ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE

by

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ABSTRACT

Antimicrobial resistance in *Streptococcus pneumoniae* has increased in recent decades at a frightening pace. The infant conjugate vaccine program, introduced in BC in 2003, is a tool for the control of invasive pneumococcal disease (IPD) with substantial potential to combat antimicrobial resistance.

The first phase of this thesis linked surveillance programs at the BC Centre for Disease Control and the National Centre for Streptococcus to evaluate recent trends in IPD incidence, serotype distribution, and antimicrobial resistance. Based on 1,288 reported cases of IPD over 2002-2005, there was a significant decrease in the incidence in children <5 years. Over half of reported cases (728/1,288) were referred for serotype and susceptibility testing. Of these, the proportion that were of vaccine-preventable serotypes declined from 68.9% to 43.8% between 2002 and 2005; in children <2 years, the decline was from 83.0% to 16.7%. The prevalence of isolates with reduced susceptibility was highest for trimethoprim-sulfamethazole (15.3% non-susceptible, 111/725 tested), penicillin (9.1%, 66/728), and erythromycin (9.1%, 66/727), and to ≥2 classes of antimicrobials (10.3%, 75/728).

Arguably, the most important driver of resistance is antimicrobial use. With access to BC’s rich province-wide prescription database, we were able to conduct a retrospective cohort study to investigate the risk posed by an individual’s prior antimicrobial consumption patterns. We obtained historical records from PharmaNet for all IPD cases reported from 2001 to February 2005 that had complete antimicrobial susceptibility testing (n=564). In multivariable modeling, use of trimethoprim-sulfamethoxazole was significantly associated with having a penicillin non-susceptible infection (OR=2.92, 95% CI:1.34-6.38). Penicillins (OR=2.12, 95% CI:1.13-3.98) or macrolides/lincosamides (OR=2.00, 95% CI:1.09-3.68) were independently associated with erythromycin non-susceptible infections, and trimethoprim-sulfamethoxazole with trimethoprim-sulfamethoxazole non-susceptibility (OR=2.09, 95% CI:1.01-4.31). Of the macrolides, only azithromycin consumption posed a significant risk for erythromycin resistance, with an etiologic fraction of over two-thirds.
The transfer of these findings into the policy realm provides timely evidence on epidemiological trends in IPD in the era of the conjugate vaccine, and informs on the risk posed by certain commonly prescribed antimicrobials. Class-specific risk measures can be used to direct targeted prescription policy action for the control of antimicrobial resistance.
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CO-AUTHORSHIP STATEMENT

The work presented in this thesis was conducted and disseminated by the Master's candidate. The co-authors of the manuscripts that comprise parts of this thesis made contributions only as is commensurate with a thesis committee of experts in a specific area as it pertains to the work. The co-authors provided direction and support. The co-authors reviewed each manuscript prior to submission for publication and offered critical evaluations; however, the candidate was responsible for the writing and the final content of these manuscripts.
1. Background

1.1. Introduction
The era of antimicrobial resistance is upon us. The increasing levels of resistance in bacteria that cause community-acquired diseases are a growing public health issue, threatening the effectiveness of current therapies and limiting capabilities for infection control\(^1\). Consequences of resistance include increased morbidity and mortality, increased treatment costs, and a critical need for new and effective pharmaceuticals\(^2\).

*Streptococcus pneumoniae* is a major etiologic cause of community-acquired pneumonia, bacterial meningitis and otitis media. Since the first occurrence of penicillin resistance in a clinical isolate was identified in 1967, drug and multidrug resistance in *S. pneumoniae* has become increasingly common worldwide\(^3\). Given the high prevalence, morbidity and mortality of pneumococcal illnesses, the increasing rates of antimicrobial resistance are of particular concern for public health.

Although antimicrobial resistance is an inevitable phenomenon resulting from naturally occurring, random mutation events, the selection pressures that promote the survival and spread of resistant organisms may be within our control. The overuse and misuse of antimicrobials is acknowledged as a major contributing factor to the emergence and proliferation of resistant organisms\(^4,5\). Accurate local surveillance is needed to direct evidence-based policy efforts to slow the spread of resistance. The purpose of this thesis project is to provide updated information on the prevalence of antimicrobial resistance in invasive pneumococcal disease (IPD), and detail the risks which are attributable to an individual’s antimicrobial consumption patterns.

This chapter is structured as follows: the first section of the literature review presents characteristics of *S. pneumoniae*, and the clinical manifestations and epidemiology of IPD. The second section describes the origins and prevalence of antimicrobial resistance in pneumococci, and the current state of knowledge of its clinical impact. The third section summarizes known risk factors for antimicrobial-resistant disease and focuses on
evidence for a key contributing factor: antimicrobial use. The fourth section details antimicrobial usage and methodology for its measurement. A sample of surveillance programs, policy efforts and interventions are presented in the fifth section. The review builds to a rationale outlining the need for the current thesis work, and the objectives and hypotheses tested herein.

1.2. Literature Review

1.2.1. Pneumococcal Disease

1.2.1.1. S. pneumoniae

*S. pneumoniae* is most commonly found in the upper respiratory tract along with other bacteria such as *H. influenzae* and *M. catarrhalis*. It is both a commensal and pathogenic bacteria; although commonly carried asymptptomatically, it is a key pathogen in upper respiratory tract infections and otitis media, as well as a main cause of serious invasive diseases including meningitis, pneumonia and bacteremia.*

The organism is a gram-positive bacteria generally found as diplococci. The bacterial surface, a polysaccharide capsule, is the main determinant of the virulence of the pathogen. At present there are 90 known capsular serotypes (grouped into 46 serogroups) though only a few serotypes are responsible for the majority of disease. Unencapsulated, or non-typable, strains also exist but are not common, and are of minor importance in causing disease.

1.2.1.2. Clinical Definitions

*Colonization*

Colonization, or carriage, refers to the time period when pneumococci reside in the host asymptptomatically. The duration of colonization may be weeks to months depending on host and pathogen characteristics, and tends to be longer in children than adults. Colonization is a dynamic process, and pneumococci of more than one serotype may colonize an individual simultaneously. Although studies of colonization have been conducted in diverse populations and use different methodology in terms of sampling and culture techniques, the general trends indicate that the prevalence of colonization
increases from the time of birth to a peak in children aged 6-24 months (up to 100% in some studies) and then decreases in older age groups\textsuperscript{7,10}. In a cross-sectional study in Alaska, rates of colonization were over 50% in children under 10 years, and only 10-20% in those over 30 years\textsuperscript{7}. Risk factors for colonization include young age, having siblings, being in daycare, being prone to acute otitis media, recent respiratory illness, low socioeconomic status, winter season, smoking, and spending time in crowded environments\textsuperscript{7,11,12}. In many instances, colonization is associated with the development of immunity for the colonizing strain. The colonization stage also facilitates transmission between individuals via aerosol droplets, and allows for the development of antimicrobial resistant strains if the colonized individual is exposed to an antimicrobial\textsuperscript{7}.

\textit{Invasive Disease.}

Invasive disease is defined as the isolation of pneumococci from blood or other normally sterile body fluid. Pneumococcal colonization does not normally result in the development of invasive disease, but infection can occur if the bacteria are transferred into a normally sterile area and immune response fails to clear the bacteria\textsuperscript{11}. Infection is more likely when the host has had recent or concurrent viral infection, malnutrition, or local damage of the mucosal lining.

The clinical manifestation of the disease depends on the site of infection. Suggested pathogenic routes from colonization to invasive disease are illustrated in Figure 1.1. Pneumococci can cause pneumonia if they proliferate in the lungs, and bacteremia if they invade the bloodstream. The pathogenic pathway to the cerebrospinal fluid resulting in meningitis is less clear\textsuperscript{7}. Common non-invasive syndromes associated with \textit{S. pneumoniae} infection are sinusitis and otitis media, which normally begin by localized spread from the nasopharynx into the sinuses or the middle ear cavity.
1.2.1.3. Epidemiology of Invasive Disease

The morbidity and mortality resulting from invasive pneumococcal disease (IPD) are significant. Health Canada estimates there are 65 cases of meningitis, 700 cases of bacteremia and 11,200 cases of pneumonia (of which 2,200 require hospitalization) each year. In the US, community-acquired pneumonia is the sixth most common cause of death, with approximately 4 million cases each year, of which 500,000 require hospitalizations (12%) and 50,000 result in death. The etiology of about 20-60% of pneumonia cases is *S. pneumoniae*. The estimated cost of treating community-acquired pneumonia exceeds US$8 billion per year. For pneumococcal meningitis, there are 3,000 - 6,000 cases each year in the US.

Since the definition of IPD relies on laboratory confirmation, reported rates of IPD are likely to underestimate the actual disease burden. The accuracy of estimates will depend on local clinical and laboratory practices and reporting guidelines. For example, prior to 2000 only pneumococcal meningitis was a reportable disease in Canada. In 2000, policy changes made all IPD reportable (including pneumococcal meningitis). From 2000 to
2002, reported IPD rates rose from 2.3/100,000 to 8.3/100,000 in BC\textsuperscript{15}, an increase that undoubtedly reflected a response to the change in reporting practices, rather than a change in IPD incidence.

Because colonization is a key step leading to infection, many of the risk factors for invasive disease are similar to colonization. IPD incidence varies greatly by demographic group. Incidence is highest in young children, especially those under 2 years of age, and in the elderly. Historically, the highest rates are in children aged 6-11 months\textsuperscript{7}. IPD in children is most likely to present as meningitis or bacteremia and is associated with case fatality rates of about 2\% in industrialized countries. In the over 65 age group, reported rates of IPD incidence in North America range from 25 to 90 cases/100,000 population\textsuperscript{7}. The clinical presentation in the elderly is most likely to be pneumonia, and it is associated with a case fatality rate of over 20\%. In BC in 2004, the overall reported incidence of IPD was about 8 cases/100,000 population, with approximately 30 cases/100,000 in children aged 1-4 years, and 15 cases/100,000 in those over 60\textsuperscript{15}. Males of all ages are at higher risk, and certain racial groups (e.g., African Americans and Native Americans in the US) have much higher incidence. Invasive disease is more common in those with lower socioeconomic status.

Environmental factors, underlying health, and lifestyle choices also have an influence on IPD incidence. For all ages, IPD is most common in the colder seasons when transmission rates are highest, although outbreaks of pneumonia or meningitis are uncommon except in institutional settings or closed and crowded communities\textsuperscript{7}. Individuals living in crowded conditions, attending daycares, or exposed to children are often found to be at higher risk, likely as a result of transmission routes and age-dependent colonization patterns. Those with co-morbidities including HIV, chronic obstructive pulmonary disease, diabetes mellitus, chronic liver disease, heart failure, coronary artery disease and chronic neurological disease are also at higher risk\textsuperscript{4}. Further risk is associated with heavy alcohol use, cigarette smoking, and exposure to secondhand smoke.
There have been changes in epidemiology of IPD in recent years since the introduction of the heptavalent vaccine (Prevnar, or PCV7). In the US, after an infant vaccine program was initiated in 2000, dramatic declines in IPD rates were observed in the under 5 population in 2001 and in 2002, and more moderate declines were noted in older age groups\textsuperscript{16,17}. Studies on the impact of the vaccine program in Canada are just beginning to be published\textsuperscript{18}.

\textbf{1.2.1.4. Serotype Distribution}

The epidemiology of the serotype distribution of \textit{S. pneumoniae} is very complex and has been described in detail in recent reviews\textsuperscript{7,8,11}. In brief, certain serotypes are more commonly associated with pneumococcal colonization or with specific types of invasive disease\textsuperscript{19}. For example, serogroups 1 and 14 are more frequently isolated from blood than other serogroups, and serogroups 6, 10, and 23 are more often isolated from cerebrospinal fluid. Serotypes 1 and 5 may be intrinsically highly virulent and commonly cause invasive disease, but are rarely isolated in colonization studies\textsuperscript{20}. There is substantial geographic variation, especially between developing and industrialized countries, though there is some suggestion that this may be an artifact of different blood sampling practices\textsuperscript{20}. Figure 1.2 shows the incidence of IPD cases by serogroup in children in the US and Western Europe. In terms of age distribution, children are generally affected by fewer serotypes then adults or the elderly. The majority of serotypes causing disease in children are covered by the heptavalent vaccine\textsuperscript{21}. 
FIGURE 1.2: Serogroup-specific incidence of invasive pneumococcal disease in young children. Source: (Rice LB, 2000)  

1.2.1.5. Disease Control

Chemotherapy

The majority of IPD cases can be managed with antimicrobial treatment. For community-acquired pneumonia, the Canadian Infectious Disease Society and the Canadian Thoracic Society have produced recommended treatment guidelines based on empirical evidence. In short, macrolides are the first-line therapeutic choice for immunocompetent outpatients. If the outpatient has chronic obstructive lung disease, the recommended treatment is a newer macrolide, either azithromycin or clarithromycin. If the patient also has a recent history of antimicrobial treatment (i.e., carries a higher risk of drug-resistant infection), the recommended treatment is either a fluoroquinolone, or as a second choice either amoxicillin/clavulanate and a macrolide, or a second generation cephalosporin and a macrolide. Guidelines are specific to the site of care; recommended treatments also exist for patients in nursing homes, in hospital wards, and in intensive care units. Similar guidelines are produced by the Infectious Diseases Society of America.

If the *S. pneumoniae* isolate's in vitro susceptibility is known then treatment should be directed accordingly. For penicillin-susceptible pathogens, oral penicillins and cephalosporins or macrolides are recommended. For intermediate-susceptible
pathogens, amoxicillin or cefuroxime is recommended, and for high-level resistance, either penicillin, cefotaxime, or ceftiraxone (i.v.) or a fluoroquinolone (levofloxacin, gatifloxacin, or moxifloxacin) is recommended. Note that for pneumococcal infections other than meningitis, even isolates with reduced susceptibility to penicillin (up to minimum inhibitory concentrations (MIC) ≤ 4 μg/mL) can be treated with high levels of β-lactam agents.

Immunization

Two vaccines are available for the prevention of pneumococcal disease. These are the 23-valent polysaccharide pneumococcal vaccine (PPV23) and the heptavalent pneumococcal conjugate vaccine (PCV7).

The PPV23 contains polysaccharide antigens for the 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F) that cause over 80% of invasive disease in adults. PPV23 has been recommended for all high-risk individuals and all persons aged 65 years and older in BC since 1998. The vaccine is administered in one dose with re-vaccination considered only for certain very high risk groups. More than 80% of healthy adults who receive PPV23 develop antibodies in less than one month, and levels remain elevated for at least five years. In older adults (>80 years) the vaccine 50-70% effective in preventing invasive disease and protection for a minimum of 3 years. The vaccine is not effective in children under 2 years, and in certain subgroups (i.e., those with significant underlying illnesses) as the polysaccharide vaccine requires a T-cell independent immune response. It provides no protection against pneumococcal colonization.

PCV7 (Prevnar) was developed to cover the seven most common pediatric serotypes (4, 6B, 9V, 14, 18C, 19F and 23F). These ‘vaccine serotypes’ accounted for 85% of invasive disease in young children in North America, pre-vaccine, and about 60-70% in Europe, as well as majority of non-invasive disease and antimicrobial resistant disease. In the conjugate vaccine capsular polysaccharides for each serotype are covalently bonded to a carrier protein (CRM197, a non-toxic mutation of diphtheria toxin). This
conjugation enhances the immunogenicity of the vaccine by stimulating a T-cell response promoted by the protein. The number of serotypes which can be included in conjugate vaccines is limited, as the presence of excessive carrier antigen can impair the antibody response. In the Kaiser Permanente clinical trial, vaccine efficacy against IPD was over 97% in subjects who received all doses. An additional benefit of the PCV7 is some degree of cross-protective immunity to vaccine-related serotypes (specifically serotypes 6A, 9A and 19A). Furthermore, PCV7 provides some protection against both colonization and non-invasive disease (i.e., acute otitis media) by vaccine serotypes in immunized individuals. This phenomenon results in reduced person-to-person transmission and provides herd immunity against colonization and invasive disease by vaccine serotypes within the larger population.

The PCV7 was licensed for use in children in Canada in 2001. In BC, a targeted immunization program of high-risk and aboriginal children aged 2 to 59 months was implemented in April 2003, and a universal immunization program began in September 2003. The vaccination is administered in four doses, at age 2, 4, 6 and 18 months. After four doses, more than 90% of health infants have detectable antibodies to all seven serotypes. Three doses are administered if vaccination is initiated after 6 months of age, two doses after 12 months, or one dose after 2 years. All children born on or after July 2003 are eligible for vaccination.

1.2.2. Antimicrobial Resistance in S. pneumoniae

1.2.2.1. Origins of Resistance

*Genetic basis of Resistance*

Antimicrobial resistance is an inevitable phenomenon in that the mutations that give rise to resistance are naturally occurring events. Mutations have been occurring for millions of years, long before the use of antimicrobials by humans. Bacterial resistance may be intrinsic (through chromosomal mutation) or acquired (through horizontal transfer). Chromosomal mutations happen randomly during DNA replication at a rate of $10^{-8}$ to $10^{-9}$ per gene. In pneumococci, mutations and the subsequent transfer of resistance to offspring ("vertical transfer") accounts for only a small proportion of observed
resistance\textsuperscript{27}. Resistance through vertical transfer, as is seen with fluoroquinolone resistance, tends to develop slowly over time\textsuperscript{22}.

The exchange of genetic information between bacteria is facilitated by the bundling of resistance determinants, and "horizontal transfer" through conjugation, transduction, or transformation\textsuperscript{28, 29}. Genes that encode for resistance are often found on plasmids, which are mobile circular or coiled genetic material that can replicate independently of the host chromosome and are easily transferred between bacterial cells or species\textsuperscript{30}. Resistance genes may also be segregated onto transposons, DNA fragments encoded with the ability to move between plasmids or chromosomes. Both plasmids and transposons often carry more than one resistance determinant. These genetic materials may be transferred through conjugation, when a DNA segment from one cell is copied and transferred to another cell during cell-to-cell contact; transduction, or phage transfer, when a virus carries genetic material from one bacteria to another; or transformation, when bacteria acquire exogenous DNA from the environment. These horizontal transfer mechanisms, as are seen in the development of macrolide resistance, can result in the rapid spread of resistance.

The effectiveness or selective pressure of an antimicrobial is a function of the antibacterial activity of the drug, the ability to accumulate adequate concentrations at the site of the infection for a long enough duration, and the efficiency of the bacteria at developing resistance to the antimicrobial mechanism of action\textsuperscript{31}. These characteristics vary between antimicrobial classes, and for drugs within classes. Genetic mechanisms for antimicrobial resistance in \textit{S.pneumoniae}, shown in Table 1.1, can include enzyme inactivation, active efflux, alternations of ribosomal targets or topoisomerases, roboxome protection, and changes in folic acid synthesis enzyme targets\textsuperscript{14}. The success of resistant organisms suggests that resistance comes at no significant fitness cost to the bacteria\textsuperscript{32, 33}. Mechanisms for key antimicrobials of interest are discussed below.
TABLE 1.1: Molecular mechanisms of antimicrobial resistance.

Source: adapted from\(^1\!\!^2\!\!^3\!\!^4\)

<table>
<thead>
<tr>
<th>Antimicrobial Class</th>
<th>Genetic Basis</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Lactams</td>
<td>Chromosomal</td>
<td>Enzyme inactivation&lt;br&gt;Decreased access to cell&lt;br&gt;Active efflux&lt;br&gt;Change in target site</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Plasmid</td>
<td>Modify target, erm (B) gene&lt;br&gt;Eflux pump, mef (A) gene</td>
</tr>
<tr>
<td>Quinolones</td>
<td>Chromosomal</td>
<td>Modify target, mutation parC of gyr A</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Plasmid</td>
<td>Ribosomal protection, tet(M) or tet(O)</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>Plasmid</td>
<td>Mutations in DHFR gene&lt;br&gt;Mutations in DHPS gene</td>
</tr>
</tbody>
</table>

Penicillin resistance in *S. pneumoniae* results from a series of mutations in the penicillin binding proteins of the bacteria. Because these proteins are the target site for many β-lactam drugs, a strain which is penicillin resistant is likely to also have developed resistance to other β-lactam drugs (i.e., amoxicillin, ceftriaxone, and cefotaxime)\(^{35}\). The penicillin resistance gene may have originated in streptococci, and was transferred by transposons to pneumococci\(^{36}\). β-lactam resistance may also occur through a mechanism which produces the β-lactamase, an enzyme which opens the four-membered lactam ring of the drug.

With macrolide resistance, there are two predominant mechanisms that vary in importance geographically and result in different resistance phenotypes. The *erm*(B) gene encodes for a conformational change in the ribosomal target site (on the 23S rRNA) which decreases the binding ability of macrolide antimicrobials. This mechanism results in high level resistance phenotype called MLS\(_B\) (MIC >32 μg/mL). It is the predominant form of macrolide resistance in Europe\(^{37}\). The other main mechanism for macrolide resistance is the *mef*(A) gene which increases efflux rates and produces low intercellular antimicrobial concentrations. The *mef*(A) gene usually results in low level macrolide resistance (MIC 2-16 μg/mL) of phenotype M and is more common in North American isolates, except in isolates from Quebec\(^{38}\). There have been an increasing number of specimens which carry both *erm*(B) and *mef*(A) genes in Asia, and more recently in the
Isolates with both resistance traits tend to belong to 1 major clone presenting as serotype 19A or 19F, and are very commonly multidrug resistant. Trimethoprim-sulfamethoxazole resistance arises from mutations which reduce the affinity for trimethoprim at its target site. Fluoroquinolone resistance results from mutations in the genes encoding two target sites for fluoroquinolone action: topoisomerase IV and DNA gyrase enzyme. These types of mutations occur rarely; however, strains that have both mutations possess high-level resistance to all fluoroquinolone agents.

The origins of the multidrug resistance phenotype are not clear. Hypothetically, multidrug resistance can develop in a number of ways: through multiple mutations within an organism, through multiple resistance determinants carried on a single transposon or plasmid, or through the acquisition of multiple genetic fragments encoding for resistance. In strains resistant to non-β-lactam antimicrobials, resistance genes for several antimicrobials have been found on the same transposon (i.e., for trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol resistance). For strains that are co-resistant to β-lactam and non-β-lactam antimicrobials, resistance does not occur by a single shared mechanism, nor does it tend to be carried on single mobile genetic element. This resistance phenotype is increasingly common but the growing rates of multidrug resistance has been attributed to a few proliferating β-lactam resistant strains which have developed other resistance traits. Examples of these are the Spanish clone, a strain of serotype 23F which is associated with resistance to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole, and often macrolides. Others are the Spanish-French clone (serotypes 6B, 9V and 14), and variants of the Spanish clone (serotypes 19A, 19B and 19F).

Proliferation of Antimicrobial Resistance
Although the development of antimicrobial resistance is natural and inevitable, human actions play a significant role in bacterial population dynamics. Key factors driving the
proliferation of antimicrobial resistant organisms are the selective pressure from antibiotic use and clonal dissemination.

First and foremost, the discovery and use of antimicrobials has had a most dramatic impact on the emergence and spread of antimicrobial-resistant organisms. The high levels of use of antimicrobials by humans (both in the community and in hospitals) and in agriculture (as growth promoters) has substantially influenced the natural selection processes. The misuse of antimicrobials, through poor prescribing practices or poor patient compliance, adds additional selection pressure. A recent paper shows that selection pressure, rather than clonal spread, is the most important determinant of pneumococcal resistance patterns in the US\textsuperscript{44}. Indeed, it has been claimed that the inappropriate use of antimicrobials is “the single most important factor influencing the spread of multiresistant \textit{S. pneumoniae}\textsuperscript{32}.

Secondly, specific aspects of pneumococcal disease facilitate the spread of resistant pneumococci. The high rates of colonization in asymptomatic individuals allows easy transmission of the respiratory pathogen\textsuperscript{45}. High-density living conditions, day-care environments and the increase in global travel in recent decades have further facilitated the spread of resistant organisms through the population. Poor infection control practices, especially in high-risk institutions such as hospitals, nursing homes and schools, are another contributing factor\textsuperscript{34}.

1.2.2.2. Defining Resistance

\textit{Antimicrobial Susceptibility Testing}

In vitro antimicrobial susceptibility is measured in terms of the MIC, the minimum concentration of the antimicrobial required to inhibit growth of the bacteria. In vitro testing is conducted by broth microdilution or E-tests according to standard procedures produced by the Clinical Laboratory Standards Institute\textsuperscript{46} and interpreted as sensitive, intermediate or highly resistant to the antimicrobial according to cutpoints, shown in Table 1.2\textsuperscript{47}. Unfortunately, there is often ambiguous use of the term “resistant” in the literature; ideally it should be clearly defined as high or intermediate resistance, or the
combination of these (generally referred to as non-susceptible). Another susceptibility
testing method is disk diffusion, but this qualitative method does not produce MIC values
and considered unreliable for β-lactam antimicrobials.

TABLE 1.2: Interpretive breakpoints for antimicrobial resistance in S. pneumoniae.
Source: (Clinical Laboratory Standards Institute, 2005)

<table>
<thead>
<tr>
<th></th>
<th>Interpretive Breakpoints (microgram/mL)</th>
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<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Penicillin</td>
<td>≤0.6</td>
</tr>
<tr>
<td>Cefotaxime*</td>
<td>≤1</td>
</tr>
<tr>
<td>Ceftriaxone*</td>
<td>≤1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>≤4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>≤2</td>
</tr>
<tr>
<td>Ofloxacine</td>
<td>≤2</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>≤0.5/0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.25</td>
</tr>
</tbody>
</table>
*for non-meninitis IPD

Multidrug resistance

Multidrug resistance (MDR) is not defined consistently in the literature, limiting
comparisons of the prevalence of MDR between studies. A fairly common definition is
non-susceptibility to three or more antimicrobials, but there are many other examples.
The SENTRY program considered MDR as isolates resistant to penicillin, erythromycin,
clindamycin, tetracycline and trimethoprim-sulfamethoxazole. A Canadian study
considered MDR as non-susceptible to three or more of the following antimicrobials:
penicillin, ceftriaxone, erythromycin, trimethoprim-sulfamethoxazole and ciprofloxacin,
and a US study defined MDR as non-susceptibility to two or more classes of
antimicrobials.

1.2.2.3. Prevalence of Resistance

Global Estimates for Antimicrobial Resistance in S. pneumoniae

In recent decades there have been alarming increases in drug and multidrug resistance in
community-acquired pathogens. Drug resistance in S. pneumoniae isolates has
developed at differing rates around the world, and the prevalence is highly variable
geographically. Recent studies report that the proportion of isolates with reduced
susceptibility to penicillin is as high as 60-80% in certain areas of Vietnam, Korea and Japan\textsuperscript{56} and greater than 50% in some European countries\textsuperscript{5}. The most recent data from SENTRY antimicrobial surveillance program, covering Europe, Latin America and North America, found 29-33\% of isolates had reduced susceptibility to penicillin (by region)\textsuperscript{41}. The proportion of isolates with either intermediate or high resistance to penicillin was 15\% in Canada in 2002\textsuperscript{51}, and high level penicillin resistance was 20\% in the US in 2001\textsuperscript{32}.

Macrolide resistance has become increasingly widespread. Results from the Alexander project for 8,882 \textit{S. pneumoniae} isolates from 26 countries over the period from 1998 to 2000 reported the overall prevalence of macrolide resistance to be 24.5\%\textsuperscript{57}. From the same project, resistance was as high as 54\% in Hong Kong in 2001\textsuperscript{58}. In 2000-01, the US arm of the PROTEKT surveillance program reported erythromycin non-susceptibility in 31\% of their 10,103 isolates, with the prevalence and mechanism of resistance highly variable geographically\textsuperscript{59}.

Resistance rates to trimethoprim-sulfamethoxazole are generally very high, with the recent SENTRY results showing to 31-50\% of isolates as non-susceptible\textsuperscript{41}. In contrast, the rates of fluoroquinolone resistance tend to be low, in the range of 0.5\%\textsuperscript{6}, though surveillance in Canada found a rise in ciprofloxacin resistance from 0\% in 1993 to 1.7\% in 1997, which coincided with increased use of the antimicrobial in adults\textsuperscript{60}.

Isolates with resistance to more than one antimicrobial are very common, and have been on the rise in North America\textsuperscript{49}. Doern and colleagues found that 22.2\% of isolates were non-susceptible to at least two of erythromycin, trimethoprim-sulfamethoxazole, chloramphenicol or tetracycline\textsuperscript{59}. The TRUST study (Tracking Resistance in the United States Today) reported an increase in isolates with resistance to 3 or more classes from 6.2\% in 1998 to 13.5\% in 2001\textsuperscript{49}, while the Alexander Project reported multidrug resistance in <1\% of isolates in 1992, rising to 17.5\% of US isolates in 2001\textsuperscript{32}. The SENTRY program found that 5.8\% of isolates over 1999-2003 were resistant to penicillin, erythromycin, clindamycin, tetracycline and trimethoprim-sulfamethoxazole\textsuperscript{41}. A
frequently observed pattern in multidrug resistance is resistance to penicillin and to macrolides, both commonly prescribed antimicrobials for the treatment of pneumococcal disease; in the PROTEKT US study, 78% of penicillin resistant isolates were also resistant to erythromycin.

While surveillance programs have found dramatic increases in the prevalence of resistance in S. pneumoniae through the 1990s, there are indications that these patterns may be changing. For example, a study in the US that evaluated trends over the period 1994 to 2003 suggested that rates of resistance to penicillin, trimethoprim-sulfamethoxazole, erythromycin and tetracycline, as well as rates of multidrug resistance may be stabilizing or declining. Recent decreases in the incidence of antimicrobial-resistant disease have been attributed in part to the pneumococcal conjugate vaccine.

**Canadian Estimates**

Detailed surveillance data for antimicrobial resistance in Canada is available online from the Canadian Bacterial Surveillance Network (CBSN). Figure 1.3 shows rates of penicillin resistance rising through the 1990s and stabilizing in recent years at around 15%. Erythromycin resistance, shown in Figure 1.4, has continued to rise. In 2005, 19% of CBSN isolates were resistant to erythromycin. Estimates for BC isolates are also available on this website, though sample sizes are small. It should be noted that sampling for this surveillance program is voluntary, not population-based, and thus results may not be representative. The most recent publication from CBSN covers 2,539 isolates collected in 2002, when 15.0% of isolates were non-susceptible to penicillin, 15.8% to erythromycin, 20.1% to trimethoprim-sulfamethoxazole and 4.3% were multidrug resistant (defined in this study as high level resistance to three or more of penicillin, ceftriaxone, erythromycin, trimethoprim-sulfamethoxazole and ciprofloxacin).
1.2.2.4. Public Health Impact

Clinical Significance of Antimicrobial Resistance

The impact of in vitro antimicrobial resistance on morbidity and mortality is difficult to quantify. The majority of studies on clinical significance have used the clearly defined outcome of mortality, although alternative measures may also be relevant outcomes (e.g., length of hospital stay, treatment cost, or frequency of complications). Another complication is that intermediate and resistant isolates are frequently categorized together.
as non-susceptible isolates in reporting, even though the clinical impact may vary with the degree of resistance.

There is good evidence that the use of standard therapies has been effective for treating pneumonia with intermediate resistance to penicillin, but at that higher levels of resistance (MIC > 4 µg/mL) complications may occur\textsuperscript{27}. A review of published studies on the management of drug-resistant pneumonias in hospitalized patients found only a single example of treatment failure with β-lactam treatment, but reported 33 treatment failures with macrolides, and 21 with fluoroquinolones\textsuperscript{64}. Another review evaluated the impact of β-lactam resistance in community-acquired pneumonia on clinical outcomes for adult patients in four cohort studies, two case control studies and one randomized control trial\textsuperscript{65}. Neither the cohort studies nor the clinical trial reported increased rates for mortality or complications in patients with non-susceptible infections, although the author noted that the majority of penicillin resistant isolates had MICs below 4 µg/mL. One of the case control studies reported a 1% treatment failure rate (5/444 patients), and the other reported four treatment failures in 41 patients, which may have been confounded by high rates of erythromycin resistance. A third review concluded that: a) for penicillin-resistant cases, there is little difference in mortality rates unless MICs increase to 4 µg/mL, b) for fluoroquinolone-resistant cases, treatment with fluoroquinolones is likely to fail and that c) that macrolide MICs of 1-8 µg/mL are clinically insignificant (as are MICs under 64 µg/mL in most cases)\textsuperscript{14}. While fewer studies have looked at outcomes other than mortality, there is some indication that the infections due to penicillin-resistant pneumonia are associated with longer hospitalizations and higher costs (due to longer stay and more expensive antimicrobial therapy)\textsuperscript{66}.

For the treatment of meningitis, antimicrobial resistance poses a real threat. Antimicrobials do not penetrate well into cerebrospinal fluid, making it hard to achieve pharmacologically effective concentrations at the infection site. Treatment failures have been documented for cephalosporin-resistant meningitis in both children and adults\textsuperscript{27}.
Part of the challenge in realizing the clinical impact may stem from limitations with the breakpoints used for in vitro susceptibility. The Clinical Laboratory Standard Institute (CLSI) interpretive cutoff points encompass a range of MIC values. For example, an isolate which has high resistance to penicillin could have an MIC as low as 2 μg/mL or an MIC over 32 μg/mL. The MIC at which the clinical impact of resistance occurs may not correspond with CLSI interpretive cutoff points. A further complication is that the clinical impact of a given MIC value varies with the site of infection, the properties of the antimicrobial (i.e., the ability of the antimicrobial to penetrate the site, the treatment regime), and the ability of the body to clear the infection. Indeed, it has been suggested that for community-acquired pneumonia, more relevant cutoffs for penicillin susceptibility are 2 μg/mL for intermediate resistance and 4 μg/mL for high resistance (as opposed to CLSI standards of 0.12-0.1 μg/mL and ≥2 μg/mL, respectively).

In conclusion, while the current levels of antimicrobial resistance in *S. pneumoniae* do not appear to have major clinical impact, the future is unknown. MIC levels in resistant isolates are rising; in time the management of resistant pneumonias using the recommended penicillins may no longer be possible and the clinical impact of drug resistance may be substantial. With limited prospects for new antimicrobials in the drug development pipeline, we need to take all preventative measures to preserve the effectiveness of our tools for disease control.

1.2.3. Risk factors for Antimicrobial-Resistant Invasive Disease

Reported risk factors for the development of resistant pneumococcal disease tend to be similar to risk factors for susceptible disease discussed in previous sections, with the addition of recent antimicrobial consumption. The relative importance of risk factors varies with the population and study design, but antimicrobial exposure is consistently important.

1.2.3.1. Demographic factors

Young age, daycare attendance and underlying disease have all been cited as risk factors for resistant disease. Recently, the international, longitudinal surveillance program
PROTEKT collected detailed demographic data and antimicrobial susceptibility for isolates of common bacteria causing community-acquired respiratory pathogens. For the 1999-2000 respiratory season they analyzed 3362 *S. pneumoniae* isolates from 25 countries and found that after adjusting for country, resistance rates were significantly higher in infants than adults (OR=1.98 for penicillin and OR=1.89 for erythromycin), and that males were less likely to have erythromycin resistant disease that females (OR=0.69). Interestingly, there was no significant difference in penicillin and erythromycin resistance patterns between inpatients and outpatients. While this may be the only international study to report on demographic variables on susceptibility patterns, it must be noted that PROTEKT collects both invasive and non-invasive isolates. In this specific report the most common specimen source was sputum, and only 15% of isolates were from blood. Unfortunately, details of previous antibiotic treatment were not collected in this study.

1.2.3.2. Antimicrobial Consumption

The overuse and misuse of antimicrobials is acknowledged as a major contributing factor to the emergence and proliferation of resistant organisms. A positive correlation between antimicrobial consumption and antimicrobial resistance in *S. pneumoniae* has been found in epidemiologic studies at the individual, regional, and national level. The following section will first discuss the proposed mechanism for this association, followed by a summary of key studies in this area. While most studies report significant associations, there are conflicting results in the literature which may stem from differences in study design, populations, disease definitions and exposure timeframes.

*Proposed mechanism for risk of antimicrobial consumption*

Resistance to antimicrobials develops in the colonization stage, and resistant invasive disease is thought to occur when infection takes place in an individual who is carrying a resistant strain. Exposure to an antimicrobial changes the ecology of the bacterial population in the nasopharynx of the person undergoing treatment. Through disease transmission, this change within an individual can affect the pneumococci composition in the wider population. The specific biological mechanism between recent antibiotic use and colonization of resistant pneumococci is not known, though several
possible mechanisms have been suggested\textsuperscript{27}. The selection hypothesis states that resistant pneumococci that were present at low density in the nasopharynx prior to antimicrobial therapy are selected for, or unmasked, during the course of treatment. Another theory is based on the replacement of sensitive pneumococci with resistant pneumococci acquired from the community, during or after antimicrobial therapy. A third theory is based on evolution of a resistant pneumococci population (i.e., through mutation) within the host as a result of the selection pressure of the antimicrobial therapy.

As an example, Lipsitch's work supports the replacement theory\textsuperscript{67}. Through mathematical modeling Lipsitch concludes that, in the case of an outbreak of resistant pneumococci, antimicrobial treatment puts an individual at higher risk for colonization with resistant organisms. The theory suggests that this occurs when the antimicrobial eradicates the individual's natural flora and creates an opportunity for colonization with the resistant bacteria, should the individual be exposed. However, in the absence of an outbreak of resistant bacteria, the individual consuming antimicrobial would not be any more likely to be colonized with resistant bacteria than with susceptible bacteria.

1.2.3.3. Epidemiological studies on consumption and resistance

Ecological Studies

Ecological studies at the level of wards, regions or countries have provided clear evidence of a positive association between antimicrobial consumption and antimicrobial resistance. A review in this area\textsuperscript{5} states that “links between antibiotic use and resistance have been consistently found at every ecological level: in patients, small human communities, different geographical areas of the same country, nationally, and internationally.” A major report in European Surveillance of Antimicrobial Consumption detailing antimicrobial use in 26 countries found a correlation between penicillin consumption and resistance\textsuperscript{68}. Further evidence supports the correlation of antimicrobial consumption with both penicillin and macrolide non-susceptible \textit{S. pneumoniae} and \textit{S. pyrogenes} isolates\textsuperscript{53}. The European Antimicrobial Resistance Surveillance Program (EARSS) used IMS consumption data and surveillance results from 11 countries to show a strong correlation ($r^2 = 0.8$) between the β-lactams and penicillin non-susceptible \textit{S.}}
pneumoniae, and a more moderate correlation for macrolide use ($r^2=0.40$)\(^9\). A large multi-site study in the US found an association between the prevalence of penicillin non-susceptible S. pneumoniae and the total number of outpatient β-lactam prescriptions, but not with the overall number of prescriptions\(^70\).

While these results from large ecological studies confirm the association, one must interpret these findings with care. Extrapolating the link to an individual level poses the risk of an ecological fallacy, as correlations at the country or regional level do not necessarily indicate a correlation at the individual level. Furthermore, ecological studies rely on surveillance programs for rates of antimicrobial resistance in an area, and surveillance of this type is often passive (or active within only a certain area or institution) and thus may not be representative of an entire region. Given these limitations, there is a need for epidemiological studies at the individual level in order to further elucidate the link between consumption and resistance.

**Case Control Studies**

Case control studies can be readily conducted in hospitals and institutions, and may compare cases with non-susceptible disease with either controls with susceptible disease, or controls with no disease. A study conducted in four surveillance regions in Canada and the US included isolates from hospitals and labs for all children aged 2-59 months with invasive pneumococcal cultures ($n = 187$) and population-based controls frequency matched by age ($n = 280$)\(^71\). In the subset of the study which compared the 52 children with high level penicillin resistant isolates to the non-diseased control group, receiving at least one course of antibiotics in the previous three months was associated with increased odds of having penicillin resistant disease (OR = 3.08, 95% CI: 1.85-7.40), as was daycare attendance and ear infection. A matched case-control study on hospitalized children (under 18 years of age) with invasive disease compared the exposures of individuals with penicillin non-susceptible isolates ($n = 24$ of intermediate resistance, $n = 12$ of high level resistance) with matched controls with susceptible isolates ($n = 108$)\(^72\). Using antibiotics in the month prior to the episode was significantly associated with non-susceptible disease (OR = 5.75, 95% CI: 2.18-15.16). Another study which age- and sex-
matched cases with penicillin non-susceptible pneumonia (n = 36) with controls having susceptible pneumonia also found that those with non-susceptible disease had received antibiotics more frequently in the past\textsuperscript{66}.

While there are other examples of case control studies that have identified antimicrobial consumption as a risk factor for resistance, applying this type of study design in this area of research has been criticized in the literature\textsuperscript{73-75}. Many of these studies exhibit methodological weaknesses in control group selection which can result in a selection bias due to systematic differences in the sampling of cases and controls. Suitable controls should differ from cases with regard to disease status, but need to be representative of the base population with respect to exposure history\textsuperscript{76,77}. When a control group with susceptible infection is selected, it is unlikely that the controls have been exposed to an active antimicrobial or else they would no longer be infected, and would not be present in the control group. This selected control group would therefore be comprised of individuals who are less likely to have been exposed to active antimicrobials, and thus have a different exposure frequency than the base population. Recent literature has suggested that this type of control group may inflate odds ratios for the risk posed by antimicrobial consumption\textsuperscript{73-75}. In the case-control study a more appropriate control group is comprised of uninfected individuals selected from the base population (for an example, see\textsuperscript{71}), although this is often constrained by economic or feasibility limitations.

\textit{Cohort Studies}

Cohort studies are another study design employed to assess the risk of antimicrobial consumption for the outcome of resistant disease at the individual level. This design does not suffer from the same selection bias as case control studies, but cohort studies have tended to be conducted in hospitals or institutions as they require exposure and outcome data for every individual in the cohort. Again, risk factors are not always consistent across study populations and different exposure/outcome definitions.

Several cohort studies have evaluated the risk posed by overall (unspecified) antimicrobial usage prior to infection in a variety of populations. An international study
of pneumococcal blood isolates from hospitalized patients in 10 countries (n = 844) found in multivariable analysis that prior antibiotic therapy (in the previous three months) was associated with greater odds (OR = 1.9, 95% CI: 1.2-2.9) for penicillin non-susceptibility. A total of 20% of patients in the study had received antibiotics in the previous three months. The only other significant risk factor was "underlying diseases or risk factors associated with immunosuppression". A study on 281 S. pneumoniae isolates from cases of bacteremia in a tertiary care hospital in San Diego, California found no significant association between prior antibiotic use and penicillin non-susceptible disease. In this study, antibiotic use was defined as having at least one in-patient or out-patient prescription filled at the hospital in the previous 90 days. Only 36 of the 281 patients had prior antibiotic use by this definition. The authors recognize this method may have underestimated antibiotic use, although no bias would be present unless there were systematic differences in the prescription sources of individuals with susceptible and non-susceptible disease.

Other cohort studies have provided more detailed information on risks posed by particular classes of antimicrobials. A study of adult and pediatric patients (n = 303) with penicillin non-susceptible bacteremia in four tertiary care hospitals in Louisiana reported an adjusted OR = 5.61 (95% CI: 3.27-9.64) for β-lactam use in the previous six months, and an OR = 2.83 (95% CI: 1.39-5.77) for macrolide use. Fluoroquinolone use in the previous six months was not associated with disease. In a study which included both invasive and non-invasive pneumococcal isolates (n=95) from five hospitals in Spain in 1993 and 1994, β-lactam use in the previous three months was not a significant risk factor for penicillin non-susceptibility in multivariable analysis, after adjusting for age, alcoholism, intravenous drug use and disease status. For multidrug resistance (defined in this study as non-susceptibility to 3 or more antimicrobials, including penicillin) previous β-lactam use was significant in non-invasive disease (OR = 7.92, 95% CI: 1.84-34.06) but not for invasive disease (OR = 1.02, 95% CI: 0.22-4.66). While this is one of the earliest studies to consider individual antimicrobial usage, the true effects have been obfuscated by the inclusion of non-invasive isolates (~1/2 of isolates).
A large study conducted by the Toronto Invasive Bacterial Diseases Network\textsuperscript{81} looked at risk factors for having antimicrobial resistant disease (as compared to intermediate or susceptible disease) in a cohort of 3,339 patients with IPD in Toronto and Peel during the period from 1995 to 2002. The authors obtained data for antimicrobial prescriptions in the three months prior to infection for 563 (16.9\%) of the participants by chart review and interviews with doctors and patients. In multivariable models, risk factors for penicillin resistant disease were use of a penicillin (OR = 2.47, 95\% CI: 1.36-4.71), use of trimethoprim-sulfamethoxazole (OR = 5.97, 95\% CI: 2.71-13.20) and use of azithromycin (OR = 2.78, 95\% CI:0.98-7.86), as well as year of infection and the absence of chronic organ disease. For erythromycin resistant infections, prior use of penicillin, trimethoprim-sulfamethoxazole and azithromycin or clarithromycin (but not erythromycin) were significant after adjusting for year of infection (OR = 1.77, 2.07, 9.93, and 3.93, respectively). For trimethoprim-sulfamethoxazole resistant infections, the use of any penicillin, trimethoprim-sulfamethoxazole, and azithromycin were significant risk factors (OR = 1.71, 4.73, and 3.49, respectively). This set of results shows class-specific risk, but also additional risk posed by prior consumption of other antimicrobial classes. In contrast, this study reports that for levofloxacin-resistant infections, the only antimicrobial class that was a significant risk factor was fluoroquinolones (OR = 12.1, 95\% CI: 4.22-35.40). Other risk factors for levofloxacin-resistant infections were living in a nursing home or hospitalization. Age was not significant in any multivariable models. Presumably these results are not biased by the limited number of individuals for which prescription history data was available, as the authors conducted additional analyses which considered all patients with missing antimicrobial histories as having not used antimicrobials, and results were not significantly different. However, this study does differ from most others as it evaluates the risk for resistant disease versus intermediate or susceptible disease, whereas it is more common for studies to consider the disease outcome as all non-susceptible disease (high or intermediate resistance).

\textit{Studies on Non-Invasive Disease}

There are also many studies which considered the risk of antimicrobial use for resistance in non-invasive disease. A large pediatric population-based study looked at erythromycin
resistance in nasopharyngeal colonization in children under 2 years of age in Greece. In total, 31% (n = 766) of children were colonized with pneumococci. The exposure of interest was β-lactam use, macrolide use, and specifically penicillin and cephalosporin use in the three months preceding the culture. The strongest association was between macrolide use in the one month prior and erythromycin resistance (OR = 5.92, p=0.001), but there was also a significant association between β-lactam use and erythromycin resistance (OR = 1.87, p=0.010). No multivariable analyses was conducted. There are many other examples of studies, mainly in children, which have also found that prior consumption of certain classes of antimicrobials poses excess risk for colonization of either penicillin or erythromycin non-susceptible *S. pneumoniae*.

### 1.2.4. Antimicrobial Consumption

The ecological studies described in the previous section provide clear evidence that areas with highest rates of antimicrobial use have the highest rates of resistance. As such, there is a need to clearly identify geographic areas with high levels of consumption. This section presents the state of knowledge on antimicrobial consumption in BC, and subsequently details methodological challenges in quantifying antimicrobial use. In brief, the World Health Organization has developed methodology facilitating direct comparisons across regions or countries, but there are remaining challenges in exposure assessment for epidemiological studies, such as the identification of the time period where consumption poses the highest risk, or the most meaningful measure of consumption (prescription count, dosage or duration).

#### 1.2.4.1. Antimicrobial consumption rates

The highest rates antimicrobial use for systemic treatment are in primary care, with over 90% of total antimicrobial use is in ambulatory care. In BC, respiratory tract infections are the most common indication for antimicrobial prescriptions (pers. comm., Mei Chong).

The British Columbia population has relatively high rates of antimicrobial consumption compared to countries such as Sweden, Germany, Denmark, the Netherlands and the
U.K.\textsuperscript{87}. Consumption rates, reported in units of defined daily doses (DDD) per 1,000 inhabitant-days (described below), are highest in ages 15-19 and those over 60, and overall are higher for females than males.

Overall rates of antimicrobial usage in BC decreased by 8.2\% from 1996 to 2000\textsuperscript{87}. In BC, $\beta$-lactam consumption accounts for the majority of antimicrobial use (42\% of DDD/1,000 inhabitant-days in 2000) and has been stable in recent years. In contrast, fluoroquinolone (specifically ciprofloxacin) consumption and new macrolide consumption (clarithromycin and azithromycin) have steadily increased.

### 1.2.4.2. Methodological challenges

**Quantifying exposure: Defined Daily Dose, Prescription Count, and Duration**

A widely used method to compare drug consumption in ecological studies is the World Health Organization Anatomical Therapeutic Chemical classification/Defined Daily Dose system\textsuperscript{88,89}. This system incorporates a conversion of the average maintenance dose per day for a drug used for its main indication in adults. Drug consumption is reported as a rate (DDD/1,000 inhabitant-days). This tool facilitates comparisons between hospitals, jurisdictions and countries and has been used widely in ecological studies\textsuperscript{5,68,69}, though its utility is generally limited to adult populations as dosage varies substantially in pediatric and adolescent populations. A recent ecological comparison used the measure of prescriptions per 1000 children to compare consumption rates in the pediatric population\textsuperscript{90}.

The majority of individual level epidemiological studies of personal antibiotic use report the number of prescriptions in a given time window, obtained either from administrative pharmacy databases or questionnaires. This measure is most commonly represented as a dichotomous variable in modeling (consumption of antimicrobial X, yes or no). One study\textsuperscript{80} also considered the duration of exposure, as number of days of antibiotic use within the time window. The authors drew on computerized inpatient and outpatient pharmacy records at study hospitals. This detail of exposure assessment could be applied with confidence in the controlled environment of hospital inpatients, but would be
questionable for outpatient prescriptions. Measures such as duration are more challenging in population-based studies as although prescriptions may be filled, one cannot be sure of compliance levels.

One pediatric study did look at multiple measures of antimicrobial exposure in assessing risk for carriage of penicillin resistant *S. pneumoniae* among 941 school students. Here the DDD for the child’s last antibiotic course was calculated using the child’s weight\(^{85}\). As a continuous variable, DDD was not associated with the outcome of penicillin non-susceptible carriage (PRSP). However, when classified as low/high dose as compared to clinical recommendations, low dose was associated with carriage of PRSP (OR = 5.9, 95% CI: 2.1-16.7). A \(\beta\)-lactam treatment course of less than five days was also associated with PRSP (OR = 3.5, 95% CI: 1.3-9.8), as was the more common binary measure of \(\beta\)-lactam use (yes/no) in the previous 30 days (OR = 2.0, 95% CI: 1.1-8.3). It is notable that although the point estimates of risk vary, all three measures for \(\beta\)-lactam exposure (use, low dose and long duration) were significantly associated with PRSP carriage.

**Time windows for Exposure Assessment**

In the literature there is a lack of consistency on the appropriate exposure window to use when evaluating the risk of antimicrobial consumption. Studies have assessed antimicrobial exposure for periods ranging from one week to one year prior to infection\(^{48, 67, 78, 80}\), likely as a result of the availability of prescription records or hospital charts.

Furthermore, based on the few studies that have considered multiple time periods, the significance of risk factors appears to vary depending on the time period used. For example, a study on resistant nasopharyngeal pneumococcal carriage\(^{82}\) assessed antimicrobial consumption prior to isolate collection through an interviewer-administered questionnaire with parents. In the analysis, the authors modeled consumption of macrolide and \(\beta\)-lactam classes in the one, two and three months prior to isolate collection and found the strongest associations between consumption and resistance in the one month time window. Ruhe and Hasbun\(^{80}\) reported univariate results for the risk
of having penicillin non-susceptible bacteremia (as compared to susceptible) from using specific classes of antibiotics in either the one or six months prior to infection. For β-lactams, penicillins, cephalosporins, and macrolides the association was strongest in the six month time window, whereas for sulfonamides the association was stronger in the one month window. The odds ratios for the two time periods were not significantly different statistically, but the differences do raise questions about what the appropriate exposure window should be.

1.2.5. Addressing Antimicrobial Resistance

1.2.5.1. National Surveillance for Antimicrobial Resistance

Canadian Bacterial Surveillance Network (CBSN)

CBSN monitors the prevalence, mechanisms and epidemiology of antibiotic resistance in Canada. CBSN is comprised of a group of clinical laboratories serving doctor’s offices and clinics, as well as community and tertiary hospitals that voluntarily submit data and bacterial isolates of community-acquired organisms. CBSN has been reporting on antimicrobial resistance trends since 1994 through publications, presentations and a website (http://microbiology.mtsinai.on.ca/research/cbsn/default.asp). While ten provinces and one territory are represented in the sample collection, about half of the isolates are from Ontario, limiting the ability to generalize findings to a national level.

National Centre for Streptococcus

The National Centre for Streptococcus (NCS) in Edmonton, Alberta has been in operation since 1992 and performs serotype and susceptibility assays on all referred invasive S. pneumoniae isolates. Results are published in annual and quarterly reports published by the National Microbiology Laboratory, available on the NCS website (http://www2.provlab.ab.ca/bugs/vlab/ncs/ncs.htm), and in peer reviewed articles. As the majority of isolates are from Alberta, the results are generally presented separately for Alberta from the rest of Canada, although in recent years there has been an increase in the number of isolates referred from BC. From a national perspective, the referral of isolates seems focused on isolates associated with specific studies or outbreaks, rather than systematic and population-based collection systems.
1.2.5.2. Policy Efforts

The World Health Organization has recommended a range of interventions for the containment of antimicrobial resistance that may be targeted to the public, prescribers, drug dispensaries, health-care systems, government, the pharmaceutical industry, and agricultural users. A few relevant examples of policy actions are discussed below.

Interventions for judicious use of antimicrobials can occur through education of both the providers and consumers. A classic example of policy action comes from Iceland, where after a rapid rise in rates of \textit{S.pneumoniae} resistance, a media campaign was launched in 1991 against the overuse of antibiotics, especially in children. From 1992-1997 there was a 35% decline in antibiotic use in children, and 10% in the overall population. This corresponded with a decrease in the prevalence of penicillin resistant isolates from 19.3% in 1993 to 14% in 1998-2000.

Looking closer to home, an educational intervention program “Do Bugs Need Drugs” was recently launched in BC (http://www.dobugsneeddrugs.org). One arm of this community education program works on the basis of educating parents through their children; workshops in Grade 2 classrooms present issues and concerns around antibiotic resistance and suggest easy steps (handwashing and appropriate use of antibiotics) to prevent antibiotic resistance from developing. The program also contains training components for health care professionals, and a media campaign targeting the general public. An evaluation of the impact of the Do Bugs Need Drugs pilot program in Alberta found that the educational intervention for Grade 2 students resulted in uptake of key messages by the parents of students, that training and resources provided to physicians, pharmacists and nurses in continuing care centers produced a statistically significant reduction in antibiotic prescribing and increases in adherence to guidelines, and that knowledge of the importance of handwashing was significantly associated with seeing the TV media campaign.
1.3 Rationale for the current work

Given today’s high antimicrobial consumption levels, the introduction of the universal infant vaccine program in 2003, and the lack of population-based estimates for antimicrobial resistance in *S. pneumoniae* isolates in this province, there is a need to conduct an investigation into the epidemiology of resistant IPD in BC. The current study draws on population-based surveillance in BC and captures serotype and resistance trends for all isolates referred to the National Centre for Streptococcus. Furthermore, while the literature supports a link between antimicrobial consumption and resistance, to our knowledge there have been no previous population-based cohort studies on risk factors for resistant pneumococcal infections. Very few epidemiological studies have been conducted in Canada, and many studies do not have detailed historical pharmacy records that allow for the investigation of the risk from class-specific antimicrobial consumption. Strengths of this study lie in the richness of the data; we have a full cohort of reported IPD cases in BC with detailed individual-level data on isolate resistance patterns from NCS and patient prescription data through PharmaNet. Our results quantify the risk of developing a resistant invasive pneumococcal infection that is specifically attributable to personal antimicrobial consumption, among those with pneumococcal infections. This information is important from a policy perspective, because if a significant amount of an individual’s risk for resistant infection stems from recent antimicrobial use then efforts to control antimicrobial resistance can be focused on patterns of human consumption.

The purpose of this study is twofold: to describe the epidemiology of antimicrobial resistant IPD in BC, and to examine and quantify the risk associated with individual antimicrobial consumption for the development of pneumococcal infections which are non-susceptible.

The hypotheses presented in the proposal of this thesis project to the Thesis Screening Panel in Health Care and Epidemiology in May 2005 were the following:

1. Reporting rates of penicillin-resistant invasive *S. pneumoniae* disease are stable over the study period.
2. Reporting rates of erythromycin-resistant invasive S. pneumoniae disease are increasing over the study period.

3. Previous personal antimicrobial consumption (overall, by class: beta-lactams, macrolides, fluoroquinolones, or by specific drug: penicillin, erythromycin) is associated with increased risk for developing resistant infection.

4. The strength of the association between antimicrobial consumption and resistance is greatest when exposure is assessed over 12 months, as compared to time windows of 3, 6 and 24 months.

5. Among individuals with invasive pneumococcal infection, greater than 50% of the drug-resistant infections in individuals who have taken antimicrobials can be attributed to their consumption of antimicrobials.

Three data sources are used in this study: reported cases of IPD from the Integrated Public Health Information System (iPHIS) at BCCDC; serotype and microbiological susceptibility testing from the National Center for Streptococcus; and outpatient antimicrobial prescription records from PharmaNet through the College of Pharmacists of BC (1995-2005). Further details on the data sources are available in Appendix I.

This work is presented as a manuscript-based thesis containing two articles prepared for peer-reviewed journals. The first (Chapter 2) is a descriptive paper on the incidence, serotype and antimicrobial resistance patterns in IPD in BC over the period 2002 to 2005. This is a particularly timely piece of work as it compares patterns before and after the introduction of the PCV7 vaccine in 2003. The second paper (Chapter 3) presents a cohort study looking at the risk associated with an individual's prior antimicrobial consumption patterns for the development of a resistant infection. The concluding chapter (Chapter 4) pulls together these results and presents the implications of these results for health policy. Appendices for the thesis contain a detailed explanation of the data sources (Appendix I), the Certificate of Approval from the Behavioural Review Ethics Board (Appendix II), the BCCDC data agreement (Appendix III) and the original abstracts submitted with each of the manuscripts (Appendix VI).
1.4 References


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40. Farrell DJ, Jenkins SG, Brown SD, Patel M, Lavin BS, Klugman KP. Emergence and spread of Streptococcus pneumoniae with erm(B) and mef(A) resistance. Emerg Infect Dis 2005; 11:851-8.


2. Epidemiology of Invasive Pneumococcal Disease in BC during the Introduction of the Conjugated Pneumococcal Vaccine

2.1. Background

The rates of antimicrobial resistance to most infectious diseases are on the rise\(^1\). Alarming increases in drug and multidrug resistance have been observed in community acquired pathogens\(^2,3\). In Canada, the overall economic burden of antimicrobial resistance is estimated at $200 million per year\(^4\).

Antimicrobial resistance in \textit{Streptococcus pneumoniae}, the leading bacterial cause of community-acquired pneumonia, bacterial meningitis and otitis media, is highly variable geographically. In North America, surveillance programs of clinical pneumococcal isolates have shown increasing rates of resistance since the 1980's with the proportion of penicillin resistant isolates at 15% in Canada in 2002\(^5\), and 25% in the United States\(^6\), accompanied by a rise in multidrug resistance\(^6\). With the high incidence, morbidity and mortality of invasive pneumococcal disease (IPD), increasing rates of antimicrobial resistance are of particular concern for public health.

The 7-valent pneumococcal conjugate vaccine (PCV7), covering 80% of serotypes causing invasive disease in children under 5 years\(^7\), was licensed for use in children in Canada in 2001. In BC, a high risk immunization program was initiated in April 2003 and the universal infant program was initiated in September 2003. Thus far, national surveillance on antimicrobial resistance in IPD have included a limited number of isolates from western Canada\(^5,8\). To obtain a more complete picture of the epidemiology of IPD in BC, we determined incidence rates from IPD case reports in the

\(^{1}\) A version of this chapter has been submitted for publication. Winters, M., Patrick, D.M, Marra, F., Buxton, J., Chong, M., Isaac-Renton, J.L., Tyrrell, G.J., Lovgren, M., Paulus, S. Epidemiology of Invasive Pneumococcal Disease in B.C. during the Introduction of Conjugated Pneumococcal Vaccine. \textit{Submitted to the Canadian Medical Association Journal, July 2006.}
reportable disease database at the BC Centre for Disease Control (BCCDC), and linked these to serotype and antimicrobial susceptibility data provided by the National Centre for Streptococcus (NCS).

2.2. Methods

Case reports
IPD, defined by the isolation of S. pneumoniae from a sterile body site, usually blood or cerebrospinal fluid, is a reportable condition in BC and has been monitored by the BCCDC since 2000. Laboratory confirmed case reports are held in the Integrated Public Health Information System (iPHIS) at the BCCDC. We extracted case reports for invasive disease (pneumococcal meningitis and other invasive pneumococcal disease) for 2002-2005. These years were selected based on a consistent case definition and pattern of referral to the NCS. Preliminary reports on incidence in BC are reported elsewhere9. The pneumococcal database steward and research board at BCCDC approved data access for this research.

Serotyping and susceptibility testing
Invasive isolates are routinely referred by BCCDC to the NCS in Edmonton, Canada, where serotyping and antibiotic susceptibility testing is performed. Serotyping was based on the quellung reaction using commercial antisera obtained from the Statens Seruminstitut, Copenhagen, Denmark. The PCV7 provides vaccine coverage for the 7 most common pediatric serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F). All other serotypes were considered non-PCV7, except for 13 isolates which were “non-typable” (i.e., no capsule could be demonstrated). Non-typable isolates were excluded from analyses. Minimum inhibitory concentrations (MICs) for 9 antimicrobials (penicillin, cefotaxime, chloramphenicol, erythromycin, levofloxacin, ofloxacin, clindamycin, trimethoprim-sulfamethoxazole and vancomycin) were determined. Testing by broth microdilution, and MIC interpretation as susceptible, intermediate or resistant was based on the guidelines provided by the Clinical and Laboratory Standards Institute that were current for each year of testing10. For this study, ‘non-susceptible’ includes isolates classified as either intermediate or resistant to an antimicrobial. Multidrug
resistance is defined as resistant to 2 or more antimicrobial classes. Since fluoroquinolone susceptibility testing at NCS changed in mid-2002 from ofloxacin to levofloxacin, the data for these antimicrobials does not cover the entire study period.

Linkage
For the purposes of this linkage, we reviewed demographic information to ensure a unique record per person in each database. The case year was defined according to the date of specimen collection. In 5% of case reports where this collection date was missing, the year was imputed based on case report date. There were 1288 iPHIS records and 928 NCS records from 2002 – 2005, which were linked by last name and date of birth. The analysis was conducted on the linked dataset stripped of personal identifiers.

Analysis
Annual cumulative incidence rates of IPD, reported as the number of cases per 100,000 population, were calculated using the P.E.O.P.L.E. 30 Population Estimates and Projections\textsuperscript{11} using all iPHIS case reports. The linked study sample consisted of 728 isolates with matching iPHIS and NCS records. Demographic and isolate characteristics between matched and unmatched records were compared by chi-square tests or, where cell size was less than 5, Fisher’s exact tests using SAS (version 9.1; SAS Institute). Chi-square tests for trends in the proportion of isolates with PVC7 serotypes and of non-susceptible isolates were conducted in Epi-Info (version 6.03; US Centers for Disease Control and Prevention). A 2-sided p-value < 0.05 was considered significant.

2.3. Results
Prior to the 2003 commencement of the universal infant program, the overall rate of IPD in BC was 8.3 cases per 100,000, with the highest rate of reported cases in 1 year olds. Between 2002 and 2005, there was a significant decrease in the incidence of IPD in children less than 5 from 54.4 to 16.2/100,000 population (p<0.001). Table 2.1 shows the rate of reported cases of IPD in BC overall, and by year of age within the less
than 5 age group. The decline in incidence was significant for ages less than 1 year, and 1, 2, and 4 years.

Overall population rates of reported IPD have been relatively stable (7.5-8.3/100,000). The rate of IPD was high in the over 65 age group, ranging from 15.8/100,000 in 2002 to 19.6/100,000 in 2005. This increase over time was not statistically significant.

In the linkage, 728 (56.5%) of IPD iPHIS reports had matching NCS records. Table 2.2 shows the distribution of matched and unmatched records according to demographic characteristics. Compared to iPHIS isolates not referred for testing, referred isolates of matched records tended to be from younger people.

In addition, 200 isolates tested at NCS did not have corresponding case reports in iPHIS. These 200 isolates tended to be from younger individuals and to be of PCV7 serotypes, but did not differ in terms of year of the case, or susceptibility to penicillin, erythromycin or trimethoprim-sulfamethoxazole (data not shown).

**Serotype Trends**

Serotype results were available for 98.2% (715/728) of matched isolates. Overall 56.6% (405/715) were of PCV7 serotypes. The isolates from children under 5 years (n=194) were of 25 different serotypes of which 76.8% (n=149) were PCV7 preventable. The other two most common serotypes from children under 5 years were 6A and 19A, accounting for 9.8% of isolates (n=19). For the isolates from population 5 years and older (n=521) there was more variability in serotype distribution with 45 different serotypes. 49.1% (n=256) were PCV7 preventable and 86.0% (n=448) were of serotypes included in the 23-valent polysaccharide vaccine (PPV23). In children <2 years, the population eligible for universal PCV7 immunization, the proportion of cases with PCV7 serotypes decreased significantly over 2002-2005 from 83.0% (39/47 cases) to 16.7% (1/6 cases) (p for trend=0.006) (Figure 2.1). Similar results are observed in the overall population (from 68.9% to 43.8%, p for trend <0.001). There were no
significant changes in the proportion of IPD cases caused by PPV23 serotypes over 2002-2005.

Antimicrobial Resistance Trends
Overall 22.8% (166/728) of matched isolates were resistant to at least one antimicrobial. The prevalence of isolates with reduced susceptibility was highest for trimethoprim-sulfamethoxazole (15.3%, 111/728), penicillin (9.1% non-susceptible, 66/728 tested), and erythromycin (9.1%, 66/727). Reduced susceptibility to other antimicrobials was low: clindamycin (4.3%, 31/727), chloramphenicol (1.0%, 7/720), cefotaxime (0.3%, 2/725), and no isolates had reduced susceptibility to vancomycin. For fluoroquinolones, 9 of 118 isolates tested were non-susceptible to ofloxacin (7.6%) and 4 of 655 tested were non-susceptible to levofloxacin (0.6%).

A total of 75 (10.5%) of isolates were found to be multidrug resistant. Of the multidrug resistant isolates, 63 (85.6%) had reduced susceptibility to trimethoprim-sulfamethoxazole, 59 (78.7%) to penicillin, and 37 (49.3%) to erythromycin.

Figure 2.2 illustrates the proportion of isolates with reduced susceptibility by age. The under 15 age group had the highest proportion of isolates with reduced susceptibility to penicillin, erythromycin, trimethoprim-sulfamethoxazole, and multiple drugs (p for trend= 0.003, <0.001, <0.001, and 0.002 respectively). Figure 2.3 illustrates the proportion of isolates with reduced susceptibility by year. There were no significant trends over 2002-2005.

Antimicrobial resistance occurred most frequently in serotypes covered by the PCV7 vaccine. Of the isolates with reduced susceptibility to penicillin, 69.2% (45/65) were of PCV7 serotypes, whereas of penicillin susceptible isolates, only 55.4% (360/650) (p=0.032) were of PCV7 serotypes. Reduced susceptibility to penicillin was also observed for non-PCV7 serotypes 19A (accounting for 20.0% of penicillin non-susceptible isolates), 35B, 6A, 15A and 1 (combined total of 30.8% of non-susceptible isolates). Similarly, of the isolates with reduced susceptibility to erythromycin, 69.7%
belong to PCV7 serotypes, whereas only 55.4% (359/648) of susceptible isolates were vaccine serotypes (p=0.025). Other serotypes accounting for a significant proportion of isolates with reduced erythromycin susceptibility were 19A (9.1%), 6A (4.5%), 12F (4.5%) and 3 (3.0%).

2.4. Interpretation

This study shows a decrease in the incidence of reported cases of IPD in younger age groups following the introduction of the heptavalent conjugate vaccine in 2003. Furthermore, it demonstrates a significant decrease in the proportion of isolates of PCV7 serotypes in children under 2 years, and in the overall population. Children under 15 years had the highest rates of isolates non-susceptible to penicillin, erythromycin, trimethoprim-sulfamethoxazole and to multiple antimicrobials.

Our most dramatic reductions in IPD occurred in those less than 2 years of age. A similar drop was observed immediately following the introduction of PCV7 in the United States in 2000. Results from the Active Bacterial Core Surveillance program for 13,568 cases of IPD in 7 states found the largest impact in children aged 1, where IPD rates fell by 69% from 168.1/100,000 in 1998/1999 to 52.3/100,000 in 2001. The authors also reported significant declines in IPD incidence in adult and elderly age groups. Subsequent studies in the US indicate that these trends have continued through 2004. Conversely, we observed a trend toward increasing IPD incidence in the over 65 age group, although this was not statistically significant. There are several possible reasons for this difference. First, the US program was introduced earlier with more opportunity for catch-up, and thus a greater chance to demonstrate herd immunity. Alternatively, there may be different levels of use of the PPV23 between BC and the US, better health status in seniors or differing surveillance techniques. Any of these reasons could limit the ability to detect an early decline in IPD in the elderly in Canada.

This is the first population-based study on antimicrobial resistance in IPD for BC. Our study showed no significant trends in antimicrobial resistance patterns by year over 2002-2005, which differs from reports of increasing prevalence of antimicrobial
resistance in preceding years in Canada\textsuperscript{5,15} and the United States\textsuperscript{6}. The Canadian Bacterial Surveillance Network (CBSN) produces estimates of the prevalence of resistance in voluntarily reported pneumococcal isolates. National estimates of erythromycin resistance show an upward trend in resistance, from 2\% of isolates in 1993 to 19\% in 2005\textsuperscript{16}. While results for BC alone are based on fewer isolates, the general trend has been similar\textsuperscript{17}. Possible reasons why we did not observe an increase in erythromycin resistance may be that this study covered only four years (a period defined by consistent reporting and referral) or that the uptake of PCV7 may be slower in BC. Also, there are differences in the study samples as CBSN includes non-invasive isolates (>50\% of isolates in 2002). With different patterns of serotype distribution and antimicrobial susceptibility between invasive and non-invasive disease\textsuperscript{18}, the origin of isolates may account for the disparity in results.

Our results show that the PCV7 vaccine serotypes are more likely to have reduced susceptibility to penicillin and erythromycin than non-vaccine serotypes. This indicates the potential for the vaccine to reduce the burden of resistant disease, especially in children, where we have shown that the proportion of non-susceptible disease is higher. Such a phenomenon has been reported in a recent study following the PCV7 program in the US\textsuperscript{14} where between 1999 and 2004 the rates of penicillin non-susceptible isolates fell by 81\% in children under 2, and by 49\% in people over 65 years. In the overall population, the rate of resistant disease caused by vaccine serotypes fell by 87\%. While it has been only 2 years since the introduction of PCV7 in BC, our study shows no increasing trend in the proportion of non-susceptible isolates, as had been reported for certain antimicrobials over 1993-2005 by CBSN\textsuperscript{16}. This shift may mark the beginning of the impact of the vaccine in Canada; future years may bring significant declines in resistant disease, in keeping with trends in US.

Surveillance results such as these are key to creating and evaluating evidence-based public health policies, but must be interpreted with care. The generalizability of these findings depends on degree of coverage of the two surveillance programs. We expected that reporting rates for IPD were high; however, this linkage revealed that 200 invasive
isolates from BC tested at NCS were not captured in the iPHIS reportable disease database. The isolates without case reports tended to be from younger individuals, and thus not surprisingly, tended to be of PCV7 serotypes. Since antimicrobial susceptibility patterns did not differ between NCS isolates with or without iPHIS reports, our results should be representative of all isolates in this regard. An additional issue is that only 56% of iPHIS case reports had isolates referred for serotype and susceptibility testing, and that referred isolates differed from non-referred isolates with respect to age. With improved rates of referral the estimates from surveillance monitoring could be increasingly accurate.

Additionally, certain questions could not be answered due to limitations of our data. The data extracted from the iPHIS system included all reported cases of IPD, but iPHIS records do not contain the clinical diagnosis (i.e., pneumonia, bacteremia) or the origin of the infection (hospital or community acquired). Finally, while the surveillance of antimicrobial resistance is important for the protection of public health, the clinical significance of in vitro resistance is not clear at present \(^1^9\)\(^-\)\(^2^2\), although as the proportion of isolates with higher MICs rises there is likely to be greater clinical impact.

In summary, this linkage of two surveillance systems provides detailed information on the epidemiology of IPD in BC. In the two years since the introduction of PCV7, there has been a dramatic reduction in the incidence in children less than 5 years and significant shifts in the distribution of serotypes away from those represented in PCV7, especially in children under 2 years. The proportion of isolates non-susceptible to penicillin, erythromycin, trimethoprim-sulfamethoxazole, and to multiple antimicrobials has not increased over 2002-2005, but the highest proportion of non-susceptible isolates is found in children under 15 years. With the introduction of the conjugate vaccine, and programs aimed to reduce antimicrobial usage in the community, continued surveillance is necessary to monitor the impact of initiatives on rates of resistant disease. Testing all invasive pneumococcal isolates for serotype and antimicrobial susceptibility would provide a more complete evidence base for public health decision-making.
Tables

TABLE 2.1: Rate of Reported Cases of Invasive Pneumococcal Disease (per 100,000)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1*</td>
<td>50.6</td>
<td>57.3</td>
<td>15.1</td>
<td>20.1</td>
</tr>
<tr>
<td>1*</td>
<td>134.7</td>
<td>105.5</td>
<td>66.5</td>
<td>14.9</td>
</tr>
<tr>
<td>2*</td>
<td>40.4</td>
<td>31.6</td>
<td>17.4</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>27.9</td>
<td>23.6</td>
<td>29</td>
<td>27.1</td>
</tr>
<tr>
<td>4*</td>
<td>22.7</td>
<td>16.2</td>
<td>11.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Whole population</td>
<td>8.3</td>
<td>7.7</td>
<td>7.5</td>
<td>7.7</td>
</tr>
</tbody>
</table>

*Chi-square test for trend significant at p<0.05
### TABLE 2.2: Distribution of IPD cases according to age, sex, and year for matched* and unmatched** iPHIS records

<table>
<thead>
<tr>
<th></th>
<th>Matched Records</th>
<th></th>
<th>Unmatched Records</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Total cases</strong></td>
<td>728</td>
<td>100</td>
<td>560</td>
<td>100</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>239</td>
<td>33</td>
<td>119</td>
<td>21</td>
</tr>
<tr>
<td>15-64</td>
<td>319</td>
<td>44</td>
<td>234</td>
<td>42</td>
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<tr>
<td>&gt;64</td>
<td>170</td>
<td>23</td>
<td>207</td>
<td>37</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>333</td>
<td>46</td>
<td>260</td>
<td>46</td>
</tr>
<tr>
<td><strong>Year of case</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>181</td>
<td>25</td>
<td>159</td>
<td>28</td>
</tr>
<tr>
<td>2003</td>
<td>191</td>
<td>26</td>
<td>124</td>
<td>22</td>
</tr>
<tr>
<td>2004</td>
<td>179</td>
<td>25</td>
<td>138</td>
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</tr>
<tr>
<td>2005</td>
<td>177</td>
<td>24</td>
<td>139</td>
<td>25</td>
</tr>
</tbody>
</table>

* isolates which have records of matching last name and date of birth in the Integrated Public Health Information System (iPHIS) at BCCDC and at the National Centre for Streptococcus (NCS).

** isolates which have records in iPHIS, but not NCS

▲ significantly different (p<0.05) distribution for matched records versus unmatched records
**Figures**

**FIGURE 2.1:** Reported cases of invasive pneumococcal disease in the population <2 years in BC over 2002-2005, by serotype status
FIGURE 2.2: Proportion of invasive pneumococcal isolates (n=564) with reduced susceptibility to penicillin, erythromycin, trimethoprim-sulfamethoxazole and multiple drug classes by age group.
FIGURE 2.3: Proportion of invasive pneumococcal isolates with reduced susceptibility to penicillin, erythromycin, trimethoprim-sulfamethoxazole and multiple drug classes over 2002-2005.
2.5. References


3. Is Individual Antimicrobial Consumption Driving Resistance?²

3.1. Background

*Streptococcus pneumoniae* is a leading cause of morbidity and mortality worldwide. It is a key pathogen in upper respiratory tract infections and acute otitis media, as well as serious invasive diseases including bacteremia and meningitis¹. In recent decades, rates of antimicrobial resistance in *S. pneumoniae* have been on the rise²,³, threatening our ability for disease control.

Ecological studies have provided substantial evidence that areas with higher rates of antimicrobial consumption have higher rates of resistant *S. pneumoniae*⁴-⁶. While there have been epidemiological studies on the association of prior consumption patterns with resistant disease at the individual level, many of these have a case-control design⁷-⁹ which may have inflated risk estimates due to methodological challenges in control group selection¹⁰. Cohort studies conducted in various populations with differing methods of measuring antimicrobial consumption have found conflicting results¹¹-¹⁵. To our knowledge, there has been only one previous cohort study in a Canadian population¹⁶. This study reported significant associations between specific antimicrobial classes and reduced susceptibility to penicillin, erythromycin, and trimethoprim-sulfamethoxazole in patients with invasive pneumococcal disease in two municipalities in Ontario.

We have conducted a population-based cohort study on all individuals with reported cases of invasive pneumococcal disease (IPD) in British Columbia (BC) over 2001-2005. Our objective was to determine the risk associated with prior individual antimicrobial consumption patterns (both overall and class-specific) for having non-susceptible IPD. This study is province-wide, and builds on the current body of knowledge by employing detailed prescription database maintained through the provincial PharmaNet program to

²A version of this chapter has been prepared for submission for publication. Winters, M., Patrick, D.M, Marra, F., Buxton, J., Chong, M., Tyrrell, G.J. Is Individual Antimicrobial Consumption Driving Resistance? For submission to the Canadian Medical Association Journal.
ascertain antimicrobial use for each individual. The BC population is a key population for study, as the high rates of antimicrobial consumption in BC relative to countries in Europe\(^\text{17}\) may be driving resistance.

### 3.2. Methods

**Setting.** This was a retrospective cohort study of all reported cases of IPD from January 2001 to February 2005 with complete antimicrobial susceptibility testing. Laboratory confirmed cases of IPD are reported to the BCCDC and stored in the Integrated Personal Health Information System (iPHIS). This study was approved by the research ethics board at UBC, and data access granted by the pneumococcal data steward at BCCDC and the College of Pharmacists of BC.

**Definitions.** IPD was defined as the isolation of pneumococci from a normally sterile body fluid. Where an individual had multiple isolates from a given individual, the first isolate with susceptibility information was considered.

Antimicrobial records from 1995-2005 were extracted from PharmaNet. This provincial population-based database held at the BC Ministry of Health contains virtually all outpatient antimicrobial prescriptions. Only records for orally administered antimicrobials in the J01 category of the WHO Anatomical Therapeutic Chemical (ATC) classification system were included in this analysis. Classes of antimicrobials were defined according to the ATC therapeutic/pharmacological subgroups (WHO level 3) where J01A= tetracyclines, J01C=penicillins, J01D=cephalosporins, J01E=sulfonamides and trimethoprim, J01F=macrolides and lincosamides, J01M=quinolones, and J01X=other antibacterials. The level 4 category J01FA was used to look exclusively at macrolides. Individual drugs of interest were identified using the chemical substance subgroups (WHO level 5), where J01CA04=amoxicillin, J01EE01=trimethoprim-sulfamethoxazole, J01FA01=erythromycin, J01FA09=clarithromycin, and J01FA10=azithromycin.
The episode date was defined as collection date of the isolate. The difference between the episode date and the date that a prescription was filled provided the number of days for the antibiotic course prior to the case. The frequency of prescriptions for each drug category was tabulated for the 3, 6, 12 and 24 months prior to the episode. A dichotomous variable (none, ≥ 1 prescription) was used for modeling purposes.

**Microbiological Testing.** Serotype and susceptibility testing for referred isolates was completed at the National Centre for Streptococcus (NCS), Edmonton, Canada. Serotyping was based on the quelling reaction using commercial antisera obtained from the Statens Serum Institut, Copenhagen, Denmark. Isolate minimum inhibitory concentrations (MICs) were determined by broth microdilution, and interpreted as either susceptible, intermediate or resistant according to the current Committee for Laboratory Standard Institute guidelines for each of the years of testing. Serotypes were classified as either vaccine serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) or other. ‘Non-susceptible’ includes isolates with either intermediate or high resistance to an antimicrobial. Multidrug resistance was defined as isolates with resistance to more than 2 antimicrobials.

The linkage of case reports with serotype and susceptibility testing records has been described elsewhere. Records were subsequently linked to the PharmaNet prescription database by the exchange of personal health numbers for unique study ID codes. The protocol involved stripping the database personal identifiers (date of birth and case date) to protect the privacy of individuals and create a fully anonymized dataset for analysis.

**Statistical Analyses.** The outcome variable was IPD non-susceptibility (Y/N) to either (i) penicillin (PNSP) (ii) erythromycin (ENSP) or (iii) trimethoprim-sulfamethoxazole (TSNSP). Univariate analyses with either Pearson’s chi-square or Fisher’s exact were used to test associations between variables in each of the time frames. Variables significantly associated with the outcome (using p < 0.10) were included in multivariable analysis. Logistic regression was used to model the association between prior antibiotic
use and the likelihood of non-susceptible IPD, with the odds ratio (OR) as a measure of this association. The main effect of interest was the consumption of specific antimicrobial classes, with patient characteristics included in the multivariable regression to obtain adjusted effects. The etiologic fraction, or attributable risk percent (AR%), was calculated as 1-1/relative risk, based on the relative risk in univariate analyses. Data manipulation was conducted with SAS, version 8.01, and statistical analyses in SPSS, version 11.0.

3.3. Results

In total 564 invasive IPD isolates had microbiological testing during the study period. Table 3.1 contains the demographic distribution and antimicrobial prescription patterns for the study population.

Overall 9.6% of isolates were non-susceptible to penicillin (PNSP), 10.1% to erythromycin (ENSP) and 16.0% to trimethoprim-sulfamethoxazole (TSNSP). Additionally, isolates were found to be non-susceptible to levofloxacin (n=3), cefotaxime (n=2), ceftriaxone (n=2), chloramphenicol (n=7), and ofloxacin (n=13), but further analyses on resistance to these antimicrobials was not conducted due to low frequencies. No isolates had reduced susceptibility to vancomycin.

In the three months prior to their IPD episode, 50.7% of individuals had at least one antimicrobial prescription, and 74.7% within the year prior. The class of antimicrobials consumed by the highest proportion of people were penicillins, followed by macrolides and lincosamides or cephalosporins (~same proportion), quinolones, and sulfonamides and trimethoprim. Over 99% of all prescriptions in the sulfonamide and trimethoprim class were for trimethoprim-sulfamethoxazole, so the risk associated with this class can be attributed specifically to trimethoprim-sulfamethoxazole.

We investigated the strength of association between non-susceptible infections and antimicrobial consumption patterns in the 3, 6, 12, and 24 months prior to infection date. Relative risk estimates were similar for consumption in the 3 and 6 months prior, but
were weaker for 12 and 24 month time periods. All subsequent univariate and multivariable analyses are based on individuals’ prescription patterns in the 6 months prior to infection.

Table 3.2 shows the frequency distribution of non-susceptible isolates according to antimicrobial consumption patterns and demographic characteristics. In univariate analysis, the use of any antimicrobial (i.e., having at least one prescription) was a significant risk factor for ENSP (RR=2.52, 95% CI: 1.33-4.75) but not for PNSP or TSNSP. Our main interest was in the risk associated with specific antimicrobial classes. Having prescriptions for penicillins or trimethoprim-sulfamethoxazole in 6 months prior to infection was significantly associated with PNSP. Prescriptions for penicillins or macrolides/lincosamides were associated with ENSP, and prescriptions for trimethoprim-sulfamethoxazole with TSNSP. In terms of patient characteristics, younger age posed a higher risk for reduced susceptibility to all antimicrobials. Males had higher risk for PNSP, and those with infections of vaccine serotypes were at higher risk for ERSP and TSNSP.

We conducted multivariable modeling to obtain risk estimates associated with the consumption of specific antimicrobial classes in the 6 months prior to infection, adjusting for confounders. Table 3.3 shows the final logistic models and the odds ratios for having a non-susceptible infection. The odds of having a PNSP infection were nearly 3 times higher for individuals who had consumed trimethoprim-sulfamethoxazole in the 6 months prior to infection, compared with those who had not (OR =2.92, 95% CI:1.34-6.38). An increased odds of ENSP was associated with consumption of penicillins (OR =2.12, 95% CI:1.13-3.98) or macrolides (OR =2.00, 95% CI:1.09-3.68). Trimethoprim-sulfamethoxazole was the only antimicrobial significantly associated with TSNSP (OR=2.09, 95% CI: 1.01-4.31). In our population, the potential confounders of gender and serotype status had no significant impact on the regression estimates for class-specific antimicrobial consumption, and thus were not included in the final models. Since the J01F class includes both macrolides and lincosamides, we ran multivariable models using the WHO level 4 code J01FA (only macrolides) to ensure that the risk estimates
were not driven by lincosamide consumption (~10% of JO1F consumption), and found no appreciable difference in risk estimates.

We also evaluated the risk associated with specific antimicrobials. Table 3.4 shows the frequency of use of erythromycin, clarithromycin, and azithromycin, the relative risk and etiologic fraction for ENSP. Erythromycin or clarithromycin consumption were not significant risk factors, but any azithromycin consumption in the six months prior was associated with over three times the risk for ENSP (RR=3.31, 95% CI: 1.87-5.85). This translates to an etiologic fraction of 70% (95% CI: 47-83%), meaning that over two-thirds of the risk of an individual developing ENSP infection is attributable to her own consumption of azithromycin.

3.4. Interpretation

This study provides evidence that an individual’s antimicrobial consumption patterns can pose excess risk for the development of IPD which is non-susceptible. Specifically, these results show that consumption of trimethoprim-sulfamethoxazole in the 6 months prior to the episode is associated with 3 times the odds of infection with a penicillin non-susceptible organism, and nearly 2 times the odds of infection with a trimethoprim-sulfamethoxazole non-susceptible organism, after adjusting for age. Both macrolide and penicillin consumption are independently associated with a higher odds of having an erythromycin non-susceptible isolate.

Our focus was on the risk posed by the consumption of specific classes of antimicrobials. Several studies have found that overall antimicrobial use is a significant risk factor while others have reported that the consumption of certain classes is important. In our population, the consumption of any antimicrobial in 6 months was associated only with ENSP, but not with PNSP or TSNSP. Additionally, we found substantial class-specific risks for non-susceptible infections associated with the use of penicillins, macrolides and lincosamides, and trimethoprim-sulfamethoxazole. Class-specific risk estimates can enable targeted policy actions for specific drugs, thereby avoiding broad
usage restrictions which may impede the inherent benefits of antimicrobial use for disease control.

A recent study\textsuperscript{16} found similar class-specific risks, although their outcome measure was slightly different. The Toronto Invasive Bacterial Disease Network (TIBDN) assessed at risk factors for resistant IPD (as compared to intermediate or susceptible IPD) in 3,339 IPD patients in two regions of Ontario. For penicillin resistant disease, consumption of a penicillin (OR=2.47) or trimethoprim-sulfamethoxazole (OR=5.97) in the 3 months prior was a significant risk factor. For erythromycin resistance, prior use of penicillin, trimethoprim-sulfamethoxazole and azithromycin or clarithromycin were significant. The research also looked at trimethoprim-sulfamethoxazole resistance, where penicillins, trimethoprim-sulfamethoxazole and azithromycin were important. This study had the benefit of a larger sample size and may have had the power to detect more risk factors, however, it had complete prescription data for only 17% of patients.

Whereas macrolide use was associated with ENSP, and trimethoprim-sulfamethoxazole use with TSNSP, penicillin consumption was not a risk factor for PNSP in multivariable analysis. Interestingly, it was trimethoprim-sulfamethoxazole consumption that was associated with PNSP. This may have been a statistical artifact due to a relatively small sample size, as both classes were associated with PNSP in the larger TIBDN study\textsuperscript{16}. However a carriage study also found that only trimethoprim-sulfamethoxazole consumption (but not penicillin or amoxicillin) was associated with PNSP, after adjusting for age, sex, and day-care attendance\textsuperscript{20}. An alternative explanation may be rooted in multidrug resistance patterns. Of the 54 penicillin non-susceptible isolates, 84.6% (44/52 tested) were also non-susceptible to trimethoprim-sulfamethoxazole. This phenotype is common worldwide; others have reported high rates of co-resistance between penicillin and trimethoprim-sulfamethoxazole\textsuperscript{22,23}. The observed association between trimethoprim-sulfamethoxazole consumption and PNSP may be explained by the selection pressure of trimethoprim-sulfamethoxazole for TSNSP; because the PNSP and TSNSP determinants of resistance often occur together, trimethoprim-sulfamethoxazole consumption also promotes resistance to penicillin.
The historical breadth of our pharmacy records enabled us to probe the effect of long-term consumption patterns. We found that the strongest association between antimicrobial consumption and non-susceptible infections was in the time periods just prior to the infection. This adds an evidence base to other studies on the risk factors for resistance which have assessed consumption only in the 6 months or less prior to infection. Because \emph{S. pneumoniae} resistance emerges during nasopharyngeal carriage and carriage is a dynamic and competitive process where strains may vary from month to month, it is intuitive that antimicrobial consumption closer to the infection would have greater impact.

The selection pressure exerted by an antimicrobial is a function of its pharmacodynamic profile. We found that overall macrolide use was associated with a higher risk of an infection which is erythromycin non-susceptible. An ecological study suggests that the consumption of specific antimicrobials may be a more important determinant for resistance than overall use, specifically within the macrolide class. In this class it has been suggested that azithromycin is much more likely to select for macrolide resistance \emph{S. pneumoniae} than clarithromycin due to its longer half life and lower potency. In our study, drug-specific risk estimates indicate that azithromycin was the main driver of erythromycin resistance; the consumption of azithromycin increased the risk of a resistant infection by over 3 times. The consumption of newer macrolides (azithromycin and clarithromycin) has been on the rise in BC in recent years. The results of our study indicate that policy action to curb azithromycin use may be one method to slow the spread of antimicrobial resistance. In this regard, the etiologic fraction is an important measure for policy makers, as it represents the expected reduction in cases among exposed individuals were the exposure to be removed. We found that over two-thirds of ENSP cases in people who consumed azithromycin could be avoided with a reduction in azithromycin consumption.

Limitations of this study include potential error in measuring antimicrobial consumption. The PharmaNet database contains records for the number of outpatient prescriptions
filled, but does not measure compliance. However, we see no reason that there would be differential compliance among patients who go on to develop resistant infections versus those with susceptible infections. Also, PharmaNet captures prescriptions in the community and in long-term care facilities but not from acute care facilities, although these likely account for less than 10% of overall antimicrobial use (J.Hutchinson, pers. comm.). Additionally, because this study was based on administrative data and limited by privacy legislation we were unable to contact individuals or collect certain variables of interest such as immunization status, clinical outcome, or origin of infection (community acquired or nosocomial). Future projects for this team may include genotyping resistant isolates to identify clonal dissemination patterns, or may investigate common indications for the prescriptions in this population in order to further guide policy action.

The results of this linkage between antimicrobial susceptibility surveillance programs and administrative prescription records shows that antimicrobial consumption is a significant risk factor for resistance at the individual level. Class-specific risk estimates and the etiologic fraction provide evidence for policy action to target specific antimicrobials, such as azithromycin, which may pose substantial threat for the proliferation of antimicrobial resistance in *S. pneumoniae*. 
### TABLE 3.1: Distribution of reported cases of invasive pneumococcal disease (n=564) according to demographic and antimicrobial consumption patterns

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>210</td>
<td>37</td>
</tr>
<tr>
<td>15-64</td>
<td>231</td>
<td>41</td>
</tr>
<tr>
<td>&gt;64</td>
<td>123</td>
<td>22</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>310</td>
<td>55</td>
</tr>
<tr>
<td><strong>Serotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV7</td>
<td>336</td>
<td>60</td>
</tr>
<tr>
<td><strong>Antimicrobial consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Penicillins</td>
<td>159</td>
<td>28</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>115</td>
<td>20</td>
</tr>
<tr>
<td>Sulfonamides and trimethoprim</td>
<td>43</td>
<td>8</td>
</tr>
<tr>
<td>Macrolides and lincosamides</td>
<td>115</td>
<td>20</td>
</tr>
<tr>
<td>Quinolones</td>
<td>87</td>
<td>15</td>
</tr>
<tr>
<td>Other antibacterials**</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

* prescriptions in 6 months prior to episode
** includes vancomycin, metronidazole, nitrofurantoin, and fosfomycin
TABLE 3.2: Distribution of *S. pneumoniae* isolates non-susceptible to penicillin (PNSP), erythromycin (ENSP) and trimethoprim-sulfamethoxazole (TSNSP) according to age, sex, serotype and antimicrobial consumption in the 6 months prior to episode

<table>
<thead>
<tr>
<th>Variable</th>
<th>% PNSP</th>
<th>p-value**</th>
<th>% ENSP</th>
<th>p-value</th>
<th>% TSNSP</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>14%</td>
<td>0.002</td>
<td>15%</td>
<td>0.024</td>
<td>24%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15-64</td>
<td>9%</td>
<td></td>
<td>6%</td>
<td></td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>&gt;64</td>
<td>3%</td>
<td></td>
<td>9%</td>
<td></td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>12%</td>
<td>0.086</td>
<td>10%</td>
<td>ns</td>
<td>18%</td>
<td>ns</td>
</tr>
<tr>
<td>female</td>
<td>7%</td>
<td></td>
<td>10%</td>
<td></td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Serotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV7</td>
<td>11%</td>
<td>ns</td>
<td>12%</td>
<td>0.047</td>
<td>18%</td>
<td>0.080</td>
</tr>
<tr>
<td>other</td>
<td>7%</td>
<td></td>
<td>7%</td>
<td></td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td><strong>Antimicrobial consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>0%</td>
<td>ns</td>
<td>17%</td>
<td>ns</td>
<td>0%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>10%</td>
<td></td>
<td>10%</td>
<td></td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>14%</td>
<td>0.038</td>
<td>18%</td>
<td>0.001</td>
<td>19%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>8%</td>
<td></td>
<td>7%</td>
<td></td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>8%</td>
<td>ns</td>
<td>12%</td>
<td>ns</td>
<td>15%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>10%</td>
<td></td>
<td>10%</td>
<td></td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Sulfonamides and trimethoprim</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>23%</td>
<td>0.005</td>
<td>14%</td>
<td>ns</td>
<td>29%</td>
<td>0.028</td>
</tr>
<tr>
<td>no</td>
<td>8%</td>
<td></td>
<td>10%</td>
<td></td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Macrolides and lincosamides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>12%</td>
<td>ns</td>
<td>17%</td>
<td>0.015</td>
<td>15%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>9%</td>
<td></td>
<td>9%</td>
<td></td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>7%</td>
<td>ns</td>
<td>7%</td>
<td>ns</td>
<td>15%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>10%</td>
<td></td>
<td>11%</td>
<td></td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td>Other antibacterials†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>10%</td>
<td>ns</td>
<td>0%</td>
<td>ns</td>
<td>10%</td>
<td>ns</td>
</tr>
<tr>
<td>no</td>
<td>10%</td>
<td></td>
<td>10%</td>
<td></td>
<td>16%</td>
<td></td>
</tr>
</tbody>
</table>

ns = not significant; PCV7 serotypes = 4, 6B, 9V, 14, 18C, 19F and 23F
* only 562 isolates had susceptibility testing for erythromycin and trimethoprim-sulfamethoxazole
** by Fisher's exact test, significance cutoff p<0.10
* chisquare test for trend, significance cutoff p<0.10
† includes vancomycin, metronidazole, nitrofurantoin, and fosfomycin
TABLE 3.3: Results of multivariable logistic regression analyses for penicillin, erythromycin, or trimethoprim-sulfamethoxazole non-susceptibility in S. pneumoniae isolates (n=564) from BC during 2001-2005

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>PNSP</th>
<th>ENSP</th>
<th>TSNSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
</tr>
<tr>
<td>Antibiotic use*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>trimethoprim and sulfonamides</td>
<td>2.92</td>
<td>1.34-6.38</td>
<td>-</td>
</tr>
<tr>
<td>penicillins</td>
<td>-</td>
<td>-</td>
<td>2.12</td>
</tr>
<tr>
<td>macrolides and lincosamides</td>
<td>-</td>
<td>-</td>
<td>2.00</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15 (ref)</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>15-64</td>
<td>0.63</td>
<td>0.35-1.15</td>
<td>0.49</td>
</tr>
<tr>
<td>&gt;64</td>
<td>0.23</td>
<td>0.08-0.67</td>
<td>0.81</td>
</tr>
<tr>
<td>Constant</td>
<td>0.14</td>
<td>-</td>
<td>0.09</td>
</tr>
</tbody>
</table>

OR=odds ratio (adjusted); CI= confidence interval; PNSP=penicillin non-susceptible, MIC >=0.12 µg/mL; ENSP=erythromycin non-susceptible, MIC >=0.5 µg/mL; TSNSP=trimethoprim-sulfamethoxazole non-susceptible, MIC >=1/19 µg/mL
*reference group for antibiotic use variables is no prescriptions in this drug class in the 6 months prior
TABLE 3.4: Relative risk (RR) and etiologic fraction (AR%) of the consumption of any macrolide, or specific macrolides in 6 months prior to infection for erythromycin resistance in *S. pneumoniae* isolates (n=564)

<table>
<thead>
<tr>
<th></th>
<th>n (%), with prescription</th>
<th>Erythromycin Resistance*</th>
<th>AR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any macrolide</td>
<td>111 (19.7%)</td>
<td>2.04 1.23-3.40</td>
<td>51%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>18 (2.3%)</td>
<td>0 na</td>
<td>na</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>65 (11.5%)</td>
<td>1.44 0.74-2.79</td>
<td>31%</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>38 (6.7%)</td>
<td>3.31 1.87-5.85</td>
<td>70%</td>
</tr>
</tbody>
</table>

*na = no individuals who consumed erythromycin had erythromycin resistant isolates
*erythromycin-resistance, MIC>=0.5 μg/mL
3.5. References


4. Final Discussion and Conclusions

4.1. Summary

This linkage study between the reportable disease surveillance program in BC, serotype and susceptibility testing conducted at the National Centre for Streptococcus (NCS), and the province-wide prescription database PharmaNet updates the epidemiological knowledge on invasive pneumococcal disease (IPD) in BC. Furthermore, it provides new information on the risk for antimicrobial resistance posed by individual antimicrobial consumption patterns.

In embarking on this project the first *a priori* hypotheses were that rates of antimicrobial resistance to penicillin would be stable over the study period and rates of macrolide resistance would rise, inkeeping with recent national trends\(^1\). Although trimethoprim-sulfamethoxazole resistance was not an element of the primary hypotheses, its inclusion is justified by the high rates of non-susceptibility observed in the BC population. In terms of the risk factors for resistance, we hypothesized that prior consumption of specific antimicrobials would be associated with increased risk of developing a resistant infection, with an attributable risk of over 50%. The *a priori* hypothesis was that this association would be strongest for consumption in the 12 months prior to the episode.

The first paper (Chapter 2) reported on the trends in incidence, serotype distribution, and antimicrobial resistance before and after the introduction of the heptavalent conjugate vaccine (PCV7) in 2003. We reported a 70% decline in the incidence of IPD in children less than 5 years of age over 2002-2005. There was also a significant change in serotype distribution, as the proportion of invasive disease caused by PCV7 vaccine-preventable serotypes decreased significantly in children less than 2 years, as well as in the overall population. In contrast with longer-term trends for Canada and BC populations\(^1,2\), we found no increase in the rates of resistance to penicillin, erythromycin, trimethoprim-sulfamethoxazole, or to multiple antimicrobials, over the study period. Finally, we reported that the highest proportion of resistant isolates occurs in the youngest age group.
The second paper (Chapter 3) moved beyond descriptive analysis to probe the risk associated with prior antimicrobial use for developing resistant disease in the cohort of individuals with IPD. We found elevated class-specific risks for commonly prescribed antimicrobials including penicillins, macrolides, and trimethoprim-sulfmethoxazole. Of the macrolides, azithromycin use posed the highest risk; over two-thirds of an individual’s risk for developing an infection with reduced susceptibility to erythromycin in those who consumed azithromycin could be attributed to that consumption.

Together these papers present an up-to-date picture of antimicrobial resistance in IPD. It appears that the previously rising rates of antimicrobial resistance in \textit{S.pneumoniae} may be stabilizing, coincident with the implementation of the new vaccine program. However, the consumption of certain antimicrobials continues to be a driver of resistance among individuals with IPD.

\textbf{4.2. Contributions}

Ecological studies have laid a foundation of evidence for the association between antimicrobial consumption and resistance rates. However, to avoid the risk of ecological fallacy and build a case toward causality, such associations must be consistent in epidemiological studies of different designs and in a variety of populations. Both case control and cohort studies on risk factors for antimicrobial resistance in IPD have been conducted. For methodological and logistical reasons outlined in the literature review, cohort studies may provide the most accurate measure of risk at the individual level. While evidence of an association exists from other recent cohort studies, the current work furthers knowledge in this area in a number of ways.

Firstly, to our knowledge this is the first population-based cohort study on antimicrobial use and resistance in IPD. Other studies have been drawn on hospitalized populations in order to facilitate exposure and outcome assessment\textsuperscript{3}. The invaluable reportable disease surveillance program and administrative prescription database in BC allowed us to investigate the risk of antimicrobial exposure in across reported cases of IPD province-wide.
Secondly, we have detailed administrative data on antimicrobial prescriptions, whereas other studies have relied on self-reported antimicrobial use or hospital records. One caveat is that the number of prescriptions filled is not necessarily synonymous with antimicrobial exposure, as patient compliance is likely less than 100%, however we see no reason why compliance rates would differ according to disease outcome. Our prescription data is highly detailed, allowing the opportunity to probe the influence of specific classes of antimicrobials, as well as the relative importance of drugs within a class. Other studies have evaluated overall antibiotic use, or certain classes, but rarely report drug-specific risks.

Additionally, we have antimicrobial prescription records for an extended time period prior to infection. While our primary interest was consumption patterns in the six months prior to infection, the richness of our data allowed investigation of the importance of consumption patterns for several years prior to infection.

Finally, given the substantial geographic variation in resistance patterns in *S. pneumoniae* \(^4\-^6\), and also in antimicrobial usage\(^7\), it is optimal to have local surveillance data for evidence-based decision making. Our results for the epidemiology of antimicrobial resistance in BC in recent years and the risk posed by antimicrobial consumption have already been communicated to policymakers at the BCCDC, to academic audiences, and to other key stakeholders through knowledge transfer activities including presentations and publications. The combined impact of this individual-level study and an ecological study being completed simultaneously by researchers at the BCCDC will provide formidable evidence of the need for changes in antimicrobial use. In addition, BC has the benefit of being one of the only provinces with a coordinated outpatient prescription database. Since this is likely the most detailed population-based study on antimicrobial resistance in Canada, it will prove to be of great use to decision-makers nation-wide.

A tangential benefit gleaned from this linkage project is new information on the overlap between the surveillance programs at BC Centre for Disease Control (BCCDC) and the
NCS. The referral of isolates from the BC Provincial Laboratory to NCS for serotype and susceptibility testing has improved markedly since the 1990’s, but remained at only 50-60% of isolates for reported cases of IPD over the period of 2002-2005. Higher rates of isolate referral may provide more accurate serotype and susceptibility results, and would increase the sample size for future epidemiological studies. Another related finding was with regards to the present rate of underreporting. Because IPD diagnosis relies on local blood sampling and lab testing procedures, there are an inherent number of invasive infections for which the etiology is never unidentified. Still, it was expected that the majority of cases with known pneumococcal etiology were captured in the reportable disease database. We found that 200 of 928 invasive isolates from BC tested at NCS had no corresponding records in the reportable disease database, indicating with certainty a significant amount of underreporting. These two outcomes have been conveyed to directors at the relevant organizations.

4.3. Limitations and Future Work

While access to administrative databases provided rich data for this project, privacy legislation and institutional restrictions also limited our analytic capabilities. The descriptive epidemiology analyses were conducted on anonymized records, with fully detailed age, health service delivery area, serotype and year variables. Prior work between the College of Pharmacists of BC and Dr. D. Patrick and Dr. F. Marra at the BCCDC paved the way for the linkage to PharmaNet data. Nonetheless, the development of the linkage protocol was an iterative process that required substantial compromise for approval by the College of Pharmacists of BC, BCCDC and the UBC Behavioural Ethics Review Board. As a result, individual demographic and serotype data was aggregated to protect the privacy of individuals, and the year of infection was not included in the final dataset, limiting our ability to adjust for temporal trends and to probe specific serotypes (i.e., 23F, a multi-drug resistant clone that is of concern worldwide). It is hoped that the timely and informative results of the current project will forge the path for smoother collaborations between these groups in the future.
Privacy limitations also prevented us from contacting individuals in order to obtain data on potentially important variables not collected by surveillance systems such as prescription compliance, immunization status, diagnosis, origin of infection (community-acquired or nosocomial) or clinical outcome. Regardless, this thesis was designed to rely on administrative data; given the timeline and budget of the project, it would not have been feasible to contact individuals in the cohort.

Future work based on this data will continue to inform methods for the control of antimicrobial resistance. Annual updates of serotype and susceptibility testing and PharmaNet records will enable continued evaluation of temporal trends. Genotyping resistant isolates may clarify trends in multidrug resistance and identify clonal dissemination patterns. Finally, there is potential to link prescriptions in this dataset to the indication for which they were prescribed, thus enabling the quantification of inappropriate prescribing (e.g., for viral infections or bronchitis). The methodology for linking PharmaNet data with records from Medical Service Plan, which contain details on the prescribing physician and indication, has already been developed at the BCCDC. An important finding of our study was the risk posed by azithromycin, a macrolide with a long terminal elimination half-life. This result is supported by a body of pharmacokinetic and pharmacodynamic literature\textsuperscript{8-10}, and the increased risk of resistance associated with long-acting macrolides has also been recently reported in an ecological study and a cohort study\textsuperscript{11,12}. A question that remains is whether long-acting drugs from other classes also pose increased risk for resistance, and whether the use of other such drugs should also be monitored carefully.

4.4. Impact on Policies for the Control of Antimicrobial Resistance

There are three important strategies for the control of disease and of the spread of resistance: vaccination (providing immunity and reducing the incidence of disease and colonization with \textit{S.pneumoniae}), prudent antibiotic use (reducing selection pressure for resistant organisms) and infection control (handwashing and other intervention methods along the transmission pathway)\textsuperscript{13}. Key results from this thesis address the first two of these areas and provide direction for policy change.
Our first chapter describes the changing epidemiology of IPD during the vaccine era. The dramatic changes observed in incidence, serotype distribution, and resistance are in accordance with the predicted impact of the vaccine, and concur with recent findings from the US\textsuperscript{14,15}. Certainly the heptavalent conjugate vaccine has demonstrated the potential to reduce morbidity and mortality from IPD in the short term. However, \textit{S.pneumoniae} is a highly adaptive species found in complex bacterial communities in the human nasopharynx. The vaccine provides immunity from infection and, to a lesser degree, colonization, by highly prevalent serotypes\textsuperscript{16}. Vaccination thus changes the ecology of the flora within the nasopharynx by creating opportunities for competing serotypes and species. There have been recent concerns around serotype replacement (the acquisition of new strains of non-vaccine serotypes) and capsular switching (the acquisition of gene cassettes that encode for a production of a new capsule, allowing virulent vaccine types to develop non-vaccine type capsules), which may affect the ability for disease control by vaccination in the future\textsuperscript{17-20}.

In addition to vaccines, advances toward the more prudent use of antimicrobials are required to control antimicrobial resistance. The risk estimates for individual consumption patterns and the etiologic fractions produced in this thesis provide evidence for targeted policy action on the appropriate use of specific antimicrobials, such as azithromycin, which may pose more substantial threat for the proliferation of antimicrobial resistance in \textit{S. pneumoniae}. While our cohort study provides evidence to policy makers to support prescription restrictions, we would like to emphasize that only ‘excessive’ use of antibiotics should be controlled. One must remember that antibiotics are a primary weapon for disease control and offer a multitude of benefits. There should be no compromise to individual or public health. However, the recommendation presented herein is that in order to curb the rapid increase in the spread of resistant organisms, and to prolong the effectiveness of currently available antimicrobials, we need to prescribe \textit{judiciously} and ensure that patients use \textit{responsibly}. The risk estimates from our cohort study for the consumption of specific classes of antimicrobials can be used for
targeted policy action, in order to minimize use of the antimicrobials which pose the highest risk.

The third area of focus for controlling the spread of antimicrobial resistance is in infection control. A substantial amount of this focus has been in hospitals and other institutions, but recent efforts have targeted infection control in the community. One example is the "Do Bugs Need Drugs" program, launched in BC in 2005, which promotes hand washing as a key message in elementary school educational programs, along with the responsible use of antimicrobials.

Surveillance is paramount to monitoring the impact of any of these policy interventions. The current project provides a timely assessment of trends in resistance during the introduction of the heptavalent vaccine, and baseline data for the evaluation of upcoming appropriate antibiotic use campaigns. Importantly, our findings revealed some degree of underreporting to the reportable disease database, and a referral rate of less than 60% of isolates to the National Centre for Streptococcus. We recommend that efforts are undertaken to refer all isolates for susceptibility testing, and that quality assurance reports are produced annually to ensure the accuracy of epidemiological trends.

Timely knowledge translation activities have meant that policy makers can already capitalize on the results of this thesis. The issue of underreporting led to the initiation of a review of the IPD surveillance system, and has recently been brought to the attention of Medical Health Officers in the province. Components of the resistance and serotypes trends since the introduction of the heptavalent vaccine have been presented at two conferences in the past year, and the full descriptive IPD epidemiology manuscript has been submitted to the Canadian Medical Association Journal. The resistance trends will also be used in one arm of the Do Bugs Need Drugs program evaluation in the coming year. Finally, the class-specific risk estimates will be communicated through BCCDC to PharmaCare along with the results of a concurrent ecological study in order to drive policy changes such as adjusted reimbursement levels which would encourage the use of "safer" antimicrobials in the population. In all, this body of work has had a substantial
impact on the knowledge on local patterns of antimicrobial resistance in IPD, and furthermore provides direct evidence for policy action to slow its spread.
4.5. References


Appendices

Appendix I: Data Sources

Integrated Public Health Information System
Invasive pneumococcal disease has been a reportable disease in BC since 1999, meaning that all confirmed cases must be reported by physicians, hospitals and laboratories to the Medical Officer of Health and collated at the BC Centre for Disease Control (BCCDC). Cases are defined by isolation of \textit{S.pneumoniae} from a normally sterile body fluid (i.e., blood, cerebrospinal fluid). Case reports are stored in the Integrated Personal Health Information System (iPHIS). Access to iPHIS case reports for research purposes requires approval by the data stewards at BCCDC.

National Centre for Streptococcus
Invasive isolates are referred from BCCDC laboratories to the National Centre for Streptococcus (NCS) in Edmonton, Alberta. Since its inception in 1992 the NCS has been testing invasive pneumococcal isolates from across Canada, but only since 2002 have there been consistent referrals of isolates from BC Serotype testing at NCS is based on the quellung reaction using commercial antisera obtained from the Statens Seruminstitut, Copenhagen, Denmark. Standard antimicrobial susceptibility testing is completed by broth microdilution, providing minimum inhibitory concentration (MICs) for a range of antimicrobials including penicillin, cefotaxime, ceftriaxone, chloramphenicol, erythromycin, levofloxacin, ofloxacin, clindamycin, trimethoprim–sulfamethoxazole and vancomycin. The MICs are interpreted as either susceptible, intermediate or resistant according to the current Committee for Laboratory Standards for each of the years of testing\textsuperscript{1}.

PharmaNet
PharmaNet is a province-wide population-based prescription drug database managed by the Ministry of Health and stewarded by the College of Pharmacists of BC. It captures virtually all outpatient prescriptions filled in BC, excepting outpatient drugs dispensed by the BC sexually transmitted diseases control programs and samples distributed by doctor’s offices.
Appendix II: UBC BREB Ethics Approval Certificate
Appendix III: BCCDC Data Utilization Application and Agreement
Part C - Records Requested (Attach a separate sheet if required)

Please list all records containing personal information to which access is requested. Access will be given only to the records listed below.

1. Invasive pneumococcal disease case reports (CD database) 2001-2005
2. Serotype and susceptibility testing from National Pneumococcal Reference Laboratory
3. Antimicrobial prescription records from PharmaNet download, 1995-2003

List variables requested: From (1) Name, Age, date of birth, sex, HSDA, case report date. From (2) Name, Age, date of birth, sex, date of test, isolate site, susceptibility profile, serotype. From (3) Study ID, sex, date of birth, all antimicrobial prescription records prior to episode date.

Part D - Agreement on Terms and Conditions of Utilization

If I am granted access to the records listed in Part C, I understand and will abide by the following terms and conditions:

1. I understand that I am responsible for maintaining the security and confidentiality of all personal information found in or taken from these records.

2. Apart from myself, only the following persons will have access to this personal information in a form which identifies or could be used to identify the individuals to whom it relates:
   - David Patrick
   - Fawziah Marra

3. Physical security will be maintained by ensuring the premises are securely locked, except when one or more of the individuals named in paragraph 2 are present. This includes the use of locking filing cabinets and secured computer network login and password.

4. Personal information contained in the records described in Part C of this form will not be used or disclosed for any purpose other than as described in Part B, nor for any subsequent purpose, without the expressed written permission of the BCCDC.

5. No personal information that identifies or could be used to identify the individuals to whom it relates will be transmitted by means of any telecommunications device, including telephone, fax or modem.

6. Individual identifiers associated with the records described in Part C will be removed or destroyed at the earliest time at which removal or destruction can be accomplished consistent with the research purpose described in Part B. At the latest (maximum 2 years), this will occur by:
   - 2005/12/31 (YYYY/MM/DD)

Any extension to this time limit must be approved in writing by the BCCDC.
Chapter 2: ABSTRACT

**Background:** Antimicrobial resistance in *Streptococcus pneumoniae* has increased in recent decades. We linked two surveillance programs to evaluate trends in incidence rates, serotype distribution, and antimicrobial resistance in invasive pneumococcal disease (IPD) since the heptavalent conjugate vaccine (PCV7) was introduced in BC in 2003.

**Methods:** We extracted case reports of IPD in BC from 2002-2005 from the reportable disease database at the BC Centre for Disease Control and linked them to serotype and antimicrobial susceptibility results from the National Centre for Streptococcus (NCS). Matched records contained patient demographics, isolate serotype and isolate susceptibility profiles for 9 antimicrobials (defined according to Clinical Laboratory Standards Institute breakpoints).

**Results:** There was a significant decrease in the incidence of IPD in children <5 from 54/100,000 population in 2002 to 16/100,000 population in 2005 (70% decrease, p<0.001). The most dramatic decline was in children aged 1 year, where the rate fell from 135/100,000 to 15/100,000 (89% decrease, p for trend <0.001). Overall, 728/1288 (56.5%) reported cases of IPD were referred for testing at NCS. For all matched cases, the proportion of isolates of PCV7 preventable serotypes decreased from 68.9% to 43.8% (p for trend <0.001) between 2002 and 2005. In children <2 years, this proportion decreased from 83.0% (39/47 cases) to 16.7% (1/6 cases) (p =0.006). The prevalence of isolates with reduced susceptibility was highest for co-trimoxazole (15.3% non-susceptible, 111/725 tested), penicillin (9.1%, 66/728), and erythromycin (9.1%, 66/727). 10.3% of isolates (75/728) were resistant to ≥2 classes of antimicrobials. There were no significant trends in the proportion of non-susceptible isolates by year. Children under 15 years of age had the highest proportion of isolates with reduced susceptibility to penicillin, erythromycin, co-trimoxazole, and to multiple antimicrobials (p for trend= 0.003, <0.001, <0.001, and 0.002 respectively).

**Interpretation:** The incidence of IPD in children <5 has decreased significantly since the introduction of PCV7. Comprehensive serotype and antimicrobial susceptibility testing provides evidence for the evaluation of immunization programs.
Chapter 3: ABSTRACT

Background: Antimicrobial use is a known driver of antimicrobial resistance. Our objective was to quantify the risk associated with consumption of specific classes of antimicrobials for the subsequent development of resistant vs susceptible invasive pneumococcal disease (IPD).

Methods: This retrospective cohort study included all BC IPD case reports from 2001 to February 2005 with antimicrobial susceptibility testing (n=564). Individual antimicrobial consumption records were obtained through the provincial PharmaNet system and categorized according to the WHO Anatomical Therapeutic Chemical classification system. We identified the risk of antimicrobial consumption patterns for infections which were non-susceptible to penicillin, erythromycin and trimethoprim-sulfamethoxazole by univariate and multivariable logistic regression analyses.

Results: Antimicrobial consumption patterns in the 6 months prior to infection had the most influence on the isolate susceptibility. In multivariable modeling, use of trimethoprim-sulfamethoxazole (OR=2.92, 95% CI:1.34-6.38) was significantly associated with having a penicillin non-susceptible infection. Penicillins (OR=2.12, 95% CI:1.13-3.98) or macrolides or lincosamides (2.00, 95% CI=1.09-3.68) were independently associated with erythromycin non-susceptible infections. Trimethoprim-sulfamethoxazole use was associated with trimethoprim-sulfamethoxazole non-susceptibility (=2.09, 95% CI: 1.01-4.31). Of the macrolides, only azithromycin posed a significant risk for erythromycin resistance.

Interpretation: Certain commonly prescribed antimicrobials pose a risk for developing infections which are resistant. Class-specific risk measures can inform targeted prescribing and policy action for the control of antimicrobial resistance.