PhD thesis

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# Preamble

I am currently writing my PhD thesis on the impact of pneumococcal vaccination in Iceland. I decided to host the thesis on github and distribute on social media. I am doing this for mostly selfish reasons. I believe I will be more motivated if my productiveness – or lack thereof, is held accountable to anyone who wishes to check. I would be grateful for any and all comments on any aspect of the thesis under construction.

# Introduction

*Streptococcus pneumoniae* is a commensal bacterium found in the nasopharynx of humans where it plays an integral role in the normal upper respiratory flora. It is also a common pathogen and is one of the most common bacterial causes of disease in humans. In classical medical texts pneumococcus is described as a Gram-positive lancet-shaped coccus that is usually found in pairs. In fact, pneumococcus is *the* Gram-positive coccus, being first bacteria that Christian Gram noted which retained the dark aniline-gentian violet stain that now bears his name (Gram [1884](#ref-Gram1884)). Pneumococcus was first isolated in 1881 by two microbiologist, George M. Sternberg in the United States and Louis Pasteur in France (Pasteur [1881](#ref-Pasteur1881); Sternberg [1882](#ref-Sternberg1881); Watson et al. [1993](#ref-Watson1993)). In both cases the sample from which the bacterium was isolated originated from healthy carriers, rather than ill patients. The causal association between this newly discovered bacterium and pneumonia was firmly established only five years later (Weichselbaum [1886](#ref-Weichselbaum1886)), and in the following decade, all clinical presentations of pneumococcal infection had been described (Robert Austrian [1981](#ref-Austrian1981)). Pneumococcus has gone by many names since its first isolation in 1881. Originally it was named *Micrococcus pasteuri* by Sternberg (Sternberg [1882](#ref-Sternberg1881)) but, by 1920 a scientific consensus was reached in which it was agreed that the official name should be *Diplococcus pneumoniae* (Winslow et al. [1920](#ref-Winslow1920)). It was not until 1974 that pneumococcus received its current name, *Streptococcus pneumoniae* (Deibel and Seeley [1974](#ref-Deibel1974)).

Pneumococcus is encapsulated by a polysaccharide coating that protects it from environmental factors. The polysaccharide capsule acts as an “invisibility cloak” to the human immune system, which is rendered unable to detect pneumococcus except through certain patterns in the oligosaccharides contained within the capsule (Tuomanen, Austrian, and Masure [1995](#ref-Epstein1995)). Based on these patterns, pneumococcus has been classified into over 97 different serotypes to date. Additionally, as the capsule contains only polysaccharides and not proteins, the immune response is T-cell independent and therefore poorly immunogenic, even after identification by the immune system (Geno et al. [2015](#ref-Geno2015b)). These characteristics are the fundamental challenges faced by scientists engineering new pneumococcal vaccines. The significance of serotypes on the development of vaccines should not be understated. The failure of the original attempt of Wright and colleagues in 1911 to prevent pneumococcal pneumonia with vaccination was indeed due to lack of knowledge about serotype-specific immunogenicity (Wright et al. [1914](#ref-Wright1914)).

All serotypes of pneumococcus have the potential to cause disease in humans. However, some are more virulent than others. The prevalence of asymptomatic carriage in the nasopharynx varies greatly by serotype as does the propensity of serotypes to cause clinical infections. Quantifying the pathogenic potential of serotypes is difficult as both their prevalence and propensity to cause disease need to be considered. With few exceptions, acquisition of a new serotype into the nasopharyngeal flora proceeds the onset of clinical disease caused by that serotype. Pneumococcal epidemiology is dominated by this effect - children act as a reservoir of asymptomatic pneumococcal carriage from which other children and adults acquire serotypes that may lead to symptomatic disease. Because of this, vaccinations that decrease the pneumococcal carriage in children have the potential of reducing the incidence of disease both in other unvaccinated children and in adults. This phenomenon is called a herd-effect, and is integral to the development of vaccination strategies to combat pneumococcal disease.

The infectious manifestations of pneumococcal disease are, broadly speaking, local infections of the respiratory tract and infections of previously sterile tissue. They range from common to uncommon, and from benign to serious. The most common infectious manifestation of pneumococcus in acute otitis media (AOM) – an infection of the middle ear. AOM is the most common reason for physician visit and for antimicrobial prescription in the paediatric population. However, the disease course is benign and rarely results in permanent disability. The pathogenesis of AOM is complex and can both be caused by viral and bacterial pathogens. Bacterial AOM is most often caused by pneumococcus and *Haemophilus influenzae*. Acute sinusitis is another common, though benign, manifestation of pneumococcal disease. Pneumococcus is also a common cause of pneumonia – the disease from which it gets its name. Pneumonia often requires hospitalization and intravenous antimicrobial treatment, and, though uncommonly, can lead to permanent disability and death. Finally, if pneumococcus gains access to normally sterile tissue, it may cause invasive infections. This includes bacteremia, an infection of the blood, and meningitis, an infection of the meninges. These infectious manifestations are grouped together as invasive pneumococcal disease (IPD). Whilst IPD is extremely uncommon, the consequences can be disastrous. The case fatality ratio from pneumococcal meningitis in Iceland is estimated at 15.3%.

Pneumococcus became an early target for vaccine development because of the broad range of disease caused by pneumococcus. The vaccine benefit can be quantified in two different ways. On one hand it can prevent uncommon but serious manifestations of disease and has the potential to prevent death and serious disability. On the other hand, it also prevents common infections that cumulatively present a large healthcare burden due to frequent physician visits, antimicrobial prescriptions as well as work hours lost by parents and caregivers. The earliest attempts to use vaccination to lessen the morbidity and mortality associated with pneumococcus date back to 1911 when Wright used whole cell innoculi to vaccinated miners in South Africa (Wright et al. [1914](#ref-Wright1914)). In the following decades, multiple animal studies showed that injection of the pneumococcal polysaccharide coating in animals protected against subsequent pneumococcal infections. On the basis of these findings, the first polysaccharide vaccine was shown to be effective (Macleod et al. [1945](#ref-Macleod1945)). This lay the foundation for modern polysaccharide pneumococcal vaccines. However, it soon became apparent that the polysaccharide vaccines were not adequately immunogenic in young children, the ill or the elderly. In response, pneumococcal conjugate vaccines were developed. The first such vaccine to be mass produced was the heptavalent Prevenar developed by Pfizer Pharmaceuticals.

Iceland is an independent island nation, isolated in the mid-Atlantic, with a homogenous populuation of roughly 330,000 individuals. The first systematic program of vaccination against pneumococcus in Iceland began in April 2011, when the 10-valent pneumococcal *Haemophilus influnzae* protein-D conjugate vaccine (Synflorix, PHiD-CV10) was introduced into the national paediatric vaccination program. The vaccine program entailed two primary doses given at three and five months of age, and a booster dose at twelve months. No catch-up program was undertaken. Prior to the introduction, no systematic vaccination effort had been undertaken in Iceland. Iceland, as other Nordic countries, has a rich legacy of national health-related registers. With a wealth of medical documentation, a unique whole-population ecological study examining the impact of systematic pneumococcal vaccination was enabled.

## Clinical manifestations of *Streptococcus pneumoniae*

~ 2 pages - Build transmission dynamics, carriage and disease mentioned in introduction - Short overview of mechanism by which individuals become colonized - Asymptomatic carriage the predecessor of infections - Non-invasive vs. invasive infections - Explain relevance of differentiating the two - list manifestations in each category building on introduction - Explain that vaccines have variable impact on different manifestations - Use examples from the two large RCTs

The relationship between pneumococcus and humans is complex. Most children are colonized by pneumococcus within the first months of life. The serotype distribution of the initial colonization is influenced by the distribution of serotypes within the family. Over the course of the child’s lifetime, they will be colonized by many different serotypes. The child’s immune system will learn to recognize the serotype and will either clear the colonization or maintain a equilibrium in which the serotype is kept within a certain limit of reproduction. Consequently, the contribution of pneumococcus to the human upper respiratory flora is in a state of constant flux. New serotypes enter while old exit, and the relative density of serotypes changes. In some cases the equilibrium between pneumococcus and the host is destabilized resulting in rapid growth of pneumococcus which results in clinical manifestations. This most commonly occurs in the upper respiratory tract where pneumococcus is generally located. This results in the common clinical manifestations of pneumococcal infections, i.e. AOM, acute sinusitis and conjunctivitis. The pathogenesis of pneumococcal pneumonia is thought to occur through micro-aspiration of upper respiratory secretions with subsequent rapid proliferation of pneumococcus in the lower respiratory tract. Invasive disease occurs when pneumococcus penetrates the host immunological defenses and proliferates in normally sterile tissue.

### Carriage

~ 3-4 pages - Define carriage; age-specific prevalence, serotype distribution - Explain that most are born carriage free - Evidence for co-carriage of different serotypes - Age at which most children acquire carriage - Risk factor: daycare, siblings, smoking etc. - Children are the main vectors of pneumococcus - Rate of clearance dependent on age - With increasing age -> increasing immunity, decreasing prevalence - Senescence and carriage in the elderly - Evidence for carriage being the predecessor infections - Evidence of asymptomatic carriage -> main spread of disease - Variable propensity of serotypes to cause disease, attack-rates - Review the Icelandic literature and changing epidemiology - Carriage prevalence - Serotype distribution - Risk factors - Describe mathematical method to estimate adult carriage from observed carriage in children.

### Acute otitis media

~ 3 - 4 pages - Define different types of otitis media; acute otitis media - Pathogens, estimated % caused by pneumococcus - Proposed mechanism by which carriage -> AOM - Epidemiology, both serotype and age - Risk factors - Burden of disease caused by AOM; health care utilization, cost - Incidence and prevalence - GP visits, antibacterial consumption, hospitalization (?) - Days of work-lost by parents - Sequelae; multiple infections, effusion, tympanostomies - Evidence of benefit of delaying 1st presentation - Review of Icelandic literature and changing epidemiology - AOM prevalence and serotype distribution - Risk factors - Associated healthcare consumption, cost - Rate of sequelae

### Pneumonia

~ 4 – 5 pages - Define: CAP, nosocomial, PP, NBPP and IPP. - Pathogens, estimated % caused by pneumococcus - Proposed mechanism by which carriage -> pneumonia - Epidemiology, both serotype and age - Risk factors - Burden of disease caused by pneumonia, health care utilization - Ways of defining severity; CURB-65 etc. - GP visits, antibacterial consumption, Hospitalization rates - Days of work lost - Mortality, sequelae - Review of Icelandic literature and changing epidemiology - Pneumococcal pneumonia prevalence and serotype distribution - Rate of hospitalization, healthcare consumption - Rate of sequelae - Risk factors

### Invasive pneumococcal disease

~ 3 -5 pages - Define different presentations of IPD: meningitis, bacteremia, etc. - Epidemiology, both serotype and age - Risk factors - Burden of disease, health care utilization - Severity - Hospitalization rates, ICU rates - Sequelae - Review of Icelandic literature and changing epidemiology - Meningitis, bacteremia, empyema, joint infection prevalence and serotype distribution - Rate of sequelae

## Pneumococcal conjugate vaccines

~ 1 -2 pages - PPSV23 original studies, downsides, immunogenicity - Development of protein conjugate vaccines, reasons - PCV7 - Higher valency PCVs

The history of pneumococcal vaccination begins in 1911 when Wright and colleagues attempted to use whole killed bacteria to vaccinate South African miners against pneumococcal pneumonia (Wright et al. [1914](#ref-Wright1914)). It should however be noted that in his original description of pneumococcus in 1881, George Sternberg observed that rabbits who were injected with his saliva mixed with alcohol and quinine did not ubiquitously die and were later resistant to re-injection with saliva (Robert Austrian [1999](#ref-Austrian1999a), Sternberg ([1882](#ref-Sternberg1881))). Sternberg had inadvertently immunized the laboratory animals against subsequent infection by injecting killed pneumococci, proving the concept 30 years before it was first attempted. The 1911 trial by Wright failed to show efficacy due to lack of knowledge of the significance of serotypes and serotype specific immunogenicity. Several trials using whole killed bacteria were published in the following two decades (Cecil [1918](#ref-Cecil1918); Lister [1916](#ref-Lister1916); Lister and Ordman [1936](#ref-Lister1936); Maynard [1913](#ref-Maynard1913)) The researchers benefited from the knowledge of serotypes which had been discovered in 1910 and used multivalent vaccines. They were however victims of underdeveloped epidemiological methodology for vaccine field trials. Due to inconsistencies in study design, the efficacy of whole bacteria pneumococcal vaccines remained fiercely debated though there seemed to be a suggestion of benefit (Robert Austrian [1999](#ref-Austrian1999a)). Vaccines based on whole killed bacteria were soon replaced with polysaccharide vaccines, following discoveries in the 1920s and 1930 of the immunogenicity of the polysaccharide capsule (Dochez and Avery [1917](#ref-Dochez1917); Finland [1931](#ref-Finland1931); Francis and Tillett [1930](#ref-Francis1930); M. Heidelberger and Avery [1923](#ref-Heidelberger1923); Schiemann and Casper [1927](#ref-Schiemann1927)). The first such trial tested a bivalent polysaccharide vaccine on 29,000 adult males in the American Civilian Conservation Corps in iterations in the 1930s (Ekwurzel et al. [1938](#ref-Ekwurzel1938)). It suffered from the same methodological problems as did the previous trials of the whole killed bacteria and its results were debated. A second large trial was conducted in the late 1930s using a tetravalent vaccine (Macleod et al. [1945](#ref-Macleod1945)). This trial built upon the experience of the previous trials and was able to show convincing efficacy against pneumococcal pneumonia. The results of this trial led to the licensure of two hexavalent polysaccharide pneumococcal vaccines in the 1940s. One was formulated for adults and the other for children, each optimized to the serotype distribution within the respective age-group. Alas, these early vaccines fell victim to unfortunate timing. Because in 1944, Tillet and colleagues showed that bacteraemic pneumococcal pneumonia could be cured by parenteral administration of benzylpenicillin (Tillett, Cambier, and McCormack [1944](#ref-Tillett1943)). Following this discovery, the medical community was stricken with a kind of nonchalance. The mortality rate of pneumococcal disease decreased sufficiently that there was no longer a perceived need for preventative vaccination. The licenses for the polysaccharide vaccines were withdrawn by the manufacturer due to lack of their use (Robert Austrian [1999](#ref-Austrian1999a)) Interest in pneumococcal vaccination re-emerged in the 1950s when it was noted that the mortality benefit of penicillin was not ubiquitous. The elderly and those who had underlying disease did not experience a decrease in their case fatality ratio (Robert Austrian and Gold [1964](#ref-Austrian1964)). This led to a redoubled effort to create a new polysaccharide vaccine. Several large randomized controlled trials were conducted in South Africa in the 1970s (R Austrian et al. [1976](#ref-Austrian1976), Smit ([1977](#ref-Smit1977))) and on the basis of these, a 14-valent pneumococcal vaccine was licensed in the United States in 1977. Its valency was increased to 23 polysaccharides in 1983 (Robert Austrian [1999](#ref-Austrian1999a)). Early in the development of pneumococcal vaccinations there was an interested in vaccinating children. Two trials were conducted in the early 1980s which attempted to use polysaccharide vaccines in young children. Neither showed benefit (Mäkelä et al. [1981](#ref-Makela1981); Sloyer, Ploussard, and Howie [1981](#ref-Sloyer1981)). This is perhaps unsurprising in light of previous trials. The first polysaccharide trial that was conducted in children in 1937 failed to detect any immunological response (Davies [1937](#ref-Davies1937)). Laboratory studies in the 1930s and 1940s revealed that the reason for this lack of efficacy was due to the thymus independent immunse response to purely sacharide antigens. These same studies showed that this could be remedied by adding a protein adjuvant, thus inducing a T-cell response. The strategy of protein conjugation saw its first success in the development of the *Haemophilus influenzae* type b vaccine. Subsequently, several different pneumococcal conjugate vaccines entered phase II and phase III clinical trials in the late 1990s. The first of which to recieve licensure was the heptavalent preparation. Higher valancy conjugated vaccined were developed and recived licensure in the new millenium.

## Impact of pneumococcal conjugate vaccines

~ 3 pages - Present evidence of magnitude of effect on VT carriage - Serotype distribution vs. carriage prevalence - Serotype replacement - Herd-effect, i.e. effect on carriage of adults and non-vaccinated

### Acute otitis media

~ 1-2 pages <- much fewer studies - Present evidence of magnitude of effect on all-cause AOM - VT vs. NVT serotypes - Serotype replacement (?)  
- Herd-effect in non-vaccinated

### Pneumonia

~ 2-3 pages - Present evidence of effect on all-cause pneumonia - VT vs. NVT serotypes - Serotype replacement (?) - Herd-effect in adults and non-vaccinated

### Invasive pneumococcal disease

~ 4-6 pages <- largest amount of studies - Present evidence of effect on IPD and subgroups; meningitis, bacteremia etc. - VT vs. NVT - Serotype replacement - Herd-effect

## Cost-effectiveness of pneumococcal conjugate vaccination

~ 3-4 pages - Present overview of literature review and critical analysis. - Recommendations of ISPOR and WHO presented, discuss importance of assumptions and methodology - Introduction to sub-chapters of lit. rev. - Explain how they will be tied in to ISPOR/WHO recommendations

### Measurement of effectiveness and choice of health outcomes

~ 1 page - Shortly explain what is meant by effectiveness and health outcomes - Tie in to ISPOR/WHO

#### Health outcomes considered

~ 2-3 pages - Describe what health outcomes were considered - Tie into actual disease burden known to be caused by pneumococcus

#### Effectiveness of PCV7

~ 3-4 pages - What effectiveness rationale is used, methods and rationale: critique - Carriage - AOM - Pneumonia - IPD

#### Effectiveness of PCV10

~2-3 pages - What effectiveness rationale is used, methods and rationale: critique - Carriage - AOM - Pneumonia - IPD

#### Effectiveness of PCV13

~ 2- 3 pages - What effectiveness rationale is used, methods and rationale: critique - Carriage - AOM - Pneumonia - IPD

### Estimating resources and cost

~1 page - Shortly explain what resources and costs mean - Direct vs. indirect - Tie in to ISPOR/WHO

# Aims

# Materials and methods

We describe our methods in this chapter.

# Results

# Discussion

Austrian, R, R M Douglas, G Schiffman, A M Coetzee, H J Koornhof, S Hayden-Smith, and R D Reid. 1976. “Prevention of pneumococcal pneumonia by vaccination.” *Transactions of the Association of American Physicians* 89: 184–94. <http://www.ncbi.nlm.nih.gov/pubmed/14433>.

Austrian, Robert. 1981. “Pneumococcus: the first one hundred years.” *Reviews of Infectious Diseases* 3 (2): 183–9. doi:[10.1093/clinids/3.2.183](https://doi.org/10.1093/clinids/3.2.183).

———. 1999. “A brief history of pneumococcal vaccines.” *Drugs & Aging* 15 Suppl 1: 1–10. <http://www.ncbi.nlm.nih.gov/pubmed/10690790>.

Austrian, Robert, and J Gold. 1964. “Pneumococcal Bacteremia with Especial Reference to Bacteremic Pneumococcal Pneumonia.” *Annals of Internal Medicine* 60: 759–76. doi:[10.7326/0003-4819-60-5-759](https://doi.org/10.7326/0003-4819-60-5-759).

Cecil, R. L. 1918. “RESULTS OF PROPHYLACTIC INOCULATION AGAINST PNEUMOCOCCUS IN 12,519 MEN.” *Journal of Experimental Medicine* 28 (1): 19–41. doi:[10.1084/jem.28.1.19](https://doi.org/10.1084/jem.28.1.19).

Davies, John A. V. 1937. “The Response of Infants to Inoculation with Type I Pneumococcus Carbohydrate.” *The Journal of Immunology*.

Deibel, RH, and HW Seeley. 1974. “Family II: Streptococcuceae.” In *Bergey’s Manual of Determinative Bacteriology*, edited by R. E. Buchanan and N. E. Gibbons, 8th ed., 490–517. Baltimore: The William; Wilkins Co.

Dochez, A Lz, and O T Avery. 1917. “The elaboration of specific soluble substance by pneumococcus during growth.” *The Journal of Experimental Medicine*. doi:[10.1084/jem.26.4.477](https://doi.org/10.1084/jem.26.4.477).

Ekwurzel, G M, J S Simmons, L I Dublin, and L D Felton. 1938. “Studies on immunizing substances in pneumococci. VIII. Report on field tests to determine the prophylactic value of a pneumococcus antigen.” *Public Health Rep* 53 (42): 1877–93.

Finland, M. 1931. “SPECIFIC CUTANEOUS REACTIONS AND CIRCULATING ANTIBODIES IN THE COURSE OF LOBAR PNEUMONIA: I. CASES RECEIVING NO SERUM THERAPY.” *Journal of Experimental Medicine* 54 (5): 637–52. doi:[10.1084/jem.54.5.637](https://doi.org/10.1084/jem.54.5.637).

Francis, T, and W S Tillett. 1930. “Cutaneous reactions in pneumonia. The development of antibodies following the intradermal injection of type-specific polysaccharide.” *The Journal of Experimental Medicine*. doi:[10.1084/jem.52.4.573](https://doi.org/10.1084/jem.52.4.573).

Geno, K. Aaron, Gwendolyn L. Gilbert, Joon Young Song, Ian C. Skovsted, Keith P. Klugman, Christopher Jones, Helle B. Konradsen, and Moon H. Nahm. 2015. “Pneumococcal Capsules and Their Types: Past, Present, and Future.” *Clinical Microbiology Reviews* 28 (3): 871–99. doi:[10.1128/CMR.00024-15](https://doi.org/10.1128/CMR.00024-15).

Gram, Christian. 1884. “Ueber die isolierte Fabung der Schizomyceten in Schnitt und Trockenpraparaten.” *Fortschritte Der Medicin* 2: 185–89.

Heidelberger, M, and O T Avery. 1923. “THE SOLUBLE SPECIFIC SUBSTANCE OF PNEUMOCOCCUS.” *The Journal of Experimental Medicine*. doi:[10.1084/jem.40.3.301](https://doi.org/10.1084/jem.40.3.301).

Lister, Frederick Spencer. 1916. “An experimental study of prophylactic inoculation against pneumococcal infection in the rabbit and in man.” *Publication of the South African Institute of Medical Research* 8: 231–87.

Lister, Frederick Spencer, and D Ordman. 1936. “The Epidemiology of Pneumonia on the Witwatersrand Goldfields and the Prevention of Pneumonia and Other Allied Acute Respiratory Diseases in Native Labourers in South Africa by Means of Vaccine.” *Journal of the American Medical Association* 106 (9): 733. doi:[10.1001/jama.1936.02770090069032](https://doi.org/10.1001/jama.1936.02770090069032).

Macleod, C M, Richard G Hodges, Michael Heidelberger, and W G Bernhard. 1945. “PREVENTION OF PNEUMOCOCCAL PNEUMONIA BY IMMUNIZATION WITH SPECIFIC CAPSULAR POLYSACCHARIDES.” *The Journal of Experimental Medicine* 82 (6): 445–65. <http://www.ncbi.nlm.nih.gov/pubmed/19871511 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2135567>.

Maynard, G D. 1913. “An enquiry into the etiology, manifestations and prevention of pneumonia amongst natives on the Rand recruited from tropical areas.” *Public South Afr Inst Med Res* 1 (0): 1–101.

Mäkelä, P H, M Leinonen, J Pukander, and P Karma. 1981. “A study of the pneumococcal vaccine in prevention of clinically acute atttacks of recurrent otitis media.” *Reviews of Infectious Diseases* 3 Suppl: S124–32. <http://www.ncbi.nlm.nih.gov/pubmed/6974386>.

Pasteur, Louis. 1881. “Note sur la maladie nouvelle provoquee par la salive d’un enfant mort de la rage.” 10. Vol. 2. Paris: I’Academie de Medicine. <https://gallica.bnf.fr/ark:/12148/bpt6k408671n>.

Schiemann, O., and W. Casper. 1927. “Sind die spezifisch pracipitablen Substanzen der 3 Pneumokokkentypen Haptene?” *Zeitschrift Fur Hygiene Und Infektionskrankheiten*. doi:[10.1007/BF02176583](https://doi.org/10.1007/BF02176583).

Sloyer, John L, John H Ploussard, and Virgil M Howie. 1981. “Efficacy of pneumococcal polysaccharide vaccine in preventing acute otitis media in infants in Huntsville, Alabama.” *Reviews of Infectious Diseases* 3 Suppl: S119–23. <http://www.ncbi.nlm.nih.gov/pubmed/7280444>.

Smit, Pieter. 1977. “Protective Efficacy of Pneumococcal Polysaccharide Vaccines.” *JAMA: The Journal of the American Medical Association* 238 (24): 2613. doi:[10.1001/jama.1977.03280250039019](https://doi.org/10.1001/jama.1977.03280250039019).

Sternberg, G M. 1882. “A fatal form of septicemia in the rabbit produced by subcutaneous injection of human saliva.” Washington: National Board of Health; U.S. Government Printing Office. <https://books.google.is/books?id=YojXubzmx3sC{\&}printsec=frontcover{\&}hl=is{\#}v=onepage{\&}q{\&}f=false>.

Tillett, W S, M J Cambier, and J E McCormack. 1944. “The Treatment of Lobar Pneumonia and Pneumococcal Empyema with Penicillin.” *Bulletin of the New York Academy of Medicine* 20 (3): 142–78. doi:[10.7326/0003-4819-60-5-759](https://doi.org/10.7326/0003-4819-60-5-759).

Tuomanen, Elaine I., Robert Austrian, and H. Robert Masure. 1995. “Pathogenesis of Pneumococcal Infection.” Edited by Franklin H. Epstein. *New England Journal of Medicine* 332 (19): 1280–4. doi:[10.1056/NEJM199505113321907](https://doi.org/10.1056/NEJM199505113321907).

Watson, David A, Daniel M Musher, James W Jacobson, and Jan Verhoef. 1993. “A Brief History of the Pneumococcus in Biomedical Research: A Panoply of Scientific Discovery Description of the Organism and Demonstration of Its Virulence.” *Clinical Infectious Diseases* 17: 913–24. doi:[10.1093/clinids/17.5.913](https://doi.org/10.1093/clinids/17.5.913).

Weichselbaum, A. 1886. “Ueber die Aetiologie der acuten Lungen-und Rippenfellentzundungen.” *Medizinische Jahrbücher.*, 483–554.

Winslow, C.-E. E, Jean Broadhurst, R. E. Buchanan, C Krumwiede, L. A. Rogers, and G. H Smith. 1920. “The Families and Genera of the Bacteria: Final Report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types.” *Journal of Bacteriology* 5 (3): 191–229. doi:[10.1086/278854](https://doi.org/10.1086/278854).

Wright, AlmrothE., W. Parry Morgan, L Colebrook, and R.W. Dodgson. 1914. “Observations ON PROPHYLACTIC INOCULATION AGAINST PNEUMOCOCCUS INFECTIONS. AND ON THE RESULTS WHICH HAVE BEEN ACHIEVED BY IT.” *The Lancet* 183 (4715): 87–95. doi:[10.1016/S0140-6736(01)56449-1](https://doi.org/10.1016/S0140-6736(01)56449-1).