VST pneumococcal disease in a community, which is similar between vaccinated and unvaccinated children (Klugmann, 2014).

In addition to analyzing herd immunity in developing nations, where socioeconomic status and vaccine accessibility are prominent determining factors for herd immunity, research studies also examined the impact of herd immunity on pneumococcal colonization and disease in different ethnic groups, including the Native American community. A study was conducted by Katherine L. O'Brien, MD, MPH at the Center for American Health analyzed the NP colonization and serotype-specific disease in the Native American community over 12 years of 7vPnC administration (Scott, 2012). Mirroring the NP Surveillance Study conducted by the Pelton team at Boston Medical Center, the purpose was to enroll individuals in a surveillance study from 2006 to 2008, where NP swab specimens and medical history information were collected on a monthly basis for 7 months (Pelton & Lapidot, 2021). Similar to the current pneumococcal surveillance study on 20vPnC, a study was undertaken to determine the pneumococcal burden before (1998-2000) and after (2001-2002) the introduction of 7vPnC. The purpose was to determine how 7vPnC altered vaccine-specific serotype presence in the Native American population after introduction, and if herd immunity could be a cause for the decline in invasive capacity of those serotypes found in 7vPnC in unvaccinated individuals (Scott, 2012). The specific objective of this was study was to understand NP serotype carriage among the Navajo and White Mountain Apache children and adults after 8 years of 7vPnC administration and to determine if long-term specific serotype prevalence has changed (Scott, 2012). Scott et. al explained that the Navajo and White Mountain Apache tribes were specifically chosen for this study due to historical high rates of NP colonization and disease as compared to the mainland population of the United States. As with the current NP Surveillance Study, researchers approached families in clinic that met certain inclusion criteria that were thought to impact pneumococcal colonization: located near the White Mountain and/or Navajo reservation, more than one child in the household was greater than 9 years old and greater than or equal to 2 people in the household would agree to participate in the study for the designated timeframe of 6 months (Scott, 2012). In addition, medical history was specifically reviewed to understand immunization record, prescribed antibiotics, admitted hospitalizations and pertinent outpatient illness in the childhood era (Scott, 2012).

In the ongoing NP Surveillance Study at Boston Medical Center led by Dr. Pelton and his team, the goal is to determine NP colonization of SP serotypes before and after the introduction of PCV 20. The only inclusion criteria for the current study includes children at or greater than the age of 2 months and less than 5 years with no facial deformity or chronic illness that would skew results. In terms of sample analysis, researchers at the Center for American Indian Health devised a specific definition for nasopharyngeal serotype prevalence: *# of serotype-specific isolations across clinic visits/total samples collected* (Scott, 2012). Ultimately, the purpose of this monumental study was to understand the invasive potentials of pneumococcal serotypes before and after 7vPnC introduction (Scott, 2012). The formula was specifically defined as *invasiveness rate = serotype IPD incidence/ serotype carriage prevalence.* Overall, 1077 eligible subjects were enrolled between March 2006 to March 2008, where a total of 868 study subjects completed 6 out of the 7 required monthly sample collection visits, and 657 study subjects completed 7 of the 7 required visits (Scott, 2012). Of importance, over 95% of enrolled subjects between the ages of 2 to less than 5 years were vaccinated with greater than or equal to 3 does of 7vPnC (Scott, 2012). In terms of actual NP specimens, a total of 6541 were collected, were 2344 (35.8%) were ruled positive for *Streptococcus pneumoniae*. After 7vPnC introduction, participants between 5 and less than 9 years old were calculated to have a carriage prevalence of 50%; children less than 5 years were calculated to have a carriage prevalence of 55.4%; adults were calculated to have a carriage prevalence significantly lower compared to any and all children participants (Scott, 2012). In comparison to pre-7vPnC introduction (1998-2000), the carriage prevalence was calculated to be 63.4%, as opposed to the aforementioned 55.4% in the midst of 7vPnC introduction, representing an 8.0% decrease in pneumococcal carriage rate among children less than 5 years old with vaccine introduction (Scott, 2012). Following a similar pattern, the carriage rate in adults in the early stage of 7vPnC introduction was 13.9%, as compared to the carriage rate of 10.9% during the main period of 7vPnC administration (2006-2008) (Scott, 2012). These statistical results support the concept of *herd immunity*, in which the younger population (children less than 5 years) were seen to have the highest carriage prevalence and over a longer time span, the older generations demonstrated a lower bacterial carriage percentage and a shift to NVST SP disease. Similarly, the Pelton team released a publication in 2014 highlighting the age-related children diagnosed with IPD with regards to both pre-13vPnC and post-13vPnC in the Massachusetts community at-large. The table below provides an extension to the general population of the United States, similar to what researchers at the Center for American Indian Health concluded about the Navajo and White Mountain tribes.

Figure 5. IPD Cases Calculation with Respect to Age and 13vPnC Vaccination (Taken with permission from Dr. Pelton from Invasive Pneumococcal Disease After Implementation of 13-Valent Conjugate Vaccine).

	Pre-PCV13, *n* (%) (*n* = 168)	Post-PCV13, *n* (%) (*n* = 85)
IPD		
<2 mo	7 (4.2)	2 (2.4)
2–23 mo	89 (53.0)	48 (56.5)
2–5 y	72 (42.9)	35 (41.2)
VT-IPD[a]		
<2 mo	3 (1.8)	0 (0)
2–23 mo	44 (26.2)	14 (16.5)
2–5 y	41 (24.4)	14 (16.5)
NVT-IPD[a]		
<2 mo	2 (1.2)	1 (1.2)
2–23 mo	24 (14.3)	27 (31.8)
2–5 y	14 (8.3)	19 (22.4)

[a] Percentages do not add up to 100% because of incomplete data.

The graph included in the paper *Impact of PCV7 Use in Native American Communities* highlights the connection to herd immunity, in which vaccine serotype (VT), serotype 6A, serotype 19A, and other non-vaccine serotype (NVT) are examined in children less than 5 years of age and compared to the percentage of carriage prevalence of each serotype as recorded in adults both before and well after 7vPnC administration in the community. The carriage prevalence for vaccine-specific serotype colonization in children have *decreased* in children less than 5 years since 7vPnC administration. In adults, the carriage prevalence percentage was even lower than children in the early stages of vaccine administration, with an expected decrease in carriage prevalence after a period of vaccine establishment in the Navajo and White Mountain tribes (Scott, 2012).

An important observation from the study statistical analysis is that not only was there SP carriage prevalence, but carriage serotype prevalence, due to vaccine introduction. This study showed that samples collected from children less than 5 years in the span before and early after release of 7vPnC demonstrated a 22.7% decrease in vaccine-specific serotype colonization after continuous 7vPnC administration (Scott, 2012). The non-vaccine-serotype carriage rate increased by 15.9% in both children and adults (Scott, 2012), as expected since the serotypes were not accounted for and consequently herd immunity could not be accounted for. Similar to the NP Surveillance Study among children at Boston Medical Center, the positive samples collected from the Navajo and White Mountain tribes were examined for serotype-specific prevalence. This information proves to be critical for disease tracking, clinical treatment and herd immunity. 43 serotypes were identified from the 233 SP isolates identified as serotype 6A and 228 serotypes identified as 6C (Scott, 2012). With regards to 7vPnC, 5 serotypes (6C, 19A, 23B, 35B and 22F) and other unidentifiable pneumococci comprised the *top 10 serotypes* that accounted for about 35% of nasopharyngeal *colonization* in this patient demographic (Scott, 2012). However, serotype 12F was not identified as among the prominent colonizing strains, but it was the most prevalent serotype in terms of samples collected from individuals with Invasive Pneumococcal Disease (Scott, 2012).

Similar to the NP Surveillance Study at Boston Medical Center, patient demographics and risk factor analysis are essential to understanding colonization prevalence and persistence within the community. For the NP Surveillance Study conducted with the Navajo and White Mountain population, researchers found that enrolled participants who confirmed they were living in a household with a child less than 9 years old who had pneumococcal colonization had a high chance of experiencing nasopharyngeal colonization (Scott, 2012). If the parent reported antibiotic use during the recent period before the clinic visit, then a lower density of nasopharyngeal colonization was noted (Scott, 2012). Supporting the idea of herd immunity, researchers also noted that increasing age groups demonstrated a decrease in colonization (Scott, 2012). Researchers found that biological males on average had a higher percentage of pneumococcal nasopharyngeal colonization. Breast-feeding history, childcare and/or school groups, household size and areas of sleeping, parent and/or guardian education level and household cigarette smokers did not exemplify significant effects on pneumococcal nasopharyngeal colonization in children less than 5 years of age (Scott, 2012). Researchers at the Center for American Indian Health found that hygiene and daily living standards, such as running water and household cleanliness can have a noticeable impact on the density of nasopharyngeal pneumococcal colonization (Scott, 2012), which may be a future direction of pneumococcal research.

The Pelton team examined the dominance of herd immunity, through a different lens in which the total reduction in IPD cases were examined by age-group and expected hospitalization risk. The article published that the greatest reduction in IPD was seen in adults aged 65 years and older who were healthy and/or had a low risk for autoimmune diseases, which contributes to the idea of long-term herd immunity (Pelton, 2019). This study was a methodical extension of the study conducted by the Center for American Indian Health on the Navajo and White Mountain tribes, in terms of examining how PCV vaccination at a younger age has important effects in the late stages of life, especially in the unvaccinated. The Pelton team evaluated changes in IPD in adults within the general population of the United States between the timeframe 2007-2015 to gauge a better understanding on 13vPnC. IPD cases, pneumococcal-attributed pneumonia and hospitalization as a result of pneumococcal-attributed pneumonia were observed over 9 years (Pelton, 2019). IPD incidence rates showed a significant decline when 13vPnC was released in the period after 7vPnC, since more serotypes were accounted for in the vaccine (Pelton, 2019). The study proved that many adults with autoimmune diseases and chronic illnesses should obtain 13vPnC immunization. Although many did not, the rates of overall IPD cases and pneumonia-induced hospitalization have decreased in favor of herd immunity protecting those at risk.

Table 1. Calculated Rates and Ratios in IPD Cases and Hospitalization by Age and Risk (Taken with permission from Dr. Pelton from Decline in Pneumococcal Disease Attenuated in Older Adults and Those with Comorbidities Following Universal Childhood PCV13 Immunization).

	Rate per 100 000 Person-years (95% CI)			Percent Decline in Rate (95% CI) From 2007–2010 to		Rate Ratios (95% CI) vs Healthy		
	2007–2010	2011–2012	2013–2015	2011–2012	2013–2015	2007–2010	2011–2012	2013–2015
Invasive pneumococcal disease								
Age/risk profile								
18–49 years								
Healthy	1.1 (1.0, 1.2)	0.6 (0.5, 0.7)	0.6 (0.5, 0.7)	43 (30, 54)	47 (36, 57)	...	...	...
At-risk	3.2 (2.6, 3.9)	2.4 (1.8, 3.1)	2.1 (1.6, 2.6)	24 (–5, 46)	35 (11, 53)	3.0 (2.4, 3.7)	4.0 (2.9, 5.5)	3.7 (2.7, 4.9)
High-risk	12.7 (10.4, 15.6)	9.3 (7.0, 12.3)	8.6 (6.7, 11.2)	27 (–3, 49)	32 (6, 51)	11.9 (9.4, 15.0)	15.3 (10.9, 21.5)	15.4 (11.3, 20.9)
50–64 years								
Healthy	2.8 (2.6, 3.1)	2.0 (1.7, 2.3)	1.9 (1.6, 2.1)	29 (16, 40)	34 (23, 44)	...	...	...
At-risk	7.0 (6.3, 7.8)	5.8 (5.0, 6.7)	6.0 (5.3, 6.7)	18 (2, 31)	15 (1, 28)	2.5 (2.2, 2.9)	2.9 (2.4, 3.6)	3.2 (2.7, 3.8)
High-risk	20.5 (18.3, 23.0)	17.3 (15.0, 20.1)	16.4 (14.4, 18.7)	16 (–2, 30)	20 (5, 33)	7.3 (6.3, 8.5)	8.7 (7.1, 10.7)	8.8 (7.4, 10.6)
65–74 years								
Healthy	4.0 (3.3, 4.9)	3.6 (2.8, 4.7)	2.7 (2.0, 3.5)	10 (–26, 35)	33 (7, 52)	...	...	...
At-risk	11.8 (10.2, 13.6)	9.9 (8.2, 12.0)	8.7 (7.3, 10.4)	16 (–7, 34)	26 (7, 41)	3.0 (2.3, 3.8)	2.7 (2.0, 3.8)	3.3 (2.4, 4.5)
High-risk	26.4 (22.6, 30.8)	25.9 (21.4, 31.2)	20.8 (17.5, 24.9)	2 (–25, 23)	21 (0, 38)	6.6 (5.1, 8.5)	7.2 (5.2, 9.9)	7.8 (5.7, 10.8)
≥75 years								
Healthy	7.1 (6.0, 8.5)	6.8 (5.3, 8.7)	6.0 (4.7, 7.7)	5 (–29, 30)	15 (–14, 37)	...	...	...
At-risk	19.2 (17.2, 21.4)	17.1 (14.7, 19.9)	18.3 (16.1, 20.8)	11 (–8, 26)	5 (–13, 20)	2.7 (2.2, 3.3)	2.5 (1.9, 3.4)	3.0 (2.3, 4.0)
High-risk	26.3 (22.9, 30.2)	24.1 (20.1, 28.9)	28.0 (24.2, 32.3)	8 (–15, 27)	–6 (–30, 13)	3.7 (3.0, 4.6)	3.6 (2.6, 4.8)	4.6 (3.5, 6.2)
All-cause pneumonia								
Age/risk profile								
18–49 years								
Healthy	44 (43, 44)	39 (38, 40)	35 (35, 36)	11 (8, 13)	19 (16, 21)	...	...	...
At-risk	238 (233, 243)	218 (212, 224)	207 (202, 212)	9 (5, 12)	13 (10, 16)	5.5 (5.3, 5.6)	5.6 (5.4, 5.8)	5.8 (5.6, 6.0)
High-risk	730 (710, 750)	704 (681, 727)	701 (681, 721)	4 (–1, 8)	4 (0, 8)	16.8 (16.2, 17.3)	18.1 (17.4, 18.8)	19.7 (19.1, 20.5)
50–64 years								
Healthy	99 (98, 101)	90 (89, 92)	88 (87, 90)	9 (6, 11)	11 (9, 13)	...	...	...
At-risk	471 (465, 478)	434 (427, 442)	429 (423, 435)	8 (6, 10)	9 (7, 11)	4.7 (4.7, 4.8)	4.8 (4.7, 4.9)	4.9 (4.7, 5.0)
High-risk	1278 (1260, 1297)	1208 (1187, 1229)	1226 (1207, 1244)	6 (3, 8)	4 (2, 6)	12.9 (12.6, 13.2)	13.3 (13.0, 13.7)	13.9 (13.5, 14.2)
65–74 years								
Healthy	220 (215, 226)	204 (197, 211)	191 (185, 197)	8 (3, 12)	13 (10, 17)	...	...	...
At-risk	979 (964, 995)	905 (887, 923)	941 (925, 957)	8 (5, 10)	4 (2, 6)	4.4 (4.3, 4.6)	4.4 (4.3, 4.6)	4.9 (4.8, 5.1)
High-risk	2081 (2046, 2118)	2005 (1962, 2049)	2124 (2087, 2162)	4 (1, 6)	–2 (–5, 0)	9.4 (9.1, 9.8)	9.8 (9.4, 10.3)	11.1 (10.7, 11.5)
≥75 years								
Healthy	880 (867, 895)	918 (899, 938)	957 (938, 975)	–4 (–7, –2)	–9 (–11, –6)	...	...	...
At-risk	2536 (2512, 2561)	2592 (2560, 2624)	2745 (2717, 2774)	–2 (–4, –1)	–8 (–10, –7)	2.9 (2.8, 2.9)	2.8 (2.8, 2.9)	2.9 (2.8, 2.9)
High-risk	3511 (3470, 3554)	3676 (3623, 3730)	3992 (3944, 4040)	–5 (–7, –3)	–14 (–16, –12)	4.0 (3.9, 4.1)	4.0 (3.9, 4.1)	4.2 (4.1, 4.3)

Abbreviation: CI, confidence interval.

Table 2. Calculated Reduction in IPD Cases and Hospitalization by Age (Taken with permission from Dr. Pelton Decline in Pneumococcal Disease Attenuated in Older Adults and Those with Comorbidities Following Universal Childhood PCV13 Immunization).

	Number of Person-years (in Millions) in 2015	Decline in Rate per 100 000 Person-years		Absolute Reduction in Cases per Year
		Percent	Absolute	
Invasive pneumococcal disease				
Age/risk profile				
18–49 years[a]	8.6	46	0.7	57
Healthy	7.5	47	0.5	38
At-risk	0.9	35	1.1	10
High-risk	0.2	32	4.1	8
50–64 years[a]	5.6	28	1.2	69
Healthy	3.8	34	1.0	37
At-risk	1.3	15	1.1	15
High-risk	0.4	20	4.1	17
65–74 years[a]	1.1	29	2.6	29
Healthy	0.5	33	1.3	7
At-risk	0.4	26	3.1	12
High-risk	0.2	21	5.6	9
≥75 years[a]	0.8	6	0.4	3
Healthy	0.3	15	1.1	3
At-risk	0.4	5	0.9	3
High-risk	0.2	−6	−1.7	−3
All-cause pneumonia				
Age/risk profile				
18–49 years[a]	8.6	18	11.0	952
Healthy	7.5	19	8.1	605
At-risk	0.9	13	31.2	288
High-risk	0.2	4	29.0	59
50–64 years[a]	5.6	10	21.5	1,205
Healthy	3.8	11	10.8	415
At-risk	1.3	9	42.2	567
High-risk	0.4	4	52.7	222
65–74 years[a]	1.1	8	22.1	244
Healthy	0.5	13	29.6	157
At-risk	0.4	4	38.7	157
High-risk	0.2	−2	−42.7	−70
≥75 years[a]	0.8	−10	−227.7	−1,878
Healthy	0.3	−9	−76.2	−210
At-risk	0.4	−8	−209.2	−750
High-risk	0.2	−14	−480.6	−918

[a]For each age group, absolute reductions in cases per year were calculated by multiplying risk-specific absolute declines in rates and risk-specific numbers of person-years in 2015 and summing products across risk groups.

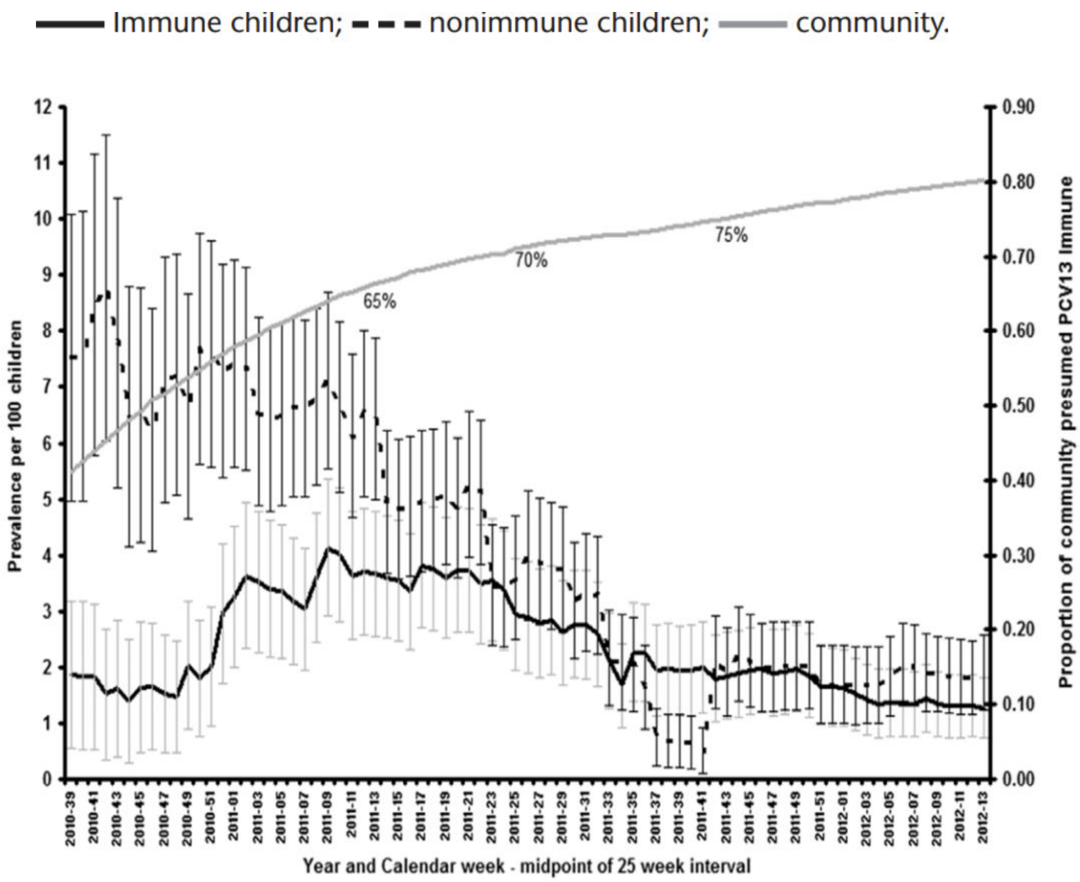
The Pelton team concluded that low vaccination rates in adults over the age of 65 with comorbidities meant that a large portion of the decline in adult IPD cases originated from the thorough immunization of children in the period of 2 months to 5 years. Individuals who were immunocompromised and/or had comorbidities that were diagnosed with IPD most likely had a weak defense system (Pelton, 2019). In terms of statistics, the team published that there was a 95% noticeable decline in VT IPD cases in children, and an 87% noticeable decline in adults following the thorough immunization of children (Pelton, 2019). In terms of overall reduction, the percent of IPD cases reduced in adults over the age of 65 years was 58%, as compared to a 95% reduction rate in children less than 5 years of age (Pelton, 2019). This observational study demonstrated that 13vPnC release, as with 7vPnC release in the previous generation, demonstrated a substantial decline in all adults within the general population of the United States, irrespective of age group, medical and vaccination history (Pelton, 2019). Adults over the age of 75 years old seemed to benefit the least from widespread vaccination, which can be attributed to a non-recoverable low state of immunity and the overtake of non-vaccine serotypes as part of IPD contraction, which is discussed in detail in the next section. Through the publication *Decline in Pneumococcal Disease Attenuated in Older Adults and Those with Comorbidities Following Universal Childhood PCV13 Immunization*, the Pelton team demonstrated that the pneumococcal conjugate vaccine is an essential component of prevention, in children and adult populations, yet more research is needed in the realm of non-vaccine serotype disease takeover.

Taken together these studies demonstrated the benefit of long-term administration of the pneumococcal conjugate vaccine, specifically 7vPnC (Scott, 2012) and 13vPnC (Pelton, 2019). More importantly, these studies enable us to adapt tools to analyze the next generations of the pneumococcal conjugate vaccines (10vPnC, 13vPnC, 15vPnC and now 20vPnC). Each of the aforementioned studies vaccine surveillance studies have contributed to the understanding of long-term defense and the establishment of *herd immunity* within groups of immunized and non-immunized individuals. Researchers at the Center for American Indian Health found that extended, routine use of 7vPnC demonstrated a significant decline in vaccine serotypes and serotype 6A (vaccine-associated) in the Navajo and White Mountain Apache demographic, where the net reduction in vaccine serotype carriage percentage was far greater than the net increase in non-vaccine serotype carriage percentage (Scott, 2012). Interestingly, this significant decline in vaccine-related-serotype pneumococcal carriage rate was not seen across all demographics in the United States, where non-vaccine serotypes were seen to have taken over for vaccine serotypes in terms of pneumococcal colonization (Scott, 2012). This result highlighted the importance of risk factors when it comes to considering the low prevalence of pneumococcal carriage in the Navajo and White Mountain communities, such as: replacement of *Streptococcus pneumonia* with species such as *Staphylococcus*, promotion of environmental factors that hinder pneumococci growth and a noticeably smaller rate of antibiotic resistance among *Streptococcus Pneumoniae* colonies (Scott, 2012). Researchers at the Center for American Indian Health were also able to make an informed hypothesis about the proportion of Invasive Pneumococcal Disease cases attributed to non-vaccine serotypes before the introduction of 7vPnC in both the Navajo and White Mountain population as compared to the general American population; they found that although the net rate of IPD has decreased in these specific Native American tribes with the serotypes accounted for in 7vPnC, the difference in IPD rates compared with that of the general American population has in fact increased due to NVT replacement of colonization (Scott, 2012).

In connection to the current NP Surveillance Study at the Pelton Lab, underlying factors such as recent *antibiotic use* that demonstrated a decrease in pediatric pneumococcal carriage and antibodies transferred to children from mothers through breast milk as protection from IPD proved to be pivotal components (Pelton, 2019). Specifically, antibiotic use and antibody transfer to children seemed to promote long-term immunity and SP carriage resistance in adults (Scott, 2012). The study also explained a trend that is expected to be carried out in future evaluations and updated generations of the pneumococcal conjugate vaccine- with the introduction of the next vaccine, there is expected to be a continued decline in vaccine-serotype carriage across all age groups, as well as cross-immunity for select non-vaccine serotypes due to the evolution of routine immunization and prominence of herd immunity (Scott, 2012). These studies also suggest a long-term but achievable goal of pneumococcal conjugate vaccine research, in which a conjugate vaccine is effectively created so as to provide protection against the understood serotypes, as well as cross-protection against all serotypes that are expected to surface, so as to protect against IPD and critical co-morbidities that can accompany colonization.

Figure 6.

Comparison trends of 13vPnC serotype prevalence in immunized and non-immunized children, plotted with the proportion of children less than 5 years in the community (Taken with permission from Dr. Pelton from Direct and Indirect Effects of PCV13 on Nasopharyngeal Carriage of PCV13 Unique Pneumococcal Serotypes in Massachusetts' Children).



The figure above demonstrates the period between July 1, 2010 and June 30, 2012, reflecting the total number of enrolled children from the first interim (407 enrollees) to the last interim (1612 enrollees). The graph demonstrates the community-level of 13vPnC in children less than 5 years in Boston, Massachusetts, which showed a significant increase in immunity from 41% to 72% at year 1 of surveillance and then 80% at year 2 of surveillance (Scott, 2012). It was demonstrated that the difference in serotype-specific carriage as attributed to 13vPnC decreased with time between the immune and non-immune as an attribute to herd immunity. As this study highlights, the overall goal of pneumococcal conjugate vaccination is to instigate anti-pneumococcal antibody production and mucosal immune response and immune memory in order to uphold immunity levels within the long-term population (Berical et. al, 2016). Pneumococcal conjugate vaccine surveillance studies have proven to be effective in understanding the immunity levels of the aging population and also the vaccine doses necessary to establish full prevention against *Streptococcus pneumoniae* and the most effective way to create future pneumococcal conjugate vaccines (Berical et. al, 2016).

COMMUNITY

What are the implications of non-vaccine serotypes in the community?

In the previous studies where herd immunity relevance was explored, researchers also found that non-vaccine serotypes seemed to have taken over vaccine-serotypes with nasopharyngeal colonization. Research published in *Human Vaccines and Immunotherapies* set out to understand the *epidemiology* of NVT before and after 13vPnC release and widespread use (Du et. al, 2021). While the previous studies on pneumococcal conjugate vaccines proved to decrease and partially eradicate colonization of SP serotypes accounted for in the vaccines, there was a noticeable increase in NP colonization prevalence of non-vaccine-specific serotypes after widespread vaccination

(Du et. al, 2021). This was a novel publication, in which researchers analyzed national surveys to understand the changes in prevalence of IPD, if any, in the time before and after 13vPnC global use. Not only did this provide insight towards VT and NVT colonization rates in the time of vaccination, but also examined the rates within different races, so as to gain a deeper understanding of epidemiology. A national survey conducted in Germany looked at IPD incidence 2 years after 13vPnC introduction, in which the incidence rate recoiled due to NVT NP colonization. The publication confirmed a 26% reduction in IPD cases, noted in the study period 2015-2016, as compared to the pre-vaccination period (Du et. al, 2021). In addition, the incidence rate of IPD cases attributed to NVTs in Germany elevated in children between the ages of 0-12 months with fluctuation in other age brackets; the incidence rate of IPD associated with non-13vPnC serotypes demonstrated a steep incline in the period from 2015-2016 (Weinberger et. al, 2018). The same trend was seen in Gambia as part of the national surveillance survey, in which the calculated incidence rate of IPD attributed to 13vPnC serotypes decreased by about 82% after a period of widespread vaccine administration in May 2011 onwards; however, the incidence rate of IPD attributed to non-13vPnC serotypes increased by about 48% in children less than 2 years, and the new rate increased approximately 47% in children less than 5 years. This study moved the conversation on vaccine science from localization to universalization. Researchers also noticed an increase in IPD cases as a result of NVTs, which has doubled since 7vPnC introduction and accounted for about 40% of cases in 2016-2017 (Mackenzie et. al, 2016). In England, the incidence rate of IPD due to non-13vPnC serotypes demonstrated a noticeable increase with an incidence ratio of 1.25 across all ages, just after 4 years of vaccine introduction in May 2011; in addition, the incidence rate of IPD caused by the outlined non-13vPnC serotypes seen in children less than 5 years was higher in study year 2013-2014 than 2012-2013, indicating the growth and perseverance of immunity from the vaccinated to unvaccinated (Du et. al, 2021). The authors noted that the IPD incidence rate due to non-vaccine serotypes has doubled since the initial release of 7vPnC and has been on the incline since. Specifically, NVTs such as 8, 9N and 12F were responsible for over 40% of IPD cases between 2016-2017 in England in children less than 5 years and adults greater than 45 years (Waight et. al, 2015). Du and the research team saw, through a national surveillance study in Denmark, a rise in cases of IPD associated with NVTs mainly identified in 13vPnC vaccinated children between the ages of 0-4 years and among elders over 65 years; in Hokkaido, the authors found that IPD cases as attributed to NVTs (10A, 11A, 15A, 23A, 35B) showed a noticeable increase by approximately 35.4% in the period between 2013-2014 (Kawaguchiya et. al, 2015). These global instances demonstrate that immunization and immunity trends are rooted in science not coincidence. Japan also demonstrated a noticeable IPD outburst after the short release and use of 13vPnC, where the rate of IPD cases as a result of non-13vPnC serotypes demonstrated a 20% increase in 2014 (Nakano et. al, 2015). Age, location and ethnicity did not affect the expected scientific outcome. In Hong Kong specifically, researchers calculated that IPD cases attributed to NVSTs increased over a short period of time, from a 2% increase (1999-2000 to 2009-2010) to a 29% increase (2010-2013), where the most pertinent 13vPnC serotypes were 15B, 15C, 6C, 15A respectively (Ho et.

al, 2015). The authors confirmed that upon the release of 13vPnC, many in-patients within the main hospitals had IPD as a result of NVTs, a trend not seen before vaccine release (Du et. al, 2021). Similarly, while Du and researchers set out to understand why the rise of NVTs sparked during and after 13vPnC release, they also wanted to understand the serotype-specific invasion potentials and if one serotype was more prominent among infected individuals. The invasive potential of serotypes was calculated as the *ratio* of IPD-serotype carriage as compared to serotype 19A (for reference); Researchers confirmed that NVTs (1, 7F and 12F) have a higher rate of invasiveness in children less than 5 years, while NVTs (6C, 15A, 15BC, 16F, 23B) have a lower rate of invasiveness than the 19A reference serotype (Du et. al, 2021). Ultimately, the epidemiology of NVTs in various countries concluded that NVTs may be the dominant isolates in SP NP samples, and the noticeable replacement of VTs with NVTs after PCV administration may signify the modification and mutation of pneumococcal-related diseases. The widespread increase in non-13vPnC serotype colonization in these diverse nations led researchers to believe that the replacement of NVTs with VTs in IPD cases was an issue that transcended racial and social boundaries; this was rooted in an issue of underlying medical history and lack of vaccine accountability for *Streptococcus pneumoniae* capsule changes. Many researchers are left to wonder whether the published surveillance studies have demonstrated that NVTs have finally been discovered or if they were always an underlying threat in communities but never accounted for.

A similar publication explored the adverse effects that introduction of the pneumococcal conjugate vaccine can cause within the microbial ecosystem and human immunity (Kwambana-Adams et. al, 2017). Although 7vPnC, 10vPnC and 13vPnC have demonstrated noticeable success, in terms of long-term public health maintenance and pneumococcal disease decline, there has been a noticeable replacement of vaccine serotypes with non-vaccine serotypes and serotype switching of pneumococcal species (Kwambana-Adams et. al, 2017). The publication highlights this growing concern within children of Gambian communities, as pneumococcal carriage and colonization rates have exceeded 90%, and 7vPnC is thought to provide protection against 63% of the known serotypes (Kwambana-Adams et. al, 2017). This significant increase in *Streptococcus pneumoniae* carriage and colonization, despite vaccine administration, demonstrates that the widespread use of the vaccine may only protect serotype-specific colonization and migration to sinuses and the middle ear passage in the infant population. To further understand *Streptococcus pneumonia* nasopharyngeal colonization and its effect on ecology, human immunity and microbiome at-large, the authors recruited over 102 infants in Gambia to then understand and categorize the amount and frequency of 7vPnC exposure (Kwambana-Adams et. al, 2017).

The study was built on separation of the 102 infants into 3 study groups, where the only differences across study groups were *birthplace*, *history of maternal vaccination* and *history of sibling vaccination*; of importance, infants placed within Group 3 where born in Gambian villages with historical 7vPnC administration and were assumed to have high historical rates of maternal and sibling immunization (Kwambana-Adams et. al, 2017). Kwambana-Adams and researchers confirmed the following group requirements and immunization amount: Group 1 (Control, at least one 7vPnC dose greater than 6 months of age), Group 2 (Direct impact of vaccination, 7vPnC doses at the ages of 2 months, 3 months and 4 months), Group 3 (Direct impact and herd immunity, 7vPnC doses at the ages of 2 months, 3 months and 4 months), where pneumococcal *carriage* was: 79% in Group 1 infants, 75% in Group 2 infants, and 82% in Group 3 infants (Kwambana-Adams et. al, 2017). On the other hand, 7vPnC serotype-carriage was 37% in Group 1 infants, 20% in Group 2 infants and 17% in Group 3 infants, which signifies that pneumococcal carriage was predominantly due to non-7vPnC serotypes (19A, 23B and 35B) as opposed to 7vPnC serotypes (Kwambana-Adams et. al, 2017). With respect to maternal vaccination history, the pneumococcal carriage rate seen in infant groups born to immunized mothers was 78%, as compared to the carriage rate seen in infant groups born to non-immunized mothers which was 83% (Kwambana-Adams et. al, 2017). In terms of serotype-specific pneumococcal carriage, infant groups from immunized mothers demonstrated a lower carriage rate (19%) in relation to infant groups from non-immunized mothers with a slightly higher carriage rate (25%) (Kwambana-Adams et. al, 2017).

Finally, Kwambana-Adams and team noted that there was no substantial difference in SP serotype-specific carriage and colonization between participants who had a vaccinated sibling as opposed to those who did not; in terms of location within Gambia, researchers found that infants born at a designated hospital had a significantly higher vaccine-specific pneumococcal carriage rate (27%) in relation to infants born in personal care (21%) (Kwambana-Adams et. al, 2017). After analyzing infants from communities in Gambia from 0-1 year through nasopharyngeal sample collection, they found that 7vPnC reduces serotype-specific colonization, yet the overall carriage and colonization rate remains above 75%, indicating that non-vaccine-specific serotypes are dominant and expansive during the infant's first 12 months (Kwambana-Adams et. al, 2017). This led researchers to conclude that even in the presence of herd immunity, receipt of 7vPnC does not substantially alter the nasopharyngeal microbiome and makeup in the sample of 102 Gambian infants, as non-vaccine serotypes have taken over the areas previously occupied by vaccine serotypes, in which the most common NVTs include: 19A, 35B and 15B (Kwambana-Adams et. al, 2017). Kwambana-Adamas and team referenced several pneumococcal surveillance studies that were previously conducted, in which the three NVTs were prominent in Gambian communities prior to 7vPnC release, indicating that vaccine administration did not create non-vaccine serotypes but certainly brought light to their existence and potential disruption to public health and immunity in future generations.

Ultimately, while 7vPnC and 13vPnC helped to significantly reduce IPD cases caused by serotypes within the released pneumococcal conjugate vaccine in many global regions, non-vaccine serotypes proved to be universally significant in replacing vaccine-serotype-associated pneumococcal colonization and disease. As a result, both tables outlined in the publication work to demonstrate a significant connection between IPD rise and NVST invasion after13vPnC introduction. This lead Kwambana-Adamas and researchers to understand that while curated vaccines may have a direct effect at eliminating a certain outcome, they may also have an effect on surfacing an underlying concern.

MECHANISMS OF CONJUGATE VACCINES

What are the mechanisms that allow the conjugate vaccine to combat colonization?

Conjugate vaccines have proved to be an effective method to both preserve immunity against bacterial capsular polysaccharides and eliminate long-term cases of bacterial meningitis caused by *Streptococcus pneumoniae* and many other encapsulated bacteria (Rappuoli R, 2018, Rappuoli et. al, 2019). The publication *On the mechanisms of conjugate vaccines* explains that the defining capsular polysaccharides of bacteria are long polymers, in which bacteria have been historically able to modify both chemically and physically (Rappuoli R, 2018, Rappuoli et. al, 2019). An important mechanism of the *conjugate* vaccine is its ability to induce antibody production against the targeted polysaccharide capsule, in order to eliminate the pathogen before widespread infection. Purified polysaccharide vaccines target bacteria such as pneumococci, but are unable to promote the protective antibody response in the infant population (Rappuoli R, 2018, Rappuoli et. al, 2019); adequate immunization of the infant population that protects against NP colonization is necessary to promote long-term and widespread immunity within a population. The publication expresses the underlying mechanics of conjugate vaccines, which explains why antibody production is essential to promote a decrease in carriage and colonization over time. After an individual has been immunized via a conjugate vaccine, the polysaccharides are absorbed by *dendritic cells* of the immune system (Rappuoli R, 2018, Rappuoli et. al, 2019). Afterwards, the capsular polysaccharides then travel to the lymph nodes, where they activate two of the most important units in the immune system, B and T cells, to initiate an immune response. T-helper cells are responsible for recognizing the bacterial-capsular polysaccharide antigen; B cells are responsible for presenting the bacterial-capsular-polysaccharide antigen; polysaccharide-specific B cells differentiate into antibody-producing plasma cells (Rappuoli R, 2018, Rappuoli et. al, 2019). Overall, this mechanistic reaction in the germinal center induces the production of high-affinity antibodies either during a response to a bacterial infection such as IPD or after immunization with PCV (Rappuoli R, 2018, Rappuoli et. al, 2019). The B cells, or receptor cells, identify and uptake small units of polysaccharides in the *light zone* of dendritic cells, or antigen-presenting cells, internalize the polysaccharide-antigens and then place them in the pocket of the major histocompatibility complex for required antigen presentation to T-helper cells (Rappuoli et. al, 2018). Afterwards, the T-helper cell becomes activated. Presentation is adequate, and the T cells can provide substantial assistance to the receptor B cells through cytokine release; the B cell receptors apply direct and rigid force so that a larger quantity of capsular polysaccharide can be retrieved from antigen-presenting dendritic cells and a stronger, more streamlined response from the T-helper cells will be in place (Rappuoli et. al, 2019). With conjugate vaccines, the goal is to produce both antibodies and memory B cells so that the host can develop long-term immunity to the bacterial polysaccharide capsule upon subsequent contact with the pathogen (Rappuoli et. al, 2019).

With regard to polysaccharide vaccines, researchers concluded that polysaccharides themselves can recruit B cells without question; polysaccharide-antigens are recognized and recruited by B cell-antibody receptors and removed from dendritic cells to then be placed in endosomes for ultimate destruction (Rappuoli et. al, 2019). However, researchers noted that once the polysaccharide-antigens were internalized in the endosome, the overall immune reaction ceased since polysaccharide-antigens could not fit within the designated major histocompatibility complex (MHC) pocket, a crucial step (Rappuoli et. al, 2019). The polysaccharide-antigens are unable to connect with T-helper cells to promote antibody and memory B cell production, which leads to B-cell apoptosis due to lack of use and compliance. Rappuoli and colleagues confirmed that pneumococcal polysaccharide vaccine (PPV) insufficiency can be mediated when polysaccharide-antigens are tightly bound and associated with carrier proteins. In other words, the weak polysaccharide antigens will be bound to strong carrier proteins in order to elicit a stronger immune response against the masked weak antigen. As a result, the B cell receptors will confidently recognize both the polysaccharides on dendritic cells and the attached carrier proteins to then produce small peptides as a result of carrier protein mechanical processing (Rappuoli et. al, 2019). Afterwards, the peptides can then be successfully transported to the MHC pocket for T cell identification and recruitment; this process leads to antibody and memory B cell production in order to familiarize the immune system with the encapsulated bacteria of interest to prepare for a rapid and successful defense when *Streptococcus pneumoniae* is encountered (see publication for illustrations). Protection is capsular-serotype specific, not protective against all serotypes. This study encouraged infectious disease researchers to continue to explore immunity by increasing the quantity of *covalent interaction* between the carrier protein (strong antigen) and polysaccharide (weak antigen). Conjugate vaccines have proved to be effective in promoting and engaging T cells, which leads to affinity maturation of antibodies, antibody-producing plasma cells and memory B cells for long-term protection and immunity, unlike the polysaccharide vaccine (Rappuoli et. al, 2018). Current studies are looking to “push the envelope” so that immunogenicity can be improved with the release of the next generations of vaccines. Researchers determined that the quantity of covalent junctions between the carrier protein and polysaccharide is an important mechanism to both present the antigen for internalization and engage T cells to aid with destruction (Rappuoli et. al, 2018).

Recently, Duarte G et. al (2022) analyzed the immunogenicity of the conjugate and polysaccharide vaccines by vaccinating women with 10vPnC and PPV23 and determined the protectiveness against *Streptococcus pneumoniae* at the corresponding stages of pregnancy (Duarte G et. al, 2022). This Surveillance Study examined the demographic and immune history of women before and after immunization (Table 3), as well as modifications, if any, in immune response and antibody production after immunization (Table 4). The results were in favor of *postpartum pneumococcal conjugate immunization* to greatly reduce common serotypes within this patient population. The study enrolled 347 pregnant women, all with HIV, in which: 115 received 10vPnC, 115 received PPV-23 and 116 were placebo (53 received 10vPnC at 24-weeks postpartum and 53 received PPV-23 at 24-weeks postpartum) (Duarte et. al, 2022). The Pelton team concluded that measured antibody concentrations were increased with both the 10vPnC and PPV-23 immunizations, with a greater trend seen postpartum (Duarte et. al, 2022).

Table 3.

Pre-vaccination immune characteristics of women

(Taken with permission from Dr. Pelton from Immunogenicity of Conjugated and Polysaccharide Pneumococcal Vaccines Administered During Pregnancy or Postpartum to Women With HIV).

Characteristic		Antepartum PCV-10 (N=38)	Antepartum PPV-23 (N=37)	Postpartum PCV-10 (N=35)	Postpartum PPV-23 (N=29)
Race	Black	24 (63%)	26 (70%)	20 (57%)	20 (69%)
	White	13 (34%)	9 (24%)	8 (23%)	6 (21%)
	Other	1 (3%)	0 (0%)	6 (17%)	3 (10%)
	Unknown	0 (0%)	2 (5%)	1 (3%)	0 (0%)
Ethnicity	Latino	36 (95%)	33 (89%)	32 (91%)	29 (100%)
	Not Latino	0 (0%)	1 (3%)	1 (3%)	0 (0%)
	Unknown	2 (5%)	3 (8%)	2 (6%)	0 (0%)
Age at randomization (years)	Mean (SD)	28 (5)	27 (7)	29 (6)	28 (7)
Gestational age at randomization (weeks)	Mean (SD)	28 (5)	27 (5)	N/A	N/A
CD4%	Mean (SD)	33 (8)	33 (10)	35 (9)	33 (11)
CD4 cells/μL	Mean (SD)	634 (217)	590 (284)	781 (297)	811 (428)
CD4>200 cells/μL	Yes	37 (97%)	35 (95%)	33 (94%)	28 (97%)
CD8 cells/μL	Mean (SD)	803 (284)	782 (392)	822 (325)	957 (302)
RNA copies/mL of plasma	Median (Q1-Q3)	39 (39-58)	39 (39-106)*	39 (39-39)	39 (39-82)
RNA <40 copies/mL	Yes	28 (74%)	22 (61%)*	30 (86%)	21 (72%)
ARV Use	Yes	38 (100%)	37 (100%)	33 (94%)	27 (93%)

Table 4.

Post-vaccination immune response of women

(Taken with permission from Dr. Pelton from Immunogenicity of Conjugated and Polysaccharide Pneumococcal Vaccines Administered During Pregnancy or Postpartum to Women With HIV).

Characteristic	Antepartum PCV-10 (N=17)	Antepartum PPV-23 (N=17)	Postpartum PCV-10 (N=18)	Postpartum PPV-23 (N=14)
Pre-vaccination IgA	0 (0,4)*	0 (0,4)	0 (0,8)	0 (0,8)
Post-vaccination IgA	0 (0,4)	0 (0,2)	1 (0,12)	0 (0,6)
Change from pre- to post- vaccination IgA	0 (-4,2)	0 (-4,2)	0 (-6,6)	0 (-6,6)
Pre-vaccination IgG	2 (0,34)	2 (0,16)	3 (0,26)	2 (0,14)
Post-vaccination IgG	3 (0,22)	4 (0,18)	5 (0,24)	2 (0, 24)
Change from pre- to post- vaccination IgG	0 (-30,6)	2 (-12,10)	0 (-12,24)	-2 (-12,18)
Pre-vaccination IFNγ	5 (0,28)	6 (0,172)	13 (0,94)	6 (0,164)
Post-vaccination IFNγ	4 (0,86)	4 (0,188)	8 (0,70)	11 (0,76)
Change from pre- to post- vaccination IFNγ	6 (-12,86)	0 (-68,68)	2 (-30,60)	2 (-88,28)
Pre-vaccination IL17A	3 (0,76)	6 (0,144)	11 (0,62)	4 (0,128)
Post-vaccination IL17A	6 (0,66)	6 (0,148)	6 (0,84)	10 (2,62)
Change from pre- to post- vaccination IL17A	1 (-26,22)	0 (-70,36)	0 (-22,62)	6 (-74,40)

* Numbers represent median (min-max) SFC/10^6 PBMC

To determine if pre-vaccination antibody concentration had a direct effect on the aforementioned results, the team analyzed pre-vaccination antibody concentration to find that a higher concentration before pneumococcal immunization resulted in an increased antibody concentration for all included serotypes (Duarte et. al, 2022). The authors found that postpartum administration of vaccines seemed to provide the most benefit to patients with chronic illnesses, such as HIV, as the percentage of CD4+ and CD8+ T cells were higher postpartum as the immune suppression that naturally accompanies pregnancy diminished (Duarte et. al, 2022). This study determined that vaccination with PPV-23 produced higher antibody concentrations in postpartum women, as opposed to 10vPnC against the serotypes found in the vaccines, but lower antibody concentrations against a specific NVT (Duarte et. al, 2022). Thus, the measured immunity of 10vPnC (conjugate vaccine) versus PPV-23 (polysaccharide vaccine) was greatest in women during the postpartum stage of this study, when cofactors such as HIV replication and CD8+ T-cells were at a low point and CD4+ T-cell concentration was high enough to ‘boost’ the response. As a result, PPV23 seems to be the most efficient vaccine for this patient demographic. However, IPD susceptibility and underlying comorbidities should be considered as important factors to consider for future vaccine design. From the initial release of 7vPnc (2000) to the release of 20vPnC in Fall 2023, research on *Streptococcus pneumoniae*, Invasive Pneumococcal Disease, vaccine development and existing comorbidities has made a significant impact on public health and global immunity now, but the previous and current studies have paved the way for the next wave of vaccine research. While vaccine companies are targeting the polysaccharide capsule for

Streptococcus pneumoniae elimination, perhaps future research can examine ways to eliminate the bacteria internally to prevent external serotype switching and modifications.

New research studies are ongoing (including at the Pelton Lab) to develop both novel capsular dependent and independent vaccines. While many research studies have proved the effectiveness of pneumococcal conjugate vaccines against vaccine-specific serotypes, it will be a challenge to create a non-pneumococcal conjugate vaccine that also considers IPD prevention. The goal should be to create a pneumococcal conjugate vaccine with pneumococcal proteins (either soluble proteins and/or polysaccharide transport proteins) to then broaden serotype coverage (Moffitt and Malley, 2016). It is equally important to consider whether this vaccine will protect immunized and non-immunized individuals alike. To account for non-PCVs, factors such as adequate dosage and existing pneumococcal colonization within patients should be researched. A serotype-independent vaccine against *Streptococcus pneumoniae* may be possible with continued research on *Streptococcus pneumoniae*, host immunity and herd immunity.

BIBLIOGRAPHY

Berical AC, Harris D, Dela Cruz CS, Possick JD. Pneumococcal Vaccination Strategies. An Update and Perspective. Annals of the American Thoracic Society, 2016 Jun;13(6):933–944. doi: 10.1513/AnnalsATS.201511-778FR.

Canada, P. H. A. of. (2021, November 1). *Government of Canada*. Canada.ca. Retrieved March 4, 2023, from https://www.canada.ca/en/public-health/services/immunization/vaccine-preventable-diseases/invasive-pneumococcal-disease/health-professionals.html

Centers for Disease Control and Prevention. (2022, January 27). *Grade: 20-valent pneumococcal conjugate vaccine (PCV20) for adults aged ≥65 years*. Centers for Disease Control and Prevention. Retrieved February 27, 2023, from https://www.cdc.gov/vaccines/acip/recs/grade/pneumo-PCV20-age-based.html

Centers for Disease Control and Prevention. (2016, April 15). *Meningitis lab manual: ID, characterization of strep pneumoniae*. Centers for Disease Control and Prevention. Retrieved March 6, 2023, from https://www.cdc.gov/meningitis/lab-manual/chpt08-id-characterization-streppneumo.html

Covid-19 testing and kids: Everything parents should know. Children's Hospital Los Angeles. (2022, May 17). Retrieved March 15, 2023, from https://www.chla.org/blog/health-and-safety-tips/covid-19-testing-and-kids-everything-parents-should-know

Duarte G, Muresan P, Ward S, Laimon L, Pelton SI, Canniff J, Golner A, Bone F, Newton L, Fenton T, Coutinho CM, João EC, Santos BR, Pilotto JH, Oliveira RH, Pinto JA, Machado ES, Kreitchman R, Chakhtoura N, Mussi-Pinhata MM, Weinberg A. Immunogenicity of Conjugated and Polysaccharide Pneumococcal Vaccines Administered During Pregnancy or Postpartum to Women With HIV. Journal of Infectious Diseases, 2022;225(6):1021-31. doi: 10.1093/infdis/jiab567. PMID: 34791324; PMCID: PMC8921998

Du QQ, Shi W, Yu D, Yao KH. Epidemiology of non-vaccine serotypes of *Streptococcus pneumoniae* before and after universal administration of pneumococcal conjugate vaccines. Human Vaccines & Immunotherapeutics. 2021 Dec 2;17(12):5628-37. doi: 10.1080/21645515.2021.1985353. Epub 2021 Nov 2. PMID: 34726580; PMCID: PMC8903918.

Etest®. bioMérieux Clinical Diagnostics. (2022, November 18). Retrieved March 15, 2023, from https://www.biomerieux-diagnostics.com/etestr#:~:text=ETEST%C2%AE%20is%20a%20predefined,tests%20(AST)%20tests%20provide.

Gram stain: What it is, purpose, procedure & results. Cleveland Clinic. (n.d.). Retrieved March 18, 2023, from https://my.clevelandclinic.org/health/diagnostics/22612-gram-stain

Janapatla RP, Su LH, Chen HH, Chang HJ, Tsai TC, Chen PY, Chen CL, Chiu CH. Epidemiology of culture-confirmed infections of Streptococcus pneumoniae (2012-2015) and nasopharyngeal carriage in children and households in Taiwan (2014-2015). Journal of Medical Microbiology. 2017 Jun;66(6):729-736. doi: 10.1099/jmm.0.000488. Epub 2017 Jun 8. PMID: 28590240

Kawaguchiya M, Urushibara N, Aung MS, Morimoto S, Ito M, Kudo K, Sumi A, Kobayashi N. Emerging non-PCV13 serotypes of noninvasive Streptococcus pneumoniae with macrolide resistance genes in northern Japan. New Microbes and New Infections. 2015 Nov 11;9:66-72. doi: 10.1016/j.nmni.2015.11.001. PMID: 26909157; PMCID: PMC4735479

Khan, Z. A., Siddiqui, M. F., & Park, S. (2019, May 3). *Current and emerging methods of antibiotic susceptibility testing*. Diagnostics (Basel, Switzerland). Retrieved March 16, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6627445/

Klugman, K. P. (2014, May 27). *Herd protection induced by pneumococcal conjugate vaccine*. The Lancet Global Health. Retrieved March 19, 2023, from https://www.thelancet.com/journals/langlo/article/PIIS2214-109X(14)70241-4/fulltext#articleInformation

Kwambana-Adams B, Hanson B, Worwui A, Agbla S, Foster-Nyarko E, Ceesay F, Ebruke C, Egere U, Zhou Y, Ndukum M, Sodergren E, Barer M, Adegbola R, Weinstock G, Antonio M. Rapid replacement by non-vaccine pneumococcal serotypes may mitigate the impact of the pneumococcal conjugate vaccine on nasopharyngeal bacterial ecology. Scientific Reports. 2017 Aug 15;7(1):8127. doi: 10.1038/s41598-017-08717-0. PMID: 28811633; PMCID: PMC5557800

L. Barth Reller, Melvin Weinstein, James H. Jorgensen, Mary Jane Ferraro, Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices, *Clinical Infectious Diseases*, Volume 49, Issue 11, 1 December 2009, Pages 1749–1755, https://doi.org/10.1086/647952

Loughlin AM, Hsu K, Silverio AL, Marchant CD, Pelton SI. Direct and indirect effects of PCV13 on nasopharyngeal carriage of PCV13 unique pneumococcal serotypes in Massachusetts' children. Pediatric Infectious Disease Journal. 2014 May; 33(5):504–510. doi: 10.1097/INF.0000000000000279. PMID: 24670957

Mackenzie GA, Hill PC, Jeffries DJ, Hossain I, Uchendu U, Ameh D, Ndiaye M, Adeyemi O, Pathirana J, Olatunji Y, Abatan B, Muhammad BS, Fombah AE, Saha D, Plumb I, Akano A, Ebruke B, Ideh RC, Kuti B, Githua P, Olutunde E, Ofordile O, Green E, Usuf E, Badji H, Ikumapayi UNA, Manjang A, Salaudeen R, Nsekpong ED, Jarju S, Antonio M, Sambou S, Ceesay L, Lowe-Jallow Y, Jasseh M, Mulholland K, Knoll M, Levine OS, Howie SR, Adegbola RA, Greenwood BM, Corrah T. Effect of the introduction of pneumococcal conjugate vaccination on invasive pneumococcal disease in The Gambia: a population-based surveillance study. The Lancet. Infectious Diseases. 2016 Jun;16(6):703-711. doi: 10.1016/S1473-3099(16)00054-2. Epub 2016 Feb 18. PMID: 26897105; PMCID: PMC4909992

Moffitt K, Malley R. Rationale and prospects for novel pneumococcal vaccines. Human Vaccines & Immunotherapeutics. 2016;12(2):383-392. doi: 10.1080/21645515.2015.1087625. PMID: 26535755; PMCID: PMC5049723

Nakano S, Fujisawa T, Ito Y, Chang B, Suga S, Noguchi T, Yamamoto M, Matsumura Y, Nagao M, Takakura S, Ohnishi M, Ihara T, Ichiyama S. Serotypes, antimicrobial susceptibility, and molecular epidemiology of invasive and non-invasive Streptococcus pneumoniae isolates in paediatric patients after the introduction of 13-valent conjugate vaccine in a nationwide surveillance study conducted in Japan in 2012-2014. Vaccine. 2016 Jan 2;34(1):67-76. doi: 10.1016/j.vaccine.2015.11.015. Epub 2015 Nov 19. PMID: 26602268

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Pelton SI, Bornheimer R, Doroff R, Shea KM, Sato R, Weycker D. Decline in Pneumococcal Disease Attenuated in Older Adults and Those With Comorbidities Following Universal Childhood PCV13 Immunization. Clinical Infectious Diseases 2019 May 17;68(11):1831-1838. doi: 10.1093/cid/ciy800. PMID: 30239637; PMCID: PMC6522679

Pelton, S. I., Lapidot , R., & Yildirim, I. (2019, November). https://mail.google.com/mail/u/0/#search/basha/FMfcgzGqQmNcmsxvmnStCcDzmfKKMbCQ?projector=1&messagePartId=0.3. *JAMA Pediatrics*.

Pneumococcal disease. National Foundation for Infectious Diseases. (2022, October 13). Retrieved February 12, 2023, from https://www.nfid.org/infectious-diseases/pneumococcal/

Pruthi, S. (2023, February 9). *Meet Our Medical editors - about this site*. Mayo Clinic. Retrieved February 22, 2023, from https://www.mayoclinic.org/about-this-site/meet-our-medical-editors

Rappuoli R, De Gregorio E, Costantino P. On the mechanisms of conjugate vaccines. Proceedings of the National Academy of Sciences USA. 2019 Jan 2;116(1):14-16. doi: 10.1073/pnas.1819612116. Epub 2018 Dec 21. PMID: 30578318; PMCID: PMC6320500

Review of *Surveillance of Pneumococcal Colonization in Children less than 5 years of age at Boston Medical Center* by Stephen Pelton, Rotem Lapidot. (2021, November 30).

Scott JR, Millar EV, Lipsitch M, Moulton LH, Weatherholtz R, Perilla MJ, Jackson DM, Beall B, Craig MJ, Reid R, Santosham M, O'Brien KL. Impact of more than a decade of pneumococcal conjugate vaccine use on carriage and invasive potential in Native American communities. Journal of Infectious Diseases. 2012 Jan 15; 205(2): 280–288doi: 10.1093/infdis/jir730. Epub 2011 Nov 29. PMID: 22128315; PMCID: PMC3244367.

Streptococcus pneumoniae - statpearls - NCBI bookshelf. (n.d.). Retrieved February 17, 2023, from https://www.ncbi.nlm.nih.gov/books/NBK470537/

Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MP, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. Lancet Infectious Diseases. 2015 May;15(5):535-543. doi: 10.1016/S1473-3099(15)70044-7. Epub 2015 Mar 20. Erratum in: The Lancet. Infectious Diseases. 2015 Jun;15(6):629. PMID: 25801458

Weinberger R, von Kries R, van der Linden M, Rieck T, Siedler A, Falkenhorst G. Invasive pneumococcal disease in children under 16 years of age: Incomplete rebound in incidence after the maximum effect of PCV13 in 2012/13 in Germany. Vaccine. 2018 Jan 25;36(4):572-577. doi: 10.1016/j.vaccine.2017.11.085. Epub 2017 Dec 16. PMID: 29258705

White Paper No. 27 I September 2015 factors influencing OD600 measurements. (n.d.). Retrieved March 15, 2023, from https://www.eppendorf.com/product-media/doc/en/148369/Detection_White-Paper_027_BioPhotometer-D30_BioSpectrometer-family_Factors-Influencing-OD600-Measurements.pdf

CURRICULUM VITAE

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