Antimicrobial Resistance: Middle Ear Study Involving Saskatchewan Native and Non-Native Children.

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Masters of Science

in the Department of Microbiology

University of Saskatchewan

Saskatoon

by

Marnie Andersen

Spring, 2000

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission to copy this thesis in any way, in whole or in part, for scholarly purposes only may be granted by the professor or professors who supervised my thesis work, or in their absence, by the Head of the Department of Microbiology and Immunology or the Dean of the College of Arts and Science. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

All requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Microbiology and Immunology
University of Saskatchewan
Saskatoon, Saskatchewan
S7N 5E5

ABSTRACT

Middle ear infections (MEI) are a significant cause of morbidity among children, particularly for those who are Native and living in northern geographic regions of Canada. The widespread use of antimicrobial agents and the dramatic rise in the number of antibiotic-resistant pathogens encountered within Saskatchewan, create concern as to whether significant differences exist between Native and Non-Native children in regards to the percentage of middle ear pathogens resistant to antimicrobial agents. The recognition that various communities may serve as reservoirs of antibiotic-resistant bacteria, has raised interest in studying the transmission of these bacteria. In order to clearly assess the prevalence of antibiotic resistance in terms of ethnicity and location, 285 middle ear aspirates were collected from Native and Non-Native children: 98 bacterial pathogens were recovered from 54 culture-positive individuals (68 % Non-Native, 32 % Native). Pulsed field gel electrophoresis (PFGE) and demographic data acquisition were methods used for studying the epidemiology of resistance. Resistance to ampicillin was found in 20 % and 100 % of *Haemophilus influenzae* and *Moraxella catarrhalis* isolates, respectively. Penicillin-resistant *Streptococcus pneumoniae* was found in 13 % of the isolates. Overall, Non-Native children seemed to harbor antimicrobial resistant pathogens more frequently, (77 %; $p=0.001$), specifically in regards to the number of ampicillin-resistant *H. influenzae* isolates (38 %; $p< 0.005$). Geographical trends in resistance were seen among children of race, however, limited demographic features could be ascribed to the risk of clonal spread of resistant pathogens. Recovery of antimicrobial resistant pathogens from children suffering from MEI continues to be prevalent. Differences in resistance rates were seen among children of different ethnic backgrounds and who were

living in various geographical communities in Saskatchewan. Further study is necessary to conclusively establish whether the differences are a true representation of antimicrobial susceptibility for MEI or due to limited sample collection.

ACKNOWLEDGEMENTS

The success of my Master's degree would have never have been achieved if it was not for the following people. I would like to thank Dr. Blondeau for taking me under his supervision and Pfizer Canada for the research grant. An appreciation goes out to Dr. Kanchana, Dr. Tan, Dr. Deneer, and Dr. Ziola for their guidance and support for making me strive for excellence. In addition, there are a number of other individuals I would like to express my gratitude to for their involvement in this project including Dr. Peter Butt, Father Mark Miller, Dr. Gore-Hickman, Dr. Spafford, Dr. Wright, Dr. Alvi, and Dr. Will.

I have been blessed with endless opportunities that I am ever grateful for. But it is all meaningless without the health and well being of my family. Their support has given me stability and made my life enjoyable in an uncertain world. Special thanks also goes out to Shantelle Borsos, Cynthia Misfeldt, and Robin Laskowski who are not only excellent people to work with, but whom I am proud to call friends. Deb Leciuk, what can I say except "thanks" for making this thesis beautiful.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
Amox	Amoxicillin
Amp	Ampicillin
Aom	Acute Otitis Media
AP-PCR	Arbitrary Primed Polymerase Chain Reaction
ARI	Acute Respiratory Infections
β	Beta
^0C	Degrees Celsius
Cf	Cefuroxime
Cef	Cefaclor
CHEF	Contour Clamped Homogenous Electric Field
CO_2	Carbon Dioxide
CFU	Colony-Forming Units
DOC	Deoxycholic Acid
DNA	Deoxyribonucleic Acid
ENT	Ear, Nose, and Throat
Ery	Erythromycin
Gati	Gatifloxacin
Kb	Kilobase Pairs
Levo	Levofloxacin
MEA	Middle Ear Aspirates
MEAs	Middle Ear Aspirations
MEI	Middle Ear Infections
MIC	Minimum Inhibitory Concentration
µl	Microlitres

ml	Mililitres
N	Native
NN	Non-Native
OME	Otitis Media With Effusion
PBP	Penicillin Binding Proteins
Pen	Penicillin
PFGE	Pulsed Field Gel Electrophoresis
PK	Proteinase K
PRSP	Penicillin Resistant Streptococcus Pneumoniae
REA	Restriction Endonuclease Analysis
RNA	Ribonucleic Acid
TBE	Tris-HCl Boric Acid EDTA Buffer
TE	Tris-HCl EDTA Buffer
SXT	Trimethoprim-Sulfamethoxazole

1.0 LITERATURE REVIEW

1.1 General Overview

After the common cold, middle ear infections (otitis media) are the most common infectious illnesses among infants and children (Bluestone, 1998; Khurana, 1995). By two years of age, approximately two-thirds of children have had at least one episode and approximately one-third have three or more episodes of acute otitis media (AOM) infections per year. If infection persist, chronic inflammation and tissue destruction may lead to learning and developing disorders, as well as, permanent hearing loss (Khurana, 1995; McCarty, 1995; McLinn, 1995).

Streptococcus pneumoniae, Haemophilus influenzae, and *Moraxella catarrhalis* are the most frequently isolated pathogens in the middle ear fluid (Barnett and Klein, 1995; McCarty, 1995; Block, 1997; Brook, 1998a). The increasing emergence of penicillin- resistant *S. pneumoniae* (PRSP), and increasing frequency of beta-lactamase producing strains of *M. catarrhalis* and *H. influenzae* are challenging the empiric therapy for AOM (Block, 1997; Brook, 1998a). During 1994 to 1995 Blondeau *et al* (1996) conducted a pediatric middle ear study and found 25% PRSP and 39% ampicillin-resistant *H. influenzae.* Because

antibiotic resistance patterns may vary regionally, a second study was performed to determine whether significant differences among PRSP isolates existed for various Saskatchewan regions. Overall northern regions demonstrated higher intermediate penicillin resistance (36.8%) compared to Saskatoon (14.3%), Regina (12.5%), and Southwestern areas (14.5%) (Blondeau *et al*, 1997).

A high incidence of AOM has been associated with selected racial groups including Native Americans, Alaskan Eskimos, and various Native Canadian subpopulations (Julien *et al*, 1987; McCullough, 1990; Salloum and Crysdale, 1990; Thomson, 1994; Harris *et al*, 1998). The majority of the Canadian subpopulations occur in northern or remote areas of Canada. The widespread use of antimicrobial agents and the dramatic rise in the number of antibiotic resistant pathogens encountered within Saskatchewan creates concern as to whether significant differences exist between Native and Non-Native children in regards to the percentage of AOM pathogens resistant to antimicrobial agents. Anecdotal evidence suggests that high illness rates among Native children may indicate greater overall drug therapy, thereby increasing the risk of developing or acquiring drug-resistant bacteria.

1.2 Otitis Media

1.2.1 Etiology

The pattern of acute otitis media infection may evolve through several phases. The early stage is often a viral infection that suppresses humoral and

cellular-immunity. This may cause greater bacterial adherence to the mucous membranes and result in swelling and edema. Complete recovery of a viral infection is encountered in most children. For people with poorly developed immune systems such as young children or the elderly, as well as those with eustachian tube dysfunction, the initial viral inflammation may lead to a secondary bacterial infection (Brook, 1998a).

The eustachian tube has at least three important physiological functions in preventing infection and accumulation of effusion in the middle ear. It provides protection against nasopharyngeal sound pressure and secretions. It also allows for adequate drainage and clearance of middle ear secretions into the nasopharynx, and permits ventilation of the middle ear to equilibrate gas pressure with atmospheric pressure (Klein, 1994). Infants are predisposed to otitis media because their eustachian tubes are shorter, wider, and lie more horizontally than those of older children. Often, the development of the valve that is responsible for opening and closing the tube is incomplete (Klein, 1994). Consequently, the secretions of the middle ear mucosa accumulate and if pathogenic bacteria that colonize the nasopharynx are present in the middle ear secretions prior to obstruction, they multiply, resulting in acute infection. If the infection does not resolve at the acute stage, a chronic infection may develop (Klein, 1994).

1.2.2 Subtypes

There are three broad types of otitis media to be considered: (1) AOM, (2) otitis media with effusion (OME) and (3) chronic otitis media with or without perforation.

AOM is often a self limited infection involving the deep surface of the tympanic membrane that lines the respiratory mucosa in the middle ear cavity. The diagnosis of AOM rests on the identification of middle ear effusion in the presence of one of the clinical signs and symptoms of acute infection. Individuals suffering from AOM may experience pain, fever, decreased hearing, vomiting, diarrhea, redness, with an altered anatomic appearance upon physical examination (McLinn, 1995; Karver, 1998). Infants between 6 and 18 months of age are the most prone to develop this condition (Khurana, 1995; Karver, 1998).

OME, also known as serous otitis media, is best defined as the presence of fluid in the middle ear without clinical signs and symptoms of an active infection (Karver, 1998). When the middle ear ventilation becomes inadequate due to eustachian tube obstruction or malfunction, pressure in the middle ear becomes relatively negative causing a serous effusion. Over several weeks mucus-secreting cells, located primarily in the eustachian tube or its orfice, migrate into the middle ear and secrete a thick, mucoid material. Classification of this condition is then termed secretory otitis media, or glue ear (Koufman,

1990). Between 10% to 20% of children will have persistent fluid that may be present for up to 3 months (Karver, 1998).

Chronic otitis media with perforation is a chronic suppurative condition where there is a perforation in the tympanic membrane characterized by a low grade chronic infection. Following an incompletely treated AOM episode, pressure within the tympanic membrane develops from the infection. Consequently the membrane ruptures and persistence of the active infection prevents healing of the original disorder (Karver, 1998). Decreased hearing and other complications such as scarring and cholesteotoma formation could occur if improperly treated. Chronic otitis media without perforation, on the other hand, is a harder condition to define, but many consider it a manifestation on the continuum between AOM and OME (Karver, 1998).

1.2.3 Prevalence of Illness

MEI are the most common conditions for which pediatric patients seek medical attention. In the United States, half of all children will have an episode before age one year and more than 80% of children have one or more episodes of otitis media by age 6 years. High incidence of otitis media occurs between the ages of 6 and 24 months (Khurana, 1995; McCarty, 1995; McLinn, 1995). According to the National Centers for Disease Control and Prevention, the estimated 24.5 million office visits in the United States for otitis media in 1990, represented a 150% increase over the number of visits made in 1975. Moreover,

the younger the child at the first episode, the higher the risk for recurrent disease (Schappert, 1992).

1.3 Aboriginal Health

1.3.1 Classification of Ancestral Groups

Currently, the Indian, Inuit and Metis peoples are recognized as Aboriginal Peoples under Section 35 of the Canadian Constitution (Report of the Royal Commission on Aboriginal Peoples, 1996).

The term First Nations is used in Canada and refers to "registered" or "status Indians". "Status" or "registered" Indians are those individuals legally recognized by the Federal Government of Canada. The Canadian government is responsible for the health, education and welfare of registered Indian People (Report of the Royal Commission on Aboriginal Peoples, 1996). The registered Indian population in Saskatchewan is made up mainly of Cree and Chipewyan origins as well as small numbers of Saulteaux, Assiniboine and Dakota origins (Senthilselvan *et al*, 1995). Some registered Indians live in reservations located in northern and southern parts of Saskatchewan, while others live off reserve in urban centers (Federation of Saskatchewan Indian Nations, 1997).

Unlike the Indian population that represents approximately 60% to 70% of the total Saskatchewan Native population, Inuit people make up 1% (Report of the Royal Commission on Aboriginal Peoples, 1996). The Inuit are separate from registered Indians, and there is no legislation comparable to the Indian Act. Nevertheless, the Federal Government provides services to these people as

if they were defined as registered Indians. Most Inuit people live in northern areas of Saskatchewan (Federation of Saskatchewan Indian Nations, 1997).

The term Metis refers to a distinct Aboriginal people who are descendants of mixed marriages between Indian and White populations. Their roots are found in association with the fur trade of the seventeenth, eighteenth and nineteenth centuries (Federation of Saskatchewan Nations, 1997). The Metis and non-status Indians have the same legal status as the rest of the Saskatchewan population. Only the province of Alberta has provided land exclusively for Metis people (Waldram *et al*, 1995). Non-registered Indians live mainly in northern parts of Saskatchewan, whereas most caucasian and Metis people live primarily in the southern part of the province where the major urban centers are located (Federation of Saskatchewan Indian Nations, 1997).

1.3.2 Magnitude of the Problem

In Canada, the Native population experiences more ill health than the rest of the Canadian population. A report by Health and Welfare Canada (1990) indicates that life expectancy for Natives is ten years less than the national average and the infant mortality rate is more than double the rate for the total Canadian population. The report goes on to state that Natives in comparison to Non-Native Canadians are at an increased risk of death from injuries and violence, suicide, diabetes, cirrhosis, and respiratory diseases. In addition, death from diseases of the respiratory system for all ages of Canadian

Aboriginals (excluding the 25 to 44 year age group) rank in the top four leading causes of death.

Acute respiratory infections (ARI) not only cause a significant number of deaths among Native people, but there is also excess morbidity associated with ARI (Young, 1983; Mao *et al,* 1986, Morrison *et al,* 1986). The relative risk ratio from various studies have indicated ARI associated morbidity in Canadian Natives is higher than Non-Native people from diseases such as pneumonia, bronchitis and influenza (Frazer-lee and Hessel, 1994; MacMillian *et al,* 1996). ARI related illness in infants and young children have been a focus of several studies (Evers and Rand, 1982; Evers and Rand, 1983; Harris *et al,* 1998). Evers *et al,* 1985, studied status Indian children in a mostly urban area of Southern Ontario. The incidence of lower respiratory disease (bronchitis, pneumonia and croup) in status Indian children was almost three times the rate of non-Indian children. There was also a tendency for multiple episodes of pneumonia and bronchitis among Indian children.

High incidence of otitis media among Native children has been reported throughout the second half of this century. Studies demonstrating this have been reported from British Columbia, Northwest Territories, Ontario, and Quebec (Evers *et al,* 1985; Julien *et al,* 1987; McCullough, 1990; Salloum and Crysdale, 1990; Thomson, 1994; Harris *et al,* 1998). High otitis media illness rates among selected racial groups including Native Americans, Alaskan Eskimos and Australian Aboriginal have also been described (Westwater and

Rebgetz, 1990; Nu, 1999). Despite the association of a high incidence of otitis media in Native children, relatively little is known about the consequences and epidemiological risk factors involved in the pathogenesis of this disease. Insufficient data exists for determining if the differences are biologically based or represent problems in access to medical care.

1.3.3 Population Projections

The Aboriginal population in Saskatchewan represents approximately 134,000 people of a total population of one million (Federation of Saskatchewan Indian Nations, 1997). Regina and Saskatoon have the highest number of Aboriginal people per capita in Canada (Heath and Welfare Canada, 1990). The 1996 census figures indicate Aboriginal people account for 7.5% of Saskatoon's population and 7% of Regina's population. By applying the current fertility, birth and death rates for the total Saskatchewan Native population, the expected population breakdown over a 50-year forecast period can be calculated. By the year 2045, the expected Aboriginal population will make up 32.5% of the total population, compared to 13.5% in 1995 (Federation of Saskatchewan Indian Nations, 1997).

The projected growth of the Native population in Saskatchewan is significant, particularly when placed alongside the Non-Native population, which has much lower fertility and birth rates. The implications of the different rates are to be found in the contrasting age profiles of the population they create. It is predicted by the year 2015, 31% of the Indian population will be

classified as young (age 0-17); 62% will fall into the labor force age group; and 6% will be considered elderly (age 65+). By contrast the Non-Native population proportions for the three categories will be 20%, 63% and 17% respectively (Federation of Saskatchewan Indian Nations, 1997). Considering the Native population may be younger overall, the prevalence of otitis media may continue to be an increasing problem for this group.

1.4 Microbiology

1.4.1 Causative Pathogens

Streptococcus pneumoniae, Haemophilus influenzae, and *Moraxella catarrhalis* are the principle pathogens in AOM (Klein, 1994; McCarty, 1995; McLinn, 1995; Bluestone, 1998). *S. pneumoniae* can be recovered from approximately 30% to 40% of patients, and the frequency of recovery of this organism tends to increase with the patient's age (Brook, 1998a). *H. influenzae* is isolated from approximately 20% to 30% of patients, and the frequency of isolation decreases with age (Block, 1997; Brook, 1998a). Organisms less frequently isolated in AOM include *Streptococcus pyogenes, Staphylococcus aureus,* and various gram-negative bacilli such as *Pseudomonas aeruginosa* and *Escherichia coli* (Brook, 1998a). Anaerobic bacteria such as *Prevotella* spp., *Porphyromonas* spp. and *Fusobacteria* spp. have also been implicated as causative middle ear pathogens (Brook, 1998a).

1.5 Management of Otitis Media

Traditionally, physicians have taken an aggressive approach to treat various types of otitis media because of the long-term consequences associated with persistent middle ear fluid, i.e. permanent hearing loss and other developmental impairments. However, the association between early otitis media and later developmental impairments has yet to be proven. For this reason, controversy exists as to the overall effectiveness in treating children with antibiotics or surgical remedies, especially in an era where the dramatic emergence of multiple drug resistant bacterial pathogens is on the rise, as are health care costs.

1.5.1 Antimicrobial Treatment

Treatment of AOM with antimicrobials is mainly empiric because eardrums of infants and young children are difficult to visualize and subtle middle ear conditions are often difficult to diagnose (Froom *et al*, 1990; Blumer, 1998). In many circumstances, middle ear aspirations (MEAs) to identify the causative pathogen(s) cannot be performed due to several reasons: (1) many clinicians lack experience with the invasive procedure of tympanocentesis; (2) limited number of clinics and laboratory services exist for properly performing MEAs and analyzing the patient specimen and (3) long lapse time can occur for obtaining laboratory results (McCarty, 1995; Bluestone, 1998; Jacobs *et al*, 1998).

Amoxicillin remains the antimicrobial drug of first choice for treatment of uncomplicated AOM. Although amoxicillin has been shown to be highly

effective against *S. pneumoniae*, it is ineffective against beta-lactamase producing strains such as *H. influenzae* and *M. catarrhalis* (Klein, 1998; Leibovitz *et al*, 1998). However, AOM associated with the latter two pathogens is more likely to resolve spontaneously (Dowell *et al*, 1999). Recent recommendations by the Centers for Disease Control and the American Academy of Pediatrics state that the use of antibiotics should be withheld in children even after a course of antibiotic therapy for AOM, because of the belief that most effusions will resolve themselves. For example, middle ear fluid generally spontaneously resolves within three months in about 60% of cases and within six months in 85% of cases (Blumer, 1998). This strategy is somewhat flawed because the pathogen of greatest concern, *S. pneumoniae*, is the least likely to resolve spontaneously and it is the most common pathogen causing AOM (Klein, 1994; Barnett and Klein, 1995; Blumer, 1998; Dowell *et al*, 1999). Some evidence suggests that increasing the dose of amoxicillin to 80 to 100 mg/kg/day in two doses may be more effective in eliminating intermediate and highly resistant PRSP than the standard dose of 40 mg/kg/day (Lister *et al*, 1997; Bluestone, 1998; Dowell *et al*, 1998). Agents other than the widely used amoxicillin include: macrolides (erythromycin, azithromycin, clarithromycin); combination drugs with sulfa components such as trimethoprim/sulfamethoxazole; second generation (cefaclor, cefprozil and cefuroxime) and third generation (cefixime, ceftibuten and ceftriaxone) cephalosporins (Barnett and Klein, 1995; Aronoff, 1996; Block, 1997; Blumer, 1998; Kaplan and Mason, 1998; Dowell *et al*, 1999).

Limited evidence as to the clinical efficacy of antibiotics over long-term use has been previously reported. Myrind *et al.* (1981) found decreased pain in the penicillin group compared with the placebo group on day two but no difference for duration of fever, otorrhea, or effusion in patients followed for up to three months. In the trial of Burke *et al* (1991), by day three, pain disappeared in 75% of the patients in the placebo group compared to 82% of those receiving antimicrobial agents. At one week, 14.7% of patients receiving placebo were treatment failures compared with 1.7% receiving an antimicrobial agent, but thereafter, no significant outcomes were found in patients followed for one year. Thus, it appears that the use of antimicrobial agents demonstrates a modest shortening of the duration and pain from an acute infection but no long-term benefits are reported.

1.5.2 Myringotomy With or Without Tube Insertion

A more aggressive treatment approach may be warranted in children with chronic middle ear fluid. The Centers for Disease Control and the American Academy of Pediatrics suggest either antimicrobial treatment or myringotomy and tympanostomy tube placement for these children (Bluestone, 1998). Myringotomy, also known as tympanocentesis, is a procedure performed to relieve pressure and allow for drainage of purulent fluid from the middle ear. Insertion of a tympanostomy tube through the myringotomy incision of the eardrum allows for adequate drainage in an otherwise blocked ear. A key issue in research into the natural history of OME is the association

between early life otitis media and later developmental impairments such as speech, learning and language development (Khurana, 1995; McCarty, 1995; Paradise, 1998). At present, long-term effects of surgery on the above have not been identified. In addition, little is known about the short term benefits of surgery other than relief from middle ear effusion (Casselbrant *et al*, 1991; Kleinman, 1994; Klein *et al*, 1994; Bluestone, 1998).

1.6 Antibiotic Resistance

1.6.1 Mechanisms of Resistance

The primary mechanism of bacterial resistance to β-lactam antibiotics for *H. influenzae* and *M. catarrhalis* isolates is the production of β-lactamases. A β-lactamase is an enzyme that binds to a β-lactam antibiotic noncovalently and hydrolyses the cyclic amide bond of the lactam ring, and then releases the altered antibiotic (Stratton, 1996). This renders the agent inactive and the micro-organism resistant. The most important β-lactamase in *H. influenzae* is TEM-1, which is found in 80% of β-lactamase positive isolates (Amyes and Gemmell, 1997). A second type of β-lactamase, designated ROB-1, has also been detected in *H. influenzae*, but is less common, accounting for 8% of all resistant organisms. *M. catarrhalis* isolates produce plasmid mediated β-lactamase enzymes designated BRO-1 and BRO-2. BRO-2 is associated with 90% or more resistant strains (Amyes and Gemmell, 1997).

PRSP has not been shown to produce β-lactamase enzymes that are associated with antibiotic resistance. Instead penicillin resistance in *S.*

pneumoniae seems to be due to alteration in penicillin binding proteins (PBP) (Wood, 1996). *S. pneumoniae* produces 5 high molecular weight PBP (1A, 1B, 2A, 2B, and 2X) and 1 low molecular weight PBP (PBP3) (Amyes and Gemmell, 1997). Alterations in the high molecular weight proteins are associated with decreased susceptibility to penicillin, whereas changes in PBP3 appear to have no role in the development of resistance (Amyes and Gemmell, 1997). The alterations found in resistant strains appear not to have risen by a process of mutation and selection, but through homologous recombination. Analysis of the sequences of genes encoding PBP in sensitive strains demonstrates that they are uniform. In contrast, the genes found in resistant isolates contain sequences of divergent DNA (Spratt, 1994). Consequently, exchange of DNA may be from another pneumococcus strain or from other streptococci species.

1.6.2 Overall Canadian Impact

The most notable trends in the bacteriology of respiratory illnesses during the past decade have been a rise in the proportion of patients infected with β-lactamase producing *H. influenzae* and *M. catarrhalis* isolates and an overall increase in drug resistant *S. pneumoniae* (Trembley *et al,* 1990; Scriver *et al,* 1994; Hoban *et al,* 1995; Blondeau *et al,* 1997; Blondeau *et al,* 1999). This is evident from results from four large surveillance studies (conducted during 1985 to 1995) indicating the percentage of ampicillin resistant *H. influenzae* isolates rose from 19% to 32% (Trembley *et al,* 1990; Scriver *et al,* 1994; Hoban *et al,* 1995; Blondeau *et al,* 1999). The prevalence of β-lactamase producing *M.*

catarrhalis isolates range from 80 to 85% (Hoban *et al,* 1995; Blondeau *et al,* 1999; Davidson *et al,* 1999). A similar increasing trend was observed among PRSP isolates. In the past, PRSP has been infrequently identified in Canada with three previous Canadian surveys reporting resistance rates of 1.5 to 2.4% (Dixon *et al,* 1977; Jette and Lamothe, 1989; Mazzulli *et al,* 1990). Recent studies now indicate that 12 to 22% of pneumococcal isolates show decreased penicillin susceptibility (Blondeau *et al,* 1996; Simor *et al,* 1996; Davidson *et al,* 1998; Blondeau *et al,* 1999). According to Davidson *et al* (1998), Saskatchewan has the highest incidence of PRSP (21%) in all of Canada.

Antimicrobial susceptibility studies for otitis media have been a focus of several North American studies. As of this date, none have been published within Canada. Results from American studies have found that up to 40% of all of nontypeable *H. influenzae* isolates and more than 90% of *M. catarrhalis* isolates from middle ear fluid are β-lactamase producers (Harold, 1995; Rodriguez *et al,* 1995; McLinn and Williams, 1996; Block, 1997). The incidence of PRSP remains variable among studies as resistant rates were found between 20% to 40% (Block *et al,* 1995; Rodriguez *et al,* 1995; Jacobs *et al,* 1998).

1.6.3 Saskatchewan Studies

Limited antimicrobial susceptibility studies have been published within Saskatchewan in regards to respiratory illnesses including otitis media. During 1995, Blondeau *et al* (1996) undertook a pilot study to generate current data on otitis media and antimicrobial resistance. Among 115 patients positive for

microorganisms, *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were frequently isolated. Susceptibility testing indicated that 39% (12/31) of *H. influenzae* isolates were β-lactamase positive and ampicillin resistant and among pneumococcal isolates 24% (4/17) showed decreased penicillin susceptibility. Of the 16 *M. catarrhalis* isolates recovered and tested, 100% were β-lactamase positive.

The high number of patients infected with PRSP poses a growing challenge to the management of otitis media because pneuococci are well known for cross resistance to alternative agents, thereby limiting patient drug therapy (Blondeau *et al*, 1995; Simor *et al*, 1996; Davidson *et al*, 1999). The situation is further complicated as the percentage of PRSP obtained from patient specimens can vary from one geographical area to another (Block *et al*, 1995; Rodriguiz *et al*, 1995; Jacobs *et al*, 1998). Blondeau *et al* (1997) tested 310 *S. pneumoniae* isolates obtained from 17 Saskatchewan medical centers located throughout Saskatchewan. Overall, northwest (16.8%) and northeast regions (20%) demonstrated higher intermediate resistance compared to southern locations (12.5% to 14.5%). Knowing that differential susceptibility profiles exist for given Saskatchewan regions may alter drug therapy for children suffering from otitis media.

1.7 Descriptive and Analytic Epidemiology

To clearly assess the emergence and spread of antimicrobial resistant pathogens, the application of a molecular strain typing method is considered along with the available clinical and epidemiological information.

1.7.1 Consent/Questionnaire Form

Construction of a consent/questionnaire form has allowed collection of additional information such as race, history of otitis media, antibiotic utilization, number of residents in one home, and places traveled. This data is normally not found on a specimen requisition sheet (see appendix D). Such demographic factors could facilitate transmission and emergence of resistant organisms (Shaw and Wendal, 1981; Embil *et al*, 1994; Block *et al*, 1995; Bluestone, 1998; Leibovitz *et al*, 1998).

1.7.2 Pulsed Field Gel Electrophoresis

1.7.2.1 Theory

One of the major limitations of DNA analysis by restriction endonuclease digestion followed by conventional agarose electrophoresis is the use of enzymes that have relatively frequent recognition sites. This creates difficulty in analyzing the resulting patterns due to the large numbers of overlapping, poorly resolved restriction fragments (<25 kb) (Arbeit, 1995). Fewer but much larger restriction fragments (10 kb to 800 kb) could be acquired by the use of an enzyme that has relatively few restriction sites. The problem of separating large restriction fragments of >25 kb cannot be readily overcome with

conventional electrophoresis. Recently, a method known as PFGE, was developed by Schwartz and Cantor (1984) to escape this size limitation. Large DNA fragments were capable of being separated by a specially designed chamber that utilizes multiple sets of electrodes that periodically alters the direction of the applied electric field. Several different methods of altering the electrophoretic conditions have been described (Schwartz and Cantor, 1986; Southern *et al*, 1987; Vollrath and Davis, 1987; Bancroft and Wolk, 1988). Some of these methods include field inversion gel electrophoresis, orthogonal field alteration gel electrophoresis, transverse field alteration gel electrophoresis and contour clamped homogenous electric field (CHEF) (Carle *et al*, 1986; Chu *et al*, 1986; Bancroft and Wolk, 1988; Smith *et al*, 1991). Among the methods previously listed, further discussion will focus on the CHEF system.

In conventional electrophoresis, application of an electric current is only applied to a gel in a single direction. The CHEF system utilizes four sets of electrodes positioned in a hexagon, creating various electric fields. The current is applied first from one set of electrodes, then shifts to the second set of electrodes for a brief period of time, and subsequently repeats the process for the third and fourth set. Each time the electrode current is applied, the DNA will attempt to reorient itself as the electrical field shifts. Longer molecules will reorient more slowly then shorter DNA sequences. Eventually, the back and forth movement results in high DNA fragment resolution.

There are a number of advantages to using PFGE as a molecular tool for studying bacterial lineages. First, the method has excellent reproducibility that can be demonstrated for a wide array of bacteria (Maslow *et al*, 1993). Reproducibility is defined as the ability to repeatedly obtain the same typing profile result with the same bacterial strain. Second, PFGE has proven to be highly discriminatory compared to other available methods for several common respiratory pathogens such as staphylococcus, *S. pneumoniae, H. influenzae,* and *M. catarrhalis* (Herman *et al*, 1995; Aparicia *et al*, 1996; Hoang *et al*, 1999). Discriminatory power refers to the ability to differentiate among unrelated strains which is determined by testing epidemiologically unrelated isolates that have proved to be indistinguishable by other techniques. Finally, the relative simplicity of the restriction profiles greatly facilitates the analysis and comparison of multiple isolates.

1.7.2.2 Application

Investigations of outbreaks of bacterial infections in hospitals and within the community often require strain-typing data to distinguish epidemic from endemic or sporadic isolates. The basic premise inherent in any molecular typing system is that epidemiologically related isolates are derived from the clonal expansion of a single precursor and share characteristics that differ from those of unrelated isolates (Maslow *et al*, 1993; Arbeit, 1995; Tenover *et al*, 1997). In other words, related isolates which are the recent progeny of a single or common precursor will have similar genotypes or banding profiles, whereas

epidemiologically unrelated isolates will have different genotypes. Determining whether isolates are the same or different is influenced by a number of inherent limitations possessed within a molecular method. Thus, variable reproducibility and discriminatory ability have been encountered among methods such as restriction endonuclease analysis (REA) and arbitrary primed polymerase chain reaction (AP-PCR) and will be further discussed.

REA uses an enzyme called an endonuclease that cleaves DNA at a particular sequence of nucleotides that may be repeated numerous times around the chromosome. The number and size of the restriction fragments generated by digesting a given piece of DNA, reflects the frequency and distribution of the restriction sites. Upon electrophoresis, hundreds of fragments can be separated, ranging from approximately 0.5-50 kilobase (kb) in length. Reproducibility has been shown to occur, however, interpretation of complex fragment profiles make this a tedious and time-consuming method (Maslow *et al*, 1993; Tenover *et al*, 1997). Furthermore REA patterns may be confounded by the presence of plasmids. Therefore, isolates that differ only in their plasmid content may be considered unrelated (Maslow *et al*, 1993).

In order to increase the ease in interpretation of hundreds of banding fragments generated by REA, additional steps can be undertaken that can be found in the method known as Southern blot. Following agarose gel electrophoresis, the separated restriction fragments are transferred onto a nitrocellulose or nylon membrane. A labeled piece of DNA or RNA (a probe) is

used to select for restriction fragments containing sequences homologous to the probe thereby limiting the number of fragments seen. Variations in the number and size of the fragments are referred to as restriction fragment length polymorphisms (Maslow *et al*, 1993; Arbeit, 1995).

One common typing method known as ribotyping involves the use of chromosomal DNA preparations and a ribosomal RNA probe. A RNA probe is used to combine with selective sequences homologous to ribosomal operon(s). Ribosomal operons are conserved clusters of genes that share related functions and are often coordinately regulated. All bacteria carry ribosomal operons and hence all bacteria are typeable (Arbeit, 1995). However, only moderate discriminatory power exists with this method due to the limited set of operons detected among bacterial species. Unlike bacteria such as *E. coli, Klebsiella* species, *Haemophilus* species, and *Staphylococcus* species that have multiple (5 to 7) ribosomal operons, other bacteria such as *M. catarrhalis, S. pneumoniae* and Mycobacteria isolates possess less numbers (Herman *et al*, 1995; Wilson *et al*, 1998; Nguyen *et al*, 1999). Bacteria possessing limited numbers of operons will often generate small numbers of band profiles that make for undesirable comparisons among isolates (Maslow *et al*, 1993). This could be overcome, in some cases, by changing the type of probe used. Currently DNA insertion element IS6110 is used to type isolates of *Mycobacterium tuberculosis* (Wilson *et al*, 1998). An insertion element is a piece of DNA that is able to move independently and can insert in multiple locations in plasmid or chromosomal

locations. This approach has proven reliable and discriminatory in differentiating outbreak strains of multi-resistant *M. tuberculosis* from other sporadic strains. For isolates with only a few copies of the insertion element, the method has relatively poor discriminatory power and must be supplemented by studies using other probes (Wilson *et al,* 1998).

Another factor affecting the interpretation of DNA fragment patterns is the choice of enzymes for preparing the chromosomal DNA digest. Ideally, restriction endonucleases should cut outside the ribosomal operons or insertion element, so that the entire sequence is present as a single fragment. However, restriction enzymes such as *Eco*RI has been shown to cut at several variable sites within the ribosomal operons thus generating short restriction fragments (Arbeit, 1995). Consequently, short sequences homologous to the probe are detected with only a weak signal compared to the signals of fragments that have more extensive homogenous sequences. This may account for such differences among epidemiologically unrelated isolates demonstrating the same pattern.

Randomly amplified polymorphic DNA assay, also referred to as AP-PCR is another alternative molecular strain typing method. This method is a variation of the PCR technique which employs a single short (10 base pair) primer that will hybridize at multiple random chromosomal locations. Amplification of the target fragment will occur if two hybridization sites are located within a few kilobases of each other, on opposite DNA strands, and in

the proper orientation. The number and location of these random sites vary among different strains. This approach is attractive because it is conceptionally simple, rapid (20 to 30 isolates can be typed per day) and suitable for use on a number of different species of bacteria (Adam *et al*, 1998; Rafferty *et al*, 1998; Dogan *et al*, 1999).

The potential speed and ease of AP-PCR must be tempered by the need to prepare samples with great care to obtain reproducibility and avoid cross contamination. For example, the reaction conditions such as variations in pH, ionic strength of the buffer used and/or the temperature or source of DNA polymerase dictates the efficiency in which primers initiate DNA synthesis (Vaneechoutte and Eldere, 1997). In addition, preparation of DNA extracts are easily contaminated by this method. Therefore, on independent amplifications of the same isolate, the number of copies generated from a particular locus can be appreciably different (Struelens *et al*, 1998). Obtaining reproducible and accurate results may be difficult with AP-PCR and as a result, the discriminatory power of this approach remains uncertain.

1.8 Summary

MEI are a significant cause of morbidity for children, especially those who are of Native descent and live in Northern regions of Canada. Most, if not all, children are often treated empirically with antibiotics because of a number of factors: (1) the eardrums of infants and young children are often difficult to visualize; (2) the eardrum findings associated with OME are often subtle and

the condition is therefore often difficult to diagnose; and (3) tympanocentesis, for determining the causative pathogen, may not be possible. Consequently over-utilization and improper utilization of antibiotics are contributing factors involved in the high prevalence of antibiotic resistant pathogens detected throughout the 1990's (Bluestone, 1998; Klein, 1998). The dramatic rise in antibiotic resistance amongst respiratory tract pathogens within Saskatchewan creates concerns as to whether there is a significant difference between antibiotic resistance in Native and Non-Native children given that respiratory tract infections occur more commonly in Native versus Non-Native people. Because antibiotic resistance patterns may vary regionally, surveillance data needs to be gathered locally. Recognition of changing patterns of antibiotic resistance in middle ear infections could affect patient management. Greater research is also needed to characterize factors contributing to the spread of antibiotic resistant pathogens in a community setting and facilitate the implementation of infection control practices.

1.9 Objectives

Given the overall problem of otitis media, a study was needed to clearly assess the etiology of this illness in terms of ethnicity, as well as the prevalence of antibiotic resistance regionally. Also the epidemiology of otitis media needs to be more fully characterrized in order to best understand the emergence and spread of resistant pathogens.

In order to carry out the concerns previously stated, 3 objectives were required: (i) to determine the prevalence of antibiotic resistance among middle ear pathogens isolated from children of Native and Non-Native ancestry, and who are living in various geographical regions within Saskatchewan; (ii) to establish the genetic relatedness of antibiotic resistant bacteria by PFGE; and (iii) to uncover possible reasons for clonal spread of resistant bacteria by collecting demographic data from children.

It is hypothesized that higher prevalence of antibiotic resistance will be detected in Northern Saskatchewan Native children when compared to Native and Non-Native children living elsewhere. Clonal spread of resistant pathogens may be linked to demographic features present in children.

2.0 MATERIALS AND METHODS

2.1 Patient Population

Patients aged 1 month to 15 years that were undergoing tympanocentesis, with or without tube insertion were enrolled in the study. All patients were seen at St. Paul's Hospital, Saskatoon, Saskatchewan, Canada, from November 1998 through February 1999.

2.1.1 Informed Consent/Questionnaire Form

Informed consent from parents and from older children was obtained before tympanocentesis or culture of drainage was performed. In obtaining informed consent, participants also agreed to answer questions regarding demographics as shown in Appendix D. The following data was obtained: age, sex, race, number of residents in one home, places traveled within the last year, past or recent history of otitis media and antibiotic utilization history. In regards to place of residence, patients were further categorized into specific geographic regions including Northern and Southern Saskatchewan, as well as the city of Saskatoon. Northern Saskatchewan areas were defined as being greater than 53 degree latitude north whereas Southern regions were classified as less than 53 degree latitude north. The consent/questionnaire form was

generated in collaboration with Father Mark Miller (ethicist at St. Paul's Hospital) and subsequently approved by the Biomedical Review Committee of St. Paul's Hospital in Saskatoon. All informed consent and questionnaires were administered by Marnie Andersen.

2.1.2 Tympanocentesis

Tympanocentesis was performed at enrollment by an otolaryngologist. Physicians were aware that the study results would not be immediately available to impact on patient care. These specialists included Doctors: Wright, Will, Alvi, Spafford, and Gore-Hickman, all practicing in Saskatoon. The procedure first involves cleaning the external ear canal of debris, then a 18 or 20 gauge spindle needle attached to a sterilized syringe was used to puncture the anteroinferior portion of the intact tympanic membrane. Fluid was aspirated into the syringe and then expelled into a sterilized centrifuge tube.

2.2 Standard Laboratory Methods

Suppliers of materials, reagents, and equipment are supplemented in 8.1.3 of Appendix C.

2.2.1 Specimen Processing

After collection of middle ear aspirates (MEA), all tubes were packaged and transported to the Antimicrobial Research Unit located at Royal University Hospital in Saskatoon within two to three hours of collection. In the microbiology laboratory, the aspirates were cultured on Tryptic soy agar media containing 5 % sheep blood and chocolate agar plates (see 8.1.1 of Appendix C).

Plates were incubated for 24 to 48 hours at 37°C with 5 % CO_2. The media, temperature and atmospheric conditions are optimal for recovery of fastidious and nonfastidous respiratory pathogens.

2.2.2 Identification of Organisms

Following inoculations, all plates were examined for growth of bacteria. Gram staining and colony morphology was used as the initial screen to identify organisms requiring further work-up. All tests selected were done according to NCCLS (National Committee for Clinical Laboratory Standards) guidelines, summarized in Table 2.1. Identification procedures are supplemented in 7.2 of appendix B.

2.2.2.1 Storage of Bacterial Isolates

All identified bacteria were stocked in 0.5 ml of skim milk in Simport cryovials. Prior to storage, the isolates were grown on Tryptic soy agar with 5% sheep blood for 18 hours in ambient air at 37°C with 5 % CO_2. One or two isolated colonies were transferred from the plate using a sterile wooden applicator stick to a cryovial containing the sterile skim milk. These vials were transferred to a -70°C freezer and stored.

2.2.3 Susceptibility Testing

There are several conventional or novel methods for performance of routine antimicrobial susceptibility testing. Common methods used within the clinical microbiology laboratory include disk diffusion test, agar dilution, Etest, and broth microdilution. For purposes of this study, only broth microdilution

Table 2.1: Selected identification tests as defined by NCCLS guidelines.

Organism	Test	Results
S. pneumoniae	gram stain	gram-positive diplococci
	colony morphology	α-hemolytic gray glistening colonies
	catalase	positive
	bile salt	soluble
	optochin	positive
H. influenzae	gram stain	gram-negative bacilli
	colony morphology	flat gray opague colonies
	porphyrin	negative
M. catarrhalis	gram stain	gram-negative coccobacilli
	colony morphology	dull yellow/gray colonies
	carbohydrate	
	fermentation - glucose	negative
	- maltose	negative
	- sucrose	negative
	- lactose	negative
	DNase	positive
	oxidase	positive
S. aureus	gram stain	gram-positive cocci
	colony morphology	β–hemolytic,yellow/white opague colonies
	catalase	positive
	Staphaurex	positive
S. pyogenes	gram stain	gram-positive cocci
	colony morphology	β–hemolytic, tiny gray/white colonies
	catalase	negative
	Path Dx Group A	positive

was performed. This method is a well-standardized, accepted and reliable reference method that is useful for research and clinical purposes.

2.2.3.1 Broth Microdilution

Minimal inhibitory concentrations (MIC) were determined as described by the NCCLS by the broth microdilution procedure. A volume of 50 µl of a 0.5 McFarland standard suspension of the test organism was diluted in the appropriate broth: Haemophilus test medium for *H. influenzae;* Todd Hewitt broth for *S. pneumoniae* and *S. pyogenes* and Mueller Hinton broth for both *M. catarrhalis* and *S. aureus* strains (200-µl total volume per well; final inoculum concentration 5 x 10^5 CFU/ml). Trays were incubated at 35°C (increased 5% CO_2 only for *S. pneumoniae* and *S. pyogenes* strains) for 16 to 20 hours prior to determining results. Before MIC for the clinical isolates were read and recorded, the growth control wells were examined for organism viability and inoculum purity. The following American Type Culture Collection (ATCC) strains were used (*S. aureus* 29213, *S. aureus* 25933, *S. pneumoniae* 49619, *H. influenzae* 49247, *H. influenzae* 49766, *E. coli* 25922, *P. aeruginosa* 27853) to confirm the acurracy of MIC each time susceptibility testing was performed. The MIC was interpreted as less than or equal to the lowest concentration of the antibiotic at which no growth was observed. Antibiotics tested in this study were as follows: ampicillin, penicillin, amoxicillin, cefaclor, cefuroxime, erythromycin, trimethoprim/sulfamethoxazole, levofloxacin, and gatifloxacin.

2.2.4 Pulsed Field Gel Electrophoresis

The process of PFGE ("DNA fingerprinting") involves embedding organisms in agarose, lysing the organism and digesting the chromosomal DNA with restriction endonucleases that cleave infrequently. Slices of agarose containing the chromosomal DNA fragments are inserted into the wells of an agarose gel, and the restriction fragments are resolved into a pattern of discrete DNA bands in the gel by a CHEF III apparatus that switches the direction of current according to predetermined conditions. The gel is stained with ethidium bromide and the DNA restriction patterns of the isolates are then compared using the BioRad Gel Doc 1000 System in order to determine their relatedness. Isolates are considered to be clonal or closely related if their macrorestriction profiles differ by one genetic event, i.e. 3 bands or less. The summary of PFGE protocols for all resistant pathogens are shown in Table 2.2. Complete details for bacterial specific PFGE protocols are described in 7.1 of Appendix B. Suppliers of all materials required for PFGE are identified in Appendix C.

2.2.4.1 Culture and DNA Preparation

Bacterial isolates were thawed and plated out on either chocolate agar or Tryptic Soy Agar (containing 5% sheep red blood cells) plates as previously described in section 2.2.2.1. From the growth plates, a bacterial suspension was made in Tris-EDTA Buffer (see Appendix A)that had an optical density equal to a 5.0 McFarland standard (approximately 15×10^8 CFU/mL). For *S.pneumoniae*

Table 2.2: Summary of PFGE protocols.

	M. catarrhalis	*S. pneumoniae*	*H. influenzae*	*S. aureus*	*s. pyogenes*
CULTURE					
- media/incubation times	BA plates/37°C, 16-20 hrs	BA Plates/37°C, 16-20 hrs, 5% CO_2	CA Plates/37°C, 16-20 hrs	BA Plates/37°C, 16-20 hrs	BA Plates/37°C, 16-20 hrs, 5% CO_2
- additional culturing	----	Transfer colonies into THB & grow to OD_{560nm}	----	----	Transfer colonies into THB & grow to OD_{560nm}
DNA PREPARATION					
- cell wash	----	PIV Buffer	----	----	PIV Buffer
- % LMPA	1.00%	1.60%	1.00%	1.30%	1.20%
- concentration of TE Buffer	0.1M Tris pH 8.0 & 0.5 M EDTA pH 8.0	0.1M Tris pH 8.0 & 0.5 M EDTA pH 8.0	0.1M Tris pH 8.0 & 0.5 M EDTA pH 8.0	1 M Tris-HCl pH 8.0 & 0.25 M EDTA pH 8.0	0.1M Tris pH 8.0 & 0.5 M EDTA pH 8.0
- lysing solution A/ incubation	0.5M EDTA, 10% Sarcosyl, 40 mg/ml PK/24 hrs @ 50°C	10mM Tris-HCl, 100mM EDTA, 0.5 % Brig, 0.2% DOC, 0.5% Sarcosyl/ 2-3 hrs @ 37°C	0.5 M EDTA, 10% Sarcosyl, 40 mg/ml PK/ 24 hrs @ 50°C	5 M NaCl, 0.25 M EDTA, 1M Tris-HCl, 10% Brig, 10% DOC, 10% Sarcosyl, 20 ug/ml Rnase, 500 U/ml Lysostaphin, 100 mg/ml lysozyme/ 48 hrs @ 50°C	10mM Tris-HCl, 1 M NaCl, 100 mM EDTA, 0.2% DOC, 0.5% Sarcosyl, 1 mg/ml Lysozyme, 20 ug/ml Rnase/6 hrs @ 37°C
- lysing solution B/ incubation	Replace with fresh Solution A/24 hrs @ 50°C	Same as Solution A except add 100 mg PK/ 24 hrs @ 50°C	Replace with fresh Solution A/24 hrs @ 50°C	0.5 M EDTA, 10% Sarcosyl, 40 mg/ml PK/ 24 hrs @ 50°C	0.25 EDTA, 1% Sarcosyl, 1 mg/ml PK/24 hrs @ 50°C
DIGESTION					
- enzyme, time	SpeI @ 37°C, 20 hrs	SmaI @ 27°C, 24 hrs	SmaI @ 27°C, 20 hrs	SmaI @ 27°C, 24 hrs	SmaI @ 27°C, 24 hrs
- run conditions	1-20 sec, 24 hrs in 1X TBE Buffer	5-30 sec, 20 hrs in 1X TBE Buffer	5-30 sec, 20 yrs in 1X TBE Buffer	5-45 sec, 20 hrs in 0.5X TBE Buffer	1-30 sec, 22 hrs in 0.5X TBE Buffer

and *S. pyogenes* isolates, colonies were first transferred to 10 ml of Todd Hewitt Broth and grown to an optical density at 560_{nm} of 0.3 to 0.5 and 660_{nm} of 0.5 to 0.6, respectively. Optical density of 1 ml of the inoculated broth was measured using a spectrophotometer. Cells were subsequently centrifuged to obtain a pellet of cells. The supernatant was removed and the cells were washed in PIV Buffer (see Appendix A) and then resuspended in 5 ml of TE Buffer. Aliquots of all bacterial-TE Buffer suspensions were mixed with an equal volume of low melting temperature agarose solution (see 7.1 of Appendix B or Table 2.2). The mixture was vortexed briefly then aspirated into either a 3 cc monoject Luer Lock syringe (for *M. catarrhalis* and *H. influenzae* isolates) or 250 µL was placed into a BioRad casting mold (for *S. pneumoniae, S. pyogenes,* and *S. aureus* isolates). The syringes and casting molds were then left at 4°C for 30 minutes. The solid agarose material obtained from the syringe was placed into a sterile petri dish and cut into 10 to 15 (approximately 3 mm in thickness) agarose plugs. Both the plugs and casting molds were added to sterile test tubes containing 5 ml of appropriate Lysis A Buffer (see Table 2.2) and incubated accordingly. At the end of the incubation period, the Lysis A Buffer was removed and replaced with 5 ml of Lysis B Buffer (see Table 2.2) and reincubated for 24 hours at 50°C in a shaking waterbath (see 7.1 of Appendix B or Table 2.2). To stop the Proteinase K action added to Lysis A and/or Lysis B solutions, a total of four rinse steps in 10 ml of TE Buffer were included in the procedure. Each rinse was for 30 minutes at room temperature with agitation.

2.2.4.2 Storage of Plugs

The TE Buffer rinse solution was removed from the plugs and replaced with 3 ml of fresh TE Buffer in Falcon multiple well tissue culture plates and stored at 4°C. The level of the TE Buffer was monitored weekly and refilled to prevent the plugs from drying.

2.2.4.3 Restriction Endonuclease Digestion

There were two procedures involved in the restriction endonuclease digestion step depending on whether the DNA was solidified in a plug or casting mold form. DNA prepared from a gel casting mold was removed from the well of the Falcon plates using a flame sterilized spatula and placed in a sterile petri plate. The mold was cut into quarters, with one quarter being transferred to a sterile 1.5 ml microcentrifuge tube. DNA prepared into cylindrical plugs were further cut in one-half using a sterilized blade and placed into a 1.5 ml microcentrifuge tube. Depending on the bacterial species a volume of the mixture (see 7.1 of Appendix B) of the manufacturer's restriction endonuclease buffer and enzyme was added to each tube. Excluding *H. influenzae* isolates, incubation times for bacteria digested with *Sma*I was 24 hours held at a constant temperature of 27°C. *Sma*I digestion for *H. influenzae* was for 20 hours at 27°C. *M. catarrhalis* isolates digested with *Spe*I required an incubation time of 20 hours at 37°C.

2.2.4.4 Preparation and Staining of Gels

A 1% pulsed field agarose gel (see Appendix A) was prepared in a 250 ml Erlenmeyer flask. Once dissolved in Tris-Boric Acid-EDTA Buffer (TBE Buffer) with heat, the molten agarose was cooled to 50-55°C and poured into a BioRad Laboratories pulsed field gel casting apparatus. After 30 minutes, the DNA digested plug molds were melted and added to the wells of the gel. The first, middle and last wells were reserved for a molecular weight marker known as BioRad lambda marker. The gel was submerged in TBE running buffer (see Appendix A) in the electrophoresis cell of a BioRad CHEF DR III System. The gel was subjected to pulsed orthogonal fields with various running conditions and times (see 7.1 of Appendix B or Table 2.2). After completion of electrophoresis, the gel was removed and placed into an ethidium bromide stain solution (see Appendix A) for 30 minutes in the dark. The gel was destained for 30 minutes in distilled water with agitation. The DNA patterns were recorded by a video camera called the BioRad Gel Doc 1000 linked to a computer system which incorporates molecular analyst software. The BioRad molecular analyst software is programmed to analyze DNA profiles in a stepwise process. This process involves (i) documentation of gels, which involves delineating and naming patterns; (ii) normalization of patterns; (iii) generation of databases and (iv) grouping or identification of patterns by quantification of their resemblance.

2.2.4.5 Analysis of Banding Profiles

The calculation of band matching coefficients was determined by the percent similarity method, also known as Dice coefficient (Herman et al, 1995). The method involves counting the number of shared DNA fragments, multiplying by two, dividing by the total number of DNA fragments in both the isolates banding profile and multiplying by 100. The resulting number gives a percentage value of the similarity between two isolates. Isolates were considered clonally related when the percent similarity was greater than 80%. Bands present outside the λ ladder molecular weight marker were not included in the analysis.

$$\%S = \frac{2n_{AB}}{n_A + n_B}$$

n_{AB} is the number of bands common for A and B, n_A refers to the total number of bands in A, n_B is the total number of bands in B, %S is the percent banding similarity between two isolates.

2.3 Data and Statistical Analysis

Univariate statistical analyses were performed comparing Native and Non-Native children, as well as children with or without specimens collected, by the chi square test, Fisher's exact test and analysis of variance depending on the data type. Analysis of variance was used for the analysis of means, and the chi square test or Fisher's exact test was used for the analysis of proportions. $P < 0.05$ was considered significant.

3.0 RESULTS:

3.1 Population Summary

During the specimen and demographic data collection period from November 1998 to February 1999, I obtained 100% consent and data collection on a total of 285 children. Two hundred and four MEA were collected from 285 children, representing a specimen collection rate of 72%. MEA not collected from 28% of patients were due to several reasons: (i) missed by doctor, (ii) absence of middle ear fluid, (iii) poor saline installation and or aspiration and (iv) insufficient specimen.

The following demographic data was collected on consent/questionnaire sheets: age; sex; race; number of residents in one home; places traveled within the last year; past or recent history of otitis media and antibiotic utilization history. Tabulation of this data is included in Tables 3.1, 3.2, and 3.3. Except for Table 3.2 (see later) and Table 3.3 (see later) no other comparisons were statistically different.

To reflect the demographic correlation in children with or without MEA, a comparison was made so as to affiliate the two data sets (see Table 3.1). Clearly the proportions of children were nearly identical among the differential data. Subdividing children by race and specimen type (children with or

Table 3.1: Demographic data comparison for children with and without specimens collected.

Demographic Characteristics	No specimen n=81	Collected Specimens n=204	p-value
Mean age (months)	46.8 ± 34.3	50.1 ± 32.9	NS
Gender			NS
Male	50 (62)	129 (63)	
Female	31 (38)	75 (37)	
Residence			NS
Saskatoon	39 (48)	101 (50)	
South Saskatchewan	25 (31)	63 (31)	
North Saskatchewan	17 (21)	40 (20)	
Mean number of residents/home	4.1 ± 1.4	4.4 ± 1.6	NS
Duration of residency			NS
Permanent residents	64 (79)	141 (69)	
Moved < 1 year	9 (11)	18 (9)	
Moved > 1 year	4 (5)	28 (14)	
Do not remember	4 (5)	17 (8)	
Regular guests			NS
Yes	35 (44)	91 (45)	
No	43 (53)	113 (55)	
Do not remember	3 (4)	0	
Traveled within last year			NS
Outside Saskatchewan	16 (20)	41 (20)	
Within Saskatchewan	4 (5)	16 (8)	
Had not traveled	61 (75)	147 (72)	
Chronic illness			NS
Asthma	19 (23)	34 (17)	
Allergies	4 (5)	10 (5)	
None	58 (72)	160 (78)	
History of ear infections			NS
Infection < 2 weeks	26 (32)	44 (22)	
Infection 1 month ago	16 (20)	32 (16)	
Infection 2 months ago	12 (15)	37 (18)	
Infection < 6 months	12 (15)	46 (22)	
Infection < 1 year	5 (6)	15 (7)	
Infection > 1 year	8 (10)	15 (7)	
Do not remember last infection	2 (2)	7 (3)	
Do not suffer from ear infections	0	8 (4)	
Antibiotic utilization for last ear infection			NS
Used antibiotics	76 (94)	189 (93)	
Had not used antibiotics	3 (4)	10 (5)	
Do not remember	2 (2)	5 (2)	
Completion of antibiotic prescription			NS
Yes	65 (80)	164 (80)	
No	8 (10)	20 (10)	
Continuing prescription	8 (10)	17 (8)	
Do not remember	0	3 (1)	

( ) = Percentage
NS = Not significant

Table 3.2: Demographic summary for Native children with and without middle ear aspirates collected.

Factors	MEA n=41	W/O MEA n=9	p-value
Age (months)			
Median	24	48	NS
Mean	36.5 ± 29.1	51.9 ± 31.3	
No. of males	31 (76)	3 (33)	p=0.014
No. of females	10 (24)	6 (67)	
Residents per home			
Mean	5.0 ± 2.5	5.1 ± 2.4	NS
Place of residence			NS
No. living in Saskatoon	17 (41)	4 (44)	
No. living in South Saskatchewan	2 (5)	0	
No. living in North Saskatchewan	22 (54)	5 (56)	
Duration of residency in present household			NS
No. living in a permanent residence	27 (66)	7 (78)	
No. who have moved < 1 year	5 (12)	1 (11)	
No. who have moved > 1 year	5 (12)	1 (11)	
No. who do not remember	4 (10)	0	
Regular guests			NS
No. with weekly guests	11 (27)	2 (22)	
No. with daily guests	9 (22)	0	
No. who don't have regular guests	20 (49)	6 (67)	
No. who were unsure when guests arrives per day or week	1 (2)	1 (11)	
Traveled within last year			NS
No. traveled outside Saskatchewan	8 (20)	0	
No. traveled within Saskatchewan	8 (20)	1 (11)	
No. who had not traveled	25 (60)	8 (89)	
Chronic illness which contribute to a respiratory illness			NS
No. who had asthma	9 (22)	1 (11)	
No. who had allergies	2 (5)	0	
No. who do not have a chronic illness	30 (73)	8 (89)	
History of ear Infections			NS
No. who had infection < 2 weeks	12 (29)	3 (33)	
No. who had infection 1 month ago	3 (7)	0	
No. who had infection 2 months ago	8 (20)	3 (33)	
No. who had infection < 6 months	11 (27)	2 (22)	
No. who had infection < 1 year	2 (5)	0	
No. who had infection > 1 year	2 (5)	1 (11)	
No. who do not suffer from ear infections	2 (5)	0	
No. who do not remember last infection	1 (2)	0	
Antibiotic utilization for last ear infection			NS
No. who had used antibiotics	38 (93)	9 (100)	
No. who had not used antibiotics	3 (7)	0	
No. who had not remembered	0	0	
Completion of antibiotic prescription			NS
No. who stated yes	33 (81)	8 (89)	
No. who stated no	2 (5)	0	
No. who were continuing their prescription	5 (12)	1 (11)	
No. who could not remember	1 (2)	0	

NS = Not significant
MEA = Middle ear aspirates
w/o = Without
No. = Number
( ) = Percentages

Table 3.3: Overall demographic data summary for Native and Non-Native children.

Factors	Native n=50 (18)	Non-Native n=235 (82)	Combined n=285
Age (months)			
Median	28	48	48
Mean	39.3 ± 29.7	51.3 ± 33.6	49.2 ± 33.2
No. of males	34 (68)	145 (62)	179 (63)
No. of females	16 (32)	90 (38)	106 (37)
Residents per home			
Mean	*5.0 ± 2.5	*4.2 ± 1.2	4.3 ± 1.5
Place of residence			
No. living in Saskatoon	21 (42)	119 (51)	140 (49)
No. living in South Saskatchewan	*2 (4)	*86 (36)	88 (31)
No. living in North Saskatchewan	*27 (54)	*30 (13)	57 (20)
Duration of residency in present household			
No. living in a permanent residence	34 (68)	171 (73)	205 (72)
No. who have moved < 1 year	6 (12)	21 (9)	27 (10)
No. who have moved > 1 year	6 (12)	26 (11)	32 (11)
No. who do not remember	4 (8)	17 (7)	21 (7)
Regular guests			
No. with weekly guests	13 (26)	74 (31)	87 (30)
No. with daily guests	9 (18)	30 (13)	39 (14)
No. who do not have regular guests	26 (52)	125 (53)	151 (53)
No. who were unsure when guests arrives per day or week	2 (4)	6 (3)	8 (3)
Traveled within last year			
No. traveled outside Saskatchewan	8 (16)	49 (21)	57 (20)
No. traveled within Saskatchewan	*9 (18)	*11 (5)	20 (7)
No. who had not traveled	33 (66)	175 (74)	208 (73)
Chronic illness which contribute to a respiratory illness			
No. who had asthma	10 (20)	43 (18)	53(19)
No. who had allergies	2 (4)	12 (5)	14 (5)
No. who do not have a chronic illness	38 (76)	180 (77)	218 (76)
History of ear infections			
No. who had infection < 2 weeks	15 (30)	55 (23)	70 (25)
No. who had infection 1 month ago	*3 (6)	*46 (19)	48 (17)
No. who had infection 2 months ago	11 (22)	38 (16)	49 (17)
No. who had infection < 6 months	13 (26)	45 (19)	58 (20)
No. who had infection < 1 year	2 (4)	18 (8)	20 (7)
No. who had infection > 1 year	3 (6)	20 (9)	23 (8)
No. who do not remember last infection	2 (4)	7 (3)	9 (3)
No. who do not suffer from ear infections	1 (2)	7 (3)	8 (3)
Antibiotic utilization for last ear infection			
No. who had used antibiotics	47 (94)	218 (93)	265 (93)
No. who had not used antibiotics	3 (6)	10 (4)	13 (5)
No. who do not remember	0	7 (3)	7 (2)
Completion of antibiotic prescription			
No. who stated yes	41 (82)	188 (80)	229 (80)
No. who stated no	2 (4)	26 (11)	28 (10)
No. who were continuing their prescription	6 (12)	19 (8)	25 (9)
No. who could not remember	1 (2)	2 (1)	3 (1)

*$p<0.05$
( ) = percentage

without MEA) revealed one discrepancy found among gender in the Native sample population (see Table 3.2). Males represented 76% of children (i.e. 31/41) with MEA, whereas 67% (i.e. 6/9) of those without MEA were female. Consequently, the values may inherently distort the true representation of the Native sample population if both data sets are combined as well as inaccurate comparisons with the Non-Native population may also be contrived. Removal of the Native group (without MEA) from the study had not significantly affected gender differences in either group. For this reason combined data was generated on children regardless of specimen type (see Table 3.3).

Non-Native children represented the greatest distribution (82%) of the sample population. Ages ranged from < 1 to > 6 years of age (mean age 49.2 $\pm$ 33.2 months) and approximately two-thirds were male. Saskatoon was the main residence for nearly half of the children, however the distribution of Native and Non-Native children located in southern and northern communities of Saskatchewan varied. Native children resided mainly in northern communities, 54%, as opposed to 13 % of Non-Native children, whereas 36% of Non-Native and 4% Native children lived in southern areas of Saskatchewan ($p<0.001$). Seventy-two percent of children lived in a permanent residence where the mean number of residents per home was higher among Native children ($p< 0.05$). The majority of children in the study who had traveled within Saskatchewan during the last year were Native ($p<0.05$). No significant differences were seen among those traveling outside Saskatchewan. Seventy-

six percent of children had not suffered from a chronic illness, such as asthma or allergies, either of which could contribute to a respiratory tract infection. Out of 285 patients, 17 (6%) reported no prior history of ear infections; 3% could not recall any while 3% had other middle ear problems, i.e. hearing deficiency. Ear infections were most frequently reported within 2 weeks prior to tympanocentesis (25%). Non-Native children accounted for a greater number of cases in one month ($p<0.05$). Ninety-three percent were prescribed antibiotics to treat their infection but only 80% were compliant in completing their prescription.

3.2 Microorganism Distribution

A total of 98 pathogenic bacteria were recovered from 54 MEA. The distribution of pathogenic bacteria retrieved from Non-Native and Native children were 68% and 32%, respectively. *H influenzae* was recovered in 40% (n=39), *M. catarrhalis* from 31% (n=30), *S. pneumoniae* from 15% (n=15), *S. aureus* from 10% (n=10) and the remaining 4% (n=4) were *S. pyogenes* isolates.

3.3 Susceptibility testing

Antimicrobial susceptibility of 98 middle ear pathogens tested against 9 antimicrobial agents is presented in Table 3.4. The overall prevalence of antibiotic resistant bacteria recovered from 54 culture positive individuals was 87%.

Table 3.4: Antimicrobial susceptibility of 98 middle ear pathogens tested against 9 antimicrobial agents.

	I.D. #	Organism	Amox	Amp	Cef	Cf	Ery	Gati	Levo	Pen	SXT
1	25R	*S. pneumo*	0.06	<0.016	2	<0.25	<0.016	0.25	0.5	0.031	1/19
2	25L	*S. pneumo*	0.06	0.031	2	<0.25	0.031	0.25	0.5	0.031	1/19
3	65R	*S. pneumo*	<0.031	<0.016	1	<0.25	0.031	0.125	0.5	<0.016	2/38
4	65L	*S. pneumo*	<0.031	<0.016	1	<0.25	0.031	0.125	0.5	<0.016	2/38
5	86R	*S. pneumo*	<0.031	<0.016	1	<0.25	0.031	0.125	0.5	<0.016	32/608
6	90R	*S. pneumo*	0.06	0.125	1	<0.25	0.031	0.125	0.5	0.06	32/608
7	150L	*S. pneumo*	1	1	64	4	>16	0.125	0.25	2	32/608
8	150R	*S. pneumo*	1	1	64	2	>16	0.125	0.25	2	32/608
9	184L	*S. pneumo*	<0.031	<0.016	0.5	<0.25	<0.016	0.125	0.25	<0.016	2/38
10	210L	*S. pneumo*	<0.031	<0.016	1	<0.25	2	0.125	0.25	<0.016	8/152
11	218L	*S. pneumo*	<0.031	<0.016	0.5	<0.25	<0.016	0.125	0.5	<0.016	1/19
12	221L	*S. pneumo*	<0.031	<0.016	1	<0.25	<0.016	0.125	0.25	<0.016	1/19
13	278L	*S. pneumo*	<0.031	<0.016	1	<0.25	<0.016	0.25	0.5	<0.016	1/19
14	282R	*S. pneumo*	<0.031	<0.016	1	<0.25	<0.016	0.125	0.25	<0.016	2/38
15	282L	*S. pneumo*	<0.031	<0.016	1	<0.25	<0.016	0.125	0.25	<0.016	1/19
16	24L	*H. influ*	0.5	0.125	2	0.5	1	0.008	0.008	0.25	0.125/2.375
17	24R	*H. influ*	0.5	0.125	2	0.5	1	0.008	0.016	0.25	0.125/2.375
18	25R	*H. influ*	0.5	0.125	1	0.25	2	0.016	0.016	0.25	8/152
19	25L	*H. influ*	0.5	0.125	1	0.25	1	0.016	0.016	0.25	8/152
20	35R	*H. influ*	>64	>64	4	0.5	1	0.008	0.008	>64	8/152
21	35L	*H. influ*	>64	>64	4	0.5	1	0.004	0.008	>64	8/152
22	47R	*H. influ*	1	0.25	16	1	4	0.004	0.008	1	8/152

Table 3.4: Continued

	I.D. #	Organism	Amox	Amp	Cef	Cf	Ery	Gati	Levo	Pen	SXT
23	47L	*H. influ*	1	0.25	16	1	4	0.004	0.008	1	8/152
24	49L	*H. influ*	1	0.125	2	0.5	2	0.008	0.016	0.25	>8/152
25	50R	*H. influ*	1	0.125	4	0.5	2	0.004	0.016	0.25	0.5/9.5
26	55L	*H. influ*	1	0.25	4	0.5	1	0.008	0.016	0.5	>8/152
27	57L	*H. influ*	0.5	0.125	2	0.5	1	0.004	0.016	0.25	>8/152
28	65R	*H. influ*	64	32	8	0.5	1	0.004	0.008	>64	0.5/9.5
29	74R	*H. influ*	1	0.25	4	0.5	1	0.004	0.008	1	8/152
30	74L	*H. influ*	1	0.25	4	0.5	1	0.004	0.008	1	8/152
31	834	*H. influ*	64	8	1	0.25	1	0.004	0.008	>64	0.06/1.19
32	83L	*H. influ*	64	8	2	0.25	1	0.004	0.008	>64	0.06/1.19
33	89L	*H. influ*	1	0.25	4	0.5	0.5	0.008	0.008	0.5	>8/152
34	100L	*H. influ*	>64	64	2	0.5	2	0.004	0.008	>64	0.5/9.5
35	140L	*H. influ*	0.5	0.125	2	0.5	1	0.008	0.016	0.25	>8/152
36	159L	*H. influ*	0.5	0.25	2	0.5	2	0.004	0.008	0.25	0.5/9.5
37	159R	*H. influ*	0.5	0.25	2	0.5	2	0.004	0.008	0.25	0.5/9.5
38	197L	*H. influ*	0.5	0.125	4	0.5	2	0.004	0.008	0.5	8/152
39	197R	*H. influ*	0.5	0.125	8	0.5	2	0.004	0.008	0.25	8/152
40	201L	*H. influ*	1	0.25	32	1	1	0.004	0.008	0.5	>8/152
41	210R	*H. influ*	1	0.125	2	0.5	1	0.008	0.016	0.25	>8/152
42	210L	*H. influ*	1	0.125	2	0.5	1	0.008	0.016	0.25	>8/152
43	212L	*H. influ*	1	0.125	8	0.5	1	0.008	0.016	0.25	0.25/4.75
44	212R	*H. influ*	1	0.125	8	0.5	1	0.008	0.016	0.25	0.25/4.75

Table 3.4: Continued

	I.D. #	Organism	Amox	Amp	Cef	Cf	Ery	Gati	Levo	Pen	SXT
45	224R	*H. influ*	1	0.125	2	0.5	2	0.004	0.008	0.25	4/76
46	224L	*H. influ*	1	0.125	2	0.5	1	0.004	0.008	0.25	4/76
47	227L	*H. influ*	0.5	0.125	4	0.5	2	0.008	0.008	1	8/152
48	250R	*H. influ*	1	0.25	4	0.5	2	0.008	0.008	0.5	0.125/2.37
49	258R	*H. influ*	1	0.125	1	0.25	1	0.008	0.016	0.25	0.06/1.19
50	258L	*H. influ*	1	0.25	1	0.25	1	0.008	0.016	0.25	0.125/2.37
51	270L	*H. influ*	1	0.25	4	1	0.5	0.008	0.008	0.5	0.125/2.37
52	272R	*H. influ*	>64	>64	64	0.5	1	0.008	0.008	>64	>8/152
53	272L	*H. influ*	>64	>64	64	0.5	0.5	0.008	0.016	>64	>8/152
54	275L	*H. influ*	0.5	0.125	8	0.5	2	0.008	0.008	0.25	>8/152
55	9L	*M. cat*	32	8	4	0.5	0.25	0.016	0.031	>8	1/19
56	41R	*M. cat*	4	2	2	0.5	0.125	0.016	0.031	>8	1/19
57	87R	*M. cat*	8	2	2	0.5	0.125	0.016	0.031	8	1/19
58	87L	*M. cat*	8	4	1	0.5	0.125	0.016	0.031	8	0.5/9.5
59	86L	*M. cat*	32	8	0.5	1	0.125	0.06	0.125	>8	0.25/4.75
60	93L	*M. cat*	32	8	2	2	0.125	0.031	0.031	>8	0.25/4.75
61	93R	*M. cat*	32	8	1	1	0.125	0.016	0.031	>8	0.5/9.5
62	109BL	*M. cat*	4	4	1	<0.25	0.125	0.031	0.031	>8	0.5/9.5
63	153R	*M. cat*	8	4	0.5	0.5	0.125	0.016	0.031	>8	0.5/9.5
64	153L	*M. cat*	1	4	<0.25	<0.25	0.125	0.008	0.016	8	0.5/9.5
65	177R	*M. cat*	64	>8	4	2	0.125	0.031	0.031	>8	0.5/9.5
66	185R	*M. cat*	8	4	0.5	0.5	<0.06	<0.016	0.031	>8	0.25/4.75

Table 3.4: Continued

	I.D. #	Organism	Amox	Amp	Cef	Cf	Ery	Gati	Levo	Pen	SXT
67	218L	*M. cat*	16	1	8	2	<0.06	0.016	0.031	8	0.25/4.75
68	223R	*M. cat*	32	8	16	2	0.25	0.031	0.031	>8	0.25/4.75
69	223L	*M. cat*	32	8	16	4	0.25	0.031	0.031	>8	0.25/4.75
70	242L	*M. cat*	16	>8	1	0.5	0.125	0.016	0.031	>8	0.5/9.5
71	242R	*M. cat*	16	8	2	1	0.125	0.016	0.031	>8	1/19
72	241L	*M. cat*	16	8	0.5	1	0.125	0.031	0.031	>8	1/19
73	241R	*M. cat*	32	8	2	0.5	0.125	0.031	0.031	>8	1/19
74	256R	*M. cat*	64	8	<0.25	0.5	0.125	0.031	0.016	>8	0.5/9.5
75	256L	*M. cat*	64	8	<0.25	0.5	0.125	0.031	0.016	>8	0.25/4.75
76	258L	*M. cat*	16	4	1	2	0.125	0.031	0.031	>8	2/38
77	258R	*M. cat*	32	8	1	2	0.125	0.031	0.031	>8	2/38
78	259R	*M. cat*	32	4	<0.25	<0.25	0.125	0.031	0.016	>8	0.5/9.5
79	259L	*M. cat*	32	2	<0.25	0.5	0.125	0.016	0.016	>8	1/19
80	274R	*M. cat*	8	8	1	0.5	0.125	0.016	0.031	>8	0.25/4.75
81	274L	*M. cat*	8	2	<0.25	0.5	0.125	0.016	0.031	8	0.25/4.75
82	278L	*M. cat*	32	8	0.5	0.5	0.125	0.031	0.031	>8	0.5/9.5
83	289R	*M. cat*	8	4	0.5	1	0.125	0.016	0.031	>8	0.25/4.75
84	289L	*M. cat*	8	4	0.5	0.5	0.125	0.031	0.031	>8	0.25/4.75
85	7R	*S.aureus*	0.5	0.06	1	1	>64	0.06	0.25	0.06	0.5/4.5
86	7L	*S.aureus*	0.5	0.06	1	1	>64	0.06	0.25	0.06	0.25/4.75
87	112BL	*S.aureus*	4	1	2	1	0.5	0.06	0.125	1	<0.125/2.375
88	112BR	*S.aureus*	4	1	2	1	0.5	0.31	0.125	1	<0.125/2.375

Table 3.4: Continued

	I.D. #	Organism	Amox	Amp	Cef	Cf	Ery	Gati	Levo	Pen	SXT
89	90R	*S.aureus*	16	8	4	1	0.5	0.06	0.125	8	<0.125/2.375
90	209R	*S.aureus*	32	0.5	1	1	0.25	0.125	0.06	>8	<0.125/2.375
91	209L	*S.aureus*	64	>8	4	1	0.5	0.06	0.06	>8	<0.125/2.375
92	241R	*S.aureus*	0.25	>8	4	1	0.5	0.125	0.125	0.016	<0.125/2.375
93	269R	*S.aureus*	64	>8	8	1	0.5	0.06	0.125	>8	<0.125/2.375
94	269L	*S.aureus*	64	>8	4	1	0.5	0.06	0.125	>8	<0.125/2.375
95	97R	*S. pyogenes*	<0.25	<0.031	<0.25	<0.25	<0.06	0.125	<0.25	0.031	<0.125/2.375
96	97L	*S. pyogenes*	<0.25	<0.031	<0.25	<0.25	0.125	0.125	0.25	<0.016	<0.125/2.375
97	112BR	*S. pyogenes*	<0.25	<0.031	<0.25	<0.25	1	0.125	0.25	<0.016	<0.125/2.375
98	112BL	*S. pyogenes*	<0.25	<0.031	<0.25	<0.25	1	0.125	0.25	<0.106	<0.125/2.375

Amox = Amoxicillin
Amp = Ampicillin
Cef = Cefaclor
Cf = Cefuroxime
Ery = Eryuthromycin
Gati = Gatifloxacin
Levo = Levofloxacin
Pen = Penicillin
SXT = Trimethoprim/sulfamethoxazole
R = Right ear
L = Left ear

3.3.1 *Streptococcus pneumoniae*

The *in vitro* activities for penicillin-sensitive and resistant *S. pneumoniae* isolates tested against 7 antimicrobial agents are summarized in Table 3.5. All penicillin-susceptible pneumococcal isolates were sensitive to other β-lactam drugs including amoxicillin, cefaclor, and cefuroxime. Decreased activity for the penicillin-susceptible *S. pneumoniae* isolates tested against erythromycin and trimethoprim-sulfamethoxazole was observed among 8% and 100%, respectively. Two out of 15 isolates had high-level penicillin resistance (>2 ug/ml) and were also resistant to amoxicillin, erythromycin, trimethoprim-sulfamethoxazole and cefaclor. All penicillin susceptible and resistant pneumococcal isolates were uniformly susceptible to cefuroxime, levofloxacin and gatifloxacin. The majority of the resistant isolates recovered, including multi-resistant *S. pneumoniae,* were isolated from Non-Native children but no significant differences were found when compared to Native children (see Table 3.6).

3.3.2 *Haemophilus influenzae*

The overall summary of the antimicrobial susceptibility data for *H. influenzae* is shown in Table 3.7. Of the 39 isolates tested against ampicillin, 15% (i.e. 6/39) produced β-lactamase and 20% (i.e. 8/39) were resistant to ampicillin. Six out of 8 strains were β-lactamase positive ampicillin resistant, while the remaining strains were β-lactamase negative ampicillin resistant. Decreased activity was also detected among strains against cefaclor (13%) and

Table 3.5: The *in vitro* activities for penicillin-sensitive and resistant *Streptococcus pneumoniae* isolates tested against 7 antimicrobial agents.

	Amox	Ery	Cf	Cef	SXT	Levo	Gati	Total
PSSP								
N	0	1	0	0	13	0	0	13
%R	0	7.7	0	0	100	0	0	
PRSP								
N	2	2	2	0	2	0	0	2
%R	100	100	100	0	100	0	0	

Amox = Amoxicillin
Ery = Erythromycin
Cf = Cefuroxime
Cef = Cefaclor
SXT = Trimethoprim/sulfamethoxazole
Levo = Levofloxacin
Gati = Gatifloxacin

Table 3.6: The percentage of antimicrobial resistant bacteria isolated from Native and Non-Native children.

	Number		Percentage											
			Ampicillin		Penicillin		Amoxicillin		Erythromycin		Cefaclor		Trimethoprim-sulfamethoxazole	
	N[a]	NN[b]	N	NN	N	NN	N	NN	N	NN	N	NN	N	NN
S. pneumoniae	2	13	---	---	0	15	0	15	0	23	0	15	100	100
H. influenzae	18	21	0*	38*	---	---	---	---	---	---	6	19	39**	81**
M. catarrhalis	6	24	100	100	---	---	---	---	0	0	0	8	0	0
S. aureus	4	6	---	---	50	83	---	---	50	0	0	0	0	0
S. pyogenes	2	2	---	---	0	0	0	0	0	100	0	0	0	0

[a] Native
[b] Non-Native
--- No NCCLS breakpoint
* <0.005
** <0.05

Table 3.7: Antimicrobial susceptibility profiles for *Haemophilus influenzae* isolates.

	Amp	Amp β+	Cf	Cef	SXT	Levo	Gati
N	8	6	0	5	24	0	0
%R	20.5	15.4	0	12.8	61.5	0	0

Amp = Ampicillin
Amp β+ = Beta-lactamase positive ampicillin resistant
Cf = Cefuroxime
Cef = Cefaclor
SXT = Trimethoprim/sulfamethoxazole
Levo = Levofloxacin
Gati = Gatifloxacin
%R = Percent resistant
Total number of *H. influenzae* isolates tested was 39.

trimethoprim-sulfamethoxazole (62%). *H. influenzae* isolates were uniformly susceptible to levofloxacin and gatifloxacin.

All ampicillin resistant *H. influenzae* isolates were found in Non-Native children with an overall resistance rate of 38% (i.e. 8/21) (see Table 3.6). Higher resistance rates to cefaclor (19%) and trimethoprim-sulfamethoxazole (81%) were also detected among Non-Native children compared to Native children with resistance rates of 6% and 39%, respectively. Chi square analysis revealed a significant difference among ampicillin-resistant and trimethoprim-sulfamethoxazole resistant *H. influenzae* isolates in children separated by race ($p<0.05$). In spite of the fact that no *H. influenzae* breakpoints exist for amoxicillin, the MIC 90 level appears high in the Non-Native group (> 64 ug/ml) compared to the MIC 90 for isolates from Native children (1 μg/ml) (data not shown).

3.3.3 *Moraxella catarrhalis*

With the exception of ampicillin, the antimicrobials were highly active against *M. catarrhalis*. Beta-lactamase activity was detected from 100% of the *M. catarrhalis* isolates and correlated with 100% reduced ampicillin susceptibility. For the other antimicrobials, resistance was rare, with only 8% intermediate resistance to cefaclor found in isolates from Non-Native children ($p> 0.05$) (see Table 3.6). All isolates were susceptible to erythromycin, cefuroxime, trimethoprim-sulfamethoxazole, levofloxacin and gatifloxacin.

3.3.4 Other Middle Ear Pathogens

The remaining resistance data was generated on *S. aureus* and *S. pyogenes* isolates. Seven out of 10 *S. aureus* isolates were resistant to penicillin and 2 isolates were resistant to erythromycin. Penicillin resistant *S. aureus* isolates were detected in 71% of the Non-Native children as compared to 29% of the Native children. Only erythromycin resistant isolates were encountered in Native children. Among the *S. pyogenes* isolates, 2 out of 4 were resistant to erythromycin and they were detected in the Non-Native group. No significant differences were found among the resistant *S. aureus* and *S. pyogenes* isolates when both groups were compared. Cefuroxime, cefaclor, trimethoprim-sulfamethoxazole, levofloxacin and gatifloxacin proved consistently active against both *S. aureus* and *S. pyogenes* isolates.

3.4 Pulsed Field Gel Electrophoresis Banding Profiles

The macrorestriction profiles obtained with enzymes *Sma*I and *Spe*I were analyzed using the percent similarity method. A percent similarity of greater than 80 % between isolates was considered significant. All isolates with decreased susceptibility to at least one antimicrobial agent were further investigated for genetic lineage. Only clonal relationships were detected among *M. catarrhalis* and *H. influenzae* isolates. Isolates demonstrating identical banding profiles (i.e. 100% banding similarity) were recovered from the same patient, but different ears (left and right ear). The remaining organisms with

unrelated DNA banding patterns included *S. pneumoniae, S. aureus* and *S. pyogenes.*

3.4.1 Related Isolates

Our survey of *M. catarrhalis* isolates was based on the analysis of 30 strains. Between 9 to 14 fragments in the molecular size range of 50 to 250 kb were generated by *Spe*I digestion (see Figure 3.1). The relationship between antibiotic-resistant *M. catarrhalis* isolates was variable with a total of 22 distinct DNA profiles detected (see Figure 3.2). Among 19 isolates a total of 4 common banding patterns was found. One of the related banding patterns was shared in a cluster of isolates including 185, 259, and 256. A second cluster included isolates 87, 93, 241 and 242. The third common banding pattern-contained isolates 41, 86, 109B and 153, while the fourth banding pattern was shared among isolates 9 and 278.

Large chromosomal restriction fragments of 25 *H. influenzae* isolates were separated by PFGE. The molecular sizes of the *Sma*I fragments ranged from 50 to 450 kb. PFGE distinguished 17 unique patterns, with an average of 8 bands (ranged 6-9) (see Figure 3.3). Only two isolates, 201 and 275, demonstrated 80% banding similarity. The remaining DNA profiles differed by more than three bands.

3.4.2 Non-related Isolates

Well-resolved patterns of 7 to11 *Sma*I fragments of approximately 50 to 300 kb in size, differentiated 11 distinct fragment types for the 15 *S. pneumoniae*

Figure 3.1: A representative pulsed field gel of *Moraxella catarrhalis* isolates after digestion with *Spe* I. All isolates have an ampicillin MIC > 0.5 μg/ml which is high resistance. The isolates in lanes in 5 and 6 show intermediate resistance against cefaclor. Lanes 5 and 6, 8 and 9, 10 and 11, illustrate identical isolates obtained from both the left and right ears of the same child. Isolates in lanes 3 and 12 represent >80% banding similarity. Isolates in lanes 8 through 11 had 90% banding similarity (values obtained from Figure 3.2). Children represented by lanes 8 through 11 were siblings, whereas no demographic characteristics connect children characterized in lanes 3 and 12. Lanes 1, 7, and 13 represent the BioRad λ molecular weight markers.

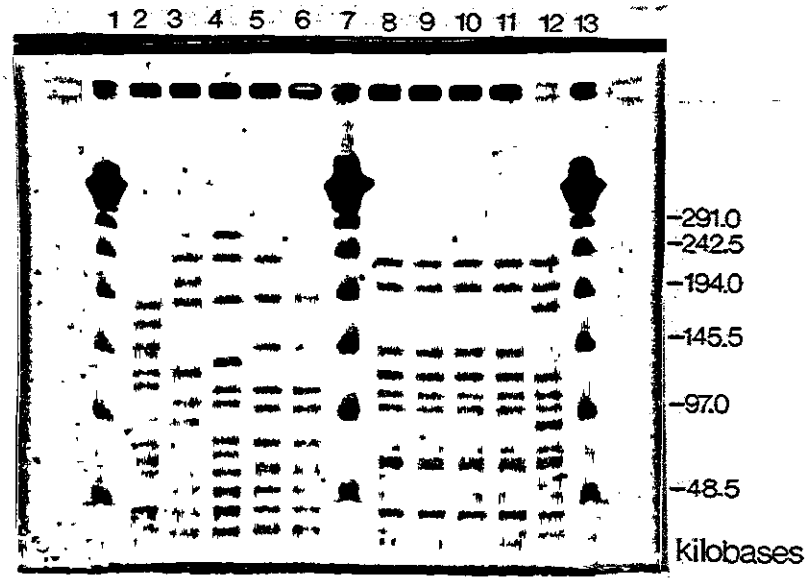


Figure 3.2: Dendrogram profiles of 30 *Moraxella catarrhalis* isolates. Analysis of fingerprints as determined by the Dice coefficient method. The scale measures similarity values, and the codes on the right give the isolate identification number. The numbers above the DNA banding patterns indicate the sizes of DNA fragments in kilobase pairs.

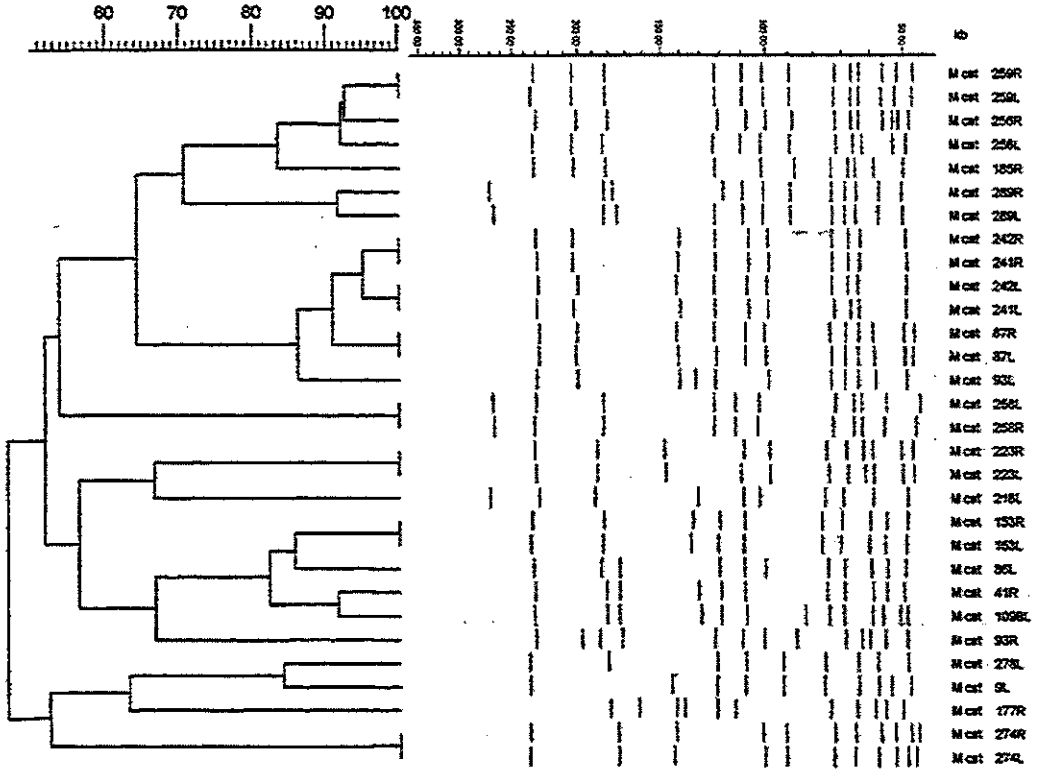
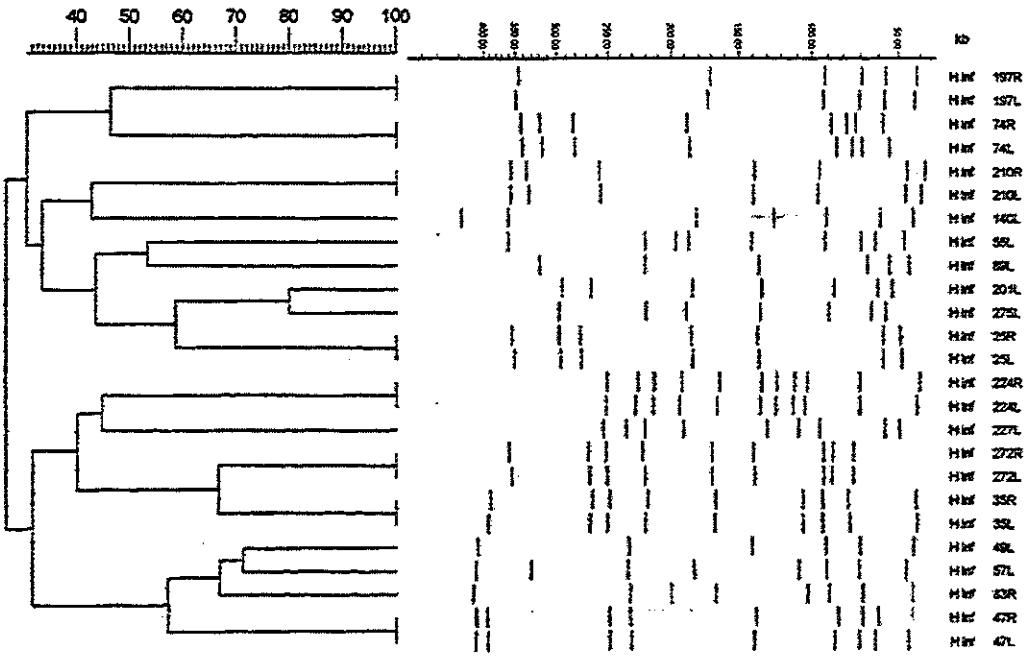


Figure 3.3: Dendrogram profiles of 25 *Haemophilus influenzae* isolates. Analysis of fingerprints as determined by the Dice coefficient method. The scale measures similarity values, and the codes on the right give the isolate identification number. The numbers above the DNA banding patterns indicate the sizes of DNA fragments in kilobase pairs.



isolates tested (data not shown). Digestion of *S. aureus* DNA with *Sma*I produced 8 to 11 fragments representing 7 distinct pattern types. Lastly, 7 to 8 fragments of approximately 50 - 300 kb were detected from two distinct fingerprints observed from 4 *S. pyogenes* isolates (data not shown).

3.5 Molecular and Demographic Epidemiology

Demographic data characteristics obtained from children harboring resistant pathogens revealed a number of significant findings (see Table 3.8). First, Non-Native children showed the greatest distribution of antimicrobial resistant pathogens (77%; $p=0.001$). Second, demographic differences among children of race occurred when place of residence was compared. Native residents of northern Saskatchewan were significantly more likely to be carriers of resistant pathogens compared to Non-Native children ($p<0.001$), whereas, greater numbers of Non-Native children living in southern areas of Saskatchewan harbored resistant pathogens ($p=0.036$). Third, Native children had a larger mean number of residents present in their household compared to Non-Native children ($p=0.019$).

High resistance rates obtained per subpopulation could not be readily linked to the spread of a predominate bacterial clone and geographical clustering among related isolates was largely absent (see Table 3.9). An exception to this was the shared banding profiles among *H. influenzae* isolates 201L and 275L. Isolate 201L was recovered from a Native child whereas isolate

Table 3.8: Demographic data summary for Native and Non-Native children harboring resistant otitis media pathogens.

Factors	Native	Non-Native	p-value
Number of isolates resistant	n=11 (23)	n=36 (77)	0.001
Age (months)			
Median	20	42	NS
Mean	21.7 ± 13.5	44.2 ± 27.3	
No. of males	8 (73)	24 (67)	NS
No. of females	3 (27)	12 (33)	
Place of residence			
No. living in Saskatoon	5 (45)	22 (61)	NS
No. living in South Saskatchewan	0	11 (30)	0.036
No. living in North Saskatchewan	6 (55)	3 (8)	<0.001
Residents per home			
Mean	5.4 ± 2.1	4.3 ± 1.0	0.019
Duration of residency in present household			NS
No. living in a permanent residence	8 (73)	24 (67)	
No. who had moved < 1 year	1 (9)	4 (11)	
No. who had moved > 1 year	0	6 (17)	
No. who do not remember	2 (18)	2 (6)	
Regular guests			NS
No. with weekly guests	4 (36)	15 (42)	
No. with daily guests	2 (18)	7 (19)	
No. who do not have regular guests	5 (45)	14 (39)	
Traveled within last year			NS
No. traveled outside Saskatchewan	2 (18)	3 (8)	
No. traveled within Saskatchewan	2 (18)	2 (6)	
No. who had not travelled	7 (64)	31 (86)	
Chronic illness which may contribute to a respiratory illness			NS
No. who had asthma	2 (18)	3 (8)	
No. who had allergies	2 (18)	4 (11)	
No. who do not have a chronic illness	7 (64)	29 (81)	
History of ear infections			NS
No. who had infection < 2 weeks	2 (18)	10 (28)	
No. who had infection 1 month ago	1 (9)	6 (17)	
No. who had infection 2 months ago	4 (36)	6 (17)	
No. who had infection < 6 months	3 (27)	6 (17)	
No. who had infection < 1 year	0	2 (6)	
No. who had infection > 1 year	1 (9)	2 (6)	
No. who do not remember last infection	0	2 (6)	
No. who do not suffer from ear infections	0	2 (6)	
Antibiotic utilization for last ear infection			NS
No. who had used antibiotics	10 (91)	31(86)	
No. who had not used antibiotics	1 (9)	3 (8)	
No. who do not remember	0	2 (6)	
Completion of antibiotic prescription			NS
No. who stated yes	8 (73)	28 (78)	
No. who stated no	1 (9)	5 (14)	
No. who were continuing prescription	2 (18)	3 (8)	

() = Percentage
NS = Not significant

Table 3.9: Demographic properties for children harboring related isolates.

Group	Patient	Age	Sex	Race	Home	#/Home	# Guests	Travel Last year	Chronic Illness	Last Ear Infection	Antibiotic Utilization	Completed Antibiotics
MC 1	185R	48	M	C	S'tn	5	5/wk	No	No	1 year	Yes	Yes
MC1	256 *	17	M	C	S.Sk	6	2/wk	No	No	2 wk	Yes	Yes
MC1	259 *	48	M	C	S'tn	4	6/d	Yes	No	2 wk	Yes	No
MC2	87 *	36	F	C	S.Sk	4	5/wk	No	No	<1 year	Yes	Yes
MC2	93L	13	M	N	S'tn	3	4/wk	Yes	No	3 mo.	Yes	Yes
MC2	241 *	36	M	C	S'tn	4	5/wk	No	Yes	2 wk	Yes	Yes
MC2	242 *	24	F	C	S'tn	4	5/wk	No	No	1 mo.	Yes	Yes
MC3	41R	13	M	C	S'tn	2	!	No	No	3 mo.	Yes	Yes
MC3	86L	14	M	C	S.Sk	3	No	Yes	No	missing	Yes	Yes
MC3	109BL	24	M	C	S'tn	4	No	No	No	3 mo.	Yes	Yes
MC3	153 *	30	M	C	S'tn	4	No	No	No	2 mo.	Yes	Yes
MC4	9L	24	M	C	S.Sk	4	No	No	No	3 mo.	Yes	Yes
MC4	278L	24	F	C	S'tn	8	No	No	No	?	?	?
HI	201L	24	M	N	S'tn	9	No	No	Yes	<2 wks	Yes	No
HI	275L	60	M	C	S'tn	4	No	No	No	2 mo.	Yes	Yes

R = Right ear
L = Left ear
wk = Week
d = Day
* = Isolates found in left and right ear
M = Male
F = Female
C = Caucasian

N = Native
S'tn = Saskatoon
SSK = South Saskatchewan
! = Unknown
? = Infection history unknown
MC = *M. catarrhalis*
HI = *H. influenzae*

Isolates with related banding profiles were identified in four clusters of *M. catarrhalis* isolates and one cluster of *H. influenzae*. Age was defined by month.

275L was obtained from a Non-Native child. Both children were residents of Saskatoon. An undisputed connection between demographic properties could not be provided among the two Saskatoon children nor those children sharing similar banding profiles living in widely separated areas.

4.0 DISCUSSION

The medical literature contains many reports of an increasing incidence of middle ear infections in children, particularly among Native children living in northern subpopulations of Canada. Management of middle ear infections often is a challenging endeavor and selecting the most appropriate antimicrobial(s) for treatment remains empiric as MEA are usually not obtained for culture. Furthermore treatment with antimicrobials has become more difficult in recent years as all of the predominant pathogens have become or are gradually developing resistance to commonly prescribed antibiotics. The recognition that various communities may serve as reservoirs of antibiotic resistant bacteria has raised interest in studying the transmission of antimicrobial-resistant bacteria. By determining that risk factors may exist in the spread of resistant pathogens will provide valuable insight into developing strategies for preventing the transmission of bacteria with resistant traits.

Specimen collection occurred during a four-month period between November 1998 to February 1999. During this period, 204 MEA were collected from 285 children. MEA were obtained mainly from Non-Native children (68%) who resided, for the most part, in two geographically distinct but continuous communities including Saskatoon and southern communities of

Saskatchewan. The remaining aspirates were recovered from Native children living predominantly within northern Saskatchewan regions (54%) and the city of Saskatoon (42%).

In total, 98 bacterial pathogens were recovered from 54 culture positive individuals. Common bacterial pathogens were recovered although an unexpected finding in the distribution of etiologic pathogens was encountered. *S. pneumoniae* was the third most common pathogen recovered from MEA, while *H. influenzae* followed by *M. catarrhalis* predominated. The marked reduction of *S. pneumoniae* isolates has been observed over the past 20 years. In the 1970's AOM was primarily a pneumococcal disease with occasional infection with *H. influenzae* and group A streptococcus (Barnett and Klein, 1995; Block, 1997). However, by 1997, approximately 40% of middle ear isolates recovered from infants and young children with AOM were *S. pneumoniae;* almost 30% were *H. influenzae* and 20% were *M. catarrhalis* isolates (Blumer, 1998). Although the incidence of *S. pneumoniae* appears to be decreasing, a marked reduction in the numbers detected from our study may have been influenced by our methodology. For example, MEA were collected in sterile centrifuged tubes. The fastidious nature of *S. pneumoniae* may require supplemented growth media in order to increase organism recovery. By placing middle ear fluid directly into a transport media may resolve the uncertainty.

In contrast, the methodology carried out in a Saskatchewan middle ear study performed during 1994 to 1995 found that *H. influenzae* was also the leading causative agent isolated from MEA. Both the 1994 to 1995 and 1998 to 1999 studies enrolled patients who were suffering from severe symptomatic AOM or chronic otitis media. Patients such as this may not represent the etiology of commonly isolated pathogens recovered from children with AOM.

National antimicrobial surveillance studies reveal that the incidence of antimicrobial-resistant pathogens including PRSP, and ampicillin resistant beta-lactamase producing strains such as *H. influenzae* and *M. catarrhalis* isolates are on the rise (Block *et al*, 1995; Harold, 1995; McLinn, 1995; Block, 1997; Brook, 1998b). A report from Saskatchewan had revealed 40% of *H. influenzae* and virtually 85% of *M. catarrhalis* strains had developed resistance to ampicillin (Blondeau *et al*, 1996). PRSP was identified in 24% of cases (Blondeau *et al*, 1997). Our study detected ampicillin resistance among *H. influenzae* and *M. catarrhalis* isolates, as well as PRSP, in rates of 20%, 100%, and 13%, respectively. Decreased resistance detected among *S. pneumoniae* and *H. influenzae* isolates could be due to the modest numbers of pathogens recovered from MEA and may underscore the results. Alternatively, the decline in antibiotic resistance may be attributed to a change in antimicrobial prescribing habits, as well as, greater compliance in antibiotic use. These factors were not investigated in this study. Explanations provided above do not account for high ampicillin

resistance rates among *M. catarrhalis* isolates, therefore, other factors should be taken into consideration.

The low incidence in the numbers of ampicillin-resistant *H. influenzae* isolates detected in our study remains unexplained. In contrast, lowered PRSP rates compared to the study conducted in 1995 could be attributed to sample population differences. The differences may be found in the distribution of children located in Saskatchewan. Geographical variation among PRSP rates has been known to occur among various North American studies. Resistance to penicillin and non-beta-lactam antibiotics of *S. pneumoniae* isolates obtained from children with invasive disease increased in two geographically distinct locations. A children's hospital in St. Louis, MO detected 26% resistance, whereas 41% was found in Atlanta, GA (Welby *et al*, 1994; Hofmann *et al*, 1995). In Northern Virginia, Rodriguez *et al* reported that PRSP was cultured from 21% of children who were diagnosed with AOM. Similarly, respiratory isolates, including isolates from MEA, collected from five regions of Saskatchewan showed considerable variation in resistance rates (Blondeau *et al*, 1997). Regional variations in high level resistance varied from 0 to 10% ($p \leq 0.012$ between two regions) and for intermediate resistance from 10 to 22%. Therefore, sample subsets of a participating population could be distinctly different between studies thus accounting for the variation in susceptibility profiles.

The pattern of increased resistance of otitis media pathogens has extended to many antimicrobial agents other than the penicillins. These include trimethoprim-sulfamethoxazole, cephalosporins and macrolides found in varying percentages in all species of bacteria tested. Resistance against cefaclor was found among *H. influenzae* and *M. catarrhalis* isolates at rates of 13% and 8%, respectively. Almost two-thirds of the *H. influenzae* isolates were trimethoprim-sulfamethoxazole resistant. In addition, 2 out of 10 *S. aureus* organisms and 2 out of 4 *S. pyogenes* isolates were resistant to erythromycin. Multi-drug resistance defined as bacteria that are resistant to 3 or more antimicrobials, was only found among *S. pneumoniae* isolates. This is a consistent finding in the emergence and spread of penicillin resistance in pneumococci. In general, pneumococci that are susceptible to penicillin remain highly susceptible to other antimicrobials. Pneumococcal isolates with intermediate or high levels of resistance to penicillin also demonstrate a concurrent decrease in susceptibilities to other β-lactam agents including cephalosporins (cefaclor, cefuroxime, cefixime), as well as trimethoprim-sulfamethoxazole and the macrolides (Block *et al*, 1995; Simor *et al*, 1996; Blondeau, 1997; Davidson *et al*, 1999).

To date, no studies have determined whether differences exist among antibiogram profiles in Native and Non-Native children with MEI. Previous studies have demonstrated that Native children are at higher risk than Non-Native children of acquiring otitis media and other upper respiratory tract

infections (Evers *et al*, 1985; Julien *et al*, 1987; McCullough, 1990; Thomson, 1994; Harris *et al*, 1998). The widespread use of antimicrobial agents and the dramatic rise in antibiotic resistance within Saskatchewan creates concern as to whether there is a significant difference between resistance rates in Native and Non-Native children. Originally, we predicted that antimicrobial resistance rates for middle ear pathogens (and to commonly used antimicrobial agents) would be higher in Native versus Non-Native children. This prediction was based on anecdotal evidence and limited collection in Saskatoon (Dr. J.M. Blondeau, personal communications; Blondeau *et al*, 1995). High illness rates of otitis media sufferers among the Native population would suggest greater overall drug therapy, thereby increasing the risk of developing or acquiring antimicrobial-resistant bacteria. Unexpectedly, our results indicated Non-Native children were significantly more likely to harbor antimicrobial resistant pathogens (77%; $p=0.001$). Out of all the resistant pathogens recovered from Non-Native children, only *H. influenzae* isolates resistant to ampicillin and trimethoprim-sulfamethoxazole ($p< 0.005$ and $p<0.05$ respectively) were found to be statistically significant when compared to the isolates obtained from Native children.

One of the main driving forces behind the progression of drug resistant bacteria is the selection pressure exerted by extensive antimicrobial use (Bacquero, 1999). When agents act upon pathogenic bacteria they also affect other bacteria. They eliminate drug susceptible bacteria that could otherwise

limit the expansion of pathogens and they simultaneously encourage the growth of resistant bystanders. Propagation of these resistant pathogenic and nonpathogenic bacteria increases the reservoir of resistance determinants in the bacterial population and greatly facilitates the likelihood that such factors will spread to other pathogens. To date, there is no definitive proof that suggests Native children are greater users of antimicrobial agents because of their high illness rates nor do they inappropriately utilize their prescription when compared to Non-Native children. This is also substantiated as no significant differences existed in the number of children who were prescribed antimicrobials nor those compliant in completing their antibiotic prescription (see Table 3.8). Higher antimicrobial resistance in the Non-Native population could not be readily explained by other potential risk factors listed in Table 3.8. Alternatively there was no intentional bias that would result in differential selection of participants to be either Native versus Non-Native in ancestry.

A possible explanation for higher antimicrobial resistance rates in the Non-Native population could be attributed to a high number of Non-Native children attending daycare. Children in daycare facilities are more likely to experience childhood infections than are children cared for at home (Wald *et al*, 1988; Khurana, 1995). There is also a greater risk of developing or acquiring antimicrobial resistant bacteria as new pathogens are brought in by other children (Khurana, 1995). Unfortunately, the initial study design failed to look

at daycare as a possible risk factor for determining differential antimicrobial resistant rates among Native and Non-Native children.

The full scope of antimicrobial resistance may not be adequately appreciated, in part, because studies that enroll selected medical centers may not completely justify the drug resistant problem for the entire population. One of the key issues in resolving selection bias is to conduct large multi-center studies which focus on obtaining greater population representation. Such centers tend to be located in highly populated areas which is characteristic of southern regions of Canada. Northern communities are smaller and distributed over large geographic areas. The area of northern Saskatchewan includes approximately one-half of the landmass of Saskatchewan (Salloum and Crysdale, 1990). Much of the area is part of the Canadian Shield, characterized by exposed bedrock, dense forest and innumerable lakes. The geography often creates communication and transportation problems that is inherent among various communities. Such communities may have nursing stations but lack services where specimens are collected and laboratory testing can be performed. Even medical centers such as LaRonge, for example, provides services for approximately 20,000 people and is considered one of the largest medical centers of the north (Saskatchewan Health, 1998). Limited services still exist for this center and this is especially true for microbiology testing. Most respiratory specimens are not collected, but if required, are often referred to other facilities where testing is routinely performed. However, the fastidious

nature of certain bacteria, such as *S. pneumoniae,* may not survive the transport. *S. pneumoniae* is one of the leading causative agents involved in otitis media and therefore it is important to properly assess the etiology of this illness. Problems such as above are barriers faced by researchers and as such larger medical centers located in southern locations are often used. It is unlikely that such centers represent the prevalence of antimicrobial susceptibility for the north.

Blondeau *et al,* 1997, further defined the problem of antimicrobial susceptibility in northern Saskatchewan including other geographical regions during a study involving collection of *S. pneumoniae* strains in 1995. Overall, Northwest and Northeast districts demonstrated higher intermediate penicillin resistance compared to Saskatoon, Regina, and Southwestern areas. Resistance rates were 16.8%, 20.0%, 14.3%, 12.5%, and 14.5%, respectively. Only high level PRSP (<3.7%) was found in Saskatoon and Regina. Critical assessment of the study revealed that respiratory specimens were obtained from both in and out patients. Inpatient specimens may confound the true representation of antimicrobial susceptibility for the general population. A selection pressure for antimicrobial resistance develops due to the extensive antimicrobial use in hospitalized settings. Bacteria can readily acquire or lose resistance determinants in a relatively short time frame (Arbeit, 1995). Including inpatient samples within a study could therefore increase or decrease the amount of antibiotic resistance detected depending upon when the specimens were collected. Furthermore larger northern health districts including

Keewatin Yathe, Mamawetan Churchill River, and Athabasca Health Authority were not enrolled for the reasons previously stated.

To better define the antimicrobial resistance problem among Saskatchewan children living in different geographical areas, all participants regardless of residence were brought to a single site for specimen collection. The potential design was to further decrease the selection bias by obtaining participants from all over Saskatchewan, and not from particular centers. Furthermore all children were classified as outpatients. Results revealed that Native children living in northern locations were more likely to harbor antimicrobial resistant pathogens compared to Non-Native children residing in the same comparable area. One possible explanation accounting for the difference could be due to large family size, which is considered a possible risk factor involved in the emergence and spread of resistant pathogens (Shaw and Wendal, 1981). In general, Native children belonged to larger families compared to Non-Native children ($p=0.019$). The reverse, however, does not occur for Non-Native children residing in southern areas of Saskatchewan that harbored a greater number of resistant pathogens ($p=0.027$). A more plausible explanation accounting for such differences could be due to the limited and unequal sample representation obtained per subpopulation. Better representation would have occurred if equal numbers of Native and Non-Native children from all regions, were included. The design bias of this study

could not have been readily overcome by the types of patients enrolled, but also due to the availability of ENT (ear, nose, and throat) specialists in the province.

Our patient selection was inherently skewed toward patients with more severe, symptomatic AOM or chronic otitis media, i.e. those with draining AOM and those projected to benefit from tympanocentesis with or without tube insertion. ENT specialists collect MEA, and their involvement was essential for proper specimen collection. ENT services are only available in Regina and Saskatoon, with the Saskatoon group being responsible for services to the largest percentage of the province (approximately 2/3 to 3/4). Conducting the study from Saskatoon was therefore necessary due to concerns associated with specimen transport while maintaining the viability of fastidious pathogens. To involve collections in Regina would not have been feasible due to the fact that the investigators needed to be present for collection of demographic data as well as ensuring that specimens would be immediately transported to the laboratory for processing. Due to the nature of this project the same individual (Marnie Andersen) needed to collect information and process specimens in order to ensure consistency with the study protocol. This becomes exceedingly evident as complete data collection dictates the direction of a molecular based epidemiological study.

The variable nature of antibiograms makes assessment of genetic lineage often difficult because antimicrobial susceptibility profiles rely on phenotypic differences. Phenotypic methods are those that characterize the products of

gene expression in order to differentiate strains (Arbeit, 1995). Properties such as bacteriophage types, biochemical profiles, antigens present on the cell's surface, and antimicrobial susceptibility profiles all are examples of phenotypic properties that can be determined in the laboratory (Maslow *et al*, 1993; Arbeit, 1995; Tenover *et al*, 1997). Because phenotypic characteristics involve gene expression, these properties all have a tendency to vary depending on changes in growth conditions, growth phase, and spontaneous mutation (Maslow *et al*, 1993; Tenover *et al*, 1997). Because of the problems with typeability, reproducibility and discriminatory power that have been associated with many phenotypic techniques, numerous genotypic techniques have been developed for molecular epidemiological studies (Maslow *et al*, 1993; Tenover *et al*, 1997).

An important aspect to any study interested in DNA analysis of an organism is the reproducibility and discriminatory power of a method. PFGE was chosen because of the excellent reproducibility and discriminatory ability obtained from various studies (Herman *et al*, 1995; Aparicia *et al*, 1996; Hoang *et al*, 1999). Similar findings occurred from our results. Reproducibility was encountered when developing *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* protocols within our laboratory. Each time isolates were tested the macrorestriction profiles remained consistent (data not shown). In regards to discriminatory power, the majority of isolates recovered from both the left and right ears of the same patient, revealed 100% banding similarity by the percent similarity method. Occasionally isolates were typed as $\geq$80% while only one

patient harbored two nonrelated *M. catarrhalis* isolates. Mixed infections have been known to occur (Arbeit, 1995; Wang *et al,* 1998) but the genetic variability of an organism, as well as, the inherent limitations of a method could be responsible for the variation detected among related isolates and thereby lowering the discriminatory power of this method.

Genetic variation among related isolates can occur unpredictably in a time span of 1 to 3 months (Maslow *et al,* 1993; Tenover *et al,* 1995; Tenover *et al,* 1997). Bacteria are capable of evolving very rapidly because of the speed of their replication. Minor modifications to enhance their survival within the environment niche can accumulate over time to produce a new pattern. For example, a single genetic event such as change in band size could be due to an insertion of DNA, or the appearance of two smaller bands where a single larger band had been seen could be the result of a random mutation in a restriction endonuclease site (Arbeit, 1995). When differences of two genetic events have occurred, results may indicate that the isolates are related, especially if they were collected over a long period of time (3 to 6 months). However, there also is a possibility that the strains are unrelated and the similarity was a result of chance (Arbeit, 1995). Often the use of a second strain typing method may clarify the relationship among the isolates examined. Due to the financial constraints of this study, a second typing method was not performed.

Technical factors may also contribute to differences seen among banding patterns. Variations in banding mobilities across a gel are due to the result of

varied agarose and buffer concentrations, gel thickness, and temperature (Swaminathar and Ghassen, 1993). Also, there is an inherent subjectivity to the decision of where bands belong in relation to one another when manually analyzing banding profiles of isolates from different gels. Our analysis utilized the BioRad molecular analyst fingerprinting software to limit subtle banding variability between gels. The program is a powerful tool for the alignment of patterns, but much of the success depends on the quality of the gels and the care and strategy of the user. For example, a global standard is selected to best represent all reference lanes that had incorporated a lambda ladder. Slight differences in migration patterns in the lambda ladder reference lanes compared to the global standard may disrupt the alignment and association of banding profiles of the adjacent lanes. Explanation such as the above, may account for variations seen in banding profiles involving *H. influenzae* isolates 201 and 275 (see Figure 3.3). It appears that there is a >3 band difference between the isolates. Manual analysis of the gel that contains both DNA profiles reveals a two band difference (data not shown). Moreover the system does not detect other problems that may be encountered with the PFGE protocol such as inadequate DNA or protein digestion.

By taking into consideration that design flaws are inherently found in PFGE, our analyses of results suggested no one predominate isolate or closely related isolate accounted for the decreased susceptibility to various antibiotics tested. Our findings indicated that although clusters of related PFGE patterns

were seen among 19 *M. catarrhalis* isolates and 2 *H. influenzae* isolates, the introduction and endemic spread of resistant pathogenic bacteria were due to a number of distinct isolates. Spread of isolates with similar banding profiles could not be linked to demographic features found among children in a consistent manner. For example, the relation between the use and misuse of antimicrobial agents and development of infection is considered to be a predisposing factor for the emergence of resistant bacteria (Brook, 1998; Leibovitz *et al,* 1998). Children varied among when the last ear infection occurred (<2 weeks to 1 year) and their compliance in completing their last antibiotic prescription (see Table 3.9). Alternatively, spread of resistant bacteria could be attributed to place of residence, exposure to family or guest contacts, and places traveled (Shaw *et al,* 1981; Baxter, 1990; MacMillan *et al,* 1996). Epidemiological evidence suggested no common connection among such factors.

4.1 Summary and Future Considerations

To date, this pediatric middle ear study remains the first epidemiological study in Canada for assessing the degree of antimicrobial resistance among Native and Non-Native children, with the potential design for understanding the emergence and spread of resistant pathogens. Results indicated that significant differences existed among children of race, especially when specific geographic locations were viewed separately. Although large family size in the Native sample population may explain higher resistance rates in the north, the

same risk factor could not be applied to Non-Native children living in southern Saskatchewan locations where high rates were found among these children.

Due to the inherent limitations of collecting MEA our patient selection consisted of children with more severe, symptomatic AOM or chronic otitis media. Further studies need to be conducted on children suffering from less severe middle ear complications of which the greater problem exists. In order to carryout such a study the following criteria should be taken into consideration: (1) obtain an adequate sample size that represents a large sociodemographically diverse population; (2) standardized diagnostic criteria for AOM; (3) immediate tympanocentesis on children diagnosed with AOM and (4) proper specimen transport to allow for greater survival of fastidious organisms. Due to limited ENT specialists in the province, tympanocentesis procedures may not be easily performed especially in Northern areas where limited medical services already exist. Nevertheless, such a study would further expand our understanding of the etiology of AOM in regards to antimicrobial resistance.

Knowing that resistance rates can vary among race and location provides valuable insight towards treatment practices. Bacteria such as *H. influenzae*, isolated from Non-Native children, were significantly more likely to be resistant to ampicillin and trimethoprim-sulfamethoxazole when compared to isolates from Native children. Although amoxicillin remains the ideal drug therapy, in situations where resistance occurs, antimicrobial agents including

ampicillin and trimethoprim-sulfamethoxazole may not be favorable alternative drug therapy for Non-Native children.

Macrorestriction analysis of resistant pathogens revealed that there were often clusters of similar isolates but most were independent due to lack of shared demographic characteristics. Future studies require a larger sample population with subsequent revisions to the data collection sheet in order to properly assess the emergence and spread of resistant pathogens. Initially the consent/demographic data form approved by the Biomedical Review Committee was constructed for the intent of obtaining information on individuals suffering from upper and lower respiratory infections. The study narrowed its focus for otitis media surveillance due to the limitations of specimen and data collection, as previously stated in section 4.0. Factors such as the incidence of AOM as single or recurrent episodes, number of office visits or surgical procedures, familial aggregation, breast feeding practices, group day care, and exposure to tabacco smoke have been implicated with the risk of otitis media and therefore should be further studied (Baxter, 1990; McCullough, 1990; Thomson, 1994; Block *et al*, 1995; MacMillian *et al*, 1996; Bluestone, 1998).

Antimicrobial resistance remains to be problematic and continued surveillance is necessary as susceptibility patterns change periodically. It is important for all communities to take part in such studies so that equal representation of the entire population can be adequately assessed. Studies

such as this may promote the necessity for additional funding for health services, especially for Saskatchewan's northern regions.

5.0 REFERENCES

Adam, C., Quin, M.M., McConnell, F., West, J., Lucey, B., Shortt, C., Cryan, B., Watson, J.B. 1998. Epidemiology and clinical impact of *P. aeruginosa* infection in cystic fibrosis using AP-PCR fingerprinting. Journal of Infectious Diseases 37:151-158.

Amyes, S.G., Gemell, C.G. 1997. Antibiotic resistance. Journal of Medical Microbiology 46:436-470.

Aparicia, P., Roman, F., Campos, J. 1996. Epidemiological characterization of *Haemophilus influenzae* using molecular markers. Enfermentation Infection Microbiology Clinica 14:227-232.

Arbeit, R.D. 1995. Laboratory procedures for the epidemiologic analysis of microorganisms. In: Manual of Clinical Microbiology, P.R. Murray *et al* (ed.), 6th ed. Washington, DC, ASM Press.

Aronoff, S.C. 1996. Antimicrobials in children and the problem of drug resistance. American Family Physicians 54:44-56.

Aronovity, G. 1998. Treatment of upper and lower respiratory tract infections: clinical trials with cefprozil. Pediatric Infectious Diseases Journal 17:S83-S88.

Bacquero, F., 1999. Evolving resistance patterns of Streptococcus pneumoniae: a link with long-acting macrolide consumption? Journal of Chemotherapy 11:35-43.

Bancroft, I., Wolk, C.P. 1988. Pulsed homogenous orthogonal field gel electrophoresis (PHOGE). Nucleic Acids Research 16:7405-7417.

Barnett, E.D., Klein, J.O. 1995. The problem of resistant bacteria for the management of acute otitis media. Pediatric Clinics of North America 42:509-517.

Baxter, J.D. 1990. What have we learned about otitis media and hearing loss by studying the native people of Canada? Journal of Otolaryngology 19:386-388.

Block, S.L., Christopher, J., Hedrick, J.A., Tyler, R.D., Smith, R.A., Keegan, E., Chartrand, S.A. 1995. Penicillin resistant *Streptococcus pneumoniae* in acute otitis media: risk factors, susceptibility patterns and antimicrobial management. Pediatric Infectious Diseases Journal 14:751-759.

Block, S.L. 1997. Causative pathogens, antibiotic resistance and therapeutic consideration in acute otitis media. Pediatric Infectious Diseases Journal 16:449-456.

Blondeau, J.M., Ashton, F.E., Isaacson, M., Yaschuk, Y., Anderson, C., Ducasse, G. 1995. *Neisseria meningitidis* with decreased susceptibility to penicillin in Saskatchewan, Canada. Journal of Clinical Microbiology 33:1784-1786.

Blondeau, J.M., Wright, G., Gore-Hickman, R., Maber, B., Spafford, P., and Will, D. 1996. Pilot Study of Pediatric Middle Ear Aspirates: Species and Antimicrobial Susceptibilities. 64th Conjoint Meeting of the Canadian Association for Clinical Microbiology and Infectious Diseases, Hamilton, Ontario, Canada, November 10-14, 1996. Abstract #Ps-70.

Blondeau, J.M., Donald, L., Vaughan, D. and the Canadian Antimicrobial Resistance Group. 1997. Regional resistance rates for penicillin resistant Streptococcus pneumoniae in Saskatchewan, Canada. 20th International Congress of Chemotherapy, Sydney, Australia, June 29 - July 3, 1997. Abstract #P039-4359.

Blondeau, J.M., Beggs, A. 1997. Antimicrobial resistant *Streptococcus pneumoniae* in Saskatchewan. Saskatchewan Medical Journal 8:16-17.

Blondeau J.M., Suter, M., Borsos, S., The Canadian Antimicrobial Study Group. 1999. Determination of the antimicrobial susceptibilities of Canadian isolates of *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*. Journal of Antimicrobial Chemotherapy 43:25-30.

Bluestone, C.D. 1998. Role of surgery for otitis media in the era of resistant bacteria. Pediatric Infectious Diseases Journal 17:1090-1098.

Blumer, J.L. 1998. Pharmacokinetics and pharmacodynamics of new and old antimicrobial agents for acute otitis media. Pediatric Infectious Diseases Journal 17:1070-1075.

Brook, I. 1998a. Microbiology of common infections in the upper respiratory tract. Primary Care 25:633-647.

Brook, I. 1998b. Microbial factors leading to recurrent upper respiratory tract infections. Pediatric Infectious Diseases Journal 17:S62-67.

Burke, P., Bain, J., Robinson, D., DonLeavy, J. 1991. Acute red ear in children: controlled trial of non-antibiotic treatment in general practise. British Medical Journal 303:558-562.

Carle, G.F., Frank, M., Olson, M.V. 1986. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. Science 232:65-68.

Casselbrant, M.L., Kaleida, P.H., Rockette, H.E., Paradise, J.L., Bluestone, C.D., Kurs-Lasky, M., Nozza, R.S., Wald, E.R. 1991. Efficacy of antimicrobial prophylaxis and of tympanostomy tube insertion for prevention of recurrent acute otitis media: results of a randomized clinical trial. Pediatric Infectious Diseases Journal 91:849-866.

Chu, G., Vollrath, D., Davis, R.W. 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science 234:1582-1584.

Davidson, R.J., the Canadian Bacterial Surveillance Network, Low, D.E. 1999. A cross Canada surveillance of antimicrobial resistance in respiratory tract pathogens. Canadian Journal of Infectious Diseases 10:128-133.

Dixon, J.M., Lipinski, A.E., Graham, M.E.P. 1977. Detection and prevention of pneumococci with increased resistance to penicillin. Canadian Medical Association Journal 117:1159-1161.

Dogan, B., Asikainen, S., Jousimies, S.H. 1999. Evaluation of two commerical kits and arbitrary primed PCR for identification and differentiation of *Actinobacillus actinomycetemcomitans, Haemophilus aprophilus* and *Haemophilus paraphrophilus*. Journal of Clinical Microbiology 37:742-747.

Dowell, S.F., Butler, J.C., Giebink, G.S., Jacobs, M.R., Jernilan, D., Musher, D.M., Rakowsky, A., Schwartz, B., The Drug Resistant *Streptococcus pneumoniae* Therapeutic Working Group. 1999. Acute otitis media: management and surveillance in an era of pneumococcal resistance – a report from the Drug Resistant *Streptococcus pneumoniae* Therapeutic Working Group. Pediatric Infectious Diseases Journal 18:1-9.

Embil, J., Ramotar, K., Romance, L., Alfa, M., Conly, J., Cronk, S., Taylor, G., Sutherland, B., Louie, T., Henderson, E., Nicolle, L.E. 1994. Methicillin-resistant *Staphylococcus aureus* in tertiary care institutions on the Canadian Prairies 1990-1992. Infection Control and Hospital Epidemiology 15:646-651.

Evers, S.E., Rand, C.G. 1982. Morbidity in Canadian Indian and non-Indian children in the first year of life. Canadian Medical Association Journal 126:239-252.

Evers, S.E. Rand, C.G. 1983. Morbidity in Canadian Indian and non-Indian children in the second year. Canadian Journal of Public Health 74:191-194.

Evers, S., Orchard, J. McCracken, E. 1985. Lower respiratory disease in Indian and non-Indian infants. Canadian Journal of Public Health 76:195-198.

Federation of Saskatchewan Indian Nations. 1997. Saskatchewan and aboriginal people in the 21st Century. Print West Publication Services, Regina: pp 10, 11, 60.

Frazer-Lec, N.J., Hessel, P.A. 1994. Acute respiratory infections in the Canadian native indian population: a review. Canadian Journal of Public Health 85:197-200.

Froom, J., Culpepper, L., Grob, P. 1990. Diagnosis and antibiotic treatment of AOM: report from International Primary Care Network. British Medical Jurnal 300:582-586.

Harold, C.N. 1995. Otitis media: antibiotic resistance of causative pathogens and treatment alternatives. Pediatric Infectious Diseases Journal 14:S51-S56.

Harris, S.B., Glazier, R., Eng, K., McMurray, L. 1998. Disease patterns among Canadian Aboriginal children. Canadian Family Physicians 44:1869-1877.

Health and Welfare Canada, Health Status of Canadian Indians and Inuit, Ottawa, ON, 1990.

Herman, P.W., Sluijter, M. Hoogenboezem, T., Heersma, H., VanBelkum, A., DeGroot, R. 1995. Comparative study of five different DNA fingerprint techniques for molecular typing of *S. pneumoniae* strains. Journal of Clinical Microbiology 33:1606-1612.

Hoang, V.U., Dulot, C., Moissenet, D., Favrova, B., Garbarg-Chenon, A. 1999. Comparison of randomly amplified polymorphic DNA analysis and PFGE for typing of *Moraxella catarrhalis* strains. Journal of Clinical Microbiology 37:450-452.

Hoban, D.J., Jona, R.N., the Canadian Ofloxacin Study Group. 1995. Canadian ofloxacin susceptibility study: a comparative study from 18 medical centers. Chemotherapy 41:34-38.

Hofmann, J., Cetron, M.S., Farley, M.M., Baughman, W.S., Facklam, R.R., Elliott, J.A., Deaver, K.A., Breiman, R.F. 1995. The prevalence of drug resistant Streptococcus pneumoniae in Alanta. New England Journal Medicine 333:481-486.

Hu, M., Galloway, J.M., Goldberg, B.W., Alpert, J.S. 1999. In: Primary Care of Native American patients. Bullerworth - Heinemann, Boston, MA:53-58.

Jacobs, M.R., Dagan R., Appelbaum, P.C., Burch, D.J. 1998. Prevalence of antimicrobial resistant pathogens in middle ear fluid: multinational study of 917 children with acute otitis media. Antimicrobial Agents and Chemotherapy 42:589-595.

Jette, L.P., Lamothe F. 1989. Surveillance of invasive *Streptococcus pneumoniae* infection in Quebec, Canada from 1984 to 1986: serotype distribution, antimicrobial susceptibility and cilnical characteristics. Journal of Clinical Microbiology 27:1-5.

Julien G., Baxter, J.D., Crago, M., Ilecki, H.J., Therien, F. 1987. Chronic otitis media and hearing deficit among native children of Kuujjvaraapik (Northern Quebec): a pilot project. Canadian Journal of Public Health 78:57-60.

Kaplan, S.L., Mason, E.O. 1998. Management of infections due to antibiotic resistant *Streptococcus pneumoniae*. Clinical Microbiology Review 11:628-644.

Karver, S. B. 1998. Otitis media. Primary Care 25:619-632.

Khurana, C. 1995. Issues concerning antibiotic use in child care settings. Pediatric Infectious Diseases Journal 14:S34-S38.

Klein, H. 1994. Otitis media. Clinical Infectious Diseases 19:823-833.

Klein, J.O. 1998. Clinical implications of antibiotic resistance for the management of acute otitis media. Pediatric Infectious Diseases Journal 17:1084-1089.

Kleinman, L.C., Kosecoff, J., Dubois, R.W., Brook, R.H. 1994. The medical appropriateness of tympanostomy tubes proposed for children younger than 16 years in the US. Journal of American Medical Association 271: 1250-1255.

Koufman, J.A. 1990. Core otolaryngology. J.B. Lippincott Company, Philadelphia, USA:93-99.

Leibovitz, E., Raiz, S., Piglansky, L., Greenberg, D., Yagupsky, P., Fuss, D.M., Leiberman, A., Dagan, R. 1998. Resistance pattern of middle ear fluid isolates in acute otitis media recently treated with antibiotics. Pediatric Infectious Diseases Journal 17:463-469.

Lister, P.D., Pong, A., Chartrand, S.A., Sanders, C. 1997. Rationale behind high dose amoxicillin therapy for acute otitis media due to penicillin non-susceptible pneumococci: support from *in vitro* pharmacodynamic studies. Antimicrobial Agents and Chemotherapy 41:1926-1932.

Locust, C.S. 1999. Overview of health programs for Canadian aboriginal people. Galloway, J.M. *et al*: In Primary Care of Native Patients, Bullerworth Heinemann, Boston, MS:17-32.

MacMillan, H.L., MacMillian, A.B., Offord, D.R., Dingle, J.L. 1996. Aboriginal health. Canadian Medical Association Journal 155:1569-1578.

Mao, Y., Morrison, H., Semenciw, R., Wigle, D. 1986. Mortality on Canadian Indian Reserves 1977-1982. Canadian Journal of Public Health 77:263-268.

Maslow, J.N., Mulligan, M.E., Arbeit, R.D. 1993. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. Clinical Infectious Diseases 17:153-164.

Mazzulli, T., Simor, A.E., Jaegar, R., Fuller, S., Low, D.E. 1990. Comparative *in vitro* activities of several new fluoroquinolones and β-lactam antimicrobial agents against community isolates of *Streptococcus pneumoniae*. Antimicrobial Agents and Chemotherapy 34:467-469.

McCarty, J.M. 1995. Bacterial susceptibility and tympanocentesis in acute otitis media. Pediatric Infectious Diseases Journal 14:S45-50.

McCullough, D.W. 1990. Chronic otitis media in the Keewatin area of the Northwest Territories. Journal of Otolaryngology 19:389-390.

McLinn, S. 1995. Double blind and open label studies of azithromycin in the management of AOM in children: a review. Pediatric Infectious Diseases Journal 14:S62-66.

McLinn, S., Williams, S.D. 1996. Incidence of antibiotic–resistant *S. pneumoniae* and beta-lactamase positive *Haemophilus influenzae* in clinical isolates from patients with otitis media. Pediatric Infectious Diseases Journal 15:S3-S9.

Morrison, H.I., Semencieu R.M., Mao, Y., Wigle, D.T. 1986. Infant mortality on Canadian Indian Reserves 1976-1983. Canadian Journal Public Health 77:269-273.

Mygrind, N., Larsen, M., Thomsen, J., Thomsen, V.F., Josefsson, K., Sorensen, H. 1981. Penicillin in acute otitis media: a double blind, placebo controlled trial. Clinical Otolaryngology 6:5-13.

Nelson, C.J., Mason, E.O, Kaplan, S.L. 1994. Activity of oral antibiotics in middle ear and sinus infections caused by PRSP: implications for treatment. Pediatric Infectious Disease Journal 13:585-589.

Nguyen, K.J., Hansen, E.J., Earinha, M.A. 1999. Construction of a genomic map of *Moraxella (Branhella) catarrhalis* ATCC 25238 and physical mapping of virulence associated genes. Canadian Journal of Microbiology 45:299-303.

Paradise, J.L. 1998. Otitis media and child development: should we worry? Pediatric Infectious Diseases Journal 17:1076-1083.

Rafferty, M.E., Baltch, A.L., Smith, R.P., Bopp, L.H., Rheal, C., Tenover, F.C., Killgore, G.E., Lyerly, D.M., Wilson, T.D., Schoonmaker, D.J., Hannett, G.E., Shayegani, M. 1998. Comparison of restriction enzyme analysis, AP-PCR and protein profile analysis typing for epidemiologic investigation of an ongoing *Clostridium difficile* outbreak. Journal of Clinical Microbiology 36:2957-2963.

Report of the Royal Commission on Aboriginal Peoples. 1996. Volume 1, pp. 12-13.

Rodriguez, W.J., Schwartz, R.H. Thorne, M.M. 1995. Increasing incidence of penicillin and ampicillin resistant middle ear pathogens. Pediatric Infectious Diseases Journal 14:1075-1078.

Salloum, S., Crysdale, W.S. 1990. Ear care for a Canadian native population. Journal of Otolaryngology 19:379-382.

Saskatchewan Health. 1998. Covered population, Corporate Information Branch ISSN 0319-5988.

Schappert, S.M. 1992. Office visits for otitis media: United States 1975-90. From Vital and Health Statistics of the Center for Disease Control/National Center for Health Statistics 214:1-18.

Schwartz, D.C., Cantor, C.R. 1984. Separation of yeast chromosome size DNAs by pulsed field gradient gel electrophoresis. Cell 37:67-75.

Scriver, D.E., Low, D.E., Simor, A.E., Toye, B., McGeer, A., Jaeger, R., The Canadian Haemophilus Study Group. 1992. Broth microdilution testing of *H. influenzae* with Haemophilus test medium versus lysed horse blood broth. Journal of Clinical Microbiology 30:2284-2289.

Scriver, S.R., Walmsley, S.L., Kau, C.L., Hoban, D.J., Brunton, J., McGeer, A., Moore, T.C., Witwicki, E., The Canadian Haemophillus Study Group. 1994. Determination of antimicrobial suseptibilities of Canadian isolates of *Haemophilus influenzae* and characterization of their β-lactamases. Antimicrobial Agents and Chemothearpy 38:1678-1680.

Senthilselvan, A., Habbick, B.F. 1995. Increased asthma hospitalizations among registered Indian children and adults in Saskatchewan, 1970-1989. Journal of Clinical Epidemiology 48:1277-1283.

Shaw, J.R., Wendal, N. 1981. Observations on the relation of environmental and behavioral factors to the occurrence of otitis media among Indian children. Public Health Report 96:342-349.

Simor, A.E., Louie, M., The Canadian Bacterial Surveillance Network. 1996. Canadian national survey of prevalence of antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae.* Antimicrobial Agents and Chemotherapy 40:2190-2193.

Smith, S.B., Heller, C., Bustamante, C. 1991. Model and computer simulations of the motion of DNA molecules during PFGE. Biochemistry 30:5264-5274.

Southern, E.M. , Anand, R., Brown, W.R.A., Fletcher, D.S. 1987. A model for the separation of large DNA molecules by crossed field gel electrophoresis. Nucleic Acids Res 15:5925.

Spratt, B.G. 1994. Resistance to antibiotics mediated by target alterations. Science 264:388-393.

Statistics Canada. 1997. Aboriginal health in Canada. Health and Welfare Canada, Ottawa ON, Cat. No. H34-35.

Stratton, C.W. 1996. The role of beta-lactamases. Antimicrobics and Infectious Disease Newsletter 15:17-23.

Struelens, M.J., Gheldre, Y.D., Deplano, A. 1998. Comparative and library epidemiological typing systems: outbreak investigations versus surveillance systems. Infection Control and Hospital Epidemiology 19:565-569.

Swaminathan, B., Ghassen, G. 1993. Molecular typing methods. In: D.H. Persing, *et al*, Diagnostic Molecular Microbiology - Principles and Applications, Washington, DC: DC Mayo Foundation.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology 33:2233-2239.

Tenover, F.C., Arbeit, R.C., Goering, R.V., The Molecular Typing Working Group for Healthcare Epidemiology of America. 1997. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infection Control and Hospital Epidemiology 18:426-439.

Thomson, M. 1994. Otitis media: How are first nation children affected? Canadian Family Physicians 40:1944-1950.

Tremblay, L.D., L'Ecyyn, J., Provencher, P., Bergeron, M.G., The Canadian Study Group. 1990. Susceptibility of *H. influenzae* to antimicrobial agents used in Canada. Canadian Medical Association Journal 143:895-901.

van Embden, J.D., Cave, M.D., Crawford, J.T., Dale, J.W., Eisenach, B., Gicquel, K., Hermans, F., Martins, C., McAdam, R., Shinnick, T.M., Small, P.M. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. Journal of Clinical Microbiology 31:406-409.

Vanuchoutte, M., Eldere, J.V. 1997. The possibilities and limitations of nucleic acid amplification technology in diagnostic microbiology. Journal of Medical Microbiology 46:188-194.

Vollrath, D., Davis, R.W. 1987. Resolution of DNA molecules greater than 5 megabases by contour-clamped homogenous electric fields. Nucleic Acids Res 15:7865-7876.

Wald, E.R., Dashefsky, B., Byers, C., Guena, N., Taylor, F. 1988. Frequency and severity of infections in daycare. Journal of Pediatrics 112:540-546.

Waldram, J.B., Herring, D.A., Young, T.K. 1995. Aboriginal health in Canada: historical, cultural and epidemiological perspectives. University of Toronto Press, Toronto, ON :10-12.

Wang, J. Sawai, T., Tomono, K., Yanagihara, K., Hirakata, Y., Matsuda, J., Mochida, C., Koga, H., Tashiro, T., Kohno, S. 1998. Infections caused by multiple strains of MRSA: a pressing epidemiological issue. Journal of Hospital Infections 39:221-225.

Welby, P.L., Keller, D.S., Cromien, J.L., Tebas, P., Storch, G.A. 1994. Resistance to penicillin and nonbeta-lactam antibiotics of *Streptococcus pneumoniae* at a childrens hospital. Pediatric Infectious Disease Journal. 13:585-589.

Westwater, A., Rebgetz, P. 1990. Epidemiology of otitis media in aboriginal children in Australia. In: Postl, B.D., Gelbert, G., *et al*, ed: Proceeding of 8th International Congress on Health. Winnipeg: University of Manitoba Press:623-625.

Wilson, S.M., Goss, S., Drobniewski, F. 1998. Evaluation of strategies for molecular fingerprinting for use in the routine work of a mycobacterium reference unit. Journal of Clinical Microbiology 36:3385-3388.

Wood, A.J. 1996. Antimicrobial drug resistance. New England Journal Medicine. 335:1445-1453.

Young, T.K. 1983. Mortality pattern of isolated Indians in Northwestern Ontario: a 10 year review. Public Health Reports 98:467-475.

6.0 APPENDIX A

6.1 Solutions and Buffers

10X TE Buffer
Dissolve 12.1g of Tris Base and 3.72 g of EDTA in 750 ml of distilled water. Bring to pH 8.0 using HCL. Adjust volume to 1 litre and autoclave.

10X TBE
Dissolve 90.8 g of Tris base, 15.4 g Boric acid, and 0.37 g disodium EDTA in 1000 ml of distilled water and autoclave.

0.5 M EDTA (8.0)
Combine 93.05 g of EDTA (disodium salt) in 400 ml distilled water. Add NaOH pellets a few at a time until EDTA is dissolved. Finish bringing the pH to 8.0 using 5M NaOH. Adjust volume to 500ml and autoclave.

10 % Sarcosyl
Dissolve 10 g of sodium sarcosinate in 70 ml distilled water. Heating the solution while stirring will help dissolve the sarcosyl. Adjust volume to 100 ml and autoclave.

10 % Brig
Dissolve 10 g in 100ml distilled water and autoclave.

10 % DOC
Dissolve 10 g in 100ml distilled water and autoclave.

Proteinase K (40 mg/ml)
Dissolve 400 mg Proteinase K in 10 ml sterile distilled water. Store at 20°C.

1% Pulse Field Agarose
Add 1.0 grams pulse field agarose to 100 ml 1X TBE. Boil until agarose is dissolved and cool to 50°C before pouring the gel.

PFGE Running Buffer
Dilute 300 ml of 10X TBE, in 2700 ml of distilled water, for a final concentration of 1X TBE.

Ethidium Bromide
Combine 40 µl stock ethidium bromide with 400 ml of distilled water.

PIV Buffer (10 mM Tris Hcl pH 8.0, 1 M NaCl)
Add 20 ml of 5M NaCl and 1 ml of Tris Hcl into distilled water for a 100 ml volume.

Skim Milk
Dissolve 200g into 1000ml of distilled water and autoclave.

Low Melting Temperature Agarose
Dissolve 1.0 g into 100ml of TE Buffer, pH 8.0. Heat to boiling for 1-1.5 minutes and cool to 50-65^{0}C in water bath.

Proteinase K Lysis Buffer
Add 20 µl of 40 mg/ml of proteinase K to 5 ml of lysis Buffer.

Rnase (20 µg/ml)
Add 0.1 ml of 10 mg/ml of Rnase into 50 ml dH20. Boil

TE Buffer
Add 5 ml of 1M Tris-HCl pH 8.0, and 8 ml of 0.25M EDTA pH 8.0 into 494 ml of distilled water.

7.0 APPENDIX B

7.1 PFGE Protocols

PFGE protocol for *H. influenzae* and *M. catarrhalis:* Organisms were grown overnight at 37°C on chocholate agar (*H. influenzae*) or Tryptic soy agar with 5 % sheep blood agar (*M. catarrhalis*). A 0.5 McFarland suspension of the organisms were suspended in 1X TE Buffer, then added to a 1.0 % low melt agarose. The suspension was aspirated into a 5 ml syringe and cooled at 4°C for 30 minutes. After solidification, agarose plugs were cut into 1/8" sections and exposed to a Proteinase K Lysis Buffer (0.5M EDTA, 10% Sarcosyl, 10 mg/ml Proteinase K, sterile distilled water), for 48 hours at 50°C in a shaking water bath. Lysis Buffer was replaced after first 24 hours with fresh Lysis solution and reincubated for another 24 hours at 50°C. Plugs were washed four times in 1X TE Buffer, 30 minutes each time. A single gel plug was cut into half's and digested with either 35 units of *Sma*I at 27°C for *H. influenzae* or *Spe*I at 37°C for *M. catarrhalis,* along with 250 µl enzyme buffer and 2,250 µl sterile distilled water. Digestion times for both organisms were 20 hours. DNA from each sample was added to 1 % Pulse Field Agarose and separated using an electric field apparatus (CHEF DR III system) set at specific run conditions: 1.0 - 26.0 second pulse for 24 hours (*H. influenzae*); 5.0 – 30.0 second pulse for 20 hours (*M. catarrhalis*). Gel was stained in ethdium bromide for 30 minutes at room temperature and destained in distilled water.

PFGE Protocol for *S. pneumoniae:* Eighteen to 24 hour colonies grown on Tryptic soy agar with 5 % sheep blood agar were inoculated in Todd Hewitt broth and incubated at 35°C with 5 % CO_2 until an optical density of 560nm was reached. Tubes were centrifuged to obtain a pellet of cells. Cells were resuspended in PIV Buffer (5M NaCl, 1M Tris-HCL pH 7.6, sterile distilled water) to wash the cells. A volume of 750 µl of the bacterial - PIV buffer suspension was added to a 1.6 % low-melting agarose and poured into plexiglass gel casting molds. After solidification, bacteria embedded in the agaroseblocks were lyzed in 100mM EDTA pH 8.0, 10mM Tris-HCl, 0.5 % Brij, 0.2 % DOC, and 0.5 % Sarkosyl, at 37°C in a shaking water bath for 2 to 3 hours. Lysis solution was removed and replaced with fresh lysis buffer with the addition of 100 mg/ml Proteinase K. Tubes were incubated at 50°C in a shaking water bath for 24 hours. Agarose molds were washed with TE buffer four times at room temperature for 30 minutes each time. One-fifth of the

agarose mold was cut from the block and digested with 35 units of *SmaI*, 20 µl *Sma*I Buffer, and 200 µl distilled water. The digested DNA plugs were placed in wells of a 1.0 % agarose gel. The digested DNA plugs were electrophoresed in a contour-clamped homogeneous electric field apparatus with initial to final time ranging from 1 to 15 seconds at 200 volts for 20 hours in 0.5X TBE Buffer.

PFGE Protocol for *S. pyogenes.* Same as *S. pneumoniae* protocol except with the following modifications. First, colonies suspended in Todd Hewitt Broth were grown to and optical density of 660nm. Second, additional reagents were added into the first lysis buffer solution which included 1M NaCl, 1 mg/ml lysozyme, and 20 µg/ml Rnase. Third, the incubation period for the first lysis solution was 6 hours. Fourth, the second lysis solution included only the following chemicals: 0.25 M EDTA, 1% Sarcosyl, and 1 mg/ml of Proteinase K. Fifth, the running conditions for the contour-clamped homogeneous electric field apparatus was 1.0 to 30.0 seconds for 22 hours.

PFGE Protocol for *S. aureus*. Organisms were grown overnight at 37°C on Tryptic soy agar with 5 % sheep blood agar. A 0.5 McFarland suspension of the organisms was suspended in 1X TE Buffer. A volume of 750 µl of the bacterial-buffer suspension was added to a 1.6 % low-melting agarose and poured into plexiglass gel casting molds. After solidification, bacteria embedded in the agarose blocks were lyzed in the following solution: 0.25 M EDTA, 1M Tris-HCl, 10% Brig, 10% DOC, 10% Sarcosyl, 20 µg/ml Rnase, 500U/ml lysostaphin, 100mg/ml lysozyme at 50°C in a shaking water bath for 24 hours. A second lysis solution (0.5 EDTA, 10 % sarcosyl, 40 mg/ml proteinase K) was added to the agarose molds at 50°C for 24 hours. Agarose molds were washed with TE buffer four times at room temperature for 30 minutes each time. One-fifth of the agarose mold was cut from the block and digested with 35 units of *SmaI*, 25 µl *Sma*I Buffer, and 200 µl distilled water. The digested DNA plugs were placed in wells of a 1.0 % agarose gel. The digested DNA plugs were electrophoresed in a contour-clamped homogeneous electric field apparatus with initial to final time ranging from 5 to 45 seconds at 200 volts for 20 hours in 0.5X TBE Buffer.

7.2 Bacterial Identification Testing

Bile Solubility Test

The bile solubility test detects the ability of bacterial cells to lyse in the presence of bile salts within a specific time and temperature. Sodium desoxycholate is the salt of purified bile acid used in the test. To test for bile solubility, one drop of the reagent is added directly onto a well isolated 18 to 24 hour colony. Incubate the plate aerobically at 35°C for 30 minutes, agar side

down. Examine the colony for disintegration and/or appearance of a partially hemolyzed area in the medium at the site where the colony was located.

Carbohydrate Utilization

Organisms utilizing carbohydrates will produce an acid environment which which exceeds the buffering capacity of the substrates, resulting in a color change in the indicator. A semisolid agar with 1 % of each of the carbohydrates: glucose, lactose, sucrose, and maltose, as well as, bromcresol purple are inoculated with a pure culture of the test organism. Incubate at 37°C for 24 to 48 hours and examine the tubes for the presence of acid.

Catalase Test

This test detects the presence of the enzyme catalase. The catalase test is performed by adding a colony on a wooden applicator stick to a suspension of 3 % H_2O_2 in a tube. The appearance of bubbles indicates that the enzyme catalase has hydrolyzed H_2O_2 into oxygen plus H_2O. No bubbles appear in a negative test result.

Cefinase Disks for β-Lactamase Production

Beta lactamase enzymes produced by bacteria break down penicillins and render the producing strains resistant to penicillins. The cefinase disk is impregnated with nitrocefin, a chromogenic cephalosporin. Colonies of the micro-organisms are rubbed onto a moistened disk. If the amide bond in the nitrocefin beta lactam ring is hydolyzed by a beta lactamase enzyme from the micro-organism, the color of the disk will rapidly change from yellow to red.

Dnase Agar

Production of the enzyme Dnase, which hydrolyzes DNA may be used to differeniate nonfermenting gram negative bacteria. The Dnase test medium contains toluidine blue complexed with DNA. Hydrolysis of DNA by the inoculated micro-organisms, after 24 to 48 hour incubation at 37°C, causes changes of structure of the dye to yield a colorless area where the inoculated organism had been applied.

Gram Stain

The gram stain is commonly used to differentiate bacteria into structural and taxonomic groups on the basis of their cell wall structure. A heat fixed smear of the organism is flooded with crystal violet for one minute then destained with tap water. A second stain, Gram's iodine, is applied to the slide for 1 minute then rinsed with water. Acetone is added briefly to the slide to decolorize the smear and immediately washed with water. A counterstain is applied to the smear with safranin for one minute. Wash in tap water and blot dry.

Optochin Susceptibility Test

Colonies of *S. pneumoniae* are inhibited by optochin (ethylhydrocupreine-hydrochloride) contained in the paper disk applied to the surface of an 5 % sheep blood agar plate. A zone of greater than or equal to 14 mm in diameter after a 24 hour incubation at 37°C with increased CO_2 is presumptive identification for *S. pneumoniae.*

PathoDx Group A

This is a rapid slide agglutination procedure for differentiating streptococci, which possess different Lancefield cell antigen groupings. In the PathoDx procedure, specific antibodies on latex particles react with, and agglutinate, streptococcal group antigen extracted from the bacterial cell wall using nitrous acid. The procedure is performed at room temperature with one or two 18 to 24 hour colonies. Agglutination within 60 seconds indicates a positive result.

Porphyrin production test

Porphyrin production test differentiates *H. parainfluenzae* from *H.influenzae. H. parainfluenzae* possesses an enzyme, porphobilinogen synthase that converts delta aminolevulinic acid into porphyrine and porphobilinogen. Kovacs reagent (p-dimethylaminobenzaldehyde) detects porphobilinogen appearing red.

Oxidase Spot Test

Oxidase is an enzyme possessed by some organisms which catalyzed the transport of electrons from donor compound (NADH) to electron receptors (O_2). In this test, the substrate paraphenylienediamine dihydrochloride serves as an artificial electron acceptor for the enzyme oxidase. The dye is oxidized and forms the colored compound indophenol blue. For this test, filter paper is saturated with the substrate. Colonies of the micro-organisms to be tested are rubbed onto the filter paper with a sterile wooden applicator stick. An immediate color change to a deep blue indicates a positive test.

Staphaurex

Staphaurex is a rapid slide agglutination procedure for differentiating staphylococci which possess coagulase and/or protein A, particularly *Staphylococcus aureus,* from staphylococci which possess neither of these factors. The Staphaurex reagent consists of polystyrene latex particles, which have been coated with fibrinogen and IgG. When mixed on a slide with a suspension of *S. aureus* organisms, reaction of clumping factor with the fibrinogen, and/or of protein A with the IgG causes rapid, strong agglutination of latex particles within 30 seconds.

8.0 APPENDIX C

8.1 Suppliers

8.1.1 Media

Chocolate Agar	PML Microbiologicals Winnipeg, MB.
Haemophilus Test Medium	PML Microbiologicals, Winnipeg, MB.
Mueller Hinton Broth	PML Microbiologicals, Winnipeg,
MB.Todd Hewitt Broth	PML Microbiologicals, Winnipeg,
MB.Tryptic Soy Agar with 5% Sheep Blood	PML Microbiologicals, Winnipeg, MB.

8.1.2 Antibiotics

Amoxicillin	Sigma-Aldrich Co., Oakville, ON.
Ampicillin	Bristol Laboratories,Montreal, Canada.
Cefaclor	Sigma-Aldrich Co., Oakville, ON.
Cefuroxime	Lilly Canada Inc., Toronto, ON.
Erythromycin	Novapharm Ltd., Toronto, ON.
Gatifloxacin	Bristol Myers, Montreal, QB.
Levofloxacin	Janssen-Ortho Inc., New York, ON.
Penicillin	Novapharm Ltd., Toronto, ON.
Trimethoprim-Sulfamethoxazole	Sigma-Aldrich Co., Oakville, ON.

8.1.3 Reagents, Chemicals, and Enzymes

Bile Salt (Sodium Desoxycholate)	Fisher Scientific Ltd., Nepean, ON.
Boric Acid	BHD Inc., Toronto, ON.
Brig-58	Sigma-Aldrich Co., Oakville, ON.
Carbohydrate sugars	Fisher Scientific Ltd., Nepean, ON.
Cefinase	VWR Scientific, Edmonton, AB.
Dnase	Fisher Scientific Ltd., Nepean, ON.
Deoxycholic Acid	Sigma-Aldrich Co., Oakville, ON.
EDTA	Sigma-Aldrich Co., Oakville, ON.
Ethidium Bromide	BioRad Laboratories, Hercules, CA.
Kovacs	PML Microbiologicals, Winnipeg, MB.
Hydogen peroxide	No Name, Saskatoon, SK.

Inoculum Water with Pluronic	Dade Diagnostic, West Sacramento, CA.
λ Ladder	New England BioLabs, Mississauga, ON.
Low Melting Temperature Agarose	BioRad Laboratories, Hercules, CA.
Lysostaphin	Sigma-Aldrich Co., Oakville, ON.
Lysozyme	Sigma-Aldrich Co., Oakville, ON.
N-laurylsarcosine	Sigma Chemical Co., St.Louis, MO.
Optochin	VWR Scientific, Edmonton, AB.
Oxidase	Fisher Scientific Ltd., Nepean, ON.
PathoDx Group A	Murex Biotech Limited, Dartford, UK.
Porphrin	PML Microbiologicals, Winnipeg, MB.
Proteinase K	Sigma-Aldrich Co., Oakville, ON.
Pulsed Field Certified Agarose	BioRad Laboratories, Hercules, CA.
Skim Milk	Fisher Scientific Ltd., Edmonton, AB.
*Sma*I	New England BioLabs, Mississauga, ON.
*Spe*I	New England BioLabs, Mississauga, ON.
Tris-HCl	Gibco BRL Technologies, Grand Island, NY

8.1.4 Disposable Plasticware

13 x 100 & 12 x 75 mm Culture Tubes	VWR Canlab, Edmonton, AB.
Falcon Multiple Well Tissue Culture Plates	VWR Canlab, Edmonton, AB.
Microcentrifuge Tube	VWR Canlab, Edmonton, AB.
Microtitre Plates	Sarstedt, St. Leonard, PQ.
3 c.c Monoject Leur Lock Syringe	Sherwood Medical, St. Louis, MO.
1-200 µl Pipette Tips	VWR Canlab, Edmonton, AB.
Sterile Plastic Petri Dish	Fisher Scientific Ltd., Edmonton, AB.
Sterile Scapel Blade	Feather Safety Razor Co., Medical Division, Japan.
Simport Cryovials	VWR Canlab, Edmonton, AB.

8.1.5 Equipment

CHEF DR III System	BioRad Laboratories, Mississauga, ON.
-70°C Freezer	Fisher Scientific Ltd., Nepean, ON.
Gel Casting Mold	BioRad Laboratories, Mississauga, ON.
Gel Doc 1000 Illuminator	BioRad Laboratories, Mississauga, ON.
Microwave Oven	Samsung, Suwon, Korea.
Pulse Field Gel Casting Apparatus	BioRad Laboratories, Mississauga, ON.
Shaking Water Bath	Mandel Scientific Co., Guelph, ON.

9.0 APPENDIX D

9.1 Consent/Questionnaire Form

*ID #*_____

Clinical Microbiology Department
Saskatoon District Health and St. Paul Hospital

Antibiotics are used to treat people with bacterial infections, but this is becoming more difficult due to the occurrence of antibiotic resistant bacteria.

The purpose of this study is to identify antibiotic resistant bacteria in those individuals with either a ear, nose, or lung infection. With your participation by answering the questions posed within this consent form, you will be increasing our understanding about the amount of antibiotic resistant bacteria present within Saskatchewan, as well as, a basic idea of how these bacteria are spread.

Those involved in this study which include your doctor, Dr. J. M. Blondeau (Supervisor of this study and Head of the Clinical Microbiology Department at Royal University Hospital) and Marnie Andersen (Master's student), will be only the people to see this consent form. **It is strongly emphasized that confidentiality will be maintained at all times!** By sharing important information about you, your health care providers will be better able to serve your medical needs.

Would you be willing to answer questions related to where you live, your medical history, and your ancestral background?

☐ **Yes** **or** ☐ **No**

For those who marked the yes box, please continue to answer questions 1 - 11 as best as you can.

---

1. What is your age?_____

2. What is your sex? (check applicable box)

☐ Male or ☐ Female

3. What is your ancestral background? (Please check off the group that best describes you).

☐ First Nation/Indian ☐ Metis ☐ Inuit
☐ NonTreaty
☐ Treaty(Treaty # optional)_____
☐ Caucasian
☐ Other (specify)_____

4. What is the usual number of residents living at your home?_____
Are there regular guests? ☐ Yes ☐ No
If yes, how many?_____

5. Where do you live? (Check and please name the place of residency in addition to stating how long you have lived there.)

☐ City_____
☐ Town_____
☐ Reserve/Band_____
☐ Village/Hamlet_____
☐ Farm_____

6. Do you have any chronic illnesses which contribute to respiratory infection? (Check the appropriate box)

☐ Yes or ☐ No

If yes, please specify.

☐ asthma ☐ allergies ☐ cystic fibrosis ☐ emphysema ☐ diabetes
☐ other_____

7. Have you suffered from any past infections, for example, infections of the ear, nose, throat, or chest? (Check the appropriate box)

☐ Yes or ☐ No

If yes, please specify what type of infection.

☐ ear infection ☐ pneumonia ☐ sinusitis (nose infection) ☐ bronchitis
☐ other (state)_____

Approximately when did the infection occur? _____

8. Have you ever used antibiotics, for example penicillin, to treat an infection? If the answer is yes, how long ago did you last take your antibiotic? (Check the appropriate box)

☐ Yes ☐ No

☐ 30 days	☐ < 5 years
☐ 6 months	☐ > 5 years
☐ 1 year	

9. Do you take all of your medication, e.g. antibiotic, as prescribed and until it is completed? (Check the appropriate box)

☐ Yes or ☐ No

10. Have you stopped taking medication (antibiotic) because of side effects? (Check the appropriate box)

☐ Yes or ☐ No

11. Have you done any significant traveling recently or in the past year? (Check the appropriate box)

☐ Yes or ☐ No

If yes, check and state where you stayed.

☐Within Saskatchewan_____

☐Within Canada_____

☐Outside Canada_____

CONSENT:

Participation in this study would be appreciated. All personal information will be kept confidential, and at no time will your identity be known to any publications such as magazines, newspapers, books, journals, internet, and other media sources such as t.v or radio. Your signature below indicates that you allow the release of demographic and medical information about you to Dr. J.M. Blondeau, as well as, Marnie Andersen. Your signature indicates that you have read and understood the information above. If you are unable to read this document, your signature indicates that this consent form has been read and explained to you.

_____

Participants signature

_____

Witness signature

_____

Date