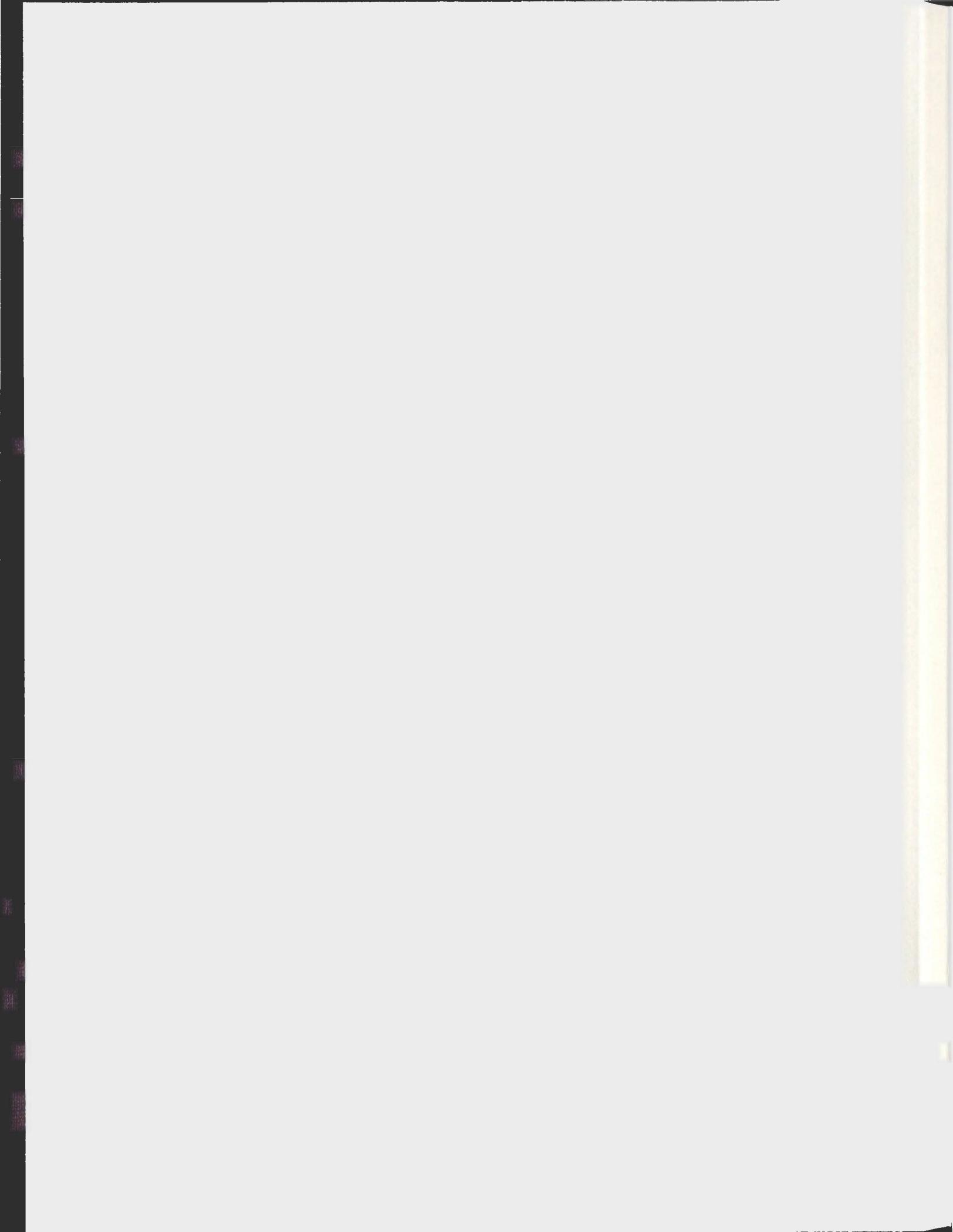


ANTIBIOTIC RESISTANCE LEVELS OF
Streptococcus pneumoniae AND ANTIBIOTIC
CONSUMPTION IN NEWFOUNDLAND AND LABRADOR

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**Antibiotic Resistance Levels of *Streptococcus pneumoniae*
and Antibiotic Consumption in
Newfoundland and Labrador**

by

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**A dissertation submitted to the Division of Community Health and Humanities
in partial fulfillment of the requirements for the degree
of Master of Science.**

Faculty of Medicine

Memorial University of Newfoundland

October 2007

St. John's

Newfoundland and Labrador

ABSTRACT

Streptococcus pneumoniae is an important human pathogen, causing potentially life-threatening infections such as pneumonia and meningitis. Until recently, it was almost uniformly susceptible to penicillin and other antibiotics. However, research during the last few years has shown a dramatic increase in the levels of resistance of *S. pneumoniae* to several classes of antibiotics. Antibiotic resistance is a rapidly evolving problem of worldwide importance. Although antibiotic resistance is related to antibiotic use, the relationship is a complex one that requires further evaluation.

Purpose: The purpose of this project was to provide baseline information on the levels of antibiotic resistance of *Streptococcus pneumoniae* for the province of Newfoundland and Labrador and to determine the relationship between different patient factors and resistance. We also compared the accuracy of various methods of susceptibility testing as well as described the trends in outpatient antibiotic consumption within Newfoundland and Labrador between 1997 and 2000.

Methods: Isolates were collected from various regions of the province between January and December 2000. Patient demographics, including sex, age, specimen source and geographic location, were submitted with the isolates. Susceptibility testing was performed according to standard protocols using both disk diffusion and the E-test system. Information on antibiotic use was provided by IMS HEALTH Canada for the years of 1997 to 2000.

Results: The levels of antibiotic resistance of *S. pneumoniae* as described by this study are comparable to the levels of resistance described elsewhere in Canada. Isolates tested during this study showed evidence of resistance to multiple antibiotics. Increased levels of resistance were shown in isolates retrieved from non-sterile sites, children and the elderly. There was little variation in the levels of resistance in isolates obtained from males and females. The E-test System was shown to provide a more specific susceptibility profile than disk diffusion. Between 1997 and 2000, the outpatient use of tetracyclines, trimethoprim and penicillins showed a steady decline while the use of cephalosporins and macrolides declined initially and then increased. Fluroquinolones were the only class of antibiotic studied that showed an increase in public consumption.

Conclusion: Limitations in isolate availability and patient information as well as a lack of information regarding regional antibiotic consumption prevented any correlations from being made between resistance levels and patient risk factors or community antibiotic use. However, this study does indicate that antibiotic resistance is a significant problem in Newfoundland and Labrador that will require further evaluation.

Acknowledgments

I would like to thank my supervisors, Dr. Bill Bavington and Dr. Jim Hutchinson, as well as the other members of my supervisory committee, Dr. Roy West and Dr. David Allison. With your guidance, this project went from conception to completion.

I would also like to express my gratitude to Dr. Sam Ratnam, Sandra March and the staff of the Newfoundland Public Health Laboratory for providing me with the resources necessary to carry out this project and helping me every step of the way.

I extend a special thanks to my parents, family and friends who supported me through the trials and tribulations of thesis preparation.

Table of Contents

<i>Chapter</i>	<i>Page</i>
Abstract	i
Acknowledgments	ii
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	3
2.1 Antibiotic Resistance	3
2.2 Environmental Issues	8
2.3 Current Research	13
2.4 Antibiotic Susceptibility Testing Methodologies	16
2.5 Drug resistant <i>Streptococcus pneumoniae</i>	19
2.6 Objectives	27
3.0 MATERIALS AND METHODS	28
3.1 Protocol	28
3.2 Antibiotic Sensitivity Testing	29
3.3 Antibiotic Utilization	30
3.4 Statistical Analysis	31

Table of Contents (Cont'd.)

<i>Chapter</i>	<i>Page</i>
4.0 RESULTS	33
4.1 Patient Demographics	33
4.2 Methodology Analysis	35
4.3 Susceptibility Profile Analysis	39
4.4 Total Antibiotic Consumption Analysis	55
5.0 DISCUSSION	58
5.1 Patient Demographics and Susceptibility Profiles	59
5.2 Methodology Comparison	67
5.3 Antibiotic Consumption	69
5.4 Limitations	71
6.0 CONCLUSION	73
7.0 REFERENCES	75
8.0 APPENDIX A	83
9.0 APPENDIX B	84
10.0 APPENDIX C	85

Table of Contents (Cont'd)

Tables & Figures

<u>Table 1:</u> Regional distribution of isolates used to determine the antibiotic susceptibility patterns of <i>S. pneumoniae</i> within Newfoundland from January 1 to December 31, 2000.	Pg 34
<u>Table 2:</u> Distribution of isolates used to determine the antibiotic susceptibility patterns of <i>S. pneumoniae</i> within Newfoundland from January 1 to December 31, 2000 on the basis of geographic location, age, sex and specimen source.	Pg 36
<u>Table 3:</u> Comparison of surveillance methodologies used to detect penicillin resistance.	Pg 37
<u>Table 4:</u> Comparison of surveillance methodologies used to detect erythromycin resistance.	Pg 38
<u>Table 5:</u> Comparison of surveillance methodologies used to detect tetracycline resistance.	Pg 40
<u>Table 6:</u> Susceptibility profile of all isolates as indicated by the E-test system received during the study period (January 1, 2000 to December 31, 2000).	Pg 41
<u>Table 7:</u> Susceptibility profile of isolates received from the Health Care Corporation of St. John's.	Pg 43
<u>Table 8:</u> Susceptibility profile of isolates received from hospital laboratories outside of St. John's.	Pg 44
<u>Table 9:</u> Susceptibility profile of isolates received from sterile sites.	Pg 45
<u>Table 10:</u> Susceptibility profile of isolates received from non-sterile sites.	Pg 47
<u>Table 11:</u> Susceptibility profile of isolates received from pediatric patients (18 years or younger).	Pg 48
<u>Table 12:</u> Susceptibility profile of isolates received from adult patients (19 - 49 years).	Pg 49
<u>Table 13:</u> Susceptibility profile of isolates received from elderly patients (50 years or greater).	Pg 50
<u>Table 14:</u> Susceptibility profile of isolates received from males.	Pg 52
<u>Table 15:</u> Susceptibility profile of isolates received from females.	Pg 53
<u>Table 16:</u> Comparison of the MICs at which 50% and 90% of the isolates were inhibited.	Pg 54

Table of Contents (Cont'd.)

Tables & Figures

Figure 1: Newfoundland antibiotic prescription rates from 1997 to 2000.

Pg 56

1.0 INTRODUCTION

People had been using molds to treat infections for more than 2500 years (World Book, 1998). Then, in 1928, Alexander Fleming observed, for the first time, a mold of the genus *Penicillium* that produced a substance that had the ability to destroy bacteria (Pelczar *et al.*, 1993). This substance, given the name penicillin, became known worldwide as 'the wonder drug' because of its effective treatment of potentially fatal infections.

Florey and colleagues showed that penicillin was an effective form of treatment for staphylococcal and streptococcal infections in February 1941 (Wilson, 1976). Their first patient was a policeman in Oxford, England who had scratched his face on a rose bush and subsequently developed local staphylococcal cellulitis followed by a secondary streptococcal infection complicated by orbital osteomyelitis and pneumonia. His initial treatment with sulfapyridine was unsuccessful. On February 12, the patient received an intravenous injection of 400 mg of crude penicillin followed by 100 mg every three hours. This treatment was continued for five days. The patient showed a rapid improvement, but after administering 4.5 gm's of penicillin the drug supply was exhausted. He lived for ten more days before succumbing to the infection. Despite the fatal result in this case, it was shown that penicillin had great promise (Wilson, 1976).

Scientists and physicians began to assume that they finally had the upper hand in the battle against microorganisms. However, less than 20 years after the discovery of penicillin, the first known evidence of bacterial resistance to penicillin was observed in a mouse model in 1943 (Schmidt and Sesler, 1943).

In the decades following Fleming's discovery, the widespread availability of penicillin and other antimicrobial compounds led to a dramatic reduction in illness and death from infectious diseases. There were great advances in controlling diseases, such as tuberculosis (Gordon *et al.*, 2000). During this time, many countries also saw improvements in sanitation, housing and nutrition, which helped in the fight against infectious diseases.

Despite the improvements in controlling illness caused by microorganisms, it has been shown in recent years that resistance to treatment with first-line drugs in bacterial pathogens ranges from zero to almost 100%. Ineffective therapy due to antimicrobial resistance has been associated with increased human suffering, lost productivity and often death (WHO, 2001). The increasing levels of drug resistance have been described as a threat to global stability and national security (WHO, 2001).

Streptococcus pneumoniae is currently a major invasive bacterial pathogen of children and older adults, which is noteworthy because of the worldwide emergence of resistance to multiple antibiotics. This project will take a closer look at the current level of antibiotic resistance in *Streptococcus pneumoniae*, also referred to as pneumococcus, within the province of Newfoundland and Labrador. It will look at how various factors may be contributing to the problem, including the prescription rates of antibiotics commonly used to treat pneumococcal infections, as well as assess the efficacy of methodologies used to determine the level of resistance in this organism.

2.0 LITERATURE REVIEW

2.1 Antibiotic Resistance

Much of the research on antibiotic resistance has been aimed at determining the mechanisms by which microbes develop resistance and the means by which organisms transmit these characteristics to subsequent generations. Bacteria acquire resistance against an antibiotic as a form of adaptation under biochemical stress. These organisms may have intrinsic/natural or acquired resistance (Bezoen *et al.*, 2001).

Intrinsic/natural resistance is inherent to the organism and is generally mediated through the shape and constituents of the organism. An example of natural resistance occurs in *Pseudomonas aeruginosa*, which has never been sensitive to chloramphenicol at any dose (Cunha, 2000). The natural resistance of *Pseudomonas* is due to the presence of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and decreased outer membrane permeability.

Acquired antibiotic resistance occurs if an organism which was once sensitive to a drug eventually becomes resistant to it (Cunha, 2000). Acquired resistance is sub-categorized by some into relative or absolute resistance. Relative acquired resistance refers to the gradual increase over time of the minimal inhibitory concentration (MIC) of a particular antibiotic. Absolute resistance, in contrast, occurs when there is a single-step mutation that occurs during or after therapy which increases the MIC of a previously susceptible isolate to extremely high levels which are unachievable using therapeutic doses (Cunha, 2000).

Acquired resistance can occur either by chromosomal mutation or DNA transfer.

Chromosomal mutations can occur within the bacterial genome at anytime, with or without the presence of antibiotic compounds in the environment. DNA transfer, in contrast, occurs when an organism receives resistance genes by taking up functional DNA from another bacterium. In both situations, organisms gain resistance to an antibiotic without selective pressure. Selective pressure refers to the environmental conditions that enhance the ability of bacteria to develop resistance to antibiotics and to proliferate.

DNA transfer can occur in three different ways. When bacteria die, the soluble DNA remains in the environment where it can be picked up by other bacteria through a process known as transformation. The second situation is through the process of transduction, which occurs when DNA is transferred between bacteria through bacteriophages, viruses that infect bacteria. The third situation occurs when there is direct cell-to-cell contact followed by the formation of a channel between organisms through which DNA, in the form of plasmids, can pass. This is referred to as conjugation.

Microbes resist antibiotics by a number of specific mechanisms including alteration of the target site, enzymatic detoxification of the antibiotic, elimination of ports of entry to the cell, production of pumps that export antibiotics out of the cell, decreased drug accumulation or the bypass of an antibiotic sensitive step (Bezeon *et al.*, 2001 and Murray, 1995). Of these specific mechanisms, changes to the target site, detoxification and decreased drug accumulation are the direct result of chromosomal mutation or may be due to the presence of a plasmid within a bacterial species (Murray, 1995). Microbes which have the ability to bypass an antibiotic sensitive step are primarily the result of acquired foreign DNA (Murray, 1995).

Natural or absolute acquired antibiotic resistance cannot be overcome by

increasing the antibiotic concentrations at clinically achievable levels (Cunha, 2000). In contrast, acquired relative resistance may be overcome by achieving blood and tissue concentrations of two to four times or greater the MIC of relative resistant isolates (Cunha, 2000).

Resistance becomes a problem when it presents as a resistant bacterial infection, not in an isolated bacterium (Cunha, 2000). The clinical problem emerges when susceptible strains are eliminated, permitting the resistant microbial flora to flourish and in some environments, to gain prominence.

Several key factors led to the increase in antibiotic resistance in the post-World War II era. First of all, there was a worldwide dismantling of the public health infrastructure, especially in the area of infectious disease surveillance (Berkelman *et al.*, 1994, Binder *et al.*, 1999 and Cohen, 1999). Complacency, decreased funding and resources and competing public health priorities led to a transfer of emphasis from infectious diseases to other areas (Cohen, 1999). These factors resulted in a weak public health surveillance system which was incapable of adequately evaluating the burgeoning problem of clinically significant antibiotic resistance. In addition, there was a disconnect between national and international public health systems which made it difficult to assess the problem on a larger scale. The recent threat of severe acute respiratory syndrome (SARS) has drawn attention to need for collective international public health action against diseases which threaten public safety, including antibiotic resistant diseases.

Then during the 1980s, major drug manufacturers turned their research away from developing new antibacterial treatments because the tools seemed to be in place for the elimination of infectious disease (Culotta, 1994). From the marketing perspective, it was

more profitable for pharmaceutical companies to turn their efforts toward other newer classes of drugs, such as those to combat chronic diseases, fungal and viral infections, that had a limited number of approved drugs, but a huge potential market. With a list of more than 100 approved antibiotics, there was a greater fight to obtain a share of the pharmaceutical market with new antibacterial agents. Research and development in this area became less profitable.

In recent years, there are a number of additional factors that are affecting the emergence and spread of resistant organisms. These factors include:

- *The development of mutations in resistance genes of bacteria.* Mutational events in bacteria were originally thought to be rare events (Cunha, 2000). However, mutations in genes that encode key metabolic functions play a key role in the development of resistance to drugs like rifampin, streptomycin, ethambutol and fluoroquinolones (Cunha, 2000). These types of mutations transform susceptible organisms into resistant ones. However, mutations in preexisting resistance genes may function to increase the level of resistance to specific antibiotics or expand the spectrum of resistance.
- *The exchange of genetic information among bacteria in which resistance genes are transmitted to new hosts.*
- *The development of environmental conditions in communities and hospitals that facilitate the development of resistant organisms.* Expanded use of antibiotics in hospitals and in sites outside hospitals (i.e., long-term care facilities, day care centers, animal feedlots and other agricultural sites) increase the selective pressure for resistant organisms to emerge in these settings.

- *The proliferation and spread of resistant bacteria.* Resistance originates as a local phenomenon but can quickly expand to global proportions. Any localized problem can be transported because international travel can carry organisms from one country to another.
- *The inability of some laboratory testing methods to detect emerging resistance phenotypes.* Recent proficiency testing studies have highlighted the difficulties that laboratories experience in detecting several of the newer bacterial resistance mechanisms with current laboratory methods (Tenover, 2001). Data from proficiency testing studies conducted by the Centre for Disease Control suggest that laboratories not only have difficulty detecting resistant strains, but often do not follow National Committee for Clinical Laboratory Standards (NCCLS) guidelines regarding the appropriate antibiotics to test and report (Tenover *et al.*, 1999 and NCCLS, 2000).

With growing concern over the impact of antibiotic resistant organisms, the Canadian Committee on Antibiotic Resistance (CCAR) released a report in 2002 which provided information on the burden that antibiotic resistance is creating for the Canadian health care system (CCAR, 2002). The CCAR estimates that drug susceptible infections currently cost between \$260 and \$553 million each year in Canada. Its model suggests that infections due to resistant organisms add \$14.2-25.5 million in direct hospitalization costs to the annual cost of health care in Canada. Screening of patients during hospital admission to detect carriers of resistant organisms add another \$10.3 million while the precautions needed to prevent the spread of resistant organisms between patients and carriers add \$15.9 million more to the budget. These figures will continue to increase as

the prevalence of drug resistance increases. Therefore, antibiotic resistance creates a substantial burden for the health care system.

2.2 Environmental Issues

Antibiotic resistance is directly related to antibiotic use (Baquero, 1996). In a bacterial population, a small number of organisms may be resistant to a particular antibiotic due to intrinsic resistance or chromosomal mutation. If their environment remains unchanged, resistance provides no advantage. However, when exposed to an antibiotic, resistant organisms will flourish while the majority of the population will be inhibited or killed.

The majority of antibiotic use actually occurs in two areas: in animals and the community. Within these areas combined, approximately 75% of the antibiotic use is thought to be of questionable therapeutic value (Harrison *et al.*, 1998).

There are three uses for antibiotics in animals: prophylaxis, treatment and growth promotion. Half of all antibiotics are used to treat animals as growth promoters in livestock and to rid cultivated produce of harmful bacteria (van den Bodaard and Stobberingh, 1999). The ideology behind their use as growth promoters is that antibiotics fed at subtherapeutic concentrations enhance the digestion of feed by reducing normal intestinal flora which competes with the host for nutrients and improve performance in food producing animals by preventing disease through the elimination of harmful intestinal bacteria (Bezoen *et al.*, 2001, McKellar, 1998 and Wegener *et al.*, 1999). The ongoing low-level dosing of antibiotics for growth and prophylaxis results in the development of resistance in bacteria in livestock by creating an environment where resistant organisms

may flourish. This selective pressure ultimately increases the likelihood of new resistant strains 'jumping' between animal species which are often kept in close proximity (WHO, 2001).

The amount of bacterial transference between animals and humans cannot be properly assessed because of a limited amount of data. However, Bezoen *et al.* (2001) suggested that there are four possible routes (two direct and two indirect) through which antibiotic resistant bacteria may be transferred between humans and animals.

- People directly in contact with animals or animal feces, such as farmers and slaughterhouse workers, are at increased risk of picking up bacteria which originated from the animal.
- There may be direct human to human spread of bacteria subsequent to human-animal exposure.
- The consumption of meat, fish, vegetables or fruit that has been contaminated with animal feces or intestinal contents may disseminate bacteria to humans.
- People who drink or use water that has been contaminated may be exposed to bacteria.

In 1997, the World Health Organization (WHO) showed concern over the excessive use of antibiotics in animals designated for human consumption as a growing risk to human health (WHO, 2001). Then in 1998, the European Union followed WHO recommendations by banning the use of antibiotics prescribed for animals that are also used as treatment for human infections and all antibiotic use for growth promotion in animals. Recent studies in Germany, Denmark and Switzerland indicate that the ban on avoparcin as a growth promoter is associated with a significant decrease in the prevalence

of vancomycin resistant enterococci in both poultry and humans (HealthCare Ontario, 2001).

The use of antibiotics in agriculture, as 'pesticides', is a common practice in many parts of Central and South America for prophylactic or therapeutic use against bacterial infections (Levy, 2001). In addition, the United States annually used approximately 300,000 pounds of antibiotics, especially tetracycline and streptomycin, as pesticides in agriculture during the 1990's (Levy, 1992 and Harrison *et al.*, 1998). The use of antibiotics in agriculture increases the geographic spread of these drugs through the distribution of produce. Antibacterial pesticides are diluted as rain and other natural events disperse them through the environment, creating widespread selective pressure for the development of resistance (Levy, 2001).

The primary contributor to antibiotic resistance is the use of these drugs in the community and hospital setting. The modern health care system is one in which health care professionals are overworked and under-informed about the problems of antibiotic resistance and are, therefore, often ill-equipped to deal with the large numbers of patients they encounter daily (WHO, 2001). Increased pressure often leads to misdiagnosis or unnecessary prescribing in an effort to prevent possible complications. It has been estimated that in North America physicians over-prescribe antibiotics by 50% (WHO, 2001). Appropriate prescribing can also be difficult when the organism is not identified, or even identifiable by culture, and/or laboratory culture results are returned several days after the initial consultation.

Add the pressure caused by patient demand to the basic pressures of our health care system and it creates quite a problem. Society seems to be suffering from a 'drug hunger'. Drug hunger has been described as the trend whereby patients are only

satisfied if a physician visit results in a prescription (Avorn *et al.*, 2000). The desire for drugs as the primary solution to society's health problems has been fueled by television, internet, magazine and newspaper advertisements (WHO, 2001). Focus groups were held with pediatricians and family physicians in Atlanta, Georgia, in 1998, to discuss their attitudes regarding antibiotic prescribing (Barden *et al.*, 1998). Most physicians agreed that their prescribing could be decreased and that parental/patient expectation was the principle reason that influenced them to prescribe antibiotics. In a 1997 study of physicians throughout Europe, they cited patient pressure as the number one reason why they prescribed the wrong antibiotics (WHO, 2001).

Patients often believe that most infections, regardless of cause, can be successfully treated with antibiotics and thus expect to receive a prescription from their physician for any perceived infection (WHO, 2001). Many randomized, placebo-controlled trials of acute bronchitis, cough and upper respiratory tract illnesses have shown that outcomes are similar between patients taking antibiotics and those given the placebo (Howie, 1976, Stott and West, 1976 and Brickfield *et al.*, 1986). However, of the patient-physician encounters for 'colds', upper respiratory tract infections and bronchitis in the United States in one year, 50% to 66% resulted in an antibiotic prescription (Gonzales *et al.*, 1997 and Metlay *et al.*, 1998). Certain patient demographic factors (female, young, white and residence in a rural area) are associated with an increased likelihood that antibiotics will be prescribed (Gonzales *et al.*, 1998). The use of antibiotic therapy when it is not warranted may create ideal conditions for the development of resistant organisms by creating an environment where resistance can be acquired through exposure to the drug or where naturally resistant organisms thrive.

The problem of resistance could be curbed by the appropriate and prudent use of antibiotics in both the community and agriculture. However, researchers need to determine the amount by which antibiotic use must be reduced and the extent to which a reduction in antibiotic consumption will affect antibiotic resistance in different microorganisms in order to establish guidelines that outline appropriate antibiotic use. There are several alarming trends in antibiotic prescribing patterns within Canada that should be noted, namely that children less than nine years of age receive proportionately more antibiotics than older individuals, family physicians write about 75% of all antibiotic prescriptions, antibiotics are often used to treat viral illnesses and second- and third-generation drugs are overused in preference to simpler ones (Wang *et al.*, 1998). The most effective approach to prevention of transmission of drug resistant pathogens is preventing the initial emergence of resistance through selected and appropriate use of antibiotics (Jarvis, 1996).

A wide variety of methods have been recommended for the improvement of antimicrobial use, including adherence to national or international guidelines, antimicrobial use evaluations, reporting of microbiology laboratory data to facilitate better antimicrobial use, antibiotic order forms, automatic stop orders, educational programs and regulation of promotional efforts by pharmaceutical representatives in the hospitals, etc. (Marr *et al.*, 1988 and Jarvis, 1996).

The selective role of antibiotic use relates not only to the total amounts being used, but also to how it is being used (Levy, 2001). Guillemot *et al.* (1998) showed that when penicillin was given in less than therapeutic doses for relatively long periods of time (five days), the patient's risk of developing penicillin-resistant *Streptococcus pneumoniae* increased. However, when the drug was used at a higher dose for shorter periods of time,

the emergence of penicillin-resistant pneumococci was much lower. Therefore, it would appear that low-level, prolonged usage of antibiotics optimally selects for bacterial resistance. If this practice were to be avoided, there would be less selective pressure for antibiotic resistant organisms.

The problem rests in the fact that the relationship between antibiotics and bacteria is not a simple one. It appears that in some geographic regions high antibiotic consumption does not lead to antibiotic resistance as rapidly as in other areas (Baquero, 1996). Levy (2001) has stated that how an amount of antibiotic is distributed among individuals within a geographic area influences the frequency of antibiotic resistant bacteria. He suggests that giving 1000 doses of an antibiotic to one individual will have considerably less ecological effect on resistance emergence than giving the same 1000 doses to 1000 individuals. This is referred to as the principle of selection density. Promotion of drug-resistant bacteria by antibiotics involves the number of individuals affected because they serve as the 'factories' of resistant bacteria and the density of these individuals in a particular environment. As the density of treated people increases, the number of resistant strains increases and ultimately, the number of susceptible strains able to survive in the environment decrease. However, in order to return to a normal ecology with susceptible microorganisms, an environment requires greater numbers of susceptible bacteria.

Studies of newly emerging resistance show that resistance in bacteria primarily arises in steps progressing from low level to high level, unless a plasmid is acquired on which resistance is already present (Levy, 1998). Initially, penicillin resistant pneumococci appeared with slightly decreased susceptibility to penicillin but over time strains have evolved to a high level of resistance. Therefore, it is important to determine the level of

resistance in a population early enough to prevent the development of high level resistance in organisms.

2.3 Current Research

Mathematical models are being used as a means of studying the emergence and spread of resistant bacteria in both the hospital and community settings. Although the number and relationship between factors contributing to resistance are complex, mathematical models often only use a subset of factors which are thought to be of prime importance (Bonten *et al.*, 2001). These models use the volume of drug use as the primary selection pressure driving changes to the frequency of resistance in the community (Austin and Anderson, 1999). However, it is difficult to determine the precise quantitative relationship between antibiotic consumption and antibiotic resistance because of a lack of longitudinal studies which record both consumption and resistance patterns (Austin and Anderson, 1999). Instead, mathematical models allow an estimation of how altering various factors, such as population genetics, transmission dynamics and antibiotic consumption, will alter the development of resistance in the community.

There has been a great deal of interest in determining the social, demographic and environmental factors which are contributing to the development and dissemination of resistant microbes globally. The main social factors which are contributing to resistance are poor patient compliance, excessive antibiotic usage in humans and animals and a lack of education regarding bacterial disease and antimicrobial therapy (Okeke *et al.*, 1999). These factors are common in both developing and industrialized countries. However, industrialized countries have more money and resources to combat the problem more effectively. Therefore, many wealthy countries have focused their efforts on fighting

infectious disease within their own borders, while failing to help eliminate them globally. However, bacteria, viruses, and parasites know no boundaries. They mutate elsewhere, become drug resistant and enter the wealthy countries via modern transportation, creating an international problem. This theory has generated much discussion throughout the world about the importance of surveillance schemes for antibiotic resistance. WHO (2001) now calls for surveillance of antibiotic resistance and antibiotic use at both local and national levels to guide clinical management and infection control, to monitor treatment guidelines, to update lists of essential drugs and to monitor the effectiveness of interventions to contain resistance. At the present time, there are no formal mechanisms or international documents that require reporting of resistance levels by individual countries. Instead, local and national resistance levels are identified through individual research projects that are published in scientific journals (WHO, 2001). Containing the problem of antibiotic resistance must involve cooperation between national governments and agencies, professional societies, non-governmental organizations and international agencies.

A major problem with resistance research is determining which studies depict the most accurate picture of resistance in a community. Within the community, the association between the increasing prevalence of resistant organisms and the severity or duration of infection is much less obvious than in the hospital setting where it has been shown to increase mortality.

There has been research directed at finding alternative forms of antimicrobial treatment (i.e., drugs, vaccines) against infections which have been shown to be caused by resistant microbes. However, bacteria are evolving at a much greater speed than new drugs are being developed. This is partially due to the fact that the pharmaceutical

industry is very competitive and thus, some companies are not willing/able to spend the time, money and effort needed to develop new antibiotics when there is no guarantee of eventual profit (Culotta, 1994). The pharmaceutical industry has reported that the research and development of antimicrobial agents take 10 to 20 years and it costs a minimum of US\$500 million to bring a single drug to market (WHO, 2001). There is also a lack of interest among companies in developing therapies for infections that primarily affect resource-poor regions of the world because of the limited potential to earn a profit (WHO, 2001).

2.4 Antibiotic Susceptibility Testing Methodologies

Susceptibility testing is performed in the laboratory and does not necessarily reflect or predict *in vivo* efficacy. Susceptibility testing is subject to great variability depending on the pathogen tested, media used, conditions of incubation and method of accessing bacterial growth. The ability to detect the emergence and global spread of resistant organisms is hampered by the weakness, or total lack, of adequate surveillance of antimicrobial resistance (Cunha, 2000). Interpretation of existing surveillance data is hampered by the multiplicity of methods used to detect resistance and by difficulties in assessing the quality of the data. Even where good quality resistance surveillance data exist, they are frequently not translated into information for public health action.

Two commonly used techniques which allow susceptibility profiles to be generated for various species of bacteria are disk diffusion and the E-test system. The agar disk diffusion method involves placing a disk impregnated with a specific concentration of antibiotic on an inoculated blood agar plate and then measuring the zone of inhibition between the middle of the disk and the edge of the bacterial growth with a ruler to

determine whether the organism is susceptible or resistant to the antibiotic. The zone of inhibition is measured in millimeters.

The E-test system is a convenient and easy method for determining the minimum inhibitory concentration (MIC) of antibiotic to which bacterial isolates are susceptible. It consists of a reagent strip, which contains a continuous concentration gradient of antibiotic that diffuse into the agar onto which the organism has been inoculated. The MIC value is read from the point where the elliptical zone of inhibition intersects the E-strip and is measured in mcg/mL. It provides an *in vitro* method for quantitative antimicrobial susceptibility testing which is more expensive than the traditional methods. However, the E-test system has become quite popular in recent years because it combines the simplicity and flexibility of disk diffusion with the opportunity to determine MIC value of a specific antimicrobial agent for a particular bacterial isolate (Kiska *et al.*, 1995 and Murray, 1995).

MIC values are used as a measurement of antibiotic resistance and are defined as the drug concentration that prevents macroscopic growth after overnight incubation of the inoculated plates at 35 °C. The readings are always taken from the area where there is a complete inhibition of all growth, including hazes and isolated colonies. If there is no inhibition along the entire strip it is interpreted that the MIC is greater than the highest value on the scale. Likewise, if the inhibition zone is below the end of the strip, it is interpreted that the MIC is lower than the lowest value on the scale. MIC value are interpreted using the National Committee for Clinical Laboratory Standards (NCCLS). NCCLS breakpoints (i.e., susceptible, intermediate and resistant) are based on achievable levels of antibiotic in the blood and the clinical data on response to treatment (NCCLS, 1997). MIC₅₀ and MIC₉₀ are given to describe the MIC value for 50% and 90% of the

isolates tested, respectively.

NCCLS categories of intermediate and resistant (sometimes referred to as high resistance) both refer to isolates which have decreased susceptibility to antibiotics. Strains with intermediate level resistance have MIC values which are 10 - 99 times greater and strains with high level resistance have MIC values which are 100 -10,000 times greater than those for susceptible strains.

When comparing methodologies, it is important to look at two measurements; sensitivity and specificity. Sensitivity is the ability of a test to identify which isolates are susceptible to an antibiotic while specificity refers to the ability to detect which isolates are resistant to an antibiotic.

Several types of interpretative error may occur when comparing methodologies. A very major error is assumed to be present when a strain is classified as resistant by the reference method, such as the E-test system, and as susceptible by the method being tested (Chaves *et al.*, 1999). If the reverse scenario occurs, whereby the strain is classified as resistant by the method being tested and susceptible by the reference method, it is considered to be a major error (Chaves *et al.*, 1999). Finally, if a third susceptibility category (intermediate resistance) has been identified, a minor error may occur. A minor error is considered to be present when strains are either (i) classified as intermediate or resistant by the reference method and, respectively, as susceptible or intermediate by the method being tested or (ii) classified as intermediate or susceptible by the reference method and, respectively, as resistant or intermediate by the method being tested (Chaves *et al.*, 1999).

Many recent studies of antibiotic resistance, especially in North America, use the E-test methodology for determining susceptibility patterns since this system is more accurate

than other testing techniques, such as disk diffusion (Lund *et al.*, 1998). Disk diffusion is sensitive, but not very specific and is generally considered acceptable as a screening test only. The E-test system is much more specific and thus, can be used both as a surveillance tool in order to detect changing levels of resistance and to generate susceptibility profiles for bacteria which will be used to guide antibiotic use. However, the E-test system is also a very expensive tool. Each E-test strip costs approximately \$1.00 while each disk costs a few cents. In hospital laboratories, where many specimens must be tested daily to determine susceptibility patterns to a number of different antibiotics, the use of the E-test system is quite costly. Since susceptibility testing is such an important component of drug selection for the treatment of infections caused by *S. pneumoniae*, it is crucial that the methodology used be both accurate and financially viable.

2.5 Drug resistant *Streptococcus pneumoniae*

Streptococcus pneumoniae, bacteria which are also known as pneumococci, are gram-positive cocci that grow in chains and are catalase negative. They produce pneumolysin, a toxin that breaks down hemoglobin into a greenish degeneration product, thereby causing alpha hemolysis on blood agar. More than 98% of pneumococcal isolates are susceptible to optochin and virtually all pneumococcal colonies are dissolved by bile salts. Nearly every clinical isolate of pneumococcus has a polysaccharide capsule, resulting in 90 known distinct capsules.

Pneumococci colonize the nasopharynx and can be isolated from 5 to 10% of healthy adults and from 20 to 40% of healthy children (Harrison's Textbook of Medicine Online, 2003). Once the organisms have colonized an adult, they are likely to persist for two to four weeks but may persist for as long as six months. Pneumococci spread from

one individual to another as a result of extensive close contact. For reasons that are unclear, certain populations, including Native Americans, Native Alaskans and African Americans, appear to be unusually susceptible to invasive pneumococcal disease (Harrison's Textbook of Medicine Online, 2003).

Pneumococci attach to human nasopharyngeal cells through the specific interaction of bacterial surface adhesions with epithelial cell receptors. Once the nasopharynx has been colonized, infection results if the organisms are carried into anatomically contiguous areas, such as the eustachian tubes or nasal sinuses, and if their clearance is hindered. The capacity to cause disease depends on two factors; 1) the ability of pneumococci to escape ingestion and killing by host phagocytic cells and 2) its ability to stimulate an inflammatory response and damage tissues.

The first isolate of *Streptococcus pneumoniae* was collected 110 years ago (Jacobs, 1992). Since then, it has been recognized to be the leading cause of community-acquired pneumonia and other infections, such as sinusitis, otitis media, meningitis and bacteremic septicemia (Tomasz, 1997). Infection with *S. pneumoniae* is the most common cause of potentially life-threatening, community acquired disease, such as meningitis and pneumonia. Each year in the United States, pneumococcal disease accounts for an estimated 3000 cases of meningitis, 50 000 case of bacteremia and 500 000 cases of pneumonia (Reichler *et al.*, 1992 and Stool and Field, 1989). Each year in Canada, it accounts for 5000 cases of bacteremia, 700 000 cases of otitis media and 12 500 cases of pneumonia resulting in hospitalization (Canadian Bacterial Surveillance Network, 2002). Before the discovery of antibiotics, the case-fatality rate for bacteremic pneumococcal pneumonia was greater than 75% (Tilghman and Finland, 1937). After the introduction of penicillin therapy for pneumococcal pneumonia, the case-fatality rate

dropped to 5% to 8% (Austrian, 1999). However, studies have recently shown that the case-fatality rate for bacteremic pneumococcal pneumonia ranges from 7% to 35% (Fine *et al.*, 1996, Kramer *et al.*, 1987, Ortqvist *et al.*, 1988, Afessa *et al.*, 1995, Marrie, 1992 and Bruyn *et al.*, 1988).

Within developed countries, invasive disease caused by this organism is a serious problem among the elderly, infants and those individuals with chronic underlying medical conditions (e.g., malignancy or human immunodeficiency virus) or immune systems that are compromised, either because of disease or immunosuppressive therapy. For patients who are immunodeficient, including those with HIV, the attack rate of pneumococcal infection is ten times that of the 'normal population' (Austrian, 1999). It has also been estimated that pneumococcal pneumonia results in the death of more than one million children each year in developing countries, approximately half of whom are less than one year of age (Buck *et al.*, 1999).

Until recently, isolates of *S. pneumoniae* were uniformly susceptible to penicillin (Borek *et al.*, 1997). Pneumococci were considered to be among the most highly penicillin-susceptible bacteria noted throughout the first quarter-century of penicillin use (Tomasz, 1997). For example, during the 1940's, three of every four persons who were treated with penicillin survived when only a decade earlier, approximately four of every five persons died of pneumococcal pneumonia (Mufson, 1998). Therefore, penicillin was prescribed by physicians as 'the drug of choice' to combat pneumococcal infections. However, things started to change during the 1960's when the first strains of pneumococci with intermediate levels of penicillin resistance began to appear.

Clinical pneumococcal resistance to penicillin was first recorded by researchers in Boston in 1965 when two of 200 strains showed minimum inhibitory concentrations (MICs)

within the intermediate resistance range (0.1 - 0.2 mcg/mL) (Appelbaum, 1992). However, the clinical significance of penicillin resistant *S. pneumoniae* was not appreciated until 1967 when Hansman and Bullen recovered a resistant isolate (MIC 0.6 mcg/ml) from the sputum of a 25-year-old female patient with hypogammaglobulinemia and bronchiectasis in Australia. Another pneumococcal isolate showing a similar pattern of resistance was recovered from an aboriginal patient in Papua, New Guinea in the same year (Gold *et al.*, 1996 and Tomasz, 1997).

Regardless of where the first resistant isolate was identified, resistant strains appeared to spread rapidly through Australia and New Guinea during the 1960's, through South Africa in the 1970's and throughout countries in Africa, Asia and Europe during the 1980's (Jernigan *et al.*, 1996). By the mid-1970's, highly resistant strains of pneumococci were being described elsewhere throughout the world (Gold *et al.*, 1996). In contrast to the first isolates which exhibited slightly elevated MIC values, the later strains were shown to have a dramatically increased MIC of penicillin as well as resistance to multiple antimicrobial agents, such as tetracycline, erythromycin, chloramphenicol, clindamycin, streptomycin and, in some cases, rifampin (Tomasz, 1997). The incidence of penicillin resistant *S. pneumoniae* increased during the succeeding years and continues to do so today.

The development and progression of penicillin-resistant strains of *S. pneumoniae* primarily occur in the community setting while resistance in other organisms, such as vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*, initially emerged as nosocomial infections (Jacobs, 1999). It has been suggested that antibiotic resistance has probably emerged in the community as a result of a variety of factors

including; clustering and overcrowding, the increased number of immunocompromised patients, an increase in the number of elderly people in the population, increased travel, the widespread use of broad spectrum antibiotics, self treatment with antibiotics, the inappropriate use of antibiotics, a lack of compliance with treatment, fewer resources for in-service training of health workers, a lack of resources for infection control and decreased funding for public health surveillance. Emphasis has been placed on excessive selective pressure (i.e., overuse of antibiotics) and close exposure to carriers for being the major factors for acquiring drug-resistant *S. pneumoniae* (DRSP) (Klugman, 1990).

Many scientists have been trying to determine the current rates of penicillin resistance in North America and so far there have been a number of interesting results. A study of 845 respiratory tract isolates from 27 United States medical centers during the late 1990's showed an increase in the prevalence of penicillin resistant *S. pneumoniae* (PRSP) of 43.8% (Doern *et al.*, 1998). The proportion of PRSP in the United States has jumped from 6.6% in 1991 - 1992 to 33.5% in 1996-1997 (Campbell and Silberman, 1998). A similar trend has been noted for Canada where PRSP was uncommon in the 1980's but has seen dramatic increases in recent years. The National Centre for *Streptococcus* in Edmonton noted an overall antibiotic resistance of 10.2% and a penicillin resistance of 7.6% for *S. pneumoniae* within Canada during 1996-1997 (Buck *et al.*, 1999). Although rates of resistance for this organism have traditionally been higher in the United States than in Canada, it appears that such differences are decreasing as resistance continues to grow rapidly in Canada (Doern *et al.*, 1998). Currently, there is no information on the rates of pneumococcal resistance in Newfoundland and Labrador.

Drug resistant *S. pneumoniae* appears to be increasing in prevalence at an alarming rate. However, it is difficult to compare how the prevalence of resistant pneumococci is changing or whether there are meaningful differences between population groups because of methodologic differences in susceptibility testing between various regions and countries. New therapeutic strategies are needed to avoid further selection of the resistant strains that are colonizing and/or infecting patients, especially among pediatric populations.

The Canadian Laboratory Centre for Disease Control (LCDC) guidelines released in 1998 stated that all *S. pneumoniae* isolates obtained from sterile body sites should be tested for antibiotic resistance. Sterile body sites include blood, cerebrospinal fluid, joint aspirates, pleural fluids, abscesses and any other site where there is clinical indication of a life-threatening infection. These isolates should be tested against penicillin (oxacillin), cefotaxime and/or ceftriaxone and vancomycin. The guidelines also stated that isolates from non-sterile sites do not require routine susceptibility testing (LCDC, 1998). However, they may be tested periodically to assess the degree of resistance of *S. pneumoniae* to various antibiotics commonly used to treat pneumococcal infections, such as penicillin (oxacillin), macrolides, cefuroxime, cefotaxime or ceftriaxone, co-trimoxazole, clindamycin or tetracycline. Incubation in CO₂ for the pneumococcal E-test improves the agreement with MIC results determined by NCCLS broth microdilution method and also, avoids the problem caused by the fact that some pneumococcal strains do not grow readily under ambient air incubation conditions (Jorgensen *et al.*, 1994).

Antibiotics which may be prescribed for pneumococcal infections include penicillin, tetracycline, erythromycin, levofloxacin, ceftriaxone and meropenem. Penicillin belongs to the antibiotic group of the same name and is known to inhibit a number of bacterial

enzymes, namely penicillin binding proteins (PBPs), which are essential for bacterial peptidoglycan synthesis. Tetracycline also belongs to the antibiotic group of the same name and inhibits protein synthesis. Erythromycin belongs to the macrolide group of antibiotics. The macrolides inhibit bacterial RNA-dependent protein synthesis. Levofloxacin is a relatively new antibiotic and belongs to the quinolone group of drugs. The quinolones target DNA gyrase, an enzyme needed for bacterial DNA synthesis. Ceftriaxone is a third generation cephalosporin. This group is known to penetrate through the outer cell envelope of gram-negative organisms. Meropenem belongs to the carbapenem group of antibiotics. Like the penicillin drugs, this group binds to PBPs, causing cell elongation and lysis.

The relationship between the frequency and type of antibiotic usage and the prevalence of pneumococcal penicillin resistance is well recognized and prescription patterns are closely linked with resistance trends (Baquero, 1996). During a 1992-1993 study of antibiotic resistance and antibiotic consumption within five countries of the European Union and the United States, it was shown that the lowest rates of Beta-lactam prescription occurred in Germany and Italy which had the lowest levels of penicillin resistant *S. pneumoniae* while the highest consumption and resistance levels occurred in Spain and France (Baquero, 1996). This illustrates the importance of studying the levels of antibiotic consumption within the community when looking at the levels of pneumococcal resistance within a population.

Baquero (1996) also showed that there are other factors which are associated with antibiotic resistant pneumococci in a population. While the prescription rates of aminopenicillins and Beta-lactams in the United Kingdom approached that of France and were higher than that of the United States, the level of penicillin resistance in the United

Kingdom was lower than that of both these countries. Therefore, the selective effect of antibiotic consumption on antibiotic resistant *S. pneumoniae* may also be dependent on the structure and density of the different populations present at a given moment in a particular area (Baquero, 1996).

In 1945, MacLeod, Hodges, Heidelberger and Bernhard reported that purified pneumococcal capsular polysaccharides were effective in preventing pneumococcal infections (MacLeod *et al.*, 1945). They found that if one were a carrier of a pneumococcal strain that was contained in the vaccine, immunization would not eliminate the carrier state. However, if a person were not a carrier before vaccination, the likelihood of becoming one after vaccination was reduced by half. A 23-valent polysaccharide pneumococcal vaccine, known as Pneumovax™, has been available for commercial use since the 1980s. The polysaccharide antigens are used to induce type-specific antibodies that enhance killing of pneumococci by phagocytic cells.

As indicated in 1945, the polysaccharide pneumococcal vaccine appears to reduce carriage and the spread of *S. pneumoniae* (McCormick, 1998). Many antibiotic resistant pneumococcal isolates belong to one of the 23 capsular types contained in the vaccine (Rossi *et al.*, 1997, Dagan *et al.*, 1996, Hortal *et al.*, 1997 and Luey and Kim, 1996).

In the early 1990s, it was estimated that 40,000 people died annually in the United States from pneumococcal infections which were vaccine preventable (Gardner and Schaffner, 1993). This was partially due to the fact that polysaccharide vaccines are not immunogenic in children less than two years of age which rendered Pneumovax™ useless in this age group. Then in 2000, a protein conjugate pneumococcal vaccine, known as Prevnar™, was introduced. Prevnar™ was aimed at reducing the incidence of invasive

pneumococcal disease in children. The conjugated pneumococcal vaccine elicits a T-cell dependent memory response and protects against seven common serotypes.

Currently, Prevnar™ is considered the vaccine of choice in children while Pneumovax™ is widely used in the adult population. Successful vaccination is indicated by a twofold antigen-specific antibody response within two to three weeks after receiving the injection.

2.6 Objectives

There has been limited research into the problem of pneumococcal antibiotic resistance or community antibiotic use in the province of Newfoundland and Labrador. This research project is intended to provide the baseline data regarding the level of antibiotic resistance of *Streptococcus pneumoniae* and antibiotic consumption for the province of Newfoundland and Labrador. The objectives of this project are to:

- 1.) To describe the resistance levels of *Streptococcus pneumoniae* within the different regions of Newfoundland and Labrador.
- 2.) To determine the relationship between various factors, including specimen source, patient age, patient sex and population density, and antibiotic resistance.
- 3.) To compare the accuracy of disk diffusion versus E-test methodology.
- 4.) To describe the trends in antibiotic consumption within Newfoundland and Labrador.

3.0 MATERIALS AND METHODS

3.1 Protocol

This research project began as a part of the existing 'Optimal Antibiotic Project' which had been granted ethical approval by the Human Investigations Committee at Memorial University of Newfoundland. The Optimal Antibiotic Project began in 1997 and was designed to measure resistance in bacteria, measure antibiotic use and measure health care resource use related to community acquired infections (Appendix C).

Ten hospital laboratories throughout the island portion of the province of Newfoundland and Labrador were invited to participate in this project at a meeting held with the laboratory directors. The sites which participated in this study included St. John's, Clarenville, Gander, Grand Falls, Corner Brook, Burin, Carbonear, Placentia and Stephenville. Laboratory selection was based on geographical location, thereby ensuring that the isolates would be received within several days in order to maintain viability of the organisms. For this reason pneumococcal isolates from laboratories in Labrador and St. Anthony were not included in this study.

Pneumococcal isolates from each of the sites were sent to the Provincial Public Health Laboratory (PHL), using a standard protocol already established by the PHL (Appendix A). The susceptibility testing of all clinically significant pneumococcal isolates began in January 1, 2000 and was completed in December 31, 2000. Clinically significant isolates were defined as those either from normally sterile sites (i.e., cerebrospinal fluid and blood) or from specimens that made contact with mucosal surfaces (i.e., sputum). The number of isolates tested was dependent on the number submitted by the

participating laboratories. The PHL annually receives approximately 80 isolates in total from the participating laboratories. However, the anticipated sample size for this study was 100 isolates.

Patients' sex, age, specimen site and geographic location were recorded.

Surveillance swabs were excluded, as were duplicates of isolates. Surveillance swabs are isolates that are taken specifically to detect the level of resistance in *S. pneumoniae* and are not clinically important for the treatment of an individual. Since surveillance swabs are not clinically significant they were eliminated from this study. Each isolate was given an identification number at the PHL which prevented any means of identifying the patient directly.

Once a bacterial isolate was submitted to the hospital laboratory, it became the property of that facility, thereby eliminating the need to request permission from the patient to use the specimen for research purposes. Instead, permission to use the pneumococcal isolate was granted by the director of the laboratory.

Each isolate was identified as *S. pneumoniae* at each participating lab based on its susceptibility to optochin prior to being sent to the PHL. *S. pneumoniae* has maintained uniform susceptibility to optochin. Therefore, in the laboratory setting an organism is identified as pneumococcus if it is susceptible to the drug.

3.2 Antibiotic Sensitivity Testing

Susceptibility testing was performed at the PHL. All isolates were tested by both systems described above for their sensitivity to the following antibiotics; penicillin, tetracycline, erythromycin and levofloxacin. If an isolate was shown to be resistant to

penicillin, its susceptibility was tested to ceftriaxone and meropenem. Bacterial suspensions were prepared in trypticase soy broth (TSB) using cultures grown under 5 % CO₂ at 35 - 36 °C on sheep blood agar (BA) plates (Appendix B). The turbidity of the suspension was adjusted to a 0.5 McFarland density standard in sterile saline or to a 1.0 McFarland standard if the isolate was mucoid. Within 15 minutes of the preparation, the inoculum was uniformly spread on fresh BA plates by using a sterile swab and the plates were allowed to dry for 10 - 15 minutes before applying the strips and disks. Each plate prepared contained a disk and E-strip containing the same antibiotic as a quality control measure. All plates were then incubated at 35 °C in 5 % CO₂ for 16 - 18 hours.

The oxacillin screen was also used as an indicator of penicillin resistance. Isolates with an oxacillin zone diameter greater than 20 mm are considered to be susceptible to penicillin and no further testing is done. However, a zone diameter of less than 20 mm does not always indicate penicillin resistance and requires further testing, in order to determine the specific MIC.

3.3 Antibiotic Utilization

Information on antibiotic use was collected from all outpatient pharmacies in Newfoundland using the structure developed by IMS HEALTH Canada. The information generated by this organization was expressed in the number of prescriptions filled within the province. IMS HEALTH was established in 1960 and currently serves as a leading source of information regarding the Canadian health care system. In addition to a variety of products and services provided by IMS HEALTH Canada, it also monitors antibiotic prescription activity by regular CompuScript audits. The IMS prescription database panel

is comprised of 4,400 pharmacies, which amounts to approximately two-thirds of all retail pharmacies in Canada. Of these pharmacies, 2,100 stores make up the CompuScript panel which then, provide monthly records of their prescription activity. This data can then be used to generate information regarding the trends in prescribing for a variety of drugs, including antibiotics, based on a classification system established by IMS HEALTH.

The internationally accepted format for describing antibiotic consumption is the World Health Organizations Anatomical Therapeutic Classification (ATC) System in Defined Daily Doses (DDD). The DDD is defined as the assumed average maintenance dose per day for a drug used on its main indication in adults. Prescription data monitored and presented in DDDs provide only an estimate of consumption and not an exact picture of actual use. Instead, this system is considered to be a pharmacoepidemiological tool which may be used to measure, analyze and influence the use of drugs and to detect changes over time. The ATC/DDD System allows comparisons between different settings, regions or even countries, which is also independent of sales prices and package size. Although the ATC/DDD System is the preferred method for describing antibiotic consumption, IMS HEALTH Canada continues to generate pharmaceutical information in number of prescriptions filled. Therefore, this study will analyze how much antibiotic was actually consumed by the Newfoundland population through the number of prescriptions filled.

3.4 Statistical Analysis

Statistical analysis was performed with SPSS (Version 8) and Microsoft Excel. Descriptive statistics were used to compare susceptibility profiles based on region, patient

age, patient sex and specimen source. Crosstabulations were used to detect isolates which exhibited multiple resistance. The antibiotic use information was compared for the different drugs of interest over a four year period.

4.0 RESULTS

4.1 Patient Demographics

There was a total of 83 isolates of *S. pneumoniae* submitted for this study between January 1 and December 31, 2000. Three of these isolates were submitted to the PHL without the appropriate patient and regional information and thus, had to be excluded from the demographic analysis. Therefore, 80 isolates were obtained with all or some of the appropriate demographic information. Of these, six isolates lacked information regarding the patient's sex, eight isolates lacked information on the source from which the specimen was taken, three lacked information on the region from where it was collected and four isolates lacked information on the age of the patient. However, all 83 isolates were used in the comparison of the two antibiotic resistance testing methodologies.

The regional distribution of 77 pneumococcal isolates included in this study is shown in Table 1. The greatest portion of isolates tested was submitted by the Health Care Corporation of St. John's (n = 54, 70.1%), followed by the Central West Health Corporation (n = 12, 15.6%). Table 1 also shows that the Central East Health Care Institutions Board and the Western Health Care Corporation submitted four isolates (5.2%) each, two isolates were obtained from the Peninsulas Health Care Corporation (2.6%) and one isolate was submitted by the Avalon Health Care Institutions Board (1.3%).

Due to the small number of pneumococcal isolates received from hospitals outside of St. John's, further analysis of the demographic data was considered on the basis of their location in relation to St. John's (i.e., hospital laboratories within St. John's

Table 1: Regional distribution of isolates used to determine the antibiotic susceptibility patterns of *S. pneumoniae* within Newfoundland from January 1 to December 31, 2000.

Region	Frequency	Percent (%)
Health Care Corporation of St. John's	54	70.1
Avalon Health Care Institutions Board	1	1.3
Peninsulas Health Care Corporation	2	2.6
Central East Health Care Institutions Board	4	5.2
Central West Health Corporation	12	15.6
Western Health Care Corporation	4	5.2
TOTAL	77	100

or outside of St. John's). Table 2 shows the distribution of isolates on the basis of patient age, sex and specimen source in relation to the geographic location of the laboratory from which they were obtained. Higher proportions of pneumococcal isolates were retrieved from children and senior citizens than other adults and non-sterile site sources predominated in both locations (Table 2). The number of isolates retrieved from males and females was approximately equal (Table 2).

4.2 Methodology Analysis

When comparing the two methodologies (disk diffusion versus E-test) used for susceptibility testing in this study, the E-test system was considered to be the 'gold standard'. Table 3 compares the use of oxacillin disks versus penicillin E-test strips for determining pneumococcal susceptibility to penicillin. The disk diffusion system classified 65 isolates as penicillin susceptible and 18 isolates as being penicillin resistant while the penicillin E-strips identified 64 isolates as susceptible and 19 as resistant (Table 3). One minor error was present since one isolate was identified as having intermediate resistance by E-test and susceptible by disk diffusion. Oxacillin disks have a sensitivity of 100% and a specificity of 94.7%.

Table 4 compares the use of disk diffusion versus E-test for determining pneumococcal susceptibility to erythromycin. The erythromycin disks categorized 79 isolates as being antibiotic susceptible and four as resistant. However, when the same isolates were tested using erythromycin E-strips (gold standard), 78 isolates were

Table 2: Distribution of isolates used to determine the antibiotic susceptibility patterns of *S. pneumoniae* within Newfoundland from January 1 to December 31, 2000 on the basis of geographic location, age, sex and specimen source .

FACTOR	GEOGRAPHIC LOCATION		TOTAL (%)
	<i>St. John's</i>	<i>Outside St. John's</i>	
Age			
=/<18 (Pediatric)	18	10	28 (36.8)
19-49 (Adult)	11	5	16 (21.1)
=/>50 (Elderly)	24	8	32 (42.1)
			76
Specimen Source			
Sterile	17	1	18 (25.0)
Non-sterile	36	18	54 (75.0)
			72
Sex			
Male	23	12	35 (47.3)
Female	28	11	39 (52.7)
			74

Table 3: Comparison of surveillance methodologies used to detect penicillin resistance.

	E-test Susceptible	E-test Resistant	Total
Disk Susceptible	64	1	64
Disk Resistant	0	18	18
Total	64	19	83

$$\text{Sensitivity} = \frac{64}{64} = 100\%$$

$$\text{Specificity} = \frac{18}{19} = 94.7\%$$

Table 4: Comparison of surveillance methodologies used to detect erythromycin resistance.

	E-test Susceptible	E-test Resistant	Total
Disk Susceptible	78	1	79
Disk Resistant	0	4	4
Total	78	5	83

$$\text{Sensitivity} = \frac{78}{78} = 100\%$$

$$\text{Specificity} = \frac{4}{5} = 80.0\%$$

classified as antibiotic susceptible and five isolates were classified as having high resistance to erythromycin (Table 4). A very major error was present since the erythromycin E-test identified one isolate as being resistant while disk diffusion classified it as susceptible. The erythromycin disks had a sensitivity of 100% and a specificity of 80.0%.

Table 5 compares the use of disk diffusion versus E-test for determining pneumococcal susceptibility to tetracycline. Using the tetracycline disks, 81 isolates were categorized as being antibiotic susceptible and two were resistant. However, using tetracycline E-strips, 80 isolates were classified as antibiotic susceptible and three isolates were classified as being resistant. Of the three resistant isolates, one was defined as having intermediate resistance and two were classified as having high resistance to tetracycline. A single minor error was present since the tetracycline E-test identified one isolate as intermediate and disk diffusion identified it as susceptible. The tetracycline disks have a sensitivity of 100% and a specificity of 66.7%.

4.3 Susceptibility Profile Analysis

Table 6 summarizes the overall levels of resistance detected in the pneumococcal isolates analyzed during this study. The greatest level of resistance was seen toward penicillin with 77.1% (n = 64) being susceptible and 22.9% (n = 19) having intermediate resistance (Table 6). Tetracycline was the only antibiotic tested to which both intermediate (n = 1, 1.2%) and high (n = 2, 2.4%) resistance was seen (Table 6). Only five isolates (6.0%) were classified as having high resistance to erythromycin and one (1.2%) was classified as having high resistance to levofloxacin (Table 6). No resistance was detected

Table 5: Comparison of surveillance methodologies used to detect tetracycline resistance.

	E-test Susceptible	E-test Resistant	Total
Disk Susceptible	80	1	81
Disk Resistant	0	2	2
Total	80	3	83

$$\text{Sensitivity} = \frac{80}{80} = 100\%$$

$$\text{Specificity} = \frac{2}{3} = 66.7\%$$

Table 6: Susceptibility profile of all isolates as indicated by the E-test system received during the study period (January 1, 2000 to December 31, 2000)

Antibiotic	Frequency - Number(%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	64 (77.1)	19 (22.9)	-	83
Tetracycline	80 (96.4)	1 (1.2)	2 (2.4)	83
Erythromycin	78 (94.0)	-	5 (6.0)	83
Levofloxacin	82 (98.8)	-	1 (1.2)	83
Ceftriaxone	19 (100)	-	-	19
Meropenem	19 (100)	-	-	19

toward ceftriaxone or meropenem.

Of the 54 isolates received from the Health Care Corporation of St. John's, 46 (85.2%) were penicillin susceptible while eight (14.8%) had intermediate resistance to penicillin (Table 7). There were 52 isolates (96.3%) classified as being susceptible and two (3.7%) with high resistance to tetracycline (Table 7). There were also 51 isolates (94.4%) with erythromycin susceptibility and three isolates (5.6%) with high resistance to erythromycin (Table 7). All of the isolates submitted by the Health Care Corporation were shown to be susceptible to levofloxacin, ceftriaxone and meropenem.

Of the 23 isolates received from the participating laboratories outside of the Health Care Corporation of St. John's, 13 (56.5%) were penicillin susceptible while 10 (43.5%) had intermediate resistance to penicillin (Table 8). There were 22 isolates (95.7%) classified as being susceptible and one (4.3%) with high resistance to tetracycline (Table 8). There were also 22 isolates (95.7%) with erythromycin susceptibility and one isolate (4.3%) with high resistance to erythromycin (Table 8). All of the isolates were susceptible to levofloxacin, ceftriaxone and meropenem.

Table 9 shows the susceptibility profile of the 20 isolates obtained from sterile sites. Of these isolates, 17 (85.0%) were penicillin susceptible and three (15.0%) had intermediate resistance to penicillin. When looking at erythromycin susceptibility, 19 (95.0%) were susceptible and one (5.0%) had high resistance. None of the pneumococcal isolates was shown to have resistance to tetracycline, levofloxacin, ceftriaxone or meropenem.

Table 10 shows the susceptibility profile of the 55 isolates obtained from non-sterile

Table 7: Susceptibility profile of isolates received from the Health Care Corporation of St. John's

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	46 (85.2)	8 (14.8)	-	54
Tetracycline	52 (96.3)	-	2 (3.7)	54
Erythromycin	51 (94.4)	-	3 (5.6)	54
Levofloxacin	54 (100)	-	-	54
Ceftriaxone	8 (100)	-	-	8
Meropenem	8 (100)	-	-	8

Table 8: Susceptibility profile of isolates received from hospital laboratories outside of St. John's

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	13 (56.5)	10 (43.5)	-	23
Tetracycline	22 (95.7)	-	1 (4.3)	23
Erythromycin	22 (95.7)	-	1 (4.3)	23
Levofloxacin	23 (100)	-	-	23
Ceftriaxone	10 (100)	-	-	10
Meropenem	10 (100)	-	-	10

Table 9: Susceptibility profile of isolates received from sterile sites

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	17 (85.0)	3 (15.0)	-	20
Tetracycline	20 (100)	-	-	20
Erythromycin	19 (95.0)	-	1 (5.0)	20
Levofloxacin	20 (100)	-	-	20
Ceftriaxone	3 (100)	-	-	3
Meropenem	3 (100)	-	-	3

sites. Of these isolates, 43 (78.2%) were penicillin susceptible and 12 (21.8%) had intermediate resistance to penicillin. Tetracycline resistance was found in three isolates (one had intermediate resistance and two were highly resistant). There were 52 isolates (94.5%) identified as being susceptible and three (5.5%) had high resistance to erythromycin. There was also one isolate (1.8%) which was resistant to levofloxacin. None of the pneumococcal isolates was shown to have resistance to ceftriaxone or meropenem.

Table 11 shows the susceptibility profile of the 29 isolates obtained from pediatric patients. Of these isolates, 24 (82.8%) were penicillin susceptible and five (17.2%) had intermediate resistance to penicillin. None of the pneumococcal isolates was shown to have resistance to tetracycline, erythromycin, levofloxacin, ceftriaxone or meropenem.

Table 12 shows the susceptibility profile of the 17 isolates obtained from adult patients. Of these isolates, 13 (76.5%) were penicillin susceptible and four (23.5%) had intermediate resistance to penicillin. There was one isolate (5.9%) with intermediate resistance and one (5.9%) with high resistance to tetracycline. High resistance to erythromycin was indicated in four isolates (23.5%). None of the pneumococcal isolates was shown to have resistance to levofloxacin, ceftriaxone or meropenem.

Table 13 shows the susceptibility profile of the 33 isolates obtained from elderly patients. Of these isolates, 23 (69.7%) were penicillin susceptible and ten (30.3%) had intermediate resistance to penicillin. There was one isolate (3.0%) which had resistance to tetracycline. None of the pneumococcal isolates was shown to have resistance to erythromycin levofloxacin, ceftriaxone or meropenem.

Table 14 shows the susceptibility profile of the 37 isolates obtained from males. Of

Table 10: Susceptibility profile of isolates received from non-sterile sites

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	43 (78.2)	12 (21.8)	-	55
Tetracycline	52 (94.5)	1 (1.8)	2 (3.7)	55
Erythromycin	52 (94.5)	-	3 (5.5)	55
Levofloxacin	54 (98.2)	-	1 (1.8)	55
Ceftriaxone	12 (100)	-	-	12
Meropenem	12 (100)	-	-	12

Table 11: Susceptibility profile of isolates received from pediatric patients (18 years or younger)

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	24 (82.8)	5 (17.2)	-	29
Tetracycline	29 (100)	-	-	29
Erythromycin	29 (100)	-	-	29
Levofloxacin	29 (100)	-	-	29
Ceftriaxone	5 (100)	-	-	5
Meropenem	5 (100)	-	-	5

Table 12: Susceptibility profile of isolates received from adult patients (19 - 49 years)

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	13 (76.5)	4 (23.5)	-	17
Tetracycline	15 (88.2)	1 (5.9)	1 (5.9)	17
Erythromycin	13 (76.5)	-	4 (23.5)	17
Levofloxacin	17 (100)	-	-	17
Ceftriaxone	4 (100)	-	-	4
Meropenem	4 (100)	-	-	4

Table 13: Susceptibility profile of isolates received from elderly patients (50 years or greater)

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	23 (69.7)	10 (30.3)	-	33
Tetracycline	32 (97.0)	-	1 (3.0)	33
Erythromycin	33 (100)	-	-	33
Levofloxacin	33 (100)	-	-	33
Ceftriaxone	10 (100)	-	-	10
Meropenem	10 (100)	-	-	10

these isolates, 28 (75.7%) were susceptible and nine (24.3%) had intermediate resistance to penicillin. Thirty-five (94.6%) were susceptible and two (5.4%) had intermediate resistance to tetracycline. There were 36 isolates (97.3%) identified as being susceptible and one (2.7%) had intermediate resistance to erythromycin. None of the pneumococcal isolates was shown to have resistance to levofloxacin, ceftriaxone or meropenem.

Table 15 shows the susceptibility profile of the 40 isolates obtained from females. Of these isolates, 30 (75.0%) were penicillin susceptible and ten (25.0%) had intermediate resistance to penicillin. When looking at tetracycline resistance, 39 (97.5%) were susceptible and one (2.5%) had high resistance. There were 37 isolates (92.5%) identified as being erythromycin susceptible and three (7.5%) had high resistance to erythromycin. None of the pneumococcal isolates was shown to have resistance to levofloxacin, ceftriaxone or meropenem.

Table 16 illustrates the MIC₅₀ and MIC₉₀ for each of the antibiotics tested. The MIC₅₀ for penicillin is 0.0012 mcg/mL, indicating the MIC at which 50% of the isolates were inhibited, while the MIC₉₀ is 0.5000 mcg/mL, indicating the MIC at which 90% of the isolates were inhibited. For the penicillin E-test system, 0.0012 mcg/mL is classified as antibiotic susceptible and 0.5000 mcg/mL is indicative of intermediate level resistance. The MIC₅₀ and MIC₉₀ for ceftriaxone are 0.1900 mcg/mL and 0.5000 mcg/mL, respectively. These two values indicate that both 50% and 90% of the isolates were inhibited at MICs categorized as antibiotic susceptible for ceftriaxone.

Table 14: Susceptibility profile of isolates received from males

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	28 (75.7)	9 (24.3)	-	37
Tetracycline	35 (94.6)	2 (5.4)	-	37
Erythromycin	36 (97.3)	1 (2.7)	-	37
Levofloxacin	37 (100)	-	-	37
Ceftriaxone	8 (100)	-	-	8
Meropenem	8 (100)	-	-	8

Table 15: Susceptibility profile of isolates received from females

Antibiotic	Frequency - Number (%)			Total
	Susceptible	Intermediate	Resistant	
Penicillin	30 (75.0)	10 (25.0)	-	40
Tetracycline	39 (97.5)	-	1 (2.5)	40
Erythromycin	37 (92.5)	-	3 (7.5)	40
Levofloxacin	40 (100)	-	-	40
Ceftriaxone	10 (100)	-	-	10
Meropenem	10 (100)	-	-	10

Table 16: Comparison of the MICs at which 50% and 90% of the isolates were inhibited

Antimicrobial Agent	MIC₅₀ (mcg/mL)	MIC₉₀ (mcg/mL)
Penicillin	0.0012	0.5
Ceftriaxone	0.19	0.5
Erythromycin	0.125	0.19
Meropenem	0.19	0.25
Tetracycline	0.125	0.25
Levofloxacin	0.75	1

The MIC₅₀ and MIC₉₀ for erythromycin are 0.1250 mcg/mL and 0.1900 mcg/mL, respectively, indicating that 50% and 90% of the isolates were inhibited at MICs categorized as antibiotic susceptible for this antibiotic. The MIC₅₀ and MIC₉₀ for tetracycline are 0.1250 mcg/mL and 0.2500 mcg/mL, respectively. Both MIC values indicate that 50% and 90% of the isolates were inhibited at MICs categorized as antibiotic susceptible to tetracycline. The MIC₅₀ and MIC₉₀ for meropenem are 0.1900 mcg/mL and 0.2500 mcg/mL, respectively, indicating that 50% and 90% of the isolates were inhibited at MICs categorized as antibiotic susceptible for meropenem. The MIC₅₀ and MIC₉₀ for levofloxacin is 0.7500 and 1.0000, respectively, thereby identifying that 50% and 90% of the isolates were inhibited at MICs categorized as antibiotic susceptible for levofloxacin.

Resistance to multiple antibiotics was demonstrated in some isolates in this study. Of the 19 isolates which were categorized as having intermediate resistance to penicillin, one isolate was also shown to have intermediate resistance to tetracycline, two had high resistance to tetracycline and three were resistant to erythromycin. Of the five isolates which were resistant to erythromycin, two were also resistant to tetracycline (one was classified as being intermediate and one was highly resistant).

4.4 Total Antibiotic Consumption Analysis

Figure 1 shows the rates of antibiotic prescription per 1000 people per day in Newfoundland and Labrador for a four year period. During the study period, penicillins were the most commonly used antibiotics. The rate of penicillin prescription steadily declined from 1.685 prescriptions per 1000 people per day in 1997 to 1.373 in 2000. Use

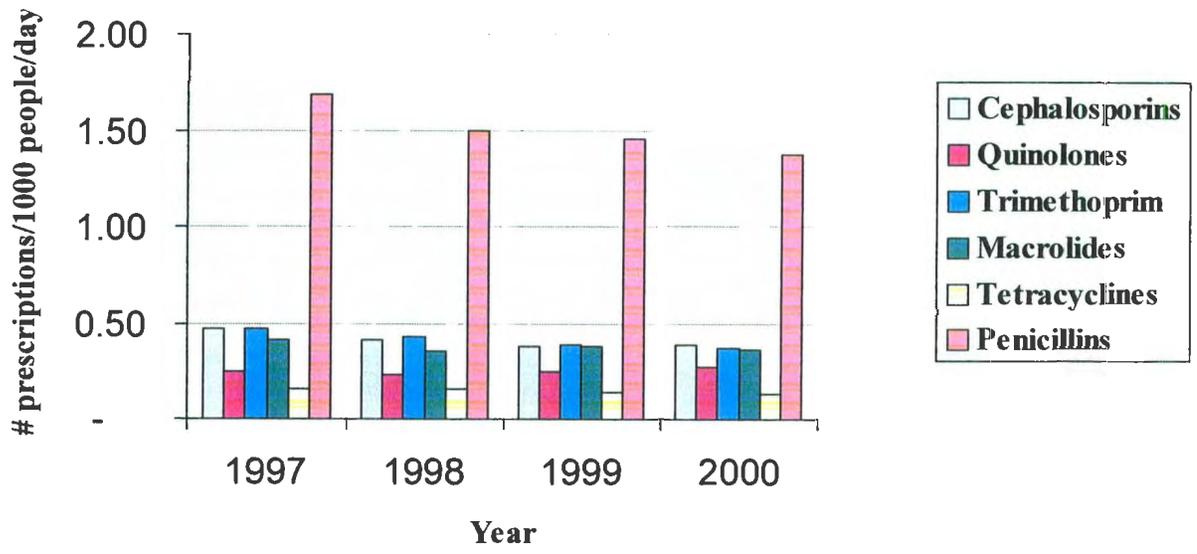


Figure 1: Newfoundland antibiotic prescription rates from 1997 to 2000

of tetracycline and trimethoprim drugs also decreased during the study period.

Tetracycline use decreased from 0.155 prescriptions per 1000 people per day in 1997 to 0.131 in 2000 while trimethoprim use dropped from 0.475 prescriptions per 1000 people per day in 1997 to 0.374 in 2000.

The only class of antibiotics which increased in rate of consumption during the four year study period were the quinolones (Figure 1). The rate of quinolone use increased from 0.246 prescriptions per 1000 people per day in 1997 to 0.273 in 2000.

The use of macrolide and cephalosporin drugs fluctuated during this period (Figure 1). Cephalosporin use declined from 0.472 prescriptions per 1000 people per day in 1997 to 0.376 in 1999 and then increased to 0.387 prescriptions per 1000 people per day in 2000. However, macrolide use dropped from 0.412 prescriptions per 1000 people per day in 1997 to 0.353 in 1998, then increased to 0.376 prescriptions per 1000 people per day in 1999, only to decrease again in 2000.

5.0 DISCUSSION

As we begin the twenty-first century, one of the most important public health issues facing society is the increasing threat of untreatable bacterial infectious diseases, especially those which exhibit antibiotic resistance. Many people fear that we are on the verge of a 'medical disaster' that would return society to the pre-antibiotic days when even small infections could become life-threatening due to a lack of effective treatments.

Newfoundland is the perfect environment in which to study changes in antibiotic resistance. It is relatively easy to collect and collate information on virtually all of the approximately 500,000 inhabitants of this province. Also, the population of Newfoundland is relatively isolated and as such, we may assume that changes in resistance rates are due to factors present within this environment rather than those introduced into it. Although this may change in the near future with the increased interest in the oil industry off the Newfoundland coast and people going to mainland Canada for work and returning for visits.

Newfoundland presents an interesting case when it comes to the issue of antibiotic resistance. As mentioned previously, the level of antibiotic resistance within a population is a function of its antibiotic consumption. However, within this province, the overall rates of resistance among clinical isolates remains relatively low despite high rates of antibiotic consumption by the people of Newfoundland. This implies that factors other than human consumption, such as population density, may be involved in the genesis and sustenance of antibiotic resistance within this province. It is difficult to verify this theory since there is limited data regarding antibiotic resistance levels within St. John's and no data for the rest

of the province.

5.1 Patient Demographics and Susceptibility Profiles

The number of isolates tested during this study (n = 83) was consistent with the annual number of pneumococcal isolates received by the PHL (n = 80) for routine susceptibility testing (Table 1).

The results of this study show that 22.9% of isolates tested were identified as having intermediate levels of resistance to penicillin (MICs, 0.12 - 1 mcg/mL) (Table 5). We had anticipated that the information collected during this study might indicate low levels of pneumococcal resistance within Newfoundland. Although this number appears to be somewhat higher than anticipated for Newfoundland, data generated during a SENTRY antimicrobial resistance surveillance program of seven Canadian institutions in 1997 showed that of the pneumococcal isolates tested 69.2% were susceptible, 21.8% were intermediate and 8.4% were highly resistant to penicillin (Doern *et al.*, 1998). It has also been shown that the pattern of resistance is not uniform across Canada (Blondeau *et al.*, 1999). Instead, resistance rates appear to increase from East to West. From the limited information here, however, the levels of intermediate resistance to penicillin in this study are comparable to the levels of resistance elsewhere in Canada.

The levels of resistance to the other antibiotics tested during this study were much lower than that of penicillin. Approximately 3.6% (1.2% with intermediate and 2.4% with high resistance) of isolates were shown to have decreased susceptibility to tetracycline (Table 5). In a study of 202 respiratory tract isolates obtained from seven hospitals across Canada, 10.2% of pneumococcal isolates in Canada were shown to be resistant to

tetracycline (Doern *et al.*, 1998), comparatively higher than the results of this study.

Only 1.2% of isolates were highly resistant to levofloxacin (Table 5). The incidence of quinolone resistance is generally thought to be fairly low, less than 5% (Campbell and Silberian, 1998). Simor *et al.* (1996) found no high level resistance to quinolone antibiotics among 1,089 Canadian isolates tested in 1994-1995 during a national survey. However, at the time of both studies, the use of quinolones against *S. pneumoniae* was quite limited. Our research indicates that the use of quinolones in Newfoundland has increased during the last several years (Figure 1). An increase in the use of quinolones may cause an increase in the level of quinolone resistance in the Newfoundland population in the near future.

Resistance to erythromycin was slightly higher at 6.0% than that of tetracycline or levofloxacin. In a 1997 study of 845 respiratory tract isolates from across the United States and 202 isolates from across Canada, macrolide resistance rates in the United States were between 10.1% and 13.0% (Doern *et al.*, 1998). However, the resistance rates in Canada were shown to be approximately one-half those seen in the United States. Thus, the level of erythromycin resistance in Newfoundland is comparable to that of the rest of Canada.

None of the isolates with resistance to penicillin was shown to have decreased sensitivity to either ceftriaxone or meropenem. A comparison of results from several recent cross-sectional studies and case control studies has shown that the majority of penicillin-intermediate strains retain full susceptibility to ceftriaxone (Wang *et al.*, 1998). The susceptibility patterns in this study are consistent with much of the research published thus far. However, if evidence is found which refutes previous findings, it may have

important implications in the future since drugs like ceftriaxone and meropenem are used as the next line of defence for infections which are caused by penicillin-resistant *S. pneumoniae*.

As seen in Table 1, the majority of pneumococcal isolates came from the Health Care Corporation of St. John's (70.1%). Of the isolates received from the Health Care Corporation of St. John's, 85.2% were susceptible to penicillin and 14.8% had intermediate resistance (Table 7). When comparing the susceptibilities of the other antibiotics, 3.7% and 5.6% were highly resistant to tetracycline and erythromycin, respectively.

The levels of antibiotic resistance indicated from the isolates received from hospital laboratories outside of St. John's are consistent with that seen within the Health Care Corporation. Of the 23 isolates tested from the laboratories outside St. John's, 43.5% had intermediate level resistance to penicillin and 4.3% had high resistance to erythromycin and tetracycline (Table 8). Although the proportion of resistant isolates is high, the small number of isolates submitted by some of the participating laboratories prevent us from making any generalizations regarding the levels of antibiotic resistance of *S. pneumoniae* within the various regions of the province.

In this study, 26.7% of isolates were retrieved from sterile sites (Table 9). Of these isolates, 15.0% had intermediate level resistance to penicillin and 5.0% were resistant to erythromycin while 100% of isolates were susceptible to tetracycline, levofloxacin, ceftriaxone and meropenem (Table 9). It is important to get an accurate measure of the degree of resistance of pneumococci found in the blood since bacteremia is one of the most serious forms of disease caused by pneumococci and one of the least responsive to

antibiotic therapy because of the overwhelming bacterial load in the blood (Jacobs, 1992).

During this study, a large number of isolates (n=55) were obtained from non-sterile sites (Table 10). Since this category of isolates also includes those from the respiratory tract and penicillin resistance is more common among isolates recovered from respiratory tract sites than from systemic sources, high levels of antibiotic resistance were expected (Kaplan, 1997 and Ball, 1999). The results of this study did confirm the hypothesis that higher levels of antibiotic resistance would be found in organisms obtained from non-sterile sites. Approximately, 21.8 % of isolates had intermediate resistance to penicillin, 5.5% were resistant to tetracycline (1.8% with intermediate resistance and 3.7% with high resistance), 5.5% had high resistance to erythromycin and 1.8% had high resistance to levofloxacin (Table 10). All isolates with penicillin resistance were susceptible to both ceftriaxone and meropenem.

A 20-year study which involved 11,407 isolates from Paris and Creteil showed that the degree of change in penicillin susceptibility is more prominent among noninvasive than invasive organisms (Geslin *et al.*, 1992). As previously stated, noninvasive organisms are those isolated from sites, such as the respiratory tract and ears, while invasive organisms are those isolated from typically sterile sites, such as blood and cerebrospinal fluid. The high level of drug resistance in organisms which reside in non-sterile sites is likely due to the fact that up to 80% of all antibiotics used in the community is prescribed to treat respiratory tract infections (Huovinen, 1998). This increase in antibiotic use will select for the growth of resistant organisms and the result in the transmission of resistant genes at these sites rather than the sterile sites where the achievable concentrations of antibiotic are generally lower (Wang *et al.*, 1998). It was shown in this study that the isolates

recovered from the non-sterile sites had higher levels of resistance than those from the sterile sites. However, the degree of penicillin resistance (i.e., intermediate) was the same in both sites. Therefore, the results of this study differ from the findings of Geslin *et al.* (1992) as mentioned above.

Approximately 36.7% of the pneumococcal isolates tested were retrieved from pediatric patients, those individuals under the age of 18 years (Table 11). It is important to monitor the antibiotic susceptibility rates of the pediatric group because this population has a decreased natural immunity compared to adults which makes them more susceptible to infectious organisms and subsequently results in high levels of antibiotic consumption. Community carriage rates are thought to be best studied in children since day care attendance and frequent empirical treatment with antibiotics are known risk factors for drug resistant pneumococcal infections (Klugman, 1990). A year long surveillance project conducted in South Africa investigated the role of age in carriage of penicillin resistant pneumococci by testing 362 nasopharyngeal isolates from children under the age of four and 521 nasopharyngeal isolates from children above the age of four (Koornhof *et al.*, 1992). Koornhof *et al.* (1992) showed that 41% of the children under the age of four carried resistant pneumococci while only 6.0% of children above the age of four were carriers, leading the authors to the conclusion that children who are less than four years of age had a significantly higher carriage of resistant organisms. This study also supports these findings. Table 11 shows that only 82.8% of isolates obtained from children were sensitive to penicillin. It is also interesting to note that these isolates did not exhibit resistance to any of the other antibiotics tested.

The high levels of antibiotic resistance in children has been attributed to the

increased frequency of single parent families as well as families where both parents work outside of the home (Cohen, 1999). This trend has resulted in greater use of daycare facilities in recent years. Within the average daycare facility, there are large numbers of children who generally have low natural immunity to bacterial infections. As a result, there is a higher incidence of infection and therefore, frequent antibiotic use within the group. These factors coupled with poor hygiene practices of children create the perfect setting for the emergence and transmission of resistant genes within the bacteria. A 1992 study in Kentucky tested nasopharyngeal swabs from 158 children who attended day care centers and compared the results to the susceptibility profile obtained from 82 children who attended a county health center (Dutchin *et al.*, 1995). Dutchin *et al.* showed that children who attended day care had a four times greater risk of acquiring a strain of *Streptococcus pneumoniae* with high-level penicillin resistance than children who do not attend such facilities. In addition, it has been suggested that children less than two years of age who also attend daycare centers have the highest risk of being colonized and infected with resistant pneumococci (Jernigan *et al.*, 1996). For the purposes of this study, it would have been interesting to see how many of the children who carried *S. pneumoniae* were also attending day-care facilities and if there was a correlation between resistance and day care within the Newfoundland population.

Like children, elderly patients also show a subsequent decrease in immunity which is related to their elevated age that put them at risk of acquiring fatal infections. For this reason, it is important that resistant isolates be identified in both children and senior citizens because the mortality rate associated with pneumococcal infections is enhanced at the extremes of life (Kanavaki *et al.*, 1994). Therefore, contracting an infection that

cannot be treated with antibiotics is especially threatening. Fortunately, both the polysaccharide or protein-conjugated pneumococcal vaccines can be used to prevent infections with resistant organisms in the elderly population.

Changing human demographics, namely the increase in the number of elderly individuals within society, have increased the dissemination of resistant organisms, such as *S. pneumoniae*, throughout a population (Cohen, 1999). Approximately 42.1% of isolates tested in this study were extracted from elderly patients, those individuals over the age of 50 years (Table 2). Of these isolates, only 69.7% were susceptible to penicillin and 97.0% were susceptible to tetracycline. These results are consistent with previous studies which have shown that elderly people are important carriers of penicillin resistant strains of *S. pneumoniae* (Jacobs, 1999). Some risk factors that have been identified as contributing to carriage of resistant organisms in the elderly population include hospitalization, prior exposure to antibiotics and tobacco use (Lund *et al.*, 1998). Aging results in a decrease in a person's natural immunity, making them more susceptible to infectious disease and increases the frequency with which a person uses antibiotics. Also, elderly people are more likely to develop chronic illnesses, such as cancer and coronary heart disease. A person's susceptibility to infectious diseases may then increase during the course of the underlying disease process while the immune system is challenged or as the person is taking some form of immunosuppressive therapy, such as chemotherapy treatment for cancer. Although the elderly patients sampled in this study were the predominant group for penicillin resistant pneumococci, we did not look at the individual risk factors that may contribute to the development or carriage of resistant isolates.

The levels of drug resistance were lower in the adult patients sampled than in the elderly group (Table 12). However, the adult population showed resistance to a wider variety of antibiotics (penicillin, tetracycline, and erythromycin) than the elderly group (penicillin and tetracycline) (Tables 12 and 13).

To date, there have been no studies which compare the differences in the susceptibility profiles of isolates retrieved from different genders. However, penicillin resistance is most common in serotypes that frequently cause disease in children and since pneumococci are easily spread from person to person by direct contact, it is anticipated that males and females who have regular contact with children are at a greater risk of picking up resistant bacteria (Gold *et al.*, 1996).

In this study, it was shown that there is very little variation in the levels of resistance between males and females. For example, 75.7%, 94.6% and 97.3% of the isolates obtained from males were susceptible to penicillin, tetracycline and erythromycin, respectively (Table 14). Likewise, 75.0%, 97.5% and 92.5% of the isolates obtained from females were susceptible to penicillin, tetracycline and erythromycin, respectively (Table 15). Therefore, the susceptibility profiles of isolates from both genders appear similar with one notable exception, the resistant isolates from females were classified as intermediate to penicillin and high to tetracycline and erythromycin while all of the resistant isolates from males were classified as intermediate. This indicates that *S. pneumoniae* isolated from females are showing greater MIC values than those from males.

There was also evidence of multiple resistance during this study. Of the isolates which were resistant to penicillin, three had decreased susceptibility to tetracycline and erythromycin, respectively. It has been shown that penicillin resistant strains are more

frequently resistant to other antimicrobial agents, such as macrolides, tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole, than strains that are susceptible to penicillin (Pradier *et al.*, 1997; Jacobv, 1994; Campbell and Silberian, 1998). However, the mechanisms responsible for resistance to these antibiotics are unrelated to those of penicillin resistance. Therefore, a single organism can develop resistance to multiple antibiotics at the same time, but through different genetic alterations.

Tetracycline, erythromycin, levofloxacin, ceftriaxone and meropenem appear to be quite active against *S. pneumoniae*. Table 16 showed that the MICs necessary to inhibit 50% and 90% of the pneumococcal isolates were identified as antibiotic susceptible. The MIC of penicillin which inhibited 50% of the isolates was in the susceptible range as well. However, the MIC of penicillin which inhibited 90% of the isolates was in the intermediate range for that antibiotic. This indicates that a high concentration of penicillin is required to eliminate most infections caused by *S. pneumoniae*.

5.2 Methodology Comparison

In this study, disk diffusion was compared to the E-test system in order to determine whether disk diffusion is accurate enough to be used in hospital settings alone. Table 3 showed that the isolates tested for penicillin resistance with the oxacillin disk classified 65 as being susceptible and 18 as being resistant. Using the penicillin E-test strips, 64 isolates were identified as being susceptible and 19 were identified as having intermediate resistance to the antibiotic (Table 3).

It has been shown that some isolates identified as resistant by the oxacillin disk-diffusion method are found to be susceptible by quantitative MIC testing while the reverse

is very uncommon (Lund *et al.*, 1998). However, during this study, it appears that both the oxacillin disk and penicillin E-test strips provide similar susceptibility profiles.

The accuracy of these methods was also compared for two other antibiotics: erythromycin and tetracycline. Using the erythromycin disks, 79 isolates were identified as being susceptible and four were identified as being resistant (Figure 2). Using erythromycin E-test strips, 78 isolates were identified as susceptible and five were identified as having high resistance (Figure 2). Similarly, 81 isolates were classified as tetracycline susceptible and two were classified as resistant using disk diffusion while 80 isolates were classified as being susceptible, one was classified as having intermediate resistance and two were classified as having high resistance when tested with tetracycline E-test strips (Figure 3). Both methods provide consistent susceptibility profiles for erythromycin and tetracycline.

During this study, disk diffusion was shown to have high values for the specificity and sensitivity measurements (Tables 3, 4 and 5). Therefore, disk diffusion appears to be a valid method for determining antibiotic susceptibility.

Based on the results of this study, it appears that disk diffusion could be used to provide a general guide for antibiotic therapy for common, uncomplicated infections. However, in the case of potentially fatal infections where patients need to receive the most effective treatment immediately, it would be important to generate the susceptibility profile through the E-test system because it gives a more specific profile than disk diffusion. Disk diffusion classifies all isolates with decreased antibiotic susceptibility as being resistant, but does not distinguish between intermediate and high levels of resistance. This information is important in determining the appropriate therapy for a serious infection and

can be generated through the E-test system.

5.3 Antibiotic Consumption

The rationale for antibiotic misuse has been based on the assumption that the risk of potential or possible bacterial infection is more hazardous to the patient than the risk from antimicrobial agents (Simmons and Stolley, 1974). In Canada, antibiotics were the second most commonly prescribed drug class, accounting for 26.3 million prescriptions in 1996 (Wang *et al.*, 1998). Wang *et al.* (1998) used the Compuscript database to generate information on antibiotic use in Canada and found that the number of prescriptions increased by 10% between 1992 and 1993, but has remained stable since then. Similarly, the purpose of this research project was to provide hard data showing exactly how much community based antibiotic consumption occurred in Newfoundland and Labrador from 1997 to 2000 for specific classes of drugs.

Amoxicillin is the most frequently prescribed antibiotic in Canada at approximately 25% of all antibiotic prescriptions (6.8 million prescriptions), followed by cephalosporins at 3.3 million prescriptions and erythromycin at 2.7 million prescriptions (Wang *et al.*, 1998). Of the drugs measured by the IMS database and shown in this study, penicillins, including amoxicillin, was the most commonly prescribed group for all infections (Figure 1). However, the use of penicillins did show a steady decline during the study period.

Newfoundland has taken an education approach to combating the problem of antibiotic misuse with the Newfoundland Optimal Antibiotic Project which began in October 1997. The purpose of the Optimal Antibiotic Project was to increase the awareness of physicians and the general public of the problem of antibiotic resistance and rational

antibiotic use. Rational antibiotic use involves taking prescribed medications correctly and curtailing overall use for the good of society. The Optimal Antibiotic Project was started with the hope that it would result in a decrease in patient demand for therapy and improve the ability of physicians to respond to patient expectations without antimicrobial therapy, thereby decreasing the population consumption of antibiotics. The information gathered on antibiotic use in this project indicates that the Optimal Antibiotic Project was a success. During the years from 1997 to 2000, the use of tetracyclines, trimethoprim and penicillins by the people of Newfoundland declined (Figure 1). Therefore, the results of this study indicate that educational programs, such as the Newfoundland Optimal Antibiotic Project, are a useful method to diminish antibiotic consumption.

An interesting trend was seen in the use of cephalosporins and macrolides which initially declined, but then showed a slight increase again (Figure 1). The use of macrolides dropped again in the last year of the study while cephalosporin use remained high. Cephalosporins contain a number of narrow- and broad-spectrum antibiotics. These agents are commonly prescribed when a physician is unsure of the pathogen causing an ailment. The high number of prescriptions for cephalosporins seen in this study was unexpected due to the implementation of the Optimal Antibiotic Project guidelines which recommended conservative use of cephalosporins.

The only group of antibiotics that increased in population use during the study were the quinolones (Figure 1). Quinolones are a relatively new class of antimicrobial agents. They were not commonly used in children because of their side-effects and were quite expensive until the mid-1990's after which time there was a decrease in cost. These agents were promoted by pharmaceutical companies during the 1990s as a means of

combating resistance to the older classes of antibiotics which had been in use for awhile. The increase in advertising and promotion by pharmaceutical companies may account for the increase in the use of these drugs during the study period.

5.4 Limitations

One of the biggest limitations to this study was the small number of isolates that was tested during the study period. Although the PHL usually receives approximately 80 pneumococcal isolates each year, we had hoped to get more isolates with active participation of the various hospital laboratories in order to ensure an accurate representation of the level of pneumococcal resistance.

There were more isolates received from the St. John's area than the rest of the island. Therefore, it was impossible to compare pneumococcal resistance levels between provincial regions. There are several factors which may have contributed to a more complete sampling of isolates from the St. John's area than elsewhere in the province. First, the institutions which comprise the Health Care Corporation of St. John's service a much larger population than the other hospitals involved in this study. Therefore, a large number of pneumococcal isolates would be expected from this area. Second, the PHL is located in St. John's and as a result, participating laboratories outside of the city have to deal with the greater expense and effort required to transport pneumococcal isolates to the PHL.

Isolates from St. Anthony and Labrador were excluded from this project because of difficulty maintaining viability of organisms during the time required for transportation. By excluding these sites, we were unable to assess the resistance rates in these areas of the

province, limiting the ability to generalize the results of this study to the province of Newfoundland and Labrador, as a whole. In addition, we were unable to assess the prevalence rates of antibiotic resistance in *S. pneumoniae* within the Aboriginal population of Labrador.

At the beginning of this research project, we had hoped to receive the antibiotic consumption information from community pharmacies throughout Newfoundland and Labrador. With this information, we would have been able to access antibiotic consumption data within the different regions of the province and perhaps correlate this information with the resistance information that had been gathered. However, the antibiotic consumption ultimately was supplied by IMS HEALTH which provided the data for the whole province, not regionally. This, in addition to the small number of isolates, prevented any correlations from being made between resistance levels and community antibiotic use.

Using the data from IMS HEALTH required us to provide antibiotic consumption information in terms of the number of prescriptions filled in relation to population. However, the internationally accepted format to describe antibiotic consumption is in terms of DDDs. Therefore, comparisons between consumption as indicated by this study and that of other published studies using the ATC/DDD System is impossible.

6.0 CONCLUSION

Community carriage of antibiotic resistant strains of bacteria is now a widespread and increasing public health concern in many parts of the world. From this standpoint, strains of *S. pneumoniae* which are resistant to penicillin are an especially serious concern for several reasons, including the fact that penicillin is still considered to be the treatment of choice against pneumococcal infections, pneumococci are easily spread from person to person and are associated with significant morbidity and mortality (Gold *et al.*, 1996). Therefore, it is important to get an idea of the prevalence of antibiotic resistance in *S. pneumoniae* on a global scale.

The purpose of this study was to provide an indicator of the level of antibiotic resistance among *Streptococcus pneumoniae* isolates in Newfoundland. It was successful in showing the overall trends in resistance to a specific series of antibiotics in this province. The rates of antibiotic resistance appear to be consistent with the trends observed elsewhere in Canada and the United States, if not lower in some cases.

Some factors have been identified which put people at risk for acquiring resistant strains of *S. pneumoniae*, include the age of the person, if the person is enrolled in or works in a day care facility, if the person has an illness which may suppress the immune system or if the person has been hospitalized in recent years (Kaplan, 1997). It has also been shown that the use of antibiotic drugs for prophylactic or therapeutic purposes in humans or for veterinary or agricultural purposes are responsible for providing the selective pressure favors the overgrowth of resistant organisms (Gold *et al.*, 1996). However, there is much more research required. For example, the specific mechanisms of resistance need to be identified, new antibiotics need to be developed, more effective

uses of the present drugs need to be discovered, the best forms of therapy need to be ascertained for infections due to multiresistant organisms and, most importantly, we need to figure out how to prevent these types of infections. Unfortunately, this research project was unable to provide any information on possible risk factors since it was not able to provide an accurate depiction of resistance levels on the basis of geographic region, patient age, patient sex or specimen source because of the small number of specimens collected during the study period.

The incidence of drug resistant *S. pneumoniae* has been shown to vary not only between communities, but also within a single community and even between different types of patients (Campbell and Silberian, 1998). For this reason, many researchers have emphasized the importance of developing a surveillance system and testing methodologies to monitor the factors which are contributing to microbial resistance as well as changing resistance patterns. This study showed that disk diffusion alone is a reasonable method for assessing microbial susceptibility in uncomplicated infections.

There have been some improvements in resistance education and awareness during the last few years. This is partially due to successful projects, such as the one in Newfoundland, which illustrate the effectiveness of educational tools as a method of reducing the problem of resistant microbes. For example, this study showed that the use of penicillins and macrolides decreased after the implementation of the Optimal Antibiotic Project. As a result, many wealthy countries are beginning to promote the judicious use of antimicrobial agents by their physicians and the public. Since the severity of the problem has been recognized and steps are being taken to correct it, we are hoping that eventually the problem of antibiotic resistance will be decreased.

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8.0 APPENDIX A

Protocol for Receiving Pneumococcal Isolates

The Newfoundland Public Health Laboratory (PHL) carries out a province-wide surveillance program for antimicrobial resistance for strains of ***Streptococcus pneumoniae* (SPN)**.

- Hospital-based Microbiology Labs across the province forward isolates of SPN, especially those isolated from normally sterile sites such as blood and CSF, to PHL via courier on blood agar plates.
- The PHL subcultures the isolates to blood agar (BA) to check for viability and purity.
- Heavy suspensions of SPN are prepared in defibrinated sheep's blood and the suspensions are frozen at -70° C to maintain viability until susceptibility test panels are performed.
- Antimicrobial MIC susceptibility tests are performed using E-test (A. B. Biodisks).

9.0 APPENDIX B

Protocol For Susceptibility Testing of *S. Pneumoniae*

Day 1

- 1.) Remove cultures to be tested from the ultrafreeze at the Newfoundland PHL.
- 2.) Allow the vials to defrost.
- 3.) Divide blood agar (BA) plates into four quadrants with marking pen and subculture each isolate onto a quadrant of the BA plate using a 10 mL loop.
- 4.) Incubate the BA plate in CO₂ at 35-36 °C for 18 to 24 hours.

Day 2

- 1.) Prepare suspensions of the test organism by dipping loop containing isolate into 0.9% normal saline or Mueller Hinton Broth. Suspension should be equivalent to 0.5 McFarland control (1.0 McFarland for mucoid strains). Suspension should be used within 15 minutes of preparation.
- 2.) Dip sterile cotton swab into suspension and rotate swab several times against the inside wall of the tube to remove excess inoculum.
- 3.) Inoculate the surface of the BA plate by streaking the plate in three planes. Plate should be uncapped for 3-5 minutes to allow excess moisture to evaporate.
- 4.) Add a single E-test strip to one BA plate for each isolate or four disks may be added to one BA plate for each isolate. One BA plate per isolate should be incubated with an optochin disk to verify that the isolate is *S. pneumoniae*.
- 5.) BA plates should be inverted and incubated for 20 to 24 hours at 35 °C in 5-7% CO₂.

Day 3

- 1.) The zone of inhibition should be measured for each strip/disk and interpreted according to NCCLS guidelines.

10.0 APPENDIX C

Optimal Antibiotic Project

The Newfoundland Optimal Antibiotic Project began in 1997 in an attempt to increase awareness of antibiotic resistance amongst physicians and the population at large as well as teach people about rational antibiotic use through a media campaign. It is a collaborative effort between physicians, pharmacists, pharmaceutical companies and government which was designed to measure resistance in clinical isolates of bacteria, antibiotic use and health care resource use related to community acquired infections.

In 2000, a proposal was generated to turn the Optimal Antibiotic Project into an ongoing program. The program would consist of the following three components:

1.) Measurement of Resistance in Clinical Bacteria

It was proposed that all hospitals with microbiology laboratories within the province of Newfoundland and Labrador measure antibiotic resistance in clinical bacterial isolates. This information would then be collated and analyzed centrally.

2.) Measurement of Resistance in "Normal" Bacteria

Since it is felt that monitoring resistance prevalence in bacteria from people outside the hospital setting is a more sensitive measure of the degree of the problem within a population, it was proposed that routine sampling of normal bacteria isolated from the general population occur in a systematic fashion. The routine sampling of these isolates would then be used for monitoring the development of resistance.

3.) Measurement of Antibiotic Use

It is important to have good measurements of antibiotic use in order to assess the efficiency of policy and how prescribing practices effect resistance prevalence in a population. It was proposed that all antibiotic information be electronically collected from community and hospital pharmacies throughout Newfoundland and Labrador. This information could then be processed centrally.

The ultimate goal of this program was to provide information which would allow the development of treatment guidelines, specific to the province of Newfoundland and Labrador, taking into account local susceptibility patterns. Regular reports would be compiled for physicians and policy makers which would allow informed decisions to be made regarding formulary recommendations. The general public would also receive regular reports providing information on local antibiotic consumption and resistance prevalence.



