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Drug resistant patterns of invasive Streptococcus pneumoniae infections in the State of Florida in 2003

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Drug Resistant Patterns of Invasive *Streptococcus pneumoniae* Infections in the State of Florida in 2003

by

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Table of Contents

List of Tables

List of Figures

List of Acronyms and Abbreviations

ABC	Active Bacterial Core Surveillance
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CSF	Cerebrospinal fluid
CSTE	Council of State and Territorial Epidemiologists
DRSP	Drug-resistant *Streptococcus pneumoniae*
HIV	Human immunodeficiency virus
IPD	Invasive pneumococcal disease
MIC	Minimum Inhibitory Concentration
NCCLS	National Committee for Clinical Laboratory Standards
OR	Odds Ratio
PCV	Pneumococcal conjugate vaccine
PPV	Pneumococcal polysaccharide vaccine
PRSP	Penicillin-resistant *Streptococcus pneumoniae*
RR	Relative Risk
SPIN	*Streptococcus pneumoniae* infections in the neonate
TMP-SMX	Trimethoprim-sulfamethoxazole

Drug Resistant Patterns of Invasive *Streptococcus pneumoniae*

Infections in the State of Florida in 2003

Michael T. Drennon

Abstract

Streptococcus pneumoniae is a major bacterial pathogen which causes pneumoniae, meningitis, otitis media, and bacteremia. Currently there are two vaccines available, Pneumococcal Polysaccharide Vaccine (PPV) for adults and the Pneumococcal Conjugate Vaccine (PCV) for children. The PCV vaccine was developed in 2000 specifically for children and infants due to the ineffectiveness of the PPV vaccine in children.

This is a cross sectional study of invasive *S. pneumoniae* in Florida during 2003. This study is designed to determine the population characteristics, clinically relevant antibiotic resistance patterns and specific risk factors for development of antibiotic resistance of invasive *S. pneumoniae.*

Participants for the study of antimicrobial resistance will be selected if they are positive for invasive *S. pneumoniae*, and have been reported to the Florida Department of Health, Bureau of Epidemiology with a laboratory specimen collection date in 2003. A total of 1056 cases were reported.

The incidence of invasive *S. pneumoniae* was calculated. Logistic regression was used to find an association between each risk factor and invasive *S. pneumoniae*. 95% Confidence Intervals were calculated to determine statistical significance.

The incidence of invasive pneumococcal disease was calculated to be 6.61 per 100,000 persons (95% CI 6.21 – 7.01). The incidence of drug resistant *S. pneumoniae* was calculated to be 3.3 per 100,000 persons (95% CI 3.03 – 3.59).The incidence of penicillin resistant *S. pneumoniae* (PRSP) was estimated to be 2.6 per 100,000 persons (95% CI 2.37 – 2.87).

Fifty percent of the cases qualified as Drug Resistant *S. pneumoniae* (DRSP), being non-susceptible to one or more antibiotics as defined by the National Committee for Clinical Laboratory Standards (NCCLS).

Age, race, gender, county and month of occurrence were evaluated as risk factors for DRSP. Only month of occurrence was determined to be a risk factor.

Compared to current studies and previous results for Florida, it appears that Florida has a decreasing incidence of antibiotic resistant *Streptococcus pneumoniae*. I believe that this is due to the use of the PCV vaccine.

Chapter One

Introduction

Invasive *Streptococcus pneumoniae* is one of the leading causes of community-acquired pneumonia, bacteremia and meningitis in the U.S. The rise of antibiotic resistance and the increased susceptibility of those with compromised immune systems have led to this bacteria becoming a serious public health problem. Those at highest risk include children less than 5 years of age, the elderly, and individuals with diseases that debilitate the immune system.

With the widespread use of antibiotics, starting in the 1940's with penicillin, there has been a steady rise in the number of resistant serotypes or strains of DRSP. The first clinical diagnosis of penicillin-resistant *S. pneumoniae* appeared in 1967 in New Guinea, and multi-drug resistant strains appeared in South Africa in 1977 (Tomasz, 1997).

The CDC reports that annually there are an estimated 175,000 hospitalized cases of pneumococcal pneumonia, 50,000 cases of pneumococcal bacteremia, and 3,000-6,000 cases of pneumococcal meningitis in the U.S. With case fatality rates as high as 30% among those with meningitis (CDC, 2003a). Additionally in children less than 5 years there are an estimated 5 million case of otitis media every year in this country. Due to the severity of the diseases caused by invasive *S. pneumoniae* and the continued rise of antibiotic resistance, several vaccines have been developed in the United States.

The first polyvalent pneumococcal vaccine was licensed in 1977, and the first conjugate pneumococcal vaccine for use in children was licensed in 2000 (CDC, 2003b). In June 1994 due to the unknown impact of DRSP the Centers for Disease Control and Prevention (CDC) convened a working group of public health professionals, laboratory experts, and physicians to devise a strategy to minimize the impact of DRSP (Jernigan, Cetron, & Breiman, 1996). The working group's strategy includes surveillance, research, prevention, and control of DRSP.

Determining risk factors that promote development of resistance, and improving programs to promote pneumococcal immunization were two of the important points addressed by the working group (Jernigan et al., 1996).

Another organization that monitors *S. pneumoniae*, The Council of State and Territorial Epidemiologists (CSTE) advised that all states should add DRSP to their list of reportable diseases in 1995. Due to this recommendation Florida added DRSP to its list of reportable diseases in mid 1996, as did two dozen other states. This information will allow for health care providers to better determine which antimicrobial therapy will be most effective in their community.

It is also important to note that invasive *S. pneumoniae* incidence varies greatly throughout the world but also in the United States. According to the CDC's Epidemiology and Prevention of Vaccine-Preventable Diseases (The Pink Book 8th Edition), incidence of invasive pneumococcal infection in the U.S. is approximately 21 cases per 100,000 (CDC, 2003b).

A study by Zangwill et al. in 1996, conducted in southern California between March 1992 and April 1995 reported incidence rates as high as 145 per 100,000 in those

less than two years of age, with a penicillin resistance rate of 14%. In 2001 a study performed in Costa Rica, reported an incidence of 2.9 per 100,000 in children less then 5 years (Ulloa-Gutierrez, Avila-Aguero, Herrera, Herrera, & Arguendas, 2003).

The following information demonstrates the importance of researching *S. pneumoniae* in Florida. Invasive pneumococcal disease is associated with antibiotic resistance and a higher incidence among children less than 5 years of age, it is important to monitor resistance patterns and incidence among this population. Knowing the trends and risk factors associated with invasive pneumococcal infections will allow the Department of Health to develop effective strategies in reducing the incidence of this disease. In addition information on antibiotic resistance patterns will be made available to the health care community. Risk factors and antibiotic resistance patterns may aid health care practitioners in selecting more effective antibiotics and developing more efficient treatment strategies for patients.

Chapter Two

Background on *Streptococcus pneumoniae*

History

Streptococcus pneumoniae, also called pneumococcus, are lancet shaped, gram positive, anaerobic bacteria (CDC, 2003b). Pasteur first documented it in 1881, from a rabies patient's saliva. Friedlander and Talamon made the association between the pneumococcus bacterium and lobar pneumonia in 1883. The discovery of the Gram stain in 1884 aided in distinguishing pneumococcal pneumonia from other types of pneumonia. There are currently 90 known serotypes, with the ten most common causing greater than 60% of worldwide invasive disease (CDC, 2003a).

Clinical Features

Streptococcus pneumoniae is one of the leading causes of pneumonia, meningitis, bacteremia, and otitis media. Additionally it can cause conjunctivitis, sepsis, sinusitis, and other diseases. Streptococci commonly inhabit the nasopharyngeal tract of humans, and can easily spread to other sites in the upper-respiratory tract to cause infection (Keyworth, 2000). In the case of infection of the central nervous system (CNS), heart valves, and joints, the circulatory system is the main means of transmission (Mandell, Bennett, & Dolin, 2000). In most cases of infection a predisposing condition exists, usually some pulmonary disease.

Pneumococcal pneumonia

Pneumococcal pneumonia is the most common form of pneumococcal infection in adults, and accounts for approximately 36% of community-acquired pneumonia and 50% of hospital-acquired pneumonia. The period of time from infection to presentation of signs and symptoms is short, lasting only one to three days. Mortality rates range from 5-7%, and may be higher in the elderly (CDC, 2003b).

Patients usually present with sudden symptoms which include high fever, shaking chills, and a productive cough. Additional symptoms can include dyspnea, tachypnea, hypoxia, tachycardia, malaise, and weakness. The patient's sputum will tend to have a rusty hue and may be abundant (Lashley & Durhan, 2000). In most cases there is a predisposing factor, which can include pulmonary disease, alcoholism, renal disease, and several other conditions (Mandell et al., 2000). Children can present with nausea, vomiting, and diarrhea, which are less common in adults. Elderly patients, those over 65 years old, may not show signs of a fever, cough, or increased sputum but tend to have a higher incidence of mental disorientation (Lashley & Durhan, 2000).

Bacteremia

Pneumococcal bacteremia accounts for more than 50,000 cases of invasive pneumococcal infection each year in the United States. The average mortality rate is 20% but in the elderly population can be as high as 60%. As with other forms of pneumococcal infection rates are higher in children, the elderly and other groups of immuno compromised individuals. Bacteremia is the most common from of invasive pneumococcal infection in children under the age of 2 years, constituting up to 70% of all

invasive pneumococcal infection in this age group (CDC, 2003b). Bacteremia frequently occurs in patients already suffering from pneumonia or meningitis. Patients usually present with temperature extremes (fever or hypothermia), tachycardia, tachypnea, hypotension and can be disoriented (Lashley & Durhan, 2000).

Meningitis

There are an estimated 3,000 to 6,000 cases of pneumococcal meningitis each year in the United States, which accounts for approximately 19% of all cases of bacterial meningitis in the U.S. Approximately 25% of patients with pneumococcal meningitis already have pneumoniae. Case-fatality rates are approximately 30% but can be has high as 80% in elderly person (CDC, 2003b).

The patient may present with headache, lethargy, vomiting, fever, seizures, and coma. Correct diagnosis is confirmed after the examination of a Gram-stained spinal fluid specimen (Mandell et al., 2000).

Otitis Media

Of all pneumococcal infections, otitis media by far accounts for the most hospital visits associated with this bacterium. Each year in the U.S. there are approximately 20 million hospital visits for otitis media, of that 5 million occur in children less than 5 years old (CDC, 2003b).

The patient will present with fever and ear pain (Lashley & Durhan, 2000). Congestion of the eustachian tube caused by a prior viral respiratory infection is thought to be a significant factor in the development of otitis media (Mandell et al., 2000). This

congestion of the eustachian tube allows for the colonization of *S. pneumoniae*, which can lead to infection.

Diagnosis

To definitively diagnose a *S. pneumoniae* infection the microorganism must be isolated from a sterile sample of body fluid (e.g. blood, spinal fluid). A gram stain will reveal Gram-positive, lancet shaped diplococci. Sputum is not considered a high-quality source, due to the normal nasopharyngeal bacteria. The quelling reaction test is used for rapid identification of pneumococci. Another test used to detect pneumococcal capsular polysaccharide antigen is Counterimmunoelectrophoresis (CIE), this method is both rapid and specific (CDC, 2003b).

Treatment

The general principles of treating a pneumococcal infection are similar to treating any other infection. First administer an antibiotic at a level adequate enough to inhibit or kill the bacteria. Second, continue treatment for an adequate period of time. Third, drain infection from closed spaces if necessary. Fourth, follow up and know what results to expect. Finally, be ready to re-evaluate treatment if observed response is not what is expected or treatment is not working (Mandell et al., 2000). There are several issues that arise in the treatment of *S. pneumoniae* that can complicate treatment. For most cases caused by pneumococcus, at the time of treatment the causative organism is unknown. In the cases where *S. pneumoniae* is considered the causative organism, antibiotic susceptibility is not known at the start of treatment. The duration of treatment necessary

to kill the bacteria is not known for most common cases of *S. pneumoniae* infections. In the cases where *S. pneumoniae* presents as otitis media or sinusitis, drainage is not usually done. In cases involving pneumococcal infections, physicians are not sure what to expect once the treatment has begun (Mandell et al., 2000).

Prevention

Developing an effective prevention strategy would be a valuable tool in reducing the burden of this disease on the population and the financial cost associated with the disease. In addition to decreasing the number of infections it would also reduce problems associated with the development of new drug-resistant strains of the bacterium.

The first pneumococcal vaccine was developed in the 1940s, but use was discontinued with the development of successful antimicrobial therapy (Lashley & Durhan, 2000). Currently there are two vaccines available, Pneumococcal Polysaccharide Vaccine (PPV) for adults and the Pneumococcal Conjugate Vaccine (PCV) for children. The PCV vaccine was developed in 2000 specifically for children and infants due to the ineffectiveness of the PPV vaccine in children.

Pneumococcal Polysaccharide Vaccine. The pneumococcal polysaccharide vaccine is composed of purified pneumococcal capsular polysaccharide. The first vaccine of this type was developed and licensed in 1977 for use in the United States; it contained 14 serotypes of the pneumococcal bacteria. In 1983 a second vaccine was licensed that contained 23 serotypes, the 23-valent polysaccharide vaccine (PPV23) contained the serotypes that account for 88% of pneumococcal disease in the U.S (CDC, 2003b).

The vaccine works by generating a serotype specific response by antibodies, that increases recognition, attachment and killing of pneumococci by leukocytes (Lashley & Durhan, 2000). A high percentage of adults (90-95%) receiving the vaccine produce antibodies to 75% of the serotypes in the vaccine. The pneumococcal vaccine is thought to be protective for up to five years in healthy individuals, but at a diminishing rate (Mandell et al., 2000). The vaccine is 60-70% effective in preventing all pneumococcal disease, but is not as effective in preventing pneumococcal pneumoniae. Effectiveness also diminishes in high risk groups, predominantly those with an underlying medical condition.

Currently there are two polysaccharide vaccines available in the U.S. Pneumovax 23 developed by Merck, and Pnu-Immune 23 by Lederle, both vaccines are very similar. The vaccine is administered by injection, either intramuscularly or subcutaneously (CDC, 2003b).

It is recommended that all adults over the age of 65 should receive the vaccine on a routine basis. Individuals who have a suppressed immune system and are over two years old are also recommended to receive the vaccine, if they are at a particular high risk to contract a pneumococcal infection. Revaccination is recommended five years after the first injection for individuals who are at high risk for developing a pneumococcal infection. Revaccination is not recommended for immuno-competent persons (CDC, 2003b).

Pneumococcal Conjugate Vaccine. Due to the ineffectiveness of PPV-23 in children less than 6 years old, a new vaccine was developed. In February of 2000 the first

pneumococcal conjugate vaccine (PCV7) was licensed in the United States. The vaccine contains capsular polysaccharide from 7 *S. pneumoniae* serotypes. The serotypes are conjugated to a nontoxic diphtheria toxin. The vaccine induces a higher antibody count and primed B-cells. These seven serotypes account for 86% of bacteremia, 83% of meningitis, and 65% of acute otitis media in U.S. children 6 years old and younger. The vaccine is administered intramuscularly (CDC, 2003b).

One cause of concern is that this vaccine only covers a few serotypes; pressing the question will additional vaccines covering different serotypes or more serotypes increase the effectiveness of the conjugated vaccine. Currently vaccines containing 9 and 11 serotypes are being developed and evaluated (CDC, 2003b).

After the four recommended doses, almost all healthy infants acquire antibodies to all 7 of the serotypes. This vaccine has been shown to reduce pneumococcal disease caused by the 7 serotypes by 97%, and reduce disease caused by serotypes not in the vaccine by 89%. The vaccine is less effective in preventing pneumonia and acute otitis media (CDC, 2003b).

Children less than 24 months old and children between the ages of 2 and 5 years who suffer from compromising medical conditions should receive this vaccine. Four doses are recommended by the CDC at 2, 4, and 6 months of age and then a booster at 12-15 months old. Children who are not vaccinated by 7 months require fewer doses, depending on their age. Re-vaccination is not recommended after the initial series (CDC, 2003b).

Epidemiology

Invasive *Streptococcus pneumoniae* occurs throughout the world with the highest incidence in the elderly, young children, and individuals with an immuno compromising condition. Due to the severity of the diseases associated with this bacterium, it is a major cause of morbidity and mortality worldwide. The burden of disease is also significant in the U.S., with high mortality rates often associated with those of compromised status (CDC, 2003b).

Age

Over all incidence in the U.S. for invasive *Streptococcus pneumoniae* was approximately 21 cases per 100,000 persons in 2002, with the disease rate decreasing due to the introduction of the conjugate vaccine (CDC, 2003c). Prior to the introduction of a vaccine the incidence was approximately 700 per 100,000 in the young adult population (Mandell et al., 2000).

Age is a significant risk factor for *Streptococcus pneumoniae* infection, considering the large discrepancies among age groups. A current study by Whiney et al., in 2003 found the following incidence rates: 17.3 per 100,000 overall, incidence rates for children less than 2 years of age was 59 per 100,000, and incidence in the adult population and those over 65 years of age was 7.6 and 49.5 per 100,000 persons respectively.

A study in the U.S. reported that of 15,860 cases, approximately 30% occurred in those over the age of 65 years, and 20% from children less than 2 years of age (Robinson, Baughman, Rothrock, Barrett, Pass et al., 2001). A hospital-based study in Catalonia,

Spain reported an incidence rate of 59.6 per 100,000 person years for children less than 2 years of age and 27.9 per 100,000 person years in persons greater than 65 years. The total incidence reported was 10.5 per 100,000 person years (Dominguez, Salleras, Cardenosa, Ciruela, Carmona et al., 2002). From these studies we can see that the two age groups most affected are persons less than 2 years of age and persons greater than 65 years of age.

Streptococcus pneumoniae infections in the neonate (SPIN) are of particular concern because Pneumococcal Conjugate Vaccine (PCV) is not administrable to infants less than 2 months old. Infections like sepsis, pneumonia and meningitis occur with low frequency in this age group, mortality associated with the infections is high. A study in the U.S. examining SPIN found that infants usually presented at 2 to 3 weeks of age with pneumonia, meningitis, or otitis media. This late onset was associated with a mortality rate of 14.3% in invasive infections (Hoffman, Mason, Schutze, Tan, Barson et al., 2003).

Gender

A study conducted in Southern California found that rates among males and females were very similar, with males (13.3 per 100,000) having a slightly higher incidence than females (11.9 per 100,000) (Zangwill, Vadheim, Vannier, Hemenway, Greenberg et al., 1996). In Austria, a recent prospective surveillance found that in hospitalized children less than 5 years old males accounted for twice as many invasive pneumococcal disease (IPD) infections as females (Rendi-Wagner, Georgopoulos, Kundi, Mutz, Mattauch et al., 2004). A study from South Africa recently reported that compared to men, women are more likely to be infected with a pediatric serotype (OR 1.59, 95% CI

1.18-2.15), and have a penicillin-nonsusceptible strain (OR 1.65, 95% CI 1.03-2.59) (Buie, Klugman, Gottberg, Perovic, Karstaedt et al., 2004).

Racial and Ethnic Distributions

Some studies have shown that prior to 2000 and the introduction of a conjugated vaccine, incidence of pneumococcal infection was higher in black Americans. A population based study conducted in Dallas County, Texas in 1995 reported incidence among black non-Hispanic to be 39 per 100,000 person years and 18 per 100,000 person years in whites. This study also reported that rates among Hispanics were similar to those in the white population (Pastor, Medley, & Murphy, 1998). A study examining the disease burden in the United States found that in 1998 incidence in the black population was 2.6 times that of the white population (Robinson et al., 2001). A study of racial differences in pneumococcal disease in Tennessee reported that in 1999 the rate of invasive pneumococcal disease in black children under 2 years of age was 340 per 100,000 persons, but in 2002 this rate had dropped 83% (Talbot, Poehling, Hartert, Arbogast, Halasa et al., 2004).

American Indians have also been shown to be at greater risk for invasive pneumococcal infections. The rate of invasive pneumococcal disease (IPD) in Navajo children less than 2 years old was 537 per 100,000 persons, between 1989 and 1996 (O'Brien, Moulton, Ried, Weatherholtz, Oski et al., 2003). Navajo adults are also at greater risk for IPD, with reported rates 2-3 times higher than those of the general American population (Benin, O'Brien, Watt, Ried, Zell et al., 2003).

Geographic Distributions

Streptococcus pneumoniae occurs worldwide. Annually, worldwide more than one million children less than 5 years old die of an invasive disease caused by *S. pneumoniae* (Bogaert, de Groot, & Hermans, 2004). The CDC reports that in the U.S. IPD contributes to more than 40,000 deaths each year (Lashley & Durhan, 2000). Incidence rates vary from country to country, and can even differ widely in countries that are adjacent to each other.

Seasonality

Similar to other infectious diseases, incidences of invasive pneumococcal infections change in a seasonal pattern. Evidence shows that there is a steady increase in the fall months, and a spike in midwinter (Dowell, Whitney, Wright, Rose, & Schuchat, 2003). Another study reported a distinct association between decreased ambient air temperature and an increase in pneumococcal infections (Kim, Musher, Glezen, Rodriguez-Barradas, Nahm et al., 1996).

Pre-existing Medical Conditions

Medical conditions that suppress the immune system greatly increase an individuals potential for pneumococcal infection: multiple myeloma, chronic lymphocytic leukemia, lymphoma, HIV infection, aplastic anemia, sickle cell disease, and any form of organ transplantation. A study conducted in 1996 in Southern California concluded that individuals with HIV (incidence: 176 per 100,000 persons) were at a

significantly greater risk than individuals in the general population (incidence: 12.5 per 100,000 persons) (Zangwill et al., 1996).

Microbiology

Streptococcus pneumonia is a gram-positive, catalase negative diplococcus which replicates in chains in liquid medium. Pneumococci produce alpha-hemolysin; this substance breaks down hemoglobin producing a green color. When grown on a blood agar plate, pneumococci are encircled in a green zone, this is indicative of a positive test. Additionally pneumococci can be identified using two other tests: catalase negativity, and solubility in bile salts or ethyl hidrocupreine (optochin) susceptibility. This last method has been shown to not be as effective due to increased resistance to optochin, microbiologist's rely more on the bile salt test (Mandell et al., 2000).

Serotypes

Streptococcus pneumoniae is normally surrounded by an external polysaccharide capsule, only in rare cases has the infection been due to a non-encapsulated form of the bacterium (Mandell et al., 2000). There are currently 90 known serotypes of *S. pneumoniae*, which are differentiated by the type of antigens on the cell capsule (Lashley & Durhan, 2000). During the 1930s serotyping was clinically relevant due to the fact that antisera were administered as therapy; this is no longer standard practice. Currently serotyping is mainly used for research purposes (Mandell et al., 2000).

There are two numbering methods used: the American and Danish systems. The American system numbers serotypes 1 to 90 in order of identification. Since strains that

frequently cause human infection were identified first and thus numbered first, explains why lowered number serotypes are often the cause of IPD. The Danish system is based on antigen similarities; group 19 includes 19F, 19A, 19B, and 19C. In the American system these serotypes would be 19, 58, 57, and 59 respectively (Mandell et al., 2000). The Danish method is the more commonly used system.

The majority of the 90 serotypes are known to cause infection, however 62% of invasive disease around the world is caused by only the 10 most common serotypes. The serotypes linked to disease are dependent upon age group, in the United States 7 serotypes (4, 9V, 14, 19F, 23F, 18C, and 6B) account for 80% of infections in children less than 6 years of age. These same serotypes account for approximately 50% of the infection in older children and adults (CDC, 2003b).

Transmission

S. pneumoniae is a human pathogen. There is no animal to human vector. Similar too many other microorganisms' pneumococci colonize the nasopharynx of asymptomatic human carriers. *S. pneumoniae* is carried in about 40% of healthy adults and carriage rates in children are even higher (Lashley & Durhan, 2000).

Transmission occurs secondary to direct person to person contact, by means of respiratory droplets. Transmission can also occur through direct oral contact and items freshly soiled with respiratory droplets (Chin, 2000). Within a household, factors such as crowding, season, upper respiratory infections, or pneumococcal infection can increase the potential for transmission. It should be noted that presence of these factors does not necessarily increase the risk of transmission within the home (CDC, 2003b).

Chapter Three

Literature Review: Invasive *Streptococcus pneumoniae*

Invasive Streptococcus pneumoniae *Case Definition*

Active Bacterial Core Surveillance defines a case of invasive pneumococcal disease as isolation of *S. pneumoniae* from a sterile source such as blood or cerebrospinal fluid (CSF). A case is defined as meningitis if *S. pneumoniae* is isolated from CSF, or if there is a clinical diagnosis and the bacterium is isolated from blood (Robinson et al., 2001).

Florida Reporting Definition

According to the Florida Department of Health Bureau of Epidemiology, diagnosis is confirmed when *S. pneumoniae* is isolated from a normally sterile site (e.g. blood, CSF). Case information is collected by county health departments and is sent to the Bureau of Epidemiology involving all invasive *S. pneumoniae* cases (Florida Department of Health, 2000).

Drug Resistant Streptococcus pneumoniae *(DRSP) Case Definition*

S. pneumoniae is considered resistant if the bacterium is resistant to one or more commonly used antibiotics. Antibiotic resistance and susceptibility is determined by antibiotic Minimum Inhibitory Concentration (MIC) breakpoints. Breakpoints for

resistance and susceptibility vary by antibiotic. Breakpoints for MIC counts are determined by NCCLS.

Drug Resistance

With the steady rise of antimicrobial resistance among strains of *S. pneumoniae* in the past decade it is now more important than ever for physicians to prescribe proper antimicrobial therapy. Where penicillin was previously the drug of choice for all pneumococcal infections, there are now areas within the U.S. that report 30% of all strains are resistant to penicillin. An additional area of concern is strains of *S. pneumoniae* that are multi-drug resistant, leading to problems with treatment.

Multi-drug resistance is defined as a strain being resistant to two or more antibiotics. Some of these multi-drug resistant strains are only susceptible to vancomycin. Antibiotics that were unnecessarily prescribed plays a large factor in the development of DRSP (CDC, 1996).

A recent 3-year study conducted in Quebec examined serotype distribution, antimicrobial susceptibility, and clinical characteristics. The findings showed that over all the incidence rates remained stable over the three year period, but there was a significant rise in nonsusceptible isolates in young children from 7.9 to 25.3%. Additionally multiple resistance was reported in 4.1% of the isolates, these isolates were resistant to β-lactams and at least two of the following antimicrobials: erythromycin, chloramphenicol, trimethoprim-sulfamethoxazole (TMP-SMX), and ofloxacin (Jette, G., Ringuette, Allard, De Wals et al., 2001).

Chenoweth et al., in 2000 conducted a review of literature on antimicrobial resistance in patients with community-acquired pneumonia. They reported that in the United States, since 1991 penicillin resistance has increased substantially, from a high of 5% (intermediate and full resistance) in 1991 to 43.8% (intermediate and full resistance) reported in 1998. Pneumococci that were resistant to penicillin were also reported to be resistant to 1st and 2nd generation cephalosporins, while 3rd generation cephalosporins (cefotaxime and cefrtiaxone) continued to be somewhat effective. Carbapenems were found to still be an effective antimicrobial in treating penicillin resistant strains of *S. pneumoniae*. Macrolide (erythromycin) resistance is reported to be between 11-14% for intermediate resistance and up to 7% in highly resistant strains. Strains that are resistant to erythromycin are commonly resistant to azithromycin. TMP-SMX resistance is increasing similarly to that of penicillin, with resistance to both often occurring in the same isolate. Vancomycin is the only antibiotic *S. pneumonia* has not yet formed a resistance to.

Considering that vancomycin is one of the last lines of defense in antimicrobial therapy for DRSP, it is important to monitor for any new developments in resistance. A recent study performed in Colombia, South America examined tolerance to vancomycin in 7 isolates of serotype 14. Tolerance is defined as the ability to inhibit growth but not completely kill the pathogen. The study found that one isolate had phenotypical characteristics of tolerance to vancomycin. This isolate showed high resistance to penicillin as well as other antibiotics (Hidalgo, Castaneda, & Arias, 2003). While tolerance occurred in only a small number of isolates, this is still a significant finding.

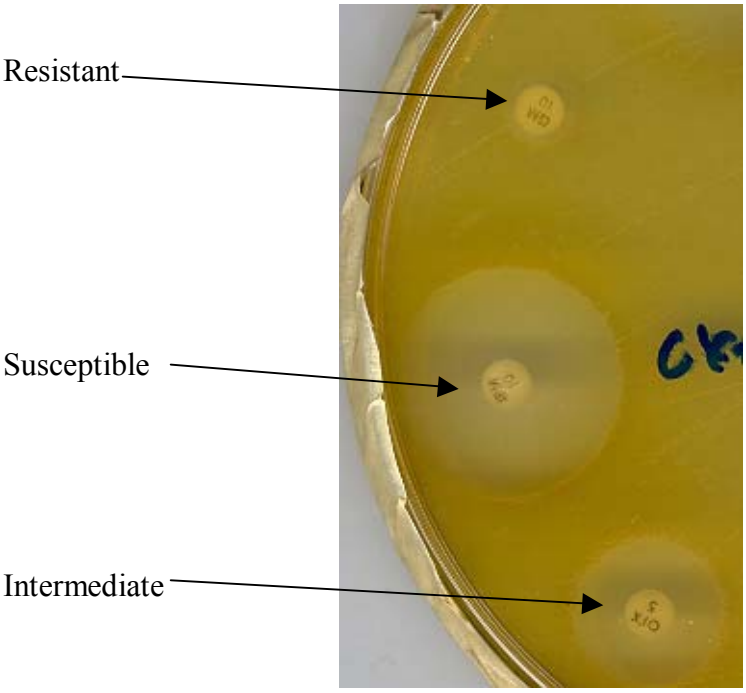
Methods of Testing Susceptibility

Methods for antimicrobial susceptibility testing of *Streptococcus pneumoniae* are determined by the NCCLS. There are several methods used to test for antimicrobial susceptibility in *Streptococcus pneumoniae*; the three most common are the Disk Diffusion method, the E-Test, and the Broth Dilution method.

Disk Diffusion Method

This method is also known as the Kirby Bauer diffusion method. This is one of the more common methods used in determining antimicrobial susceptibilities. In this method, small paper disks, 6mm in size, are infused with a standard amount of antibiotic and placed on an agar plate that has been inoculated with *S. pneumoniae*. NCCLS suggests that Mueller-Hinton agar containing 5% sheep blood, incubated at 35 ^{o}C; 5% CO_2; for 20 to 24 hours be used to grow *S. pneumoniae*. For screening methods a disk infused with 1µg of Oxacillin to determine penicillin resistance, but does not determine quantitative susceptibility (Kiska, Kerr, Jones, Chazotte, Eskridge et al., 1995). Zone size and specific antibiotic content per disk is determined by NCCLS. A large zone of inhibited growth is indicative of susceptible (S) strain of the bacteria, inhibited growth that falls between the breakpoints for susceptible and resistant is deemed to be intermediate (I), and if the growth zone is less than the breakpoint then it qualifies as resistant (R). (Figure 1).

Figure 1: Kirby Bauer Disk Diffusion: Zones of Inhibition



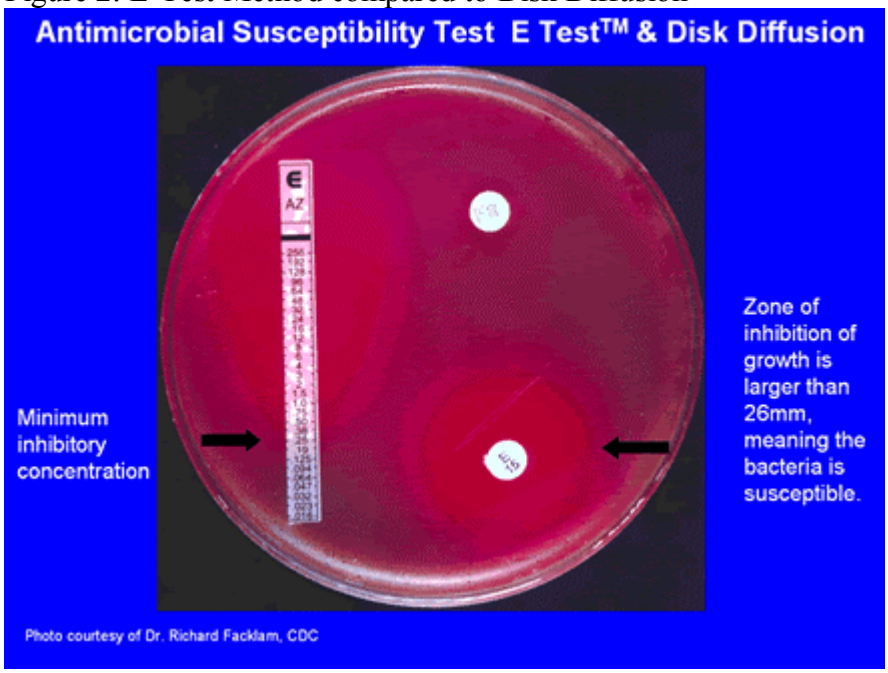
Source: (Sachais, 1997)

E-Test

The E-Test method, manufactured by AB biodisk (Culver City, Calif.), is similar to the disk diffusion method in that a plastic strip is infused with the antibiotic. This strip in addition has a gradient that allows for precise and accurate reading of the results. (Figure 2) The manufacture's recommendations for growth of *S. pneumoniae* are the same as NCCLS for the disk diffusion method. NCCLS also determines the breakpoints for the E-Test method. The strip is read where the inhibited growth intersects the E Test strip. The E-Test strip is marked in one half $\log_2$ increments. One study notes that it is

important to take care and read where pneumococcal growth stops and not the area of alpha-hemolysis (Jorgensen, Ferraro, McElmeel, Spargo, Swenson et al., 1994).

Figure 2: E-Test Method compared to Disk Diffusion



Source: (Facklam, 2003)

Broth Dilution Method

NCCLS recommends using cation-adjusted Mueller-Hinton broth, supplemented with 5% lysed horse blood for pneumococci. *S. pneumoniae* that will be used to inoculate the microdilution trays is grown on sheep blood agar plates for 24 hours in 5% CO_2. A final inoculum density of 5 x 10^5 CFU/ml is placed in the microdilution wells and incubated for 20-24 hours at 35 ^oC in ambient air (Jorgensen et al., 1994).

Susceptibility Testing

In February 2000 the CDC reported the results of a survey used to determine susceptibility testing practices for *S. pneumoniae* in the United States. The survey was used to determine: which susceptibility tests were used, whether laboratories were following NCCLS guidelines, which antimicrobials were being tested, and how results were being reported to clinicians. The laboratories were chosen by their participation in the CDC's Emerging Infections Program, Active Bacterial Core Surveillance (ABC). Of the 659 labs questioned most were using proper NCCLS methods. The results showed that the majority of laboratories used the E-Test for penicillin, the disk diffusion method for Fluoroquinolones, and broth microdilution for other antimicrobials. The majority of laboratories reported the results of the test to clinicians as an interpretation (i.e. susceptible, intermediate, or resistant). Additionally most laboratories (70%) reported the minimum inhibitory concentration (MICs) to the clinician, while only 5% reported the zone diameter (Gelling, Rothrock, Vugia, Shillam, Burnite et al., 2002).

With the increase in incidence of multi-antibiotic resistance and the increase in penicillin resistance among *S. pneumoniae* strains there have been several studies done to evaluate tests that are both accurate and quick. Macias et al., in 1994 compared the effectiveness of the E-Test against the standard broth microdilution method in determining penicillin resistant strains of *S. pneumoniae*. The results found that while the E-Test is a reliable method there are some issues; a difference in the dilution schemes of the two tests, and E-Test results are on a linear scale and not directly comparable to results from the dilution method.

Interpretation of Test Results

Results for the above tests are determined by NCCLS zone diameter and MIC breakpoints for each antibiotic. Each year the susceptibility breakpoints are reviewed and updated if necessary. In January of 2003 NCCLS updated the breakpoints of *S. pneumoniae* for cefotaxime and cefrtiaxone; these new breakpoints were made to differentiate between strains causing meningitis and nonmeningeal diseases. MIC breakpoints for penicillin were not changed because penicillin breakpoints are also used to predict resistance in other penicillins, cephalosporins, and carbapenems (Daily, Farley, Jorgensen, Barrett, Thompson et al., 2004).

Epidemiology of Drug Resistant Streptococcus pneumoniae

Due to the rise of antibiotic resistance among isolates of *S. pneumonia* and the severity of the diseases it causes largely contribute to the importance of monitoring the resistance patterns and risk factors for DRSP. It is important to determine trends and risk factors of the disease to aid health care workers in developing effective strategies in combating *S. pneumoniae*. Geographic and seasonal trends are important to monitor to keep track of when and where disease occurs. Risk factors include but are not limited to; age, race, previous antibiotic use, day care attendance, and recent hospitalization.

Geographic and Seasonal Distribution

Streptococcus pneumoniae occurs world wide, with a varying incidence rates not only in other countries but within the United States as well. The following studies illustrate varying rates throughout the world. An epidemiologic study in Southern

California evaluating the effectiveness of PCV, reported incidence rates of 145/100,000 in children less than 2 years old, 72/100,000 in children less than 5 years old and 32/100,000 in adults over the age of 65. At the same time there was no increase in penicillin resistance, however there was an increase in high-level resistance from 4% to 21% (Zangwill et al., 1996). Massachusetts children less than 7 years old presenting for routine well visits were tested for pneumococcal colonization, 26% were found to be colonized with *S. pneumoniae*. One hundred and sixty-six of the isolates were tested for susceptibility, 33% were found to be susceptible to penicillin, 14% resistant to cephalosporins, and 22% resistant to erythromycin (Finkelstein, Huang, Daniel, Rifas-Shiman, Kleinman et al., 2003).

Incidence rates for children less than 2 years old in Austria were 14.5/100,000 and an overall incidence rate of 13.7/100,000 persons. 21.4% of *S. pneumoniae* isolates showed intermediate resistance, while none were found to have full resistance. 32.1% of the isolates had developed full resistance to clarithromycin, and 33.9% had developed resistance to erythromycin (Rendi-Wagner et al., 2004).

Very low rates of penicillin resistance (2.6% of isolates) have been reported in children less than 2 years old in Tasmania Australia, additionally no high resistance found to 3rd generation cephalosporins (Christie, Coleman, Wan, Jacobs, & Carapetis, 2002).

A recent study in Switzerland had the most striking geographic difference; an outbreak of a specific strain of *S. pneumoniae* caused a large increase in prevalence (6.8 to 19.7%) in the west region of the country. While the east region of Switzerland had a slight decrease in prevalence of 7.7 to 5.2%. Penicillin susceptibility was 90% for those

less than 17 years old, and 81% for children less than 2 years (Muhlemann, Matter, Tauber, & Bodmer, 2003). These susceptibly numbers are slightly higher than those of other studies, indicating that resistance is not yet a significant problem in Switzerland.

Seasonal studies have shown that there is an increase in pneumococcal infections each winter. Dowell et al., in 2003 reported that there is a distinct fall increase in pneumococcal infection and a midwinter peak. These patterns are consistent across seven U.S. geographical regions. The study also reported that there was no distinct temperature pattern associated with disease. The seasonal peak between children and adults differed, while the adults displayed a distinct peak in mid-winter children displayed a broader and flatter peak. Additional studies have also shown this midwinter peak in pneumococcal disease. These studies have also reported a significant association between respiratory viruses and SO_2 levels, and an increase in pneumococcal infections (Kim et al., 1996).

Risk Factors

Age. Young children and adults over the age of 65 have been shown to be at a greater risk of developing a pneumococcal infection. These two age groups are at a higher risk because their immune systems are more susceptible.

A hospital based study in Catalonia, Spain evaluating incidence rates of *S. pneumoniae* over a three year period (January 1997-December 1999) reported 1218 pneumonias positive for *S. pneumoniae*. Of these case 6.8% were in children less than 2 years, and 45.7% in adults over 65 years (Dominguez et al., 2002).

Decreased susceptibility to penicillin was found in 37% of *S. pneumoniae* isolates obtained during an 8-year study of children less than 5 years old in Uruguay, South America (Camou, Palacio, Di Fabio, & Hortal, 2003).

A Brazilian prevalence study examining *S. pneumoniae* resistance to penicillin in a hospital from July 1991 to December 1992 and 1994, found that 21.8% of isolates had intermediate resistance to penicillin. The study also reported that being less than 4 years old (OR 3.53, 95% CI: 1.39-8.96) was associated with decreased susceptibility (Levin, Sessegolo, Teixeira, & Barone, 2003).

A 1995 Dallas county, Texas study of invasive pneumococcal disease found that the highest incidence of IPD occurred in children less than 2 years old (136/100,000 person years) and adults over 65 years (80/100,000 person years). Twenty percent of the isolates were reported to be non susceptible to penicillin, with resistance rates higher in individuals <2 years and >65 than in those 2-29 or 30-64 years old (Pastor et al., 1998).

Race and Ethnicity. Studies have shown white race is associated with DRSP. In Baltimore, MD white individuals were found to account for the majority of penicillin resistant *S. pneumoniae* (PRSP) infections. The authors note that the "incidence of PRSP, which is a product of the incidence of pneumococcal infection and the proportion of disease due to PRSP", was higher among black individuals (Albanese, Roche, Pass, Whitney, McEllistrem et al., 2002).

A 1995 Atlanta study found that while incidence of invasive pneumococcal infection was higher in blacks, whites were actually at a higher risk of DRSP (Risk Ratio 1.66). The authors state that better access to health care and abuse of antibiotics among

whites, probably contributes to the higher risk among that population (Hofmann, Cetron, Farley, Baughman, Facklam et al., 1995).

Previous Antibiotic Use. Previous antibiotic use has been shown to be one of the primary risk factors in contributing to antimicrobial resistance in pneumococci (Chenoweth, Saint, Martinez, Lynch III, & Fendrick, 2000). The increase use in Fluoroquinolones and several other drugs has led to the increase in mutations among *S. pneumoniae* strains resulting in resistance (Goldstein & Garabedian-Ruffalo, 2002). In rural Kentucky previous antibiotic treatment was found to be independently predictive ($P<0.0001$) of PRSP, in patients with acute otitis media (AOM) (Block, Harrison, Hendrick, Tyler, Smith et al., 1995).

A 1998-1999 Switzerland study using nationwide surveillance data, ran analysis on 1179 pediatric (<17 years) pneumococcal isolates to determine risk factors for pneumococcal infection. This study found, using logistic regression, that age and recent antibiotic therapy were risk factors for carriage of penicillin non-susceptible *S. pneumoniae* (PNSP) (Muhlemann et al., 2003).

Day Care Attendance. A Massachusetts study conducted in 2001 reported day care attendance as an independent risk factor (OR: 3.9) for PNSP (Finkelstein et al., 2003). Recent day care attendance was determined to be a risk factor (OR 3.79) in children between 2-59 months, in North America (Levine, Farley, Harrison, Lefkowitz, McGeer et al., 1999).

Younger siblings of day-care attendees are at an increased risk of exposure to DRSP. Givon-Lavi et al. found that carriage of *S. pneumoniae* and antibiotic resistance was high among both day-care attendees and their younger siblings. Strains that are included in the 7-valent vaccine, and are associated with antibiotic resistance, were significantly higher in the younger siblings (Givon-Lavi, Fraser, Porat, & Dagan, 2002).

Studies of Incidence of DRSP

Several studies, examining *S. pneumoniae* incidence and drug resistance strains of the bacteria, have been conducted not only in the U. S. but in other countries throughout the world. A number of those studies are summarized here, to further emphasize the importance of this growing problem.

A 1994 Atlanta based study found an overall incidence rate of invasive disease to be 30 cases per 100,000 persons. Penicillin-resistant rates were 6/100,000 for whites and 11/100,000 among blacks. Penicillin resistance occurred in 25% of the isolates. Penicillin often coincided with other antibiotics in multi-resistant strains. Young children were found to be at a higher risk of DRSP infection compared to older patients (6% vs. 2%, RR of 2.27, 95% CI: 0.84-6.14). Penicillin resistance was reported to be a greater issue among whites than blacks (32% vs. 19%, RR 1.66, 95% CI: 1.18-2.32) (Hofmann et al., 1995).

From July 1998 to August 1999, meropenem efficacy was evaluated in a tertiary children's hospital in the southern United States. The study found that pneumococci that have high resistance to penicillin and cefotaxime also had high resistance to meropenem.

Twenty-seven of 29 penicillin resistant strains were also resistant to meropenem (Buckingham, Davis, & English, 2002).

An 11 year study in Barcelona, Spain examining incidence among children found that 37.1% of the isolates had intermediate resistance to penicillin and 8.6% demonstrated high resistance. Additionally 32.4% of the isolates were resistant to erythromycin (Pineda, Fontanals, Larramona, Domingo, Anton et al., 2002).

Trimethoprim-sulphamethoxazole (TMP-SMZ) is a common antibiotic used in HIV/AIDS patients, to prevent infection with *Pneumocystis carinii*. Several studies have been conducted throughout the world to determine if long-term use increases risk of infection with TMP-SMZ resistant strains of bacteria.

Using ABCs data from 1998-1999 Fry et al., in 2003 concluded that long term therapy with TMP-SMZ does not increase an HIV/AIDS patient's chance of becoming infected with a TMP-SMZ resistant strain of *S. pneumoniae*. A study in Zimbabwe also concluded that HIV status did not increase a person's risk of DRSP, but did report that 50% of isolates were resistant to TMP-SMZ among HIV positive individuals. This study also reported that penicillin resistance occurred in 50% of strains in HIV seropositive individuals, compared to only 16% of isolates in HIV seronegative patients (Gwanzura, Pasi, Nathoo, Hakim, Gangiadzo et al., 2003).

Prevention and Control Measures of DRSP

Cost associated with the diseases caused by DRSP is an important factor to take into consideration when discussing prevention and control. Cost of treatment for adults has been found to be higher among patients with an *S. pneumoniae* infection resistant to

penicillin as opposed to a susceptible strain. The increased cost is associated with a longer length of stay and more expensive antibiotic, cefrtiaxone versus penicillin G (Quach, Weiss, Moore, Rubin, McGeer et al., 2002).

An Austrian study conducted between July 1996 and October 1998, evaluating the impact of PPV23 on geriatric patients, found that the majority of patients developing pneumonia had not received the vaccine. The study found that the vaccine significantly (OR 0.279; $p<0.0001$) reduced the risk of pneumonia. Wagner et al., in 2003 also found that vaccination significantly (OR 0.331; $p<0.0001$) reduced death associated with pneumonia.

Chapter Four

Rational of Study and Research Objectives

Rationale of Study

Considering that invasive pneumococcal disease is associated with antibiotic resistance and a higher incidence among children less than 5 years of age, it is important to monitor resistance patterns and incidences among this population. Knowing the trends and risk factors associated with invasive pneumococcal infections will allow the Department of Health to develop effective strategies in reducing the incidence of this disease. In addition information on antibiotic resistance patterns will be made available to the health care community. Risk factors and antibiotic resistance patterns may aid health care practitioners in selecting more effective antibiotics and developing more efficient treatment strategies for patients.

Invasive *S. pneumoniae* is a reportable disease in Florida and many other states. Laboratory reports with antibiograms are sent to the Department of Health, Bureau of Epidemiology, by the county health departments. Antibiograms have proven to be a simple and accurate way to estimate prevalence of antibiotic-resistant pneumococci (Van Beneden, Lexau, Baughman, Barnes, Bennett et al., 2003).

Objectives

The objectives of this study are:

1. Define the demographics of cases of invasive *S. pneumoniae*, in Florida in 2003.
2. Determine the clinically relevant antibiotic resistance patterns of *S. pneumoniae*, as listed by the National Committee for Clinical Laboratory Standards (NCCLS. 1999), in Florida in 2003.
3. Determine if age, gender, race, region and seasonal variation are risk factors for development of antibiotic resistance among invasive *S. pneumoniae* cases in Florida during 2003.
4. Determine the incidence rate of *S. pneumoniae* in Florida in 2003 and compare the incidence rate to reported data from 1999 and 2000 for the entire state to determine if significant changes have occurred.

Chapter Five

Methods

Study Design

This is a cross-sectional study of *Streptococcus pneumoniae* during 2003 in the state of Florida. Due to the lack of a control group only descriptive analysis will be performed. Cross-sectional studies are used to examine a specific disease within a population (Gordis, 2000). Data for 2003 was reported to the Bureau of Epidemiology by county health departments and used to form the data set for this study.

Institutional Review Board (IRB) approval was received, from the University of South Florida and the Florida Department of Health, in September 2004 prior to any analysis of data. Patients were not contacted at any time during this study. All information was obtained from laboratory reports and antibiograms provided by the Department of Health, Bureau of Epidemiology. Personal identifiers were removed from the data set prior to analysis, to comply with IRB requirements for exemption.

Study Population

The study population is comprised of 1056 invasive *S. pneumoniae* cases that were reported to Florida Department of Health (FDOH) Bureau of Epidemiology for 2003, as having a positive laboratory diagnosis of invasive *S. pneumoniae*. Ninety nine percent of reported cases were residents of Florida; the remaining 1% had no county of

residence reported. All cases will be used in the analysis of incidence, but missing values will be accounted for during the analysis of risk factors.

Inclusion Criteria

Cases of invasive *Streptococcus pneumoniae* were included in this study only if they were reported to the Bureau of Epidemiology and they met the States definition for a case of invasive *S. pneumoniae*. According to the FDOH Bureau of Epidemiology, diagnosis is confirmed when *S. pneumoniae* is isolated from a normally sterile site (e.g. blood, cerebrospinal fluid).

Data Sources

The dataset for this study was compiled from two different sources: (1) laboratory results from state labs, and (2) antibiograms received with the lab reports that were sent to the Bureau of Epidemiology. The majority of the reports were complete, with enough information necessary to construct the dataset.

Data Collection

Lab reports were sent to the Bureau of Epidemiology throughout 2003 and into 2004. The lab reports were reviewed by the Regional Epidemiologist to determine if the cases met the case definition of an invasive *S. pneumoniae* infection. The dataset was constructed at the Bureau of Epidemiology and all files were kept in the locked office of the Regional Epidemiologist for safekeeping. All identifying information was removed from the dataset prior to analysis.

Data Management

The Department of Health (DOH) database was constructed using Microsoft Excel. The database includes information provided by laboratory reports and antibiograms provided by county health departments. The information included in the database consists of date of report, county and county number, case number, ICD9 codes, last name, date of birth, age, source of the infection, type of infection, antibiotic resistance, gender, race, ethnicity, zip code, date of recognized infection, origin of the disease, occupation of the patient, and week number event occurred. The personal identifiers were removed from the database prior to analysis and leaving the Department of Health. The data was then analyzed with Statistical Analysis Software (SAS)

Definition of Variables

Variables that were analyzed in this study include: antibiotics, county of residence, gender, race, ethnicity, age, and month (season) the event occurred. All variables were determined by the lab reports turned into the DOH. There are 67 counties in the state of Florida; all counties are required to report cases of invasive *S. pneumoniae* to the State Health Department. Twenty counties either failed to report any or had no cases of invasive *S. pneumoniae*. Of the remaining 47 counties, the majority of cases were reported from counties with populations greater than 450,000. Gender was reported as; Male, Female, or Unknown. Race was reported as: American Indian/Native Alaskan, Asian/Pacific Islander, Black, White, Other, or Unknown. Ethnicity was reported as either Hispanic or Non-Hispanic. Age was collected in years, months, and days. Age was then divided into age groups (in years); <1, 1-2, 3-4,0-4, 5-17, 18-34, 35-49, 50-64, and

≥65, for comparison to previous Florida studies (Florida Department of Health, 2000). Finally the month in which the event occurred was used.

Data Analysis

Descriptive epidemiology was used to describe patterns of *S. pneumoniae* infection and antibiotic resistance by class of antibiotic from January 1, 2003 to December 31, 2003. Variables analyzed included age, gender, race, county, and month (seasonal variation), to determine risk factors for *S. pneumoniae* becoming resistant to various antibiotics.

The population was divided into age groups as described by the Florida Department of Health (Florida Department of Health, 2000). Incidence of infection (per 100,000) was calculated for each age group using the current census projections for population data. Considering that incidence of *S. pneumoniae* is lowest among those between 5-17, this age group was used as a reference group to demonstrate the impact of the bacterium on those less than 4 years old and individuals over 65 years old.

Due to the potential for dilution of the data, only counties with populations over 450,000 were included in the analysis of risk factors for antibiotic resistance. These counties include: Brevard, Broward, Miami-Dade, Duval, Hillsborough, Orange, Palm Beach, Pinellas, and Polk. This population limit was chosen, because these counties make up the majority (>70%) of cases reported.

Frequency was calculated excluding any missing values. Assuming that any missing values are missing at random, these values would have no effect on the outcome. Logistic regression was used to find association between each risk factor and resistance

among invasive *S. pneumoniae* cases. Logistic regression was chosen due to the fact that the dependent variable (resistance) has a binary outcome (either resistant or susceptible). Logistic regression is an effective way to determine the relative importance of the independent variables. p-Values and 95% Confidence Intervals were calculated to determine statistical significance. Goodness of fit and residuals were examined to determine how well the variables fit the final model. These residual plots are located in appendix A. When outliers were controlled for, there was no change in how well variables fit the model. Statistical analysis was performed using the SAS statistical software.

Chapter Six

Results

The results chapter is divided into three major sections. The first will cover incidence and epidemiologic characteristics of invasive *S. pneumoniae* disease, the second will cover the clinically relevant antibiotic resistance patterns of *S. pneumoniae*, and the third will cover risk factors for antibiotic resistance among invasive *S. pneumoniae* cases.

In the first section the demographics of the whole study population will be examined, using all 1056 cases of invasive *S. pneumoniae*. The second section will examine the portion of the cases that reported resistance to any of the 16 antibiotics that were included in the dataset. The third section will use the cases from Florida counties with populations over 450,000 persons to determine if county, gender, race, age or month are risk factors for resistance.

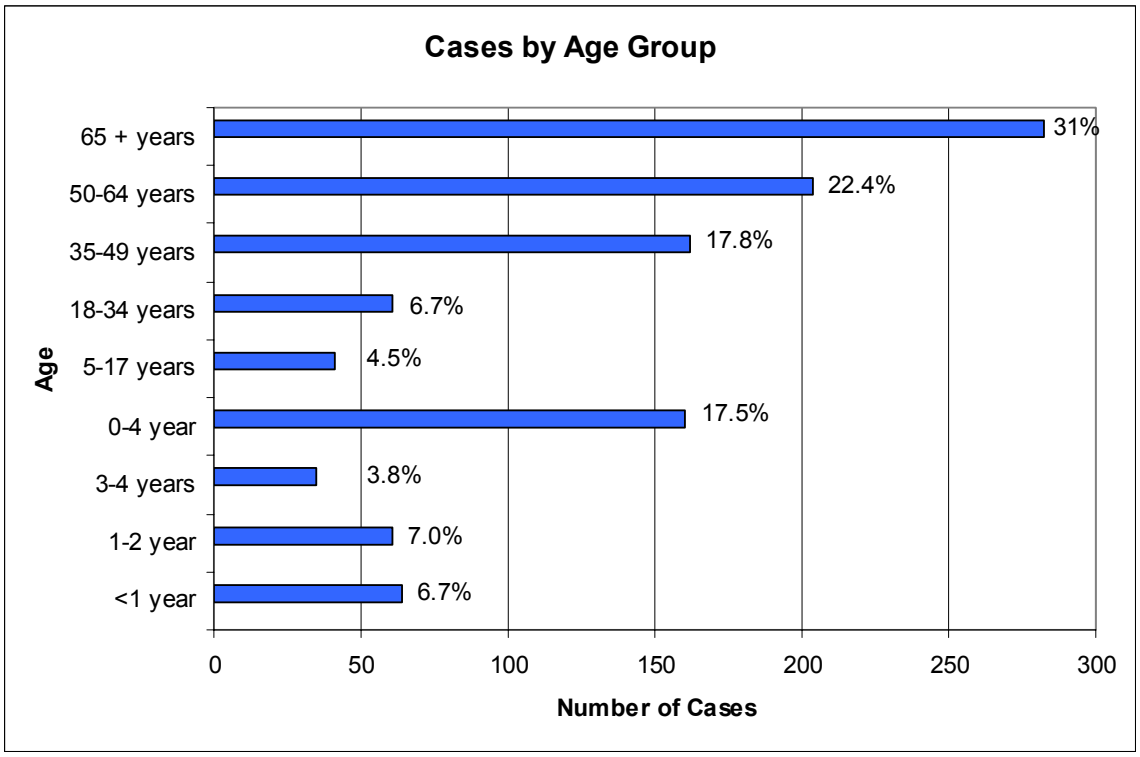
Characteristics of the Study Population

Descriptive analysis was performed on all 1056 culture-confirmed cases of invasive *S. pneumoniae* reported during 2003 to the Florida Department of Health, Bureau of Epidemiology, allowing for characterization (Objective 1) of the study population. Cases were reported from the entire state, including 47 of 67 counties.

Age

Of the 1056 reported cases 910 reported age data. 133 reported no age type. By reporting no age type it was impossible to determine if a reported age was months or years. In addition 13 reported no age. The following graph (Figure 3) illustrates the distribution by age group for all Invasive *S. pneumoniae* cases, with age reported, in Florida 2003. The graph also illustrates the percentage of cases accounted for by age group.

Figure 3: Distribution of invasive *S. pneumoniae* cases by age group, Florida 2003



Source: *S. pneumoniae* dataset, Florida 2003

Age groups were also examined to determine if there were any differences in percentages when looking at race, county, and penicillin resistance. The following table

illustrates the comparisons. County's demonstrated similar percentages of cases by age group compared to that of the total population. Black race had a higher percentage (27.3%) among those 35 to 49 years old compared to the same age group for the total population (17.8%). Black race also had a lower percentage (11.6%) among those 65 years and older compared to the overall population (31%). White race displayed slightly lower percentages among those less than 5 years of age while Black race demonstrated slightly higher percentages among the same age groups, when both were compared to the overall population. The majority of penicillin resistant cases occurred among individuals less than 4 years of age and those over 35 years of age.

Table 1: Comparison of Age, Race, and Penicillin Resistance by percentage of population, Florida 2003

Age Group in years	Number in group	% of Total Population	Number (%) by Race		% by Penicillin Resistance
			Black	White	
<1	61	6.7	19 (7.8)	38 (6.5)	7.56
1-2	64	7.0	21 (8.7)	29 (4.9)	10.47
3-4	35	3.8	13 (5.4)	15 (2.6)	6.98
0-4	160	17.5	53 (21.9)	82 (14.0)	25.01
5-17	41	4.5	10 (4.1)	25 (4.3)	2.91
18-34	61	6.7	27 (11.2)	31 (5.3)	5.81
35-49	162	17.8	66 (27.3)	82 (14.1)	16.28
50-64	204	22.4	58 (23.9)	135 (23.2)	18.02
65+	282	31.0	28 (11.6)	228 (39.1)	31.98
Total	910	100.0	242 (100)	583 (100)	

Source: *S. pneumoniae* dataset, Florida 2003

The following chart (Table 2) illustrates the distribution of cases by age group and incidence for each age group. Over half (54%) of *S. pneumoniae* cases were adults over the age of 50 and children less than five years of age accounted for nearly 20%.

Table 2: Percentage and Incidence of *S. pneumonia*e cases, Florida 2003

Age Group	Number In Age Group	% of Total Cases	Incidence Rate Per 100,000
<1 year	64	6.7	34.21
1-2 year	61	7.0	16.27
3-4 years	35	3.8	9.11
0-4 year	160	17.5	16.91
5-17 years	41	4.5	1.52
18-34 years	61	6.7	1.79
35-49 years	162	17.8	4.54
50-64 years	204	22.4	8.02
≥ 65 years	282	31.0	10.04
Total	910	100.0	

Source: *S. pneumoniae* Database, Florida 2003

Gender

Gender information was available on all 1056 cases in the study population. Males (n=577) made up 55% of the population. This proportion differs slightly from the overall proportion of males to females in Florida, in which females account for 51.2% of the population and males account for 48.8%, according to U.S. census information for 2000. Table 3 lists each age group by gender. The information in table 3 illustrates that while females account for a greater proportion of the over all population, proportions of males to females was similar among all age groups except those over 65 years of age.

Table 3 also illustrates that male's account for a higher percentage of *S. pneumoniae* cases among all age groups except those over 65 years, and females only account for 0.6% more cases. Table 4 displays frequency and percentage of *S. pneumoniae* by age group, sex, and race. Age groups <1, 1-2, and 3-4 don't add to the total percentage of cases for the combined age groups due to rounding of decimal places.

Table 3: Percentage of *S. pneumoniae* cases per age group by gender, Florida 2003

Age Group in years	% of Study Population		% of Florida Population	
	Male	Female	Male	Female
<1	4.1	2.6	0.6	0.6
1-2	4.7	2.3	1.2	1.1
3-4	2.0	1.9	1.2	1.2
0-4	10.8	6.8	3.0	2.9
5-17	3.0	1.5	8.7	8.2
18-34	3.4	3.3	10.9	10.5
35-49	10.1	7.7	11.0	11.3
50-64	12.1	10.3	7.6	8.4
65+	15.2	15.8	7.6	10.0
Total	54.5	45.5	48.8	51.2

Source: *S. pneumoniae* data, Florida 2003

Table 4: Frequency and Percentage of invasive *Streptococcus pneumoniae* cases by age group, sex and race, in Florida 2003

Age Group (years)	Number In Age Group	% of Total Cases	Males		Females		Black		White	
			#	% of Total Pop.	#	% of Total Pop.	#	% of Total Pop.	#	% of Total Pop.
<1	61	6.7%	37	4.1%	24	2.6%	19	2.1%	38	4.2%
1-2	62	6.8%	42	4.6%	20	2.2%	21	2.3%	28	3.1%
3-4	35	3.8%	18	2.0%	17	1.9%	13	1.4%	15	1.6%
0-4	158	17.4%	97	10.7%	61	6.7%	53	5.8%	81	8.9%
5-17	41	4.5%	27	3.0%	14	1.5%	10	1.1%	25	2.7%
18-34	61	6.7%	31	3.4%	30	3.3%	27	3.0%	31	3.4%
35-49	162	17.8%	92	10.1%	70	7.7%	66	7.3%	82	9.0%
50-64	204	22.4%	110	12.1%	94	10.3%	58	6.4%	135	14.8%
65+	284	31.2%	138	15.2%	144	15.8%	28	3.1%	229	25.2%
Total	910	100.0%	495	54.4%	413	45.4%	242	26.6%	583	64.1%

Source: *S. pneumoniae* data, Florida 2003

Racial and Ethnic Differences

Racial information was available on 953 of the 1056 cases. Of these 70.5% (672) were white, 28.1% (268) were African-American, less than one percent were Asian, and less than one percent reported Other as race.

Ethnicity data was reported for 842 of the 1056 cases. Of these Non-Hispanic was reported by 84.2% (709) of the cases, and Hispanic was reported by 15.7% (133) of the cases. Examining ethnicity by gender, Hispanic males accounted for the majority of case (63%) where ethnicity was reported. The following chart (Table 5) illustrates the distribution of *S. pneumoniae* cases and penicillin resistant cases by ethnicity. The chart demonstrates that for both Hispanics and non-Hispanics the majority of cases occur in those less than four years of age and those over 50 years of age. Similar results were found for penicillin resistance.

Table 5: Frequency and percentage of *S. pneumoniae* cases by Ethnicity, in Florida 2003

Age Group in years	Number of cases (%) by Ethnicity		Penicillin Resistance (I + R) N (%) by Ethnicity	
	Hispanic	Non-Hispanic	Hispanic	Non-Hispanic
<1	16 (13%)	43 (6%)	8 (13%)	29 (9%)
1-2	16 (13%)	36 (6%)	9 (14%)	21 (7%)
3-4	7 (6%)	22 (3%)	5 (8%)	13 (4%)
0-4	37 (32%)	101 (15%)	22 (35%)	63 (20%)
5-17	10 (8%)	27 (4%)	5 (8%)	11 (4%)
18-34	4 (3%)	50 (8%)	2 (3%)	24 (8%)
35-49	13 (11%)	114 (18%)	6 (10%)	52 (17%)
50-64	24 (20%)	140 (22%)	10 (16%)	67 (22%)
≥65	32 (26%)	210 (33%)	18 (28%)	89 (29%)
Total	122	642	63	306

Source: *S. pneumoniae* data, Florida 2003

Geographic Distribution

County information was available on 1043 of the 1056 cases. Counties with populations over 450,000 were examined more closely, to get a clearer picture of age distribution in counties with large populations. The analysis of incidence by age group and county will be examined more closely in later sections. Counties with an asterisk (*) had too few cases to calculate an accurate confidence limit. The following table (table 6) displays the number of *S. pneumoniae* cases and incidence for all 67 counties.

Table 6: Number of *S. pneumoniae* cases and incidence by county, Florida 2003

COUNTY	#	POP	# of *S. pneumoniae* cases	Incidence Per 100,000	Confidence Limits	
					Lower	Upper
Alachua	1	223,578	16	7.2	3.6	10.7
* Baker	2	23,424	1	4.3	--	--
Bay	3	155,193	9	5.8	2.0	9.6
Bradford	4	26,928	0	0.0	0.0	0.0
Brevard	5	505,711	40	7.9	5.5	10.4
Broward	6	1,731,347	74	4.3	3.3	5.3
Calhoun	7	12,921	0	0.0	0.0	0.0
* Charlotte	8	153,392	1	0.7	--	--
Citrus	9	126,458	8	6.3	1.9	10.7
Clay	10	157,502	0	0.0	0.0	0.0
Collier	11	286,634	14	4.9	2.3	7.4
Columbia	12	60,244	4	6.6	0.1	13.2
Dade	13	2,341,167	147	6.3	5.3	7.3
DeSoto	14	33,879	0	0.0	0.0	0.0
Dixie	15	13,982	0	0.0	0.0	0.0
Duval	16	817,480	69	8.4	6.4	10.4
Escambia	17	295,886	24	8.1	4.9	11.4
Flagler	18	62,206	5	8.0	1.0	15.1
* Franklin	19	10,003	1	10.0	--	--
Gadsden	20	45,134	0	0.0	0.0	0.0
* Gilchrist	21	15,633	3	19.2	--	--
Glades	22	11,165	0	0.0	0.0	0.0
* Gulf	23	15,247	1	6.6	--	--

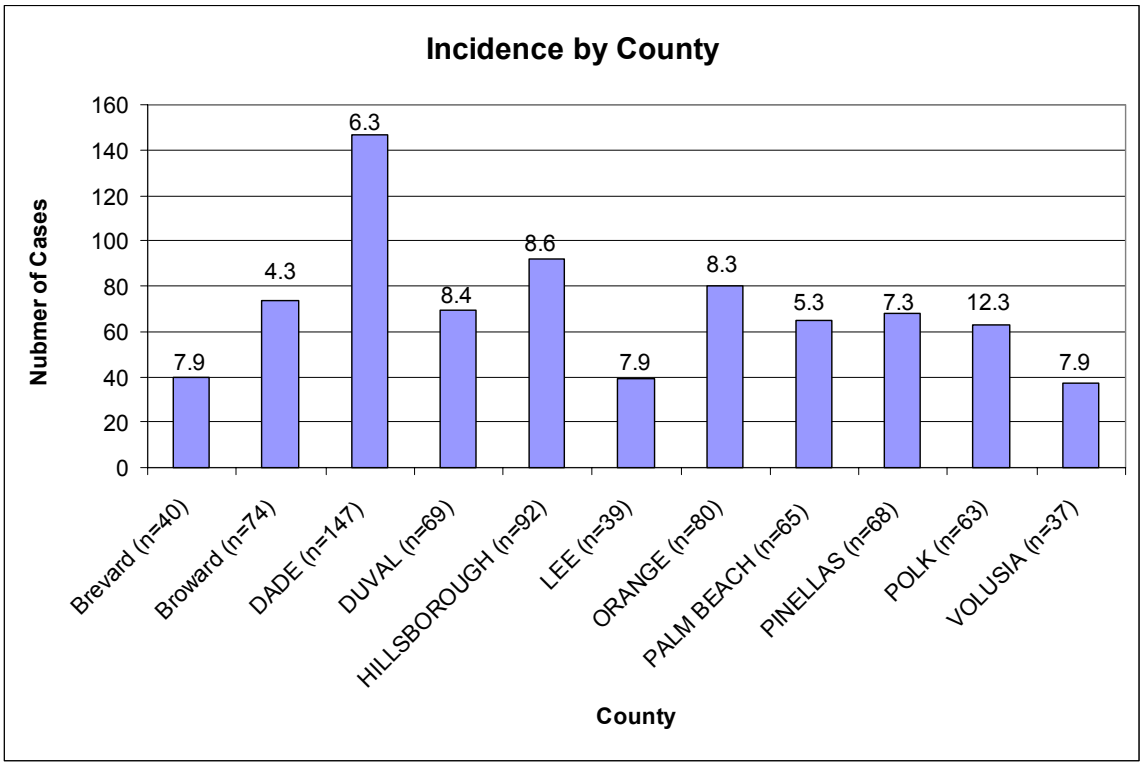
Hamilton	24	13,917	0	0.0	0.0	0.0
* Hardee	25	27,659	2	7.2	--	--
Hendry	26	37,064	5	13.5	1.6	25.3
Hernando	27	143,449	6	4.2	0.8	7.5
* Highlands	28	91,051	2	2.2	--	--
Hillsborough	29	1,073,407	92	8.6	6.8	10.3
Holmes	30	18,986	0	0.0	0.0	0.0
Indian River	31	120,463	1	0.8	-0.8	2.5
* Jackson	32	46,508	1	2.2	--	--
* Jefferson	33	14,037	2	14.2	--	--
Lafayette	34	7,333	0	0.0	0.0	0.0
Lake	35	245,877	20	8.1	4.6	11.7
Lee	36	492,210	39	7.9	5.4	10.4
Leon	37	242,577	10	4.1	1.6	6.7
Levy	38	36,270	0	0.0	0.0	0.0
Liberty	39	7,315	0	0.0	0.0	0.0
Madison	40	18,766	0	0.0	0.0	0.0
Manatee	41	286,804	7	2.4	0.6	4.3
Marion	42	280,288	13	4.6	2.1	7.2
Martin	43	135,122	4	3.0	0.1	5.9
* Monro	44	78,940	1	1.3	--	--
Nassau	45	61,625	0	0.0	0.0	0.0
Okaloosa	46	178,104	7	3.9	1.0	6.8
Okeechobee	47	37,481	0	0.0	0.0	0.0
Orange	48	964,865	80	8.3	6.5	10.1
Osceola	49	205,870	7	3.4	0.9	5.9
Palm Beach	50	1,216,282	65	5.3	4.0	6.6
Pasco	51	388,906	24	6.2	3.7	8.6
Pinellas	52	926,146	68	7.3	5.6	9.1
Polk	53	510,458	63	12.3	9.3	15.4
Putnam	54	71,841	11	15.3	6.2	24.4
Santa Rosa	55	133,092	5	3.8	0.5	7.1
Sarasota	56	346,793	17	4.9	2.6	7.2
Seminole	57	386,374	20	5.2	2.9	7.5
* St. Johns	58	142,869	2	1.4	--	--
St. Lucie	59	213,447	11	5.2	2.1	8.2
* Sumter	60	58,875	3	5.1	--	--
Suwannee	61	36,695	0	0.0	0.0	0.0
Taylor	62	19,415	0	0.0	0.0	0.0
Union	63	14,002	0	0.0	0.0	0.0
Volusia	64	468,663	37	7.9	5.3	10.4
Wakulla	65	26,131	0	0.0	0.0	0.0
* Walton	66	46,373	1	2.2	--	--
Washington	67	21,604	0	0.0	0.0	0.0

FLORIDA		17,019,068	1043	6.1	5.8	6.5

Source: *S. pneumoniae* data, Florida 2003

Counties with populations over 450,000 were highlighted in the previous table and include Brevard (505,711), Broward (1,731,347), Miami-Dade (2,341,167), Duval (817,480), Hillsborough (1,073,407), Lee (492,210), Orange (964,865), Palm Beach (1,216,282), Pineallas (926,146), Polk (510,458) and Volusia (468,663). Together these counties make up over 70% of reported cases in 2003. The following figure (Figure 4) shows the number of cases reported in the previous counties. Incidence (per 100,000) is indicated at the top of each column.

Figure 4: Geographic distribution and incidence (per 100,000) of *S. pneumoniae* in counties with populations over 450,000, Florida 2003

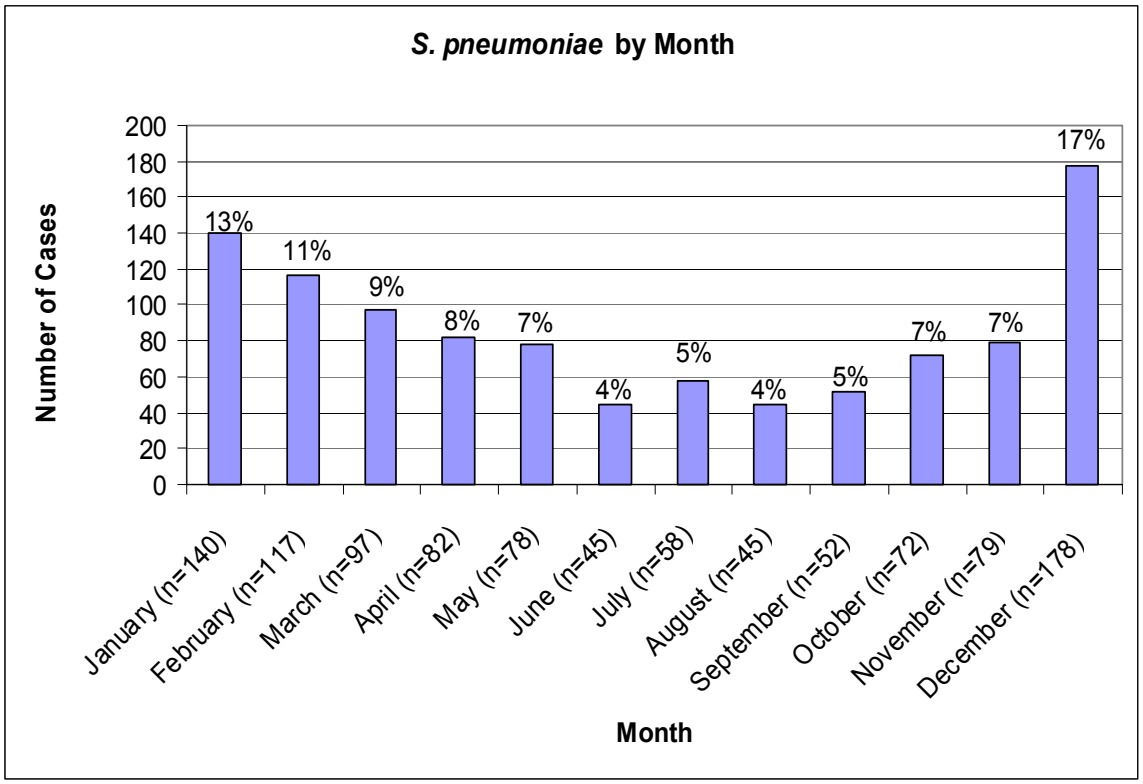


Source: *S. pneumoniae* data, Florida 2003

Seasonality

The majority of invasive *S. pneumoniae* cases (1043 of 1056) reported a date of infection. This data was used to analyze seasonal variation of *S. pneumoniae*. The following figure (Figure 5) shows the monthly distribution of cases. From this chart one can see a lull in the warmer months, and then a steady increase starting in fall and finally peaking in December. The majority of cases 41% occur between December and February.

Figure 5: Percentage of *S. pneumoniae* cases by month, Florida 2003



Source: *S. pneumoniae* data, Florida 2003

Table 7: Reported antibiotic resistance rates of *S. pneumoniae*, Florida 2003

Class	ANTIBIOTIC	TOTAL NUMBER	% of Total Cases	Antibiotic Susceptibility				
				N (%) S	N (%) MS	N (%) I	N (%) R	N (% I + R)
Penicillins	**Penicillin**	882	84%	441 (50%)	15 (2%)	252 (28%)	174 (20%)	452 (48%)
Macrolides	**Erythromycin**	626	59%	414 (66%)	0 (0%)	11 (2%)	201 (32%)	212 (34%)
	Azithromycin	174	16%	212 (64%)	0 (0%)	8 (5%)	54 (31%)	62 (36%)
	Total	698	66%	456 (65%)	0 (0%)	15 (2%)	227 (33%)	242 (35%)
Cephalosporins	**Cefotaxime (3rd)**	512	48%	463 (90%)	2 (0.4%)	31 (6%)	16 (3%)	47 (9%)
	Ceftriaxone (3rd)	635	60%	599 (94%)	2 (0.3%)	24 (4%)	10 (2%)	34 (6%)
	Total (3rd)	792	75%	726 (92%)	4 (0.1%)	49 (6%)	17 (2%)	66 (8%)
	Cefuroxime (2nd)	177	17%	119 (67%)	0 (0%)	11 (6%)	47 (27%)	58 (33%)
Lincosamides	**Clindamycin**	341	32%	274 (80%)	0 (0%)	32 (9%)	35 (10%)	67 (19%)

N=Number
3rd=third generation cephalosporins, 2nd=second generation cephalosporins
Percent of total cases is out of 1056
S=susceptible, MS=mild susceptible, I=intermediate, R=resistant

Source: *S. pneumoniae* data, Florida 2003

Table 7: Reported antibiotic resistance rates of *S. pneumoniae*, Florida 2003

Class	ANTIBIOTIC	TOTAL NUMBER	% of Total Cases	Antibiotic Susceptibility				
				N (%) S	N (%) MS	N (%) I	N (%) R	N (% I + R)
Carbapenem	**Meropenem**	69	7%	55 (80%)	0 (0%)	8 (11%)	6 (9%)	14 (20%)
	Imipenem	123	12%	96 (78%)	10 (8%)	13 (11%)	4 (3%)	17 (14%)
Fluoroquinolones	**Ofloxacin**	139	13%	127 (91%)	0 (0%)	10 (7%)	2 (1%)	12 (8%)
	Levofloxacin	342	32%	335 (98%)	0 (0%)	2 (1%)	5 (1%)	7 (2%)
Tetracycline	**Tetracycline**	359	34%	312 (87%)	0 (0%)	6 (2%)	41 (11%)	47 (13%)
Glycopeptide	**Vancomycin**	797	75%	797 (100)%	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Other	**Chloramphenicol**	292	28%	258 (88%)	0 (0%)	2 (1%)	32 (11%)	72 (12%)
	TMP/SMX	529	50%	331 (63%)	0 (0%)	40 (7%)	158 (30%)	198 (37%)
	Rifampin	84	8%	84 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

N=Number

3rd=third generation cephalosporins, 2nd=second generation cephalosporins

Percent of total cases is out of 1056

S=susceptible, MS=mild susceptible, I=intermediate, R=resistant

Source: *S. pneumoniae* data, Florida 2003

Clinically Relevant Antibiotic Resistance Patterns

Clinically relevant antibiotic resistance patters (Objective 2) were determined by analyzing several antibiotics. Antibiotics examined in this study included Penicillin, Azithromycin, Cefotaxime, Ceftriaxone, Cefuroxime, Chloramphenicol, Clindamycin, Erythromycin, Imipenem, Levofloxacin, Meropenem, Ofloxacin, Rifampin, Tetracycline, Trimethoprim-Sulfamethoxazole, and Vancomycin. The previous table (Table 7) shows each of the antibiotics examined and the results from susceptibility testing.

Penicillin Susceptibility

The overall rate of penicillin resistant *S. pneumoniae* (PRSP) in Florida for 2003 was 48.30% which is slightly lower than what was reported for Florida in 2000 (56.8%) (Florida Department of Health, 2000). The following table (Table 8) presents the breakdown of penicillin susceptibility by age group. The table shows that all age groups had penicillin resistance (both intermediate and fully resistant) greater than 41%, with the highest percentage of resistance occurring in ages four years and younger. Additionally the table shows that elevated incidence occurs in children less than four years old and adults over the age of 50 years. The highest incidence of resistance occurred in children less than one year of age (20.3 per 100,000), out of 186,977 children less than one year of age. Overall incidence rates for the population (15,982,378) were found to be 2.6 per 100,000.

Table 8: Percentage and incidence (per 100,000) of PRSP by age group, Florida 2003

AGE GROUPS			Invasive *Streptococcus pneumoniae* cases						
			Penicillin Susceptibility						
			Susceptible (S)			Intermediate 'I' and Resistant 'R'			
Age (years)	Cases In Age Group	% of Total Cases	Number Susceptible	% of Total	% of Age Group	Number Resistant	% of Total	% of Age Group	Incidence of Pen R
<1	50	7%	12	2%	24%	38	5%	76%	20.3
1-2	58	8%	20	3%	34%	36	5%	62%	9.6
3-4	31	4%	9	1%	29%	22	3%	71%	5.7
0-4	139	19%	41	6%	29%	96	13%	69%	10.1
5 - 17	34	5%	15	2%	44%	19	3%	56%	0.7
18 - 34	50	7%	23	3%	46%	27	4%	54%	1.4
35 - 49	130	18%	64	9%	49%	66	9%	51%	1.8
50 - 64	162	22%	68	9%	42%	94	13%	58%	3.7
≥65	223	30%	108	15%	48%	117	16%	52%	4.2
TOTAL	738	100%	319	43%		419	57%		2.6

Source: *S. pneumoniae* data, Florida 2003

Drug Susceptibility

Resistance information was provided for several antibiotics for this study. Table 7 shows each of the antibiotics examined. For each antibiotic the number of cases that were tested for susceptibility is listed, as well as the susceptibility results. Of the 1056 cases tested for resistance 535 (51%) were resistant to one or more antibiotics, and thus classified as drug resistant *S. pneumoniae* (DRSP) as defined by the CDC.

The antibiotics were divided into 8 major classes; Penicillins, Macrolides Cephalosporins, Fluoroquinolones, Lincosamides, TMP/SMX, Tetracycline and Glycopeptides. Table 9 summarizes *S. pneumoniae* cases resistant to specific antibiotics

Table 9: Intermediate and fully resistant strains of *Streptococcus pneumoniae* for various antibiotics, by age group, Florida 2003

Age Group	Penicillin		Erythromycin		Cefotaxime (3rd)		Ceftriaxone (3rd)		Levofloxacin		TMP/SMX		Tetracycline	
	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)	No. In Age Group	I + R (%)
<1	50	38 76%	34	22 65%	28	6 21%	32	0 0%	10	0 0%	27	14 52%	18	5 28%
1-2	56	36 64%	37	19 51%	35	5 14%	43	5 12%	15	0 0%	28	15 54%	17	2 12%
3-4	31	22 71%	18	5 28%	23	3 13%	25	3 12%	8	1 13%	17	7 41%	9	2 22%
0-4	137	96 70%	89	46 52%	86	14 16%	100	8 8%	33	1 3%	72	36 50%	44	9 20%
5 - 17	34	19 56%	21	4 19%	22	2 9%	28	1 4%	11	0 0%	18	7 39%	10	0 0%
18 - 34	50	27 54%	35	9 26%	27	3 11%	34	3 9%	22	0 0%	32	12 38%	22	2 9%
35 - 49	130	66 51%	94	28 30%	78	8 10%	90	4 4%	52	0 0%	85	36 42%	58	10 17%
50 - 64	162	94 58%	122	46 38%	94	9 10%	117	10 9%	67	0 0%	102	41 40%	67	8 12%
≥ 65	225	117 52%	162	75 46%	122	11 9%	164	8 5%	103	6 6%	136	62 46%	96	17 18%
TOTAL	738	419 57%	523	208 40%	429	47 11%	533	34 6%	288	7 2%	445	194 44%	297	46 15%

Source: *S. pneumoniae* data, Florida 2003

by age group. Vancomycin is a glycopeptide, and was not reported in the table due to the fact that no cases reported either intermediate or high levels of resistance. These specific antibiotics were chosen because more data was available on these drugs.

Multi-Drug Resistance

Combinations of drug resistance were analyzed for those records with reported susceptibility for the antibiotics (N=154): penicillin, ceftriaxone, erythromycin, tetracycline, TMP/SMX, and chloramphenicol. The most common pattern of multi-drug resistance was observed for resistance to penicillin, erythromycin, and TMP/SMX. This information is displayed in Table 10.

Table 10: Isolation of *Streptococcus pneumoniae* with various patterns of antibiotic resistance, Florida 2003

	Pattern of Resistance						Total Number (%)
	Penicillin	Ceftriaxone	Erythromycin	Tetracycline	TMP/SMX	Chloramphenicol	
1*	R	S	R	S	R	S	23/154 (14.9)
2	R	S	S	S	S	S	20/154 (13)
3	R	S	S	S	R	S	10/154 (6.5)
4	R	S	R	S	S	S	8/154 (5.2)
5	S	S	S	S	R	S	6/154 (3.9)
6*	R	S	R	R	R	R	4/154 (2.6)

* Resistance to 3 or more classes
Resistance includes both 'I' intermediate and 'R' resistant strains
TMP/SMX=trimethoprim-sulfamethoxizole

Source: *S. pneumoniae* data, Florida 2003

Macrolides

Erythromycin resistant strains of *S. pneumoniae* have been shown to also exhibit resistance to penicillin and other Macrolides including azithromycin. Of the 626 isolates tested for erythromycin susceptibility 222 (33.87%) were resistant (either intermediate or fully resistant). Of these 99 (49%) were fully resistant to penicillin and 79 (39%) displayed intermediate resistance to penicillin. Azithromycin susceptibility was reported on 36 (16.21%) of the 222 erythromycin cases. Of these isolates 32 (88.8%) displayed resistance to erythromycin.

Cephalosporins

The cephalosporins that were analyzed for this study were Cefotaxime, Ceftriaxone are third generation cephalosporins and Cefuroxime which is a second generation cephalosporin. These antibiotics have been shown to display low levels of resistance (<10%) in penicillin susceptible strains, with some increased percentage of resistance (~50%) in PRSP strains (Florida Department of Health, 2000). 512 isolates were tested for susceptibility of Cefotaxime, 47 (9.17%) were reported as being resistant (I and R). Of these that were also tested for penicillin susceptibility, 44 (93.61%) were resistant (I and R). 635 isolates were tested for susceptibility to Ceftriaxone, of these 34 (5.35%) were resistant. Of these 34 strains, 33 (97%) demonstrated resistance to penicillin. Susceptibility was also reported for Cefuroxime; 58 (32.76%) of 177 isolates were found to be resistant. Of these 58 cases, 57 (98.27%) were also found to have intermediate or full resistance to penicillin.

Fluoroquinolones

Levofloxacin and Ofloxacin are Fluoroquinolones that were examined in this study. Ofloxacin is a first generation, while Levofloxacin is a second generation. Ofloxacin is not the best choice of treatment due to its poor susceptibility performance (Florida Department of Health, 2000). Levofloxacin is currently an appropriate treatment choice for outpatients or admitted patients with *S. pneumoniae* pneumonia (Shah, Gidudice, Griesback Jr, Morley, & Vasoya, 2004).

Susceptibility to Levofloxacin was reported for 342 cases. Of these 342 cases seven (2%) were classified as being resistant (I and R). Cases that were tested for both Penicillin and Levofloxacin yielded three of seven (42.8%) cases resistant (I and R) to both.

Trimethoprim Sulfamethoxazole

Trimethoprim Sulfamethoxazole (TMP/SMX) is reported to have elevated resistance levels along with Penicillin resistance. This drug is not the most appropriate choice in treatment of *S. pneumoniae*.

Susceptibility of TMP/SMX was reported for 529 cases. Among these 529 cases 198 (37.43%) were reported as resistant (I and R). Cases that were tested for both Penicillin and TMP/SMX, 248 reported resistance to penicillin and 186 (75%) reported co-resistance to TMP/SMX.

Tetracycline

Tetracycline resistance has been on the rise in Florida since 1979 (Florida Department of Health, 2000). There were 359 cases with data for tetracycline, resistance was reported in 47 (13.09%). Cases reporting tetracycline and resistance to penicillin numbered 176, of these 43 (24.43%) reported co-resistance to tetracycline.

Vancomycin

Currently there have been no reports of a case of *S. pneumoniae* being resistant to vancomycin (Pallares, Fenoll, & Linares, 2003). A recent study in Mississippi reported two cases of *S. pneumoniae* being tolerant to vancomycin (Gillis, White, Whitehurst, & Sullivan, 2005).

Susceptibility for vancomycin was reported for 797 cases. 100% were susceptible to vancomycin.

Risk Factors for Antibiotic Resistance

The variables that were examined as potential risk factors (Objective 3) for antibiotic resistance include; gender, race, age group, and month. Logistic regression was performed to determine which if any of these factors posed a risk. The following table (Table 11) summarizes all results from the logistic regression model.

Table 11: Maximum Likelihood Estimates and Confidence Limits from logistic regression, Florida 2003

Variable		Maximum Likelihood Estimates		p-value	Odds Ratio	95 % Wald Confidence limits	
		Estimate	Standard Error			Lower	Upper
Gender	Male	0.0365	0.0915	0.6896	1.076	0.752	1.54
Race	White	0.0503	0.3167	0.8739	0.902	0.569	1.364
	Other	-0.2038	0.6064	0.7368	0.699	0.115	4.247
Age Group	≤ 1 year	0.65	0.391	0.0965	3.077	0.969	9.766
	1-2 year	0.2609	0.3523	0.459	2.085	0.704	6.173
	3-4 year	0.1558	0.4576	0.7336	1.877	0.526	6.699
	18-34 years	0.0677	0.3202	0.8326	1.719	0.61	4.845
	35-49 years	-0.394	0.2164	0.0687	1.083	0.447	2.628
	50-64 years	-0.1824	0.2027	0.368	1.338	0.562	3.188
	≥ 65 years	-0.0839	0.1933	0.6644	1.477	0.627	3.478
Female gender, Black Race, 5-17 year olds, and July were used as reference groups							

Source: *S. pneumoniae* data, Florida 2003

Table 11: Maximum Likelihood Estimates and Confidence Limits from logistic regression, Florida 2003

Variable		Maximum Likelihood Estimates		p-value	Odds Ratio	95 % Wald Confidence limits	
		Estimate	Standard Error			Lower	Upper
Month	January	0.3623	0.2786	0.1965	4.747	2.142	10.517
	February	-0.1244	0.2626	0.6357	2.918	1.345	6.327
	March	2.8474	0.9307	0.0022	56.972	7.21	450.152
	April	1.2373	0.4472	0.0057	11.387	3.808	34.05
	May	0.6833	0.3962	0.0846	6.543	2.399	17.842
	June	0.0599	0.396	0.8797	3.508	1.29	9.542
	August	-0.3598	0.3614	0.3194	2.305	0.9	5.904
	September	-0.3611	0.3382	0.2855	2.303	0.943	5.623
	October	-1.0027	0.2779	0.0003	1.212	0.548	2.68
	November	-1.3011	0.2767	<0.0001	0.899	0.406	1.993
	December	-0.846	0.232	0.0003	1.418	0.686	2.929
Female gender, Black Race, 5-17 year olds, and July were used as reference groups							

Source: *S. pneumoniae* data, Florida 2003

Age was analyzed comparing all age groups to 5-17 year olds. The 5-17 year age group was used as the reference group because it demonstrated the lowest incidence. The likelihood of DRSP for age less than 1 (OR=3.1 CI 0.97 - 9.8), age 1-2 years (OR = 2.1 CI 0.70 - 6.2) and age greater than 65 years (OR = 1.5 CI 0.63 - 3.5). These results were not significant.

Race was analyzed using Black race as the reference group. No association was found between race and DRSP.

For gender, males posed no greater risk than females (OR = 1.1 CI 0.8-1.5). These results were also found to be non-significant.

Using the binary logit model, only month of occurrence was found to be a significant factor ($p<.0001$). Using July as the reference group, all other months were examined for increased risk of resistance. Cases occurring any month except November were more likely to have a resistant strain. The only significant results occurred in October (OR 1.2, 95% CI 0.5 – 2.6), November (OR .9, 95% CI 0.4 – 2.0), and December (OR 1.4, 95% CI 0.7 – 2.8). These odd ratios do not demonstrate a dramatic increase in the likelihood of DRSP among these months.

Another analysis was preformed, dividing the months into four seasons: Spring (March, April, and May), summer (June, July, and August), autumn (September, October, and November) and winter (December, January, and February). Using logistic regression, there was a significant difference between the likelihood of DRSP for spring (OR 0.1, 95% CI 0.05 - 0.2). Once again the OR did not demonstrate a dramatic increase in the likelihood of DRSP during spring.

Incidence of Streptococcus pneumoniae

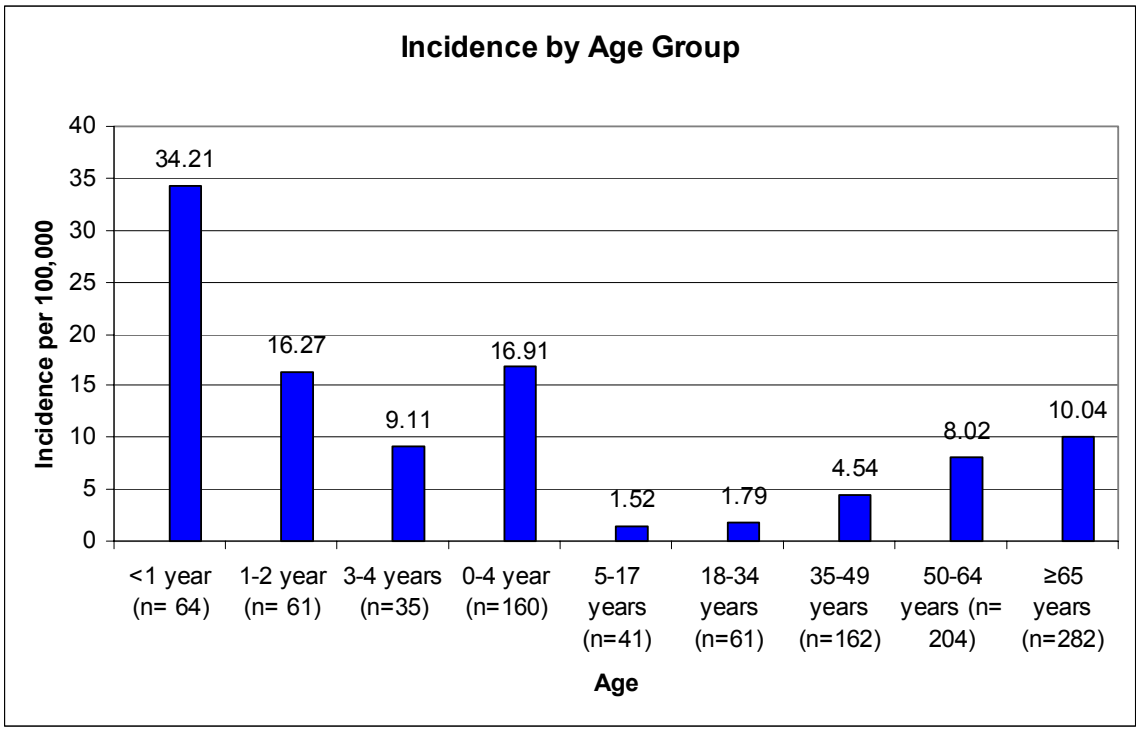
For the calculation of incidence of *S. pneumoniae* (Objective 4), all cases reported to the state Bureau of Epidemiology were included. Cases reported covered the entire year of 2003 for the state of Florida.

The incidence of invasive pneumococcal disease was calculated to be 6.61 per 100,000 persons (95% CI 6.21 – 7.01).

Incidence by Age group

Incidence was calculated using 2003 population estimates obtained from the United States Census Bureau. Incidence rates for all age groups are shown in Figure 6. Incidence was highest among children less than one (34.21/100,000). Incidence was also elevated among children less than 4 years of age and in adults over 65.

Figure 6: Incidence of *S. pneumoniae* by age group, Florida 2003



Source: *S. pneumoniae* data, Florida 2003

Incidence by Gender and Race

Incidence among males, 7.4 per 100,000 persons (95 % CI 6.8 – 8.0) was higher than that of females, 5.9 per 100,000 (95% CI 5.3 – 6.4).

Incidence among black race, 11.5 per 100,000 (95% CI 10.1 – 12.9), was twice that of whites, 5.4 per 100,000 (95% CI 5.0 – 5.8).

Chapter Seven

Discussion

Limitations of the Study

This is the only population-based *S. pneumoniae* data source that includes the entire state of Florida. So there are several reporting issues that can cause limitations in the analysis of the data.

Selection bias may be introduced due to incorrect reporting by hospitals; there is the potential for accurate reporting of resistant cases and under reporting of case that report no resistance. This potential discrepancy in reporting would resort in the over estimation of antibiotic resistance. Furthermore laboratories and providers that fail to report cases of invasive *S. pneumoniae* to the Department of Health or laboratories that file incomplete reports can introduce bias. The lack of a quality assurance program to insure data quality is also a limitation.

Unfortunately for this study it is impossible to create an accurate control group, because of the possibility of incomplete reporting. Considering that the main goal of this study was descriptive analysis of the study population, one of the significant limitations was the lack of reporting of vaccine coverage. Vaccine coverage information would be valuable in determining efficacy of the vaccine in reducing incidence.

A limitation in the determination of risk factors and calculation of incidence is the potential for missing cases and missing variables. Both of these factors contribute to underestimation of risk factors and incidence.

Strengths of the Study

Considering that invasive pneumococcal disease is associated with antibiotic resistance and a higher incidence among children less than 5 years of age, it is important to monitor resistance patterns and incidence among this population. One of the strengths of this study is that it examines cases on a state wide level, not just by county. This aids in determining problem areas within the state.

This study defines the trends and risk factors associated with antibiotic resistance among invasive pneumococcal infections. Applying this information will allow the Department of Health to develop effective strategies in reducing the incidence of this disease. In addition antibiotic resistance patterns were defined and will be made available to the health care community.

Risk factors and antibiotic resistance patterns may aid health care practitioners in selecting more effective antibiotics and developing more efficient treatment strategies for patients.

Interpretation of Results

Characteristics of the Study Population

Comparing the results from this study with previous studies there are several occurrences of interest.

The proportion of males was 54.6%. This is consistent with the number of males with infection in comparison with previous years in Florida, males accounted for 52.3% of cases in 2000 and 53.3% of cases in 1999 (Florida Department of Health, 2000). When examining Hillsborough County alone, males accounted for a lower percentage (53%) when compared to a 1997 study which reported 58% (Walker & Sanderson, 1998). A survey of Central Florida from July 1997 thru March 1999 reported 55.9% of cases were male, which is higher than the 50% reported for 2003. These proportions are consistent with previous analysis of data for the U.S. in which males accounted for 55% of cases (Robinson et al., 2001).

Overall incidence of infection was 6.6/100,000 for 2003, this is a decrease from that reported by the state in 2000 (11.2 per 100,000). The highest incidence of infection occurs in children less than 4 years of age and adults over 65. Incidence was especially high among children <1 year of age (34.21/100,000). This number was lower than the Robinson study found in 1995-1998, which reported a pre-vaccine incidence of 166.9/100,000, in children less than 2 years (Robinson et al., 2001). The incidence is also lower than the CDC's report for 2003, of 39.3/100,000, for persons <1 year of age (CDC, 2004).

Children less than 5 years of age accounted for 18% of cases. This is a decrease from previous Florida reports, in 2000 40% of the cases were reported in children less

than 5 years old (Florida Department of Health, 2000). Hillsborough County also showed a similar decrease in percentage among those less than 5 years of age, 22% in 2003 down from 42% in 1997 (Walker & Sanderson, 1998). A survey of central Florida form July 1997-March 1999 reported 42% of cases occurred among children less than 5 years of age, which is greater than the 17% that was reported in 2003. Once again this is most likely due to the introduction of the PCV vaccine.

The results from this study also demonstrated whites accounting for a higher rate (71.5%) of cases and lower incidence 5.39/100,000 (95% CI 4.98-5.8), where race was reported. It is interesting to note that even though blacks made up a smaller proportion, incidence was higher among this group 11.48/100,000. This is possibly due to a higher proportion of cases occurring among blacks aged 35 to 49 years, in which studies have shown lower vaccine coverage compared to those over 65 years of age. These results are consistent with previous studies (Pastor et al., 1998) and (Robinson et al., 2001).

Seasonal variation is consistent with other reports, with the highest number of cases being reported during the winter months of November through February. These findings are consistent with results from the Kim and Dowell studies. Both of which reported peaks in the number of cases in the winter months, especially December (Kim et al., 1996) (Robinson et al., 2001).

Findings of Invasive Streptococcus pneumoniae

The overall incidence for invasive *S. pneumoniae* was calculated using 2003 population estimates for Florida, this information is illustrated in table 5. The incidence was 6.13 per 100,000 Florida residents (95% CI 5.76-6.50). This incidence is lower than the 11.2/100,000 that was reported in Florida in 2000 (Florida Department of Health, 2000). Florida's overall incidence is also lower than that which was reported by the CDC for 2003, 13.9/100,000 persons (CDC, 2004). Hillsborough county's incidence was 8.6 per 100,000 (95% CI 6.8-10.3) which was higher than that of the entire state. Although incidence in Hillsborough county was down from the 16.9 per 100,000 reported in 1997 (Walker & Sanderson, 1998). The incidence found in this study shows a similar trend to what was reported by Whitney in 2003, a decreasing trend in incidence since the introduction of the pneumococcal polysaccharide vaccine (PCV) in 2000 (Whitney, Farley, Hadler, Harrison, Bennett et al., 2003).

This study found an estimated incidence for children less than one year of age to be 34.21 per 100,000 children in Florida in this age group. Population data from 2000 was used to calculate incidence for age groups and other demographics. This is similar to the 39.3/100,000 persons incidence that was reported by the CDC for 2003 (CDC, 2004). Children less than 1 year of age in the Whitney study had an incidence of 59.0/100,000 persons in 2001 (Whitney et al., 2003). Walker and Sanderson found in Hillsborough County for 1997 that children less than four years of age had an incidence of 142.7 per 100,000 (95% CI 114.0-171.4). This study found an incidence of 16.9 per 100,000 (95% CI 14.3-19.5) in the same age group for Hillsborough county. These decreases in incidence are most likely due to the use of the PCV vaccine.

Findings of Antibiotic Resistance

This study found that 19.7% of cases were fully resistant to penicillin. When cases that showed intermediate resistance are added the percentage jumps to 48.3%. The Florida Department of Health reported in 2000 that the overall rate of penicillin resistant *S. pneumoniae* (PRSP) was 56.8%. A study of Hillsborough County during 1997 reported PRSP rates of 38%. A survey conducted of Miami-Dade County in 1998, of community acquired infections also reported PRSP rates of 38%. Another study conducted of central Florida from July 1997 thru March 1999, found PRSP rates for community acquired infections to be 38%. These resistance percentages include both intermediate and fully resistant cases.

Children less than four years of age had the highest rate (61%) resistant to penicillin. This rate is slightly lower than the 65% that was reported in Florida for 2000. These decreases in DRSP rates are most likely due to the PCV vaccine.

In addition to penicillin resistance, this study examined multiple resistant cases. Penicillin/Erythromycin/Trimethoprim Sulfamethoxazole had 23 cases, but made up the highest percentage of multiresistant patterns. These results are similar to a study by Walker in Hillsborough County in 1998, which had the combination of Penicillin/Erythromycin/Trimethoprim Sulfamethoxazole occurring most frequently (Walker & Sanderson, 1998).

Summary of Chance, Bias and Confounding

This study was a descriptive analysis with minor analytic analysis of invasive *S. pneumoniae* cases in Florida, for 2003. Due to missing values and a small data set it was difficult to make any statistically significant association involving risk factors for DRSP.

Information bias was a potential issue in the analysis too. In regards to antibiotic susceptibility hospitals most likely reported more antibiotic resistant cases than susceptible cases. This likely resulted in the overestimation of resistance.

Conclusions

This study demonstrates that Florida is experiencing a decrease in the proportion of resistance among reported cases of invasive *S. pneumoniae*. Considering the recent introduction of a vaccine for children, this trend is a good sign. These results are consistent with current findings that show a decrease in both those less than four years of age and those over 65 (Centers for Disease Control and Prevention, 2005). In addition to the reduction in resistance since 2000 there was also a decline in incidence. Not only is the incidence for 2003 (6.13/100,000) less than that reported in 2000 (11.2/100,000), it is also about half of that reported by the CDC for the U.S. in 2003 (13.9/100,000). This reduction in incidence is potentially another sign that the vaccines could be having their desired effect.

Reporting measures should continue and in addition be expanded to include testing for specific isolates and vaccination history. This step should be taken to determine if the vaccines are reducing the targeted isolates, and also to determine if new isolates are taking their place. Following recommendations from the CDC and NCCLS,

susceptibility testing should be conducted for all isolates from normally sterile sites. In addition to penicillin a broad spectrum cephalosporin should be tested to continually monitor changes in resistance.

There is a definite need to continually monitor this disease in the State of Florida. In conclusion with increased vaccine coverage and appropriate antibiotic use, DRSP cases might continue to decline.

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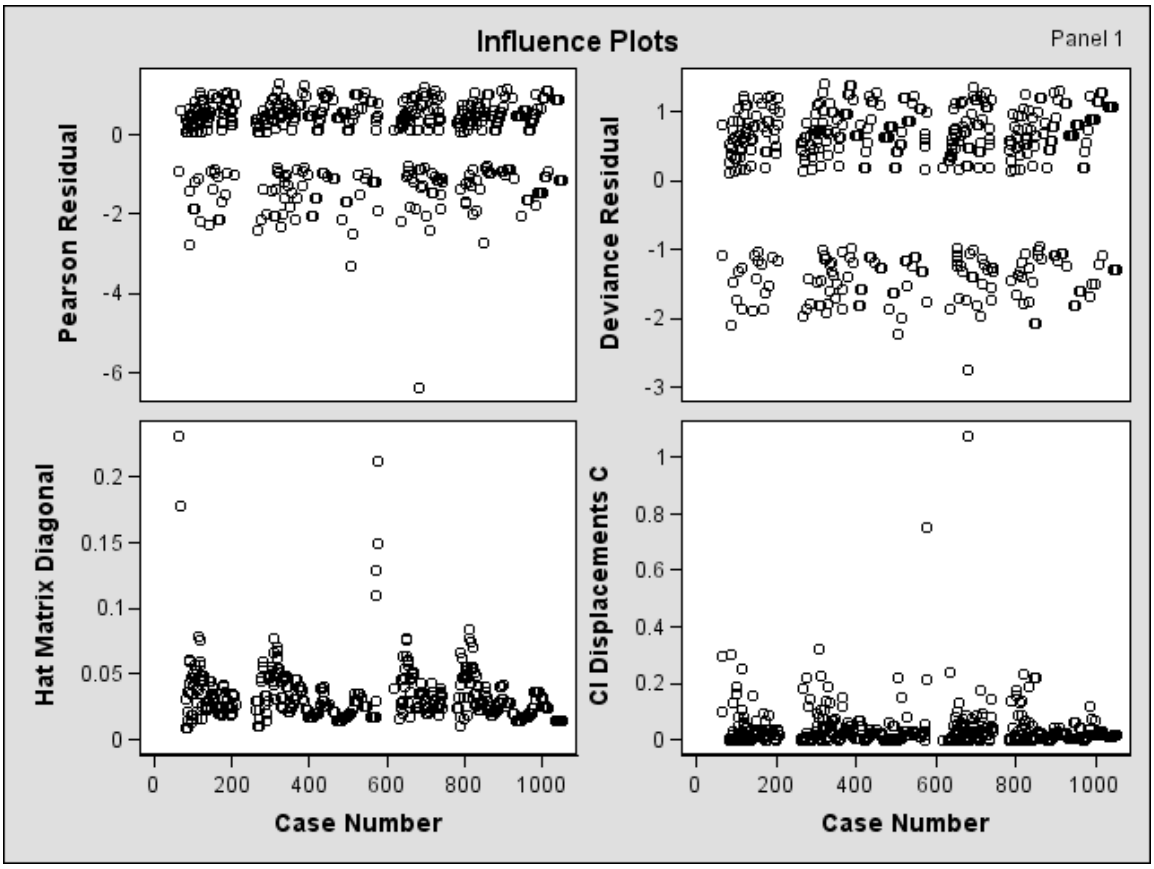
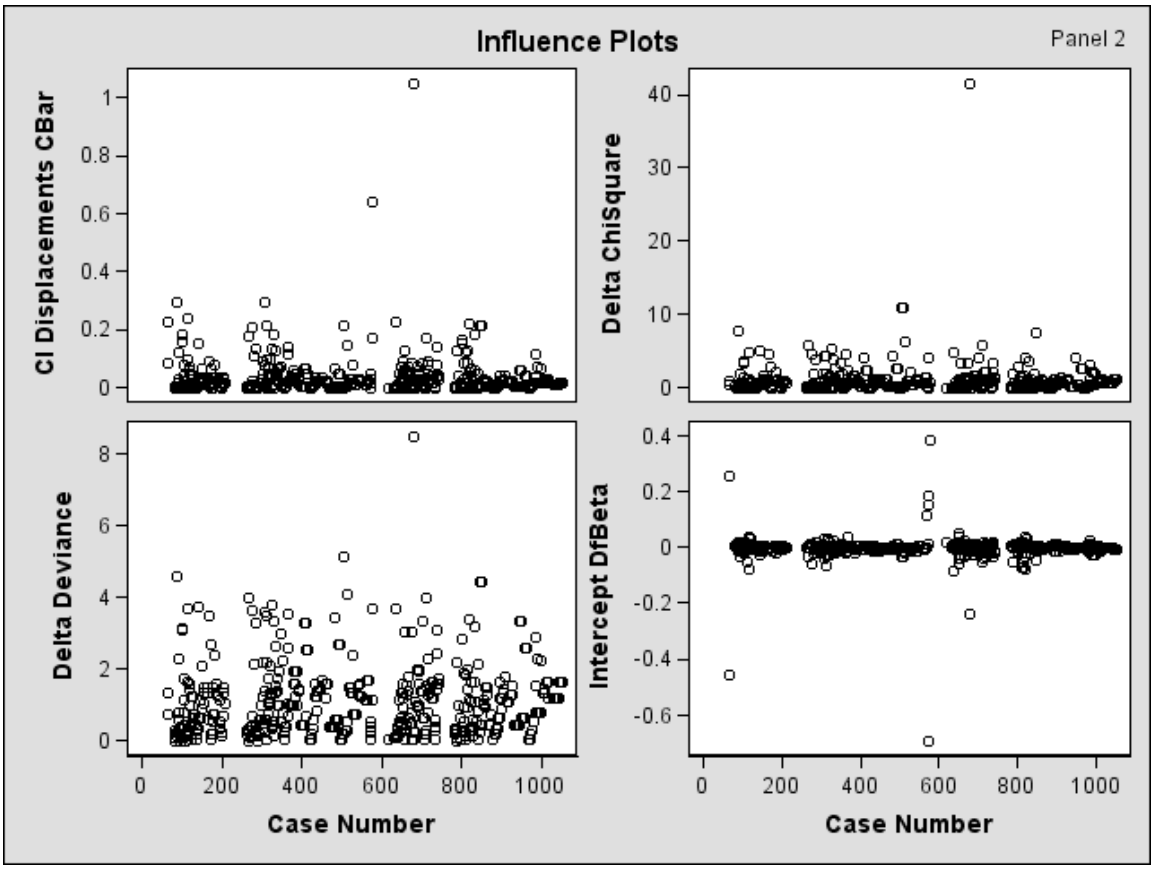
Appendices

Appendix A: Residual Tables and Influence Plots



Source: *S. pneumoniae* data, Florida 2003



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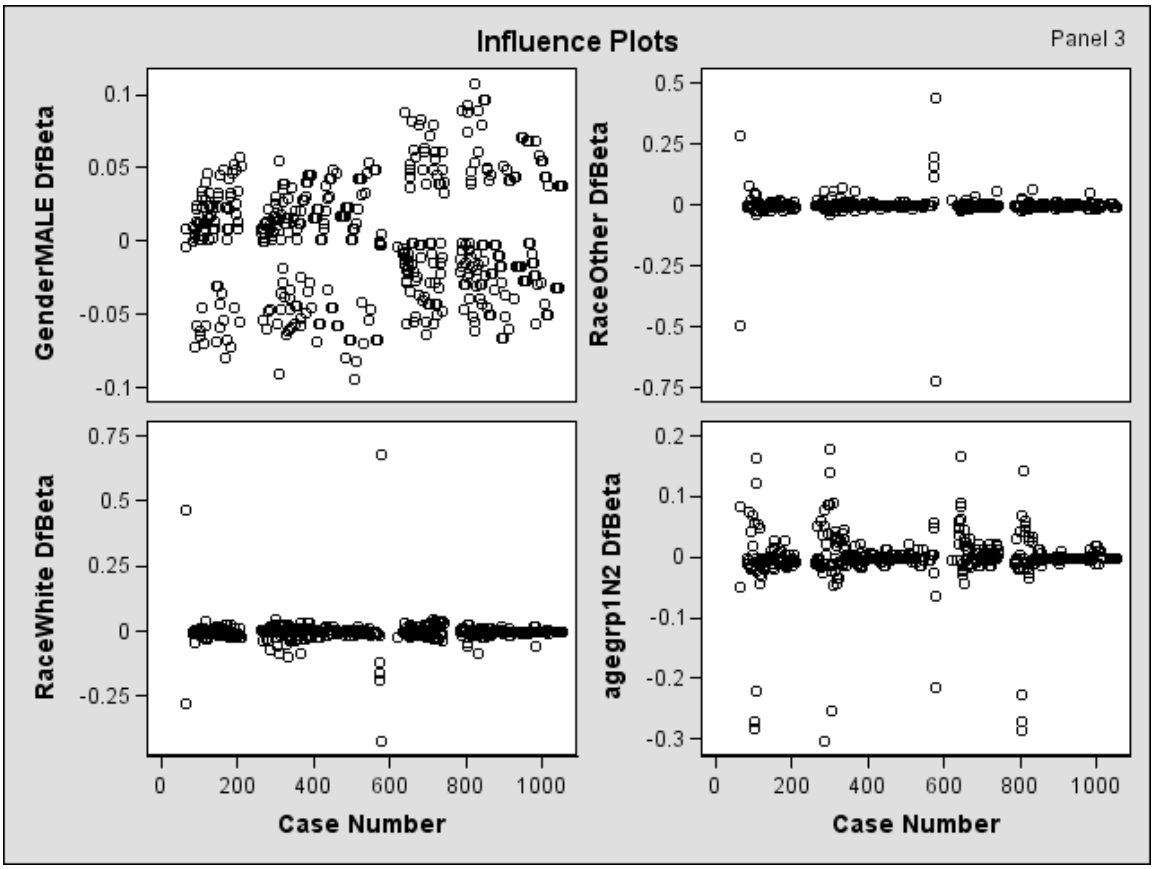

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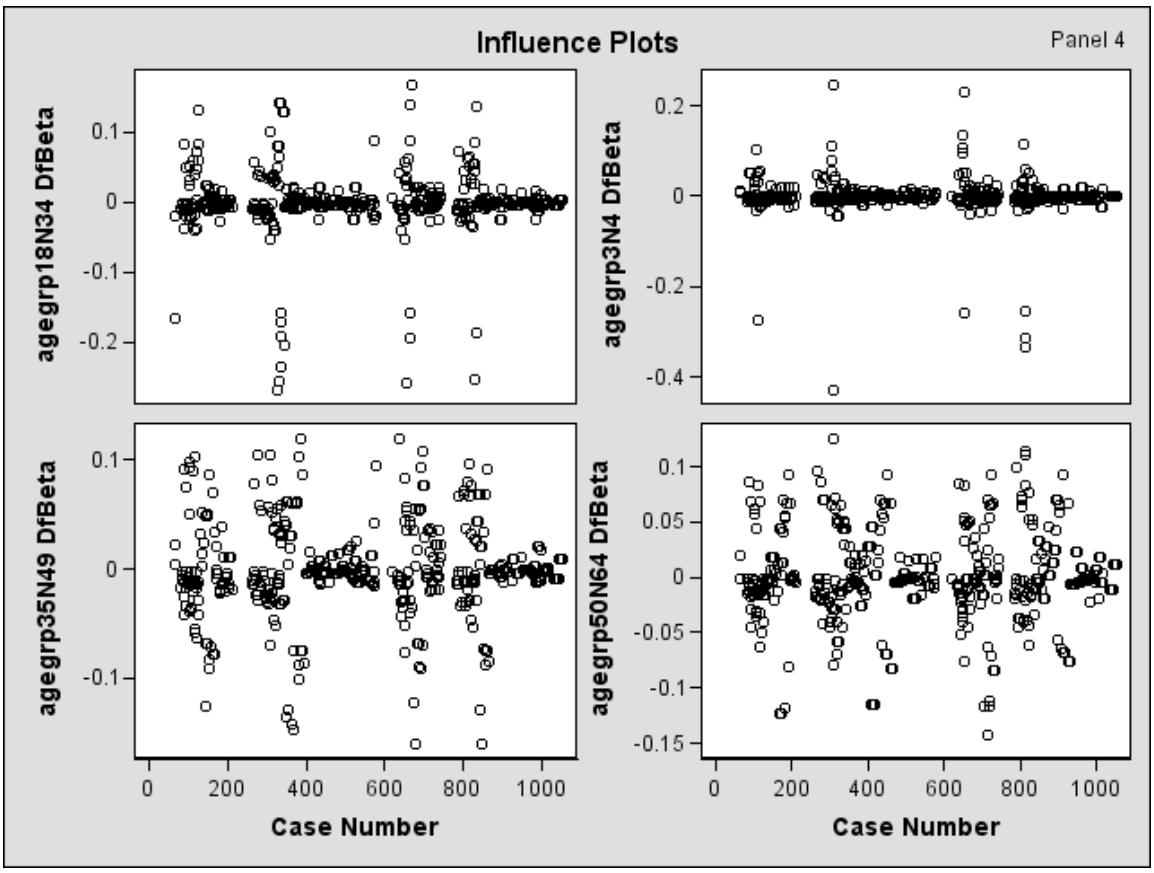

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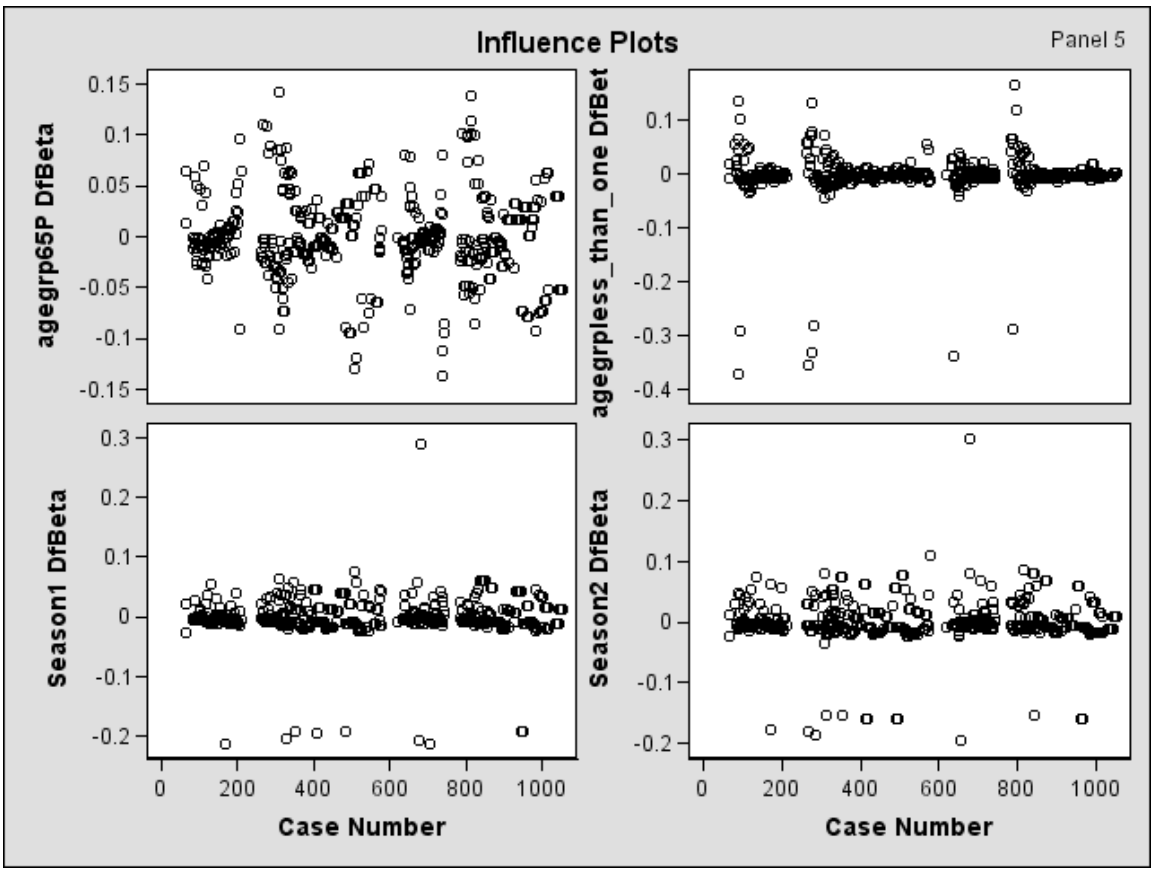

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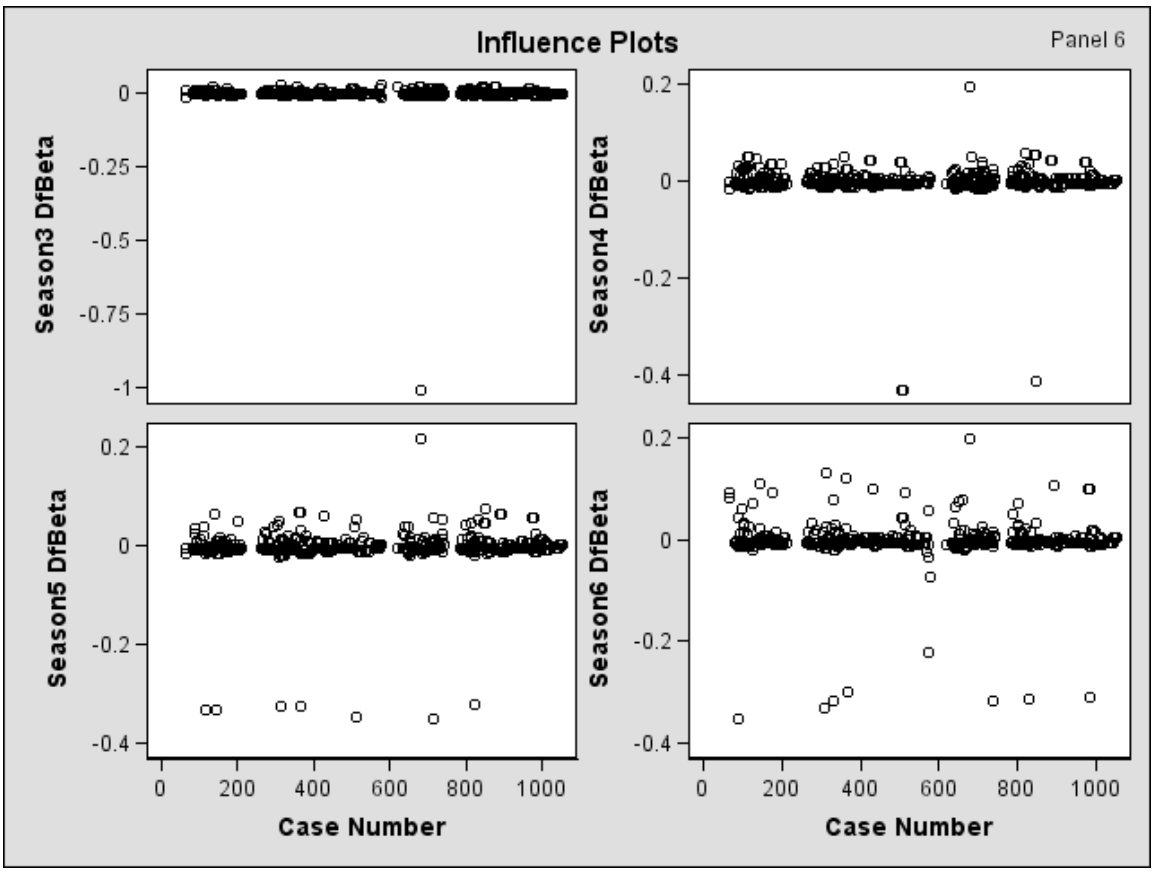

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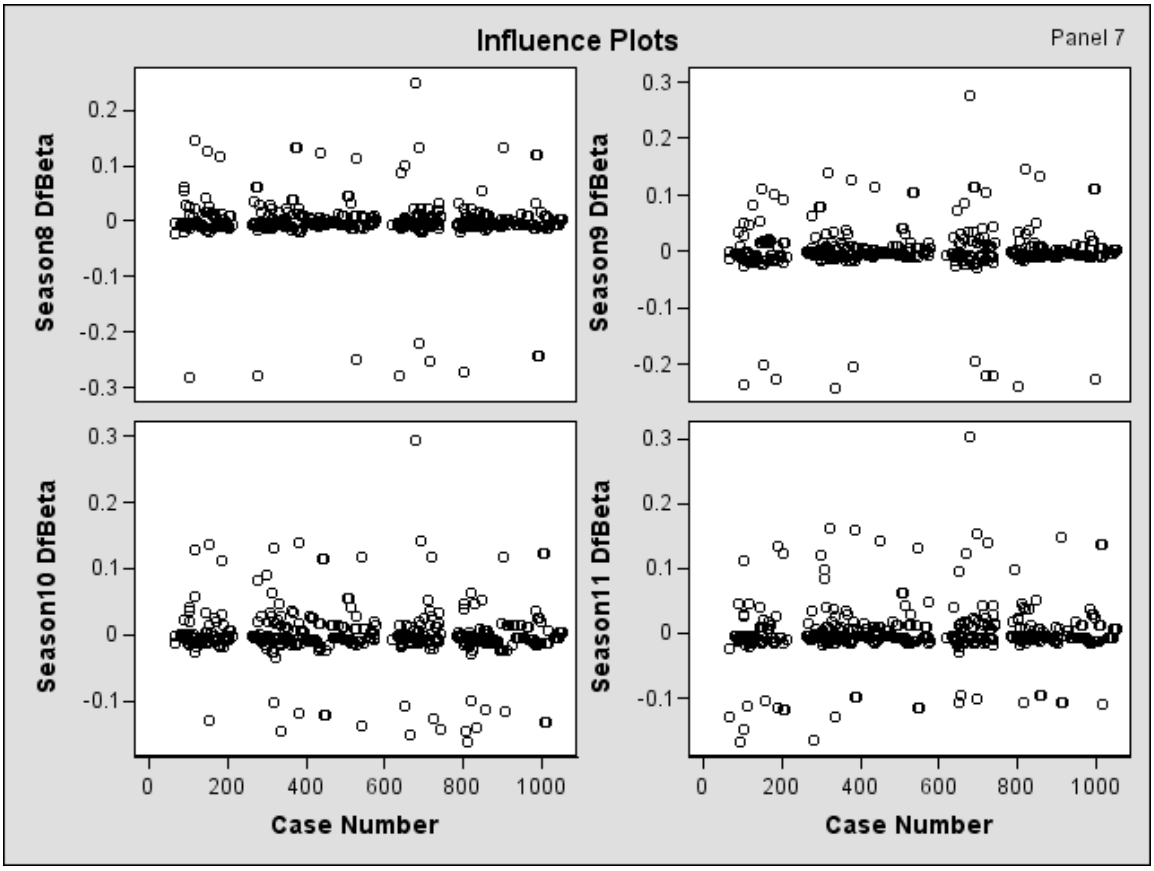

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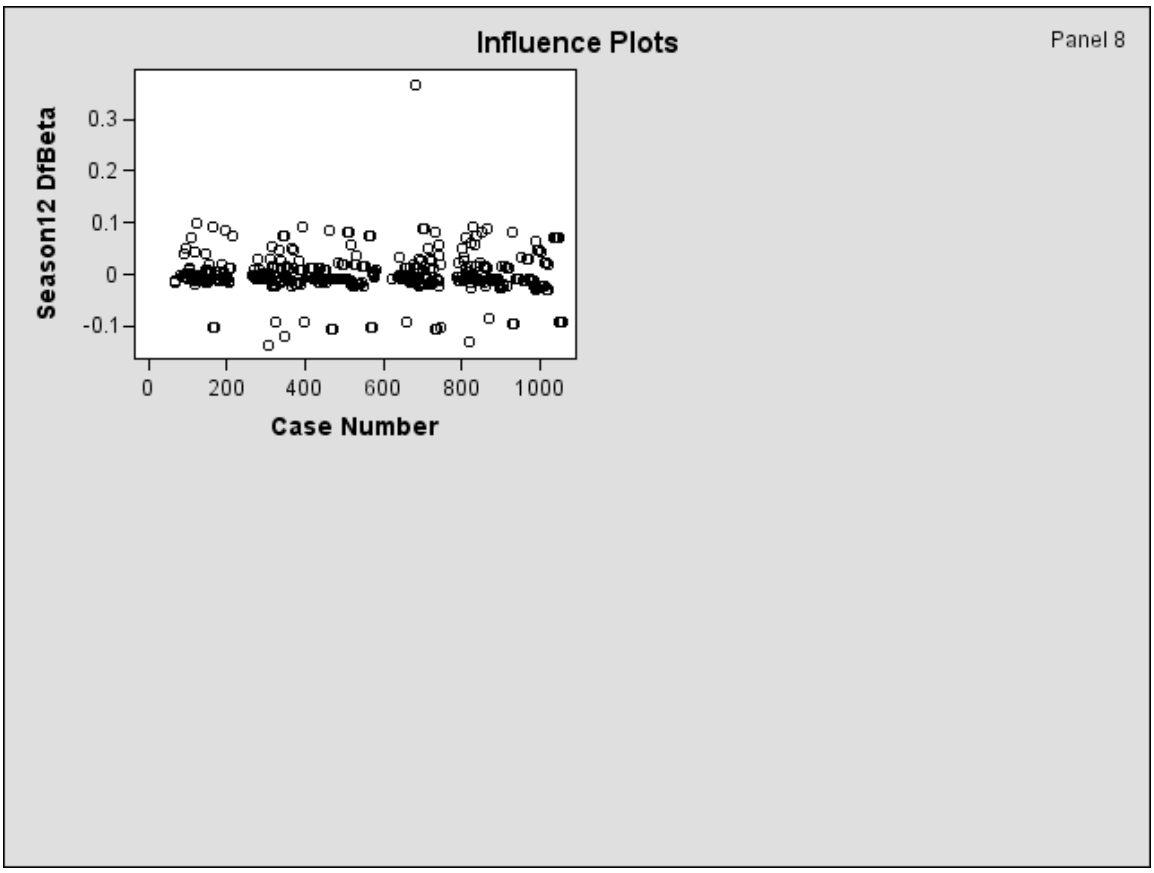

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