Methicillin-resistant *Staphylococcus aureus* in Canadian hospitals from 1995 to 2007: a comparison of adult and pediatric inpatients.

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements for the Master's of Science degree in Epidemiology

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ABSTRACT

The literature directly comparing the epidemiology of MRSA among adult and pediatric hospitalized patients is strikingly minimal. The objective of this thesis was to identify any differences between these two patient groups. The Canadian Nosocomial Infections Surveillance Program MRSA data (1995 to 2007: n=1,262 pediatric and 35,907 adult cases) were used to compare MRSA clinical and molecular characteristics and rates. Hospital characteristics were modeled using repeated measures Poisson regressions. The molecular and epidemiological characteristics of MRSA differed significantly between adults and children. Compared to children, MRSA in adults was more likely to be healthcare-associated, colonization, SCC*mec* type II, PVL negative, and resistant to most antibiotics. Rates of MRSA in Canada increased in both populations over time but were significantly higher in adults. The hospital characteristics associated with increased MRSA rates differed in adult and pediatric facilities. Implications for infection prevention and control strategies are discussed.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Doug Coyle for supporting me throughout my entire Master's program. Dr. Coyle was very approachable and always helpful for advice on all aspects of my Master's.

I would like to express my sincere gratitude to Dr. Aboubakar Mounchili and Linda Pelude for their help and guidance with my thesis work and for always making themselves available to answer my questions.

I would like to thank Dr. Caroline Quach for all of her help in providing feedback and contributing her clinical knowledge to my project. I would like to especially thank her providing many additional educational opportunities.

I would like to recognize Denise Gravel for presenting the opportunity to complete my thesis at the Public Health Agency of Canada (PHAC).

This project would not have been possible without the work of the entire Canadian Nosocomial Infections Surveillance Program (CNISP) in collecting, reporting, and preparing the MRSA dataset over 13 years and for granting me access to the dataset. I would also like to thank all members of the CNSISP for the opportunity to present my work at the annual meeting and for providing their feedback on my project.

I had the incredible opportunity to present my work at the Association for Medical Microbiology and Infectious Disease (AMMI) in Canada annual conferences where I received feedback from professionals in this field and was able to attend educational presentations. I would especially like to thank AMMI Canada and the University Of Ottawa Department of Epidemiology for their financial support which made my trip to present at the conference possible.

I would like to recognize the Ontario Government, the Public Health Agency of Canada and the University of Ottawa for the funding of my thesis work.

Thank you to my family and friends for their support.

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List of Acronyms & Abbreviations

Acronym or Abbreviation	Definition
MRSA	Methicillin-Resistant *Staphylococcus aureus*
S. aureus	*Staphylococcus aureus*
CNISP	Canadian Nosocomial Infections Surveillance Program
PHAC	Public Health Agency of Canada
CHEC	Canadian Hospital Epidemiology Committee
NML	National Microbiology Laboratory
HAISE	Healthcare Associated Infection Surveillance and Epidemiology
HA	Healthcare-Associated
HAI	Healthcare-Associated Infections
CA	Community-Associated
CCDIC	Centre for Communicable Disease and Infection Control
NICU	Neonatal Intensive-Care Unit
PICU	Pediatric Intensive-Care Unit
ICU	Intensive-Care Unit
FTE	Full-time equivalent
ICP	Infection-Control Professionals
Ped(s)	Pediatric(s)
MLST	Multilocus sequence type
ST	Sequence type
CC	Clonal complex
PFGE	Pulsed-field gel electrophoresis
SCC*mec*	Staphylococcal cassette chromosome mec
PCR	Polymerase chain reaction
SSTI and SSTIs	Skin and soft-tissue infection(s)
UTI	Urinary-tract infection
PVL	Panton-Valentine Leukocidin
SAB	*Staphylococcus aureus* bacteraemias
GLMEM	Generalized Mixed Effects Models
GEE	Generalized Estimating Equations

Definitions

MRSA cases were classified as "healthcare-associated" or "community-associated" based on two definitions: the surveillance definition and the laboratory strain definition.

<table>
<tr><th colspan="2">Surveillance Definition of HA-MRSA and CA-MRSA</th></tr>
<tr><td colspan="2">The source of MRSA was determined wherever possible based on the infection control professional's clinical judgment. The judgment was made based on the patient's chart using the following definitions:</td></tr>
<tr><th>Healthcare-Associated MRSA</th><th>Community-Associated MRSA</th></tr>
<tr><td>Hospital stay of greater than 72 at the time of positive MRSA culture hours (Protocols changed slightly each year, some used 48 hours but mostly used 72 hours)
Presence of established healthcare-associated risk factors. Information assessed included: previous MRSA status, date of admission, total length of hospital stay, prior hospitalization or admission to another healthcare facility (admission within the past 12 months), and where the patient was admitted from (e.g. long-term care facility)</td><td>Hospital stay of less than 72 hours at the time of positive MRSA culture (Protocols changed slightly each year, some used 48 hours but mostly used 72 hours)
MRSA cases where the patient had no established healthcare-associated risk factors and:
(i) was hospitalized < 72 hours (more recent protocols use < 48 hours);
(ii) had no previous history of MRSA;
(iii) had no medical devices such as urinary catheters, IV lines, feeding tubes, tracheostomy, dialysis access, etc.
(iv) had no history of hospitalization, surgery, or dialysis in the 1 year preceding the MRSA-positive culture;
(v) was not a resident of a long-term care facility in the 1 year preceding the MRSA-positive culture.</td></tr>
</table>

<table>
<tr><th colspan="2">Laboratory Strain Definition of HA-MRSA and CA-MRSA</th></tr>
<tr><td colspan="2">Cases were separated into HA- or CA-MRSA based on the strain types that have been classified in the literature as "typically health care associated" and "typically community associated" MRSA strains (Golding, 2008; Christianson, 2007; Otto et al., 2010; Nichol et al., 2011)</td></tr>
<tr><th>Healthcare-Associated MRSA</th><th>Community-Associated MRSA</th></tr>
<tr><td>CMRSA 1, 2, 3/6, 4, 5, 8, and 9, Danish CO-MRSA, European, ST398, ST88, ST97, USA1000-China/Taiwan, and USA1100-SWP/Oceania</td><td>CMRSA 7 (USA400) and CMRSA10 (USA300)</td></tr>
</table>

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See biographies in Appendix A.

Type of thesis: Monograph

Thesis Category: 2. Secondary analysis of existing datasets

CHAPTER 1: Introduction

Background

Infections that occur while receiving medical care, also known as healthcare-associated infections (HAI), are of increasing concern in Canada and worldwide as they are estimated to affect hundreds of millions of people worldwide each year and lead to prolonged hospital stays, increased healthcare costs, antimicrobial resistance and increased morbidity and mortality (WHO, 2010). Antimicrobial resistance is rapidly increasing in all countries and is associated with greater mortality along with concern over the decreasing number of effective therapies (Conly, 2002).

Staphylococcus aureus (*S. aureus*) is a bacterial pathogen, which asymptomatically colonizes the skin and mucosal surfaces of healthy humans and can cause infections ranging from wound infections to osteomyelitis, endocarditis, bacteremia, etc. *S. aureus* can be acquired in the community or in hospital settings and is often associated with the insertion of medical devices such as those used for hemodialysis, venous catheterization, indwelling catheters, or surgeries with artificial prostheses (CDC, 2011). Since the initial use of antibiotics in the 1940s, resistant strains of *S. aureus* have emerged, starting with Penicillin-resistant, followed by Methicillin-resistant, and finally Vancomycin-resistant strains (WHO, 2011). *S. aureus* is particularly successful in the acquisition and expression of antibiotic resistance, explaining in

part its high associated burden of disease worldwide (Otto, 2010). Groups of people known to be at increased risk for *S. aureus* colonization compared to the general population include healthcare workers, injection drug users, chronic haemodialysis patients, and individuals with diabetes and/or skin conditions (Loeb *et al*., 2008). This is important given that colonization is a suspected risk factor for infection with *S. aureus*. (Loeb *et al*., 2008). *S. aureus* infections are currently being reported as the leading cause of healthcare- acquired infections worldwide and an increasing percentage of these infections are methicillin-resistant (Lo and Wang, 2011; Otto, 2010; CDC, 2011; WHO, 2011). As such, MRSA infections place a significant burden on the healthcare system and are associated with increased morbidity and mortality as well as healthcare resource utilization (WHO, 2011).

There has been less emphasis on the burden and impact of MRSA in pediatric settings and to our knowledge, no direct comparison of the epidemiology of MRSA infections in adult and pediatric patients has been conducted. Adult, pediatric and neonatal surveillance data has not been published separately for Canada. However Simor *et al*. (2010) published Canadian MRSA surveillance data that included all age groups and more recently, Matlow *et al*. (2012) published Canadian data specific to pediatric patients. In addition, an extensive literature review revealed few articles directly comparing the epidemiology or strains of MRSA in adult versus pediatric patients and those that did were often of poor quality. This is a significant gap in the literature since "worldwide, pediatricians advocate that children should be managed differently from adults" (Chang, 2005). Thus the importance of these analyses which aim to examine the epidemiology of MRSA infections in pediatric and adult patients from the same surveillance

sample and highlight any differences that may better inform prevention and treatment programs specific for each age group.

MRSA Epidemiology

Initially, MRSA infections were found primarily in health care settings among patients with particular risk factors, indicating it had not yet achieved a virulence potential high enough to cause disease in healthy individuals (Lo and Wang, 2011; Otto, 2010). Methicillin-resistant *Staphylococcus aureus* was first reported in the 1960s (Cuddy, 2008; Bassetti *et al*., 2009; Crawford and Daum, 2010). The traditional risk factors associated with MRSA included invasive procedures such as surgery, dialysis or the insertion or presence of a medical device, prolonged or recent hospitalization, admission to the ICU, chronic disease, malignancy, organ transplantation, diabetes, and prolonged or repeated exposure to antimicrobials (Lo and Wang, 2011; Coello *et al*., 1997; Laupland, 2008; Fukuta *et al*., 2012; Herold *et al*., 1998).

In the early 1980s, reports of MRSA among adult patients without the traditional risk factors started to emerge in the United States (Saravolatz *et al*., 1982; Nelson and Wilson, 1985). These cases of antibiotic-resistant *Staphylococcus aureus* acquired outside of health care settings were referred to as "community-acquired MRSA" (CA-MRSA). Retrospective investigations of medical records identified cases of CA-MRSA as early as the 1980s in the US (Herold *et al*., 1998) and Australia (Riley and Rouse, 1995). Reports of community-acquired MRSA among children were first published in the 1990s in Australia, the United States, Canada

(Udo *et al*., 1993; O'Brien *et al*., 1999; O'Brien *et al*., 2004; Frank, 1999; Adcock *et al*., 1998; CDC, 1999; Suggs *et al*., 1999; Siegel *et al*., 1999; Embil *et al*., 1994; Shahin *et al*., 1999) to the early 2000s (Hussain *et al*., 2000; Naimi *et al*., 2001; Groom *et al*., 2001; Leman et al., 2002; Fey *et al*., 2003). However, the origin of CA-MRSA remains controversial. Some believe CA-MRSA was a result of healthcare contact with HA-MRSA which then spread to the community, whereas other theories suggest that CA-MRSA emerged separately in the community (Naimi *et al*., 2003; Aires de Sousa and de Lencastre, 2004).

The definition of healthcare-associated MRSA is based on several factors, including the length of stay in hospital prior to positive MRSA culture (generally greater than 48 or 72 hours - varies in different publications), previous MRSA positive status, and the presence of traditional healthcare- associated risk factors. With regard to community-acquired MRSA (CA-MRSA), the definition often varies, but the most accepted clinical or surveillance definition is that developed by the Centers for Disease Control and Prevention (CDC) in 2000, which defines CA-MRSA infection as a case diagnosed within 48 hours of hospitalization or an outpatient that lacks traditional risk factors for MRSA infection such as: the receipt of hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time culture samples were obtained, or previous isolation of MRSA (Lo and Wang, 2011). In addition to surveillance or clinical definition, both HA- and CA-MRSA have been differentiated based on the isolated strain types (specific strains are described in the microbiology section) as described by the CDC (2006) and several

other authors including Simor *et al*. (2010), David *et al*. (2006), Buckingham *et al*. (2004), and Buescher (2005).

In addition to the concerns over the emergence of MRSA among a previously healthy, young population (Fridkin *et al*., 2005), there has been recent emphasis on the increasing numbers of severe illness associated with MRSA being reported (Arnold *et al*., 2006; Gonzalez *et al*., 2005). CA-MRSA causes a wide range of infections ranging from skin and soft tissue infections to severe invasive diseases including sepsis, necrotizing pneumonia, necrotizing fasciitis and disseminated invasive osteomyelitis. (Lo and Wang, 2011; Otto, 2010). This is of considerable importance given that CA-MRSA has now been reported in children and adults all over the world (Naimi *et al*., 2003; Otto, 2010). Compared to HA-MRSA strains, CA-MRSA strains have been shown to have high epidemiological success, high fitness, increased virulence and the ability to disseminate in the community and predominate over methicillin-susceptible strains of *S. aureus*. (Baba *et al*., 2002; Naimi *et al*., 2003; Lo and Wang, 2011; Otto, 2010). CA-MRSA has been implicated in severe fatal diseases such as the Waterhouse-Friderichsen syndrome in children and necrotizing fasciitis and pneumonia, all of which are only rarely reported in traditional hospital strains (Baba *et al*., 2002; Otto, 2010). Significant epidemiological and clinical data provide evidence that specific pro-inflammatory and cytolytic toxins (alpha-toxin, phenol-soluble modulins (PSMs), and Panton-Valentine Leukocidin (PVL)) may be largely responsible for the virulence and disease severity of CA-MRSA (Otto, 2010; Lo and Wang, 2011). In sum, the emergence of a more virulent MRSA in young and previously healthy individuals justifies continued MRSA surveillance and further studies to address the issue.

MRSA Microbiology

Methicillin-resistant *Staphylococcus aureus* are gram-positive cocci bacteria classified in the Micrococcaceae family (Cohen, 1986). Isolates are usually confirmed as MRSA using polymerase chain reaction (PCR) to detect *nuc* and *mecA* genes (Simor *et al*., 2010). MRSA can also be confirmed by a culture-based assay on chromogenic agar (ChromAgar). Molecular typing of MRSA is done using pulsed-field gel electrophoresis (PFGE) and using PCR to type the staphylococcal cassette chromosome *mec* (SCC*mec*) (Simor *et al*., 2010).

Laboratory characterization has revealed that there are distinct differences in the microbiological characteristics of CA-MRSA and HA-MRSA including different PFGE clonal groups, SCC*mec* variants, exotoxin gene profiles, and antimicrobial susceptibility profiles (Naimi et al., 2003; Chen *et al*., 2006; Lo and Wang, 2011). MRSA strains generally recognised as CA-MRSA include CMRSA7 (also known as USA400, ST1 (Sequence Type), CC1 (Clonal Complex)) and CMRSA10 (also known as USA300, ST8, CC8), while CMRSA1 (USA600, ST45 or CC45), CMRSA2 (USA100, USA800, ST5, or CC5), CMRSA3/6 (ST241 or ST239), CMRSA4 (USA200 or ST36), and CMRSA5 (USA500, ST1, or CC1) constitute the HA-MRSA group. The CMRSA7 (USA400) lineage has been predominant in Canada and Alaska, while in the US, CMRSA7 (USA400) has been almost entirely replaced by CMRSA10 (USA300 or ST8) strains (Otto, 2010). Although other CA-MRSA strains can cause severe invasive infection, CMRSA10 (USA300) has been found to be the most virulent and transmissible (Otto, 2010; Bassetti, 2009). Moreover, CMRSA 10 (USA300) strain is increasingly being isolated in hospital cases of MRSA infection, contributing significantly

to the continued increase of hospital-related cases. Typically HA-MRSA strains are persisting in hospital settings, while CA strains appear to be adding to the burden within healthcare settings (Otto, 2010; Naimi *et al*., 2003).

The production of pro-inflammatory and cytolytic toxins is a factor that affects the virulence of different MRSA strains (Otto, 2010; Lo and Wang, 2011). Panton-Valentine Leukocidin (PVL) is a cytotoxin associated with MRSA that has been found to be correlated with severe skin infections and abscess formation (Lo and Wang, 2011). It is a nonlethal form of leukocidin, a molecule produced by *S. aureus* that lyses leukocytes. Epidemiological and clinical evidence both suggest that PVL may be the origin of the high virulence of CA-MRSA, however this remains a highly debated issue (Otto, 2010; Lo and Wang, 2011). Although PVL has been associated with CA-MRSA strains in many studies, some laboratory studies found no evidence of a role of PVL in CA-MRSA pathogenesis. (Lo and Wang, 2011).

Antimicrobial Resistance

Antimicrobial resistance (AMR) is defined by the WHO as "resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive" (2012). The microorganism gains the ability to withstand antimicrobial drugs (such as antibiotics) when it mutates or gains a resistance gene. Antimicrobial resistance is often the consequence of antimicrobial misuse and consequently, standard treatments become ineffective (WHO, 2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a *S. aureus* that became resistant to beta-lactam antibiotics

(i.e., first-line antibiotics that include oxacillin, penicillin, and amoxicillin) (CDC, 2011). The two main mechanisms of resistance in MRSA are the production of β-lactamases (enzymes that destroy β-lactam antibiotics) and, most importantly, the alteration of penicillin-binding proteins (PBP) (Mulligan *et al*., 1993). PBPs are membrane-bound enzymes that are essential to the bacterial cell's survival and are the targets of β-lactam drugs. Alterations in these enzymes mean that the antibiotics cannot bind to the cell, which means that antibiotics cannot stop the cell wall synthesis and thus become ineffective (Mulligan *et al*., 1993). The most common gene responsible for PBP modification in MRSA is the chromosomal gene, *mecA*, which encodes an altered penicillin binding protein called PBP2a (Loeb *et al*., 2008; Mulligan *et al*., 1993). In addition to being resistant to β-lactam antibiotics, many strains of MRSA are also resistant to other classes of antibiotics due to various mechanisms, which will not be described here (Mulligan *et al*., 1993).

CHAPTER 2: LITERATURE REVIEW

LITERATURE REVIEW METHODS

There are very few articles that directly compare the epidemiology or microbiology of MRSA in adult and pediatric patients. An extensive literature review for articles that compared MRSA among adult and pediatric patient populations was conducted. The initial search in Ovid Medline yielded 1,037 articles and the search in the Cochrane Library yielded 62 reviews (See Appendix B for literature process diagram). These articles were screened by title, then by abstract and full-text using a set list of inclusion and exclusion criteria (see Appendix C). Only 18 articles and none of the reviews met the inclusion criteria.

The articles were quality appraised using the SIGN (Scottish Intercollegiate Guidelines Network) critical appraisal checklists as guidelines. There are six available SIGN checklists, each specific to the type of study (review, cohort, case-control, RCT, diagnostic, economic – See Appendix D for a sample). We made some modifications as the majority of studies comparing characteristics of MRSA in pediatric and adult patients do not strictly follow the typically study designs (comparison of clinical or laboratory characteristics rather than outcomes or exposures). Using the SIGN rating system, articles were rated as “High Quality”, “Acceptable Quality”, or “Low Quality” evidence. Among the 18 articles included, the majority were of low quality or they only assessed a limited number of differences in the epidemiology of MRSA between adult

and pediatric patients. Thirteen articles included both adult and pediatric patients, 4 included only pediatric patients and made comparison with existing literature on adult patients, and 1 article was a commentary. Of the 13 articles that included both adult and pediatric populations, 8 were classified as "low quality" using the SIGN critical appraisal tools. The main limitations of these studies included: small sample sizes, methodological flaws (such as a lack of control for confounding factors, or other risks for bias), lack of statistical analyses, limited generalizability (for example only studying patients with a very specific MRSA infection type like rhinosinusitis) limited differentiation between MRSA and MSSA or minimal comparisons between adults and children (Huang and Hung, 2006; Frank *et al*., 1999; Saiman *et al*., 2003; Saito *et al*., 1998; Shangmuganatha *et al*., 2005; Sadoyama *et al*., 2000, Warner *et al*., 2009; Marais *et al*., 2009). The remaining 5 articles were deemed to be "acceptable quality" comparison studies (David *et al*., 2006; Park *et al*., 2007; Naimi *et al*., 2003; Santos *et al*., 2010; Hudson et al., 2012); however, each had some limitations and none included nationwide data that compared both the clinical and microbiological characteristics of adult and pediatric MRSA patients. The 4 studies which included only pediatric patients and compared their results with previously reported adult MRSA studies were limited by the fact that the adult and pediatric patients were not from the same study populations and this may have introduced bias in the comparisons (Denniston *et al*., 2006; Khairulddin *et al*., 2004; Suryati and Watson, 2002; Wolf *et al*., 2010). Overall, the literature review yielded no study conducted in Canada.

Overall, the literature review for articles comparing adult and pediatric MRSA supported the need for the current study. The majority of the findings described in the literature review

are hypothesis generating due to small sample sizes and weak methodologies. Among the relevant studies, there was no Canadian article comparing adult and pediatric patients. To our knowledge, the current study will be the first to directly compare both the epidemiological and microbiological characteristics (including strain typing and AMR) in adults and children hospitalized during the same time period in geographically representative Canadian acute care centres.

SUMMARY OF THE LITERATURE

This section summarizes the findings from the articles included in the literature review of the differences between MRS in adults and children. The findings are grouped by subject.

Prevalence and Incidence of MRSA

Summary: Prevalence and Incidence of MRSA in Adults vs. Children

- The studies comparing MRSA prevalence or incidence were conducted in the United States, United Kingdom, Taiwan, Brazil, and Australia
- Most studies found higher prevalence or incidence rates of MRSA (both colonization and infection) among adult patients compared to children
- Neonatal patients were found to have higher proportions or rates of MRSA than pediatric patients, and more similar rates to adult patients
- Among pediatric cases of *S. aureus* bacteraemias in the UK (from 1990 to 2000), the proportion of MRSA was found to increase the most in infants. This was likely due to increasing numbers of premature infants in neonatal units.
- One study of Australian bacteraemia patients found similar proportions of MRSA among

adults and children
- One small study in Taiwan found a higher proportion of MRSA among pediatric *S. aureus* rhinosinusitis cases than adult cases

The majority of the studies comparing adult and pediatric patients included a comparison of the incidence, or prevalence of MRSA or the proportion of *S. aureus* caused by MRSA strains. These included studies from the United States, United Kingdom, Taiwan, Brazil, and Australia, however there were no Canadian studies. Overall, most studies found a higher prevalence of MRSA (both colonization and infection) among adult patients compared to children (Kairam 2011; Denniston *et al*., 2006; Huang and Hung, 2006; Khairulddin *et al.*, 2004; Santos *et al*., 2010; Wolf *et al*., 2010; Tillotson *et al*., 2008; Hudson *et al*., 2012). However, one found similar proportions of MRSA bacteraemia in Australian adult and pediatric patients (Suryati and Watson, 2002) and another found a higher proportion of MRSA among pediatric S. aureus rhinosinusitis compared to adults (Huang and Hung, 2006).

Frank *et al.* (1999) reported all *S. aureus* isolates recovered from inpatients and outpatients at the University of Illinois Hospital (516-bed tertiary care facility with 86 pediatric beds) from 1987 to 1997. They found 14-32% of the 983-1,525 *S. aureus* isolates were MRSA. The authors extracted demographic, microbiological, and clinical data from medical records for pediatric patients from whom MRSA was isolated. The number of cases per year and the clindamycin susceptibilities of the isolates were presented. Adult patients had higher numbers of total MRSA cases than pediatric although only absolute numbers were presented and not rates. The authors reported increases in pediatric MRSA isolates after 1993 in spite of generally stable rates of MRSA overall among patients at the University of Illinois Hospital from 1987 and

1997 (Frank *et al.*, 1999). They largely attributed the observed rising pediatric MRSA rates to increases in community-associated MRSA in this pediatric population (Frank *et al.*, 1999).

Cases of *S. aureus* bacteraemia (SAB) at Birmingham Heartlands Hospital in the United Kingdom (68 bed children's unit, no patients with malignancy or PICU, neonatal unit with 5 ITU cots, 3 HDU cots and 20 special care cots) from 1993 to 2003 were reviewed and compared with UK adult MRSA bacteraemia rates in the literature (Denniston *et al*., 2006). Patients were divided into paediatric or neonatal based on the unit where they received care, not based on age, and all patients were <16 years. Five of 51 pediatric cases of *S. aureus* bacteraemia were MRSA and 8 of the 33 cases of neonatal SAB were MRSA over the 10-year study period (Denniston *et al*., 2006). Compared to the UK national MRSA surveillance which reported that 40% of adult SAB is due to MRSA, the current review found 13.4% of pediatric SAB was associated with MRSA (27% neonatal, 5.8% paediatric). Therefore the rates of MRSA bacteraemia appeared to be much lower in children compared to adults in the UK. However, neonatal rates were much higher than pediatric rates suggesting hospitalized neonates may be at a higher risk for MRSA bacteraemia among pediatric patients (Denniston *et al*., 2006).

Adult and pediatric clinical MRSA isolates were collected from 2008-2009 at 30 hospitals in California and they observed higher infection rates among adults. The estimated annual MRSA infection rate in adults was 119/100,000 compared to 22/100,000 in children (Hudson et al., 2012).

Adults and children with acute rhinosinusitis caused by various bacterial strains, including *S. aureus* and MRSA, at a hospital in Taiwan were compared for bacteriological and drug

susceptibility characteristics (methods described in more detail under the risk factor section below) (Huang and Hung, 2006). They found a MRSA prevalence of 30% (16 of 53) among all *S. aureus* isolated, and an overall MRSA prevalence of 3% (16 of 601) among acute rhinosinusitis cases. In the adult patients, 23% (7 of 30) of the *S. aureus* isolates were MRSA and in the pediatric patients, 39% (9 of 23). The overall prevalence of MRSA in acute rhinosinusitis patients was 2% (7 of 292) in adults and 3% in children (9 of 309). (Huang and Hung, 2006). On the whole, there was a higher a prevalence of MRSA rhinosinusitis among pediatric patients than adult patients in this Taiwanese hospital.

The Health Protection Agency Communicable Disease Surveillance Centre (HPACDS) in England and Wales collected surveillance data on *S. aureus* bacteraemia from 1990 to 2001 (Khairulddin *et al.,* 2004). Khairulddin *et al.* (2004) reviewed pediatric MRSA bacteraemia cases from this surveillance system and found 376 reports of MRSA bacteraemia among children, of which 53% were infants less than 12 months. They reported increases in both the absolute number of cases and the proportion of MRSA among *S. aureus* bacteraemia in this population over the surveillance period, from 4 (1%) in 1990 to 77 (13%) in 2000 (Khairulddin *et al.*, 2004). The most drastic increase in the proportion of MRSA was observed in infants, from 1% in 1990 to 15% in 2000 and 13% in 2001 (Khairulddin *et al.*, 2004). The proportion of MRSA increased over the surveillance period while the number of MSSA cases among children and infants remained relatively stable. The authors proposed that these trends may be attributable to increases in MRSA among premature infants in neonatal units (Khairulddin *et al.*, 2004). Finally, Khairulddin *et al.* (2004) compared the observed pediatric rates with previously reported adult

rates of MRSA bacteraemia and noted that although not yet as high as the rates in adults, pediatric rates are increasing. This is concerning not only because MRSA bacteraemia is associated with increased length of hospital stay and mortality rates but the authors raised the concern that pediatric rates may follow the trends observed previously in adults (Khairulddin *et al.*, 2004).

A cohort of randomly selected patients admitted to emergency and clinical wards in a hospital in Porto Alegre, Brazil, was screened for MRSA colonization and SCC*mec*A detection by anterior nares swabs at admission and weekly thereafter (Santos *et al*., 2010). A total of 297 adult (patients above 14 years old) and 176 pediatric (14 years old or less) patients, selected within 72 hours of admission to the 749-bed tertiary-care hospital from 2006 to 2007 (Santo *et al*., 2010) were included in this study. Clinical characteristics and risk factor information were obtained through patient interviews, from assistant physicians and medical residents, and from electronic medical records. Sixteen adults were colonized (5%) and 2 infected with MRSA, while 3 pediatric patients were colonized (2%) and 1 was infected with MRSA at admission (all cases were community-acquired MRSA since all admission screening cultures were taken within 72 hours of admission) (Santo *et al*., 2010). The incidence rate of hospital-associated MRSA (positive culture more than 72 hours after admission) was 5.5/1,000 patient-days for adults (20 adults were colonized during hospitalization) and 1.1/1,000 patient-days for children (2 children were colonized) (Santo *et al*., 2010). For patients who had negative cultures at admission but acquired MRSA colonization during hospitalization, the median number of days to positive culture was greater in adults than in children (15 and 8 days respectively) (Santo *et al*., 2010).

Overall, despite a relatively high risk pediatric population (70% had been admitted to a hospital in the last year, 19% had a neoplastic disease) the authors found a relatively low incidence of MRSA colonization among this Brazilian pediatric cohort (Santo *et al*., 2010). The authors found this to be consistent with another study conducted in Brazil which also reported a low prevalence of MRSA in hospitalized children less than 5 years of age (1%) in this time period (Lamaro-Cardoso *et al*., 2007). The incidence among adult patients reported by Santos et al., 2010, on the other hand, was relatively high.

A retrospective review of the charts of *S. aureus* bacteraemia cases from 1994 to 1998 at a children's hospital in Sydney, Australia was compared with data in the literature on MRSA bacteraemia in adult patients (Suryati and Watson, 2002). The hospital was originally a 230-bed tertiary care facility which expanded to 300-beds in 1995 and has a NICU and a PICU (20-beds each) (Suryati and Watson, 2002). Overall 47,485 blood cultures were performed, 142 cases of *S. aureus* bacteraemia occurred in 135 pediatric patients (data was available for 140 episodes), and 20 cases were due to MRSA. (Suryati and Watson, 2002). MRSA remained a primarily nosocomial pathogen in the pediatric bacteraemia cases in this study, with only 2 cases of community-associated MRSA bacteraemia observed over the 5-year study period (Suryati and Watson, 2002). Twenty-seven percent of the nosocomial *S. aureus* bacteraemia cases were caused by MRSA in children admitted to this Australian hospital (Suryati and Watson, 2002). This was comparable to what was reported by other studies on adult bacteraemia including Steinberg *et al*. (1996) who reported that MRSA accounted for 32% of nosocomial *S. aureus* bacteraemia cases at a 500-bed acute care hospital in Atlanta, Georgia, U.S.A. from 1990 to

1993 and Panlilio *et al*. (1992) who reported that between 15 and 38% (for hospitals with >200 beds and ≥ 500 beds, respectively) of *S. aureus* isolates reported to the US National Nosocomial Infections Surveillance System in 1991 were MRSA.

In 2006, the susceptibility data for all *S. aureus* isolated from children at tertiary care pediatric hospitals in Australia were analysed. The proportions of MRSA were compared between hospitals and states as well as with published studies of *S. aureus* isolated in Australian adult patients (Wolf *et al*., 2010). All inpatients and outpatients less than 17 years of age with a positive *S. aureus* isolate were included and routine infection control screening samples were excluded. A total of 7275 *S. aureus* isolates from 4779 pediatric patients at 7 hospitals were included in the study and 188 patients had documented bacteraemia (Wolf *et al*., 2010). The published studies on adult *S. aureus* isolates in Australia included a 2004 survey of adult hospital outpatients (Nimmo et al., 2006) and a 2005 survey of adult hospital inpatients (Nimmo *et al*., 2007). The prevalence of MRSA among *S. aureus* isolates at different hospital sites ranged from 10% (41/399) to 22% (155/699) for adult outpatients (Nimmo *et al*., 2006) and from 23% (80/355) to 43% (358/825) for adult inpatients (Nimmo *et al*., 2007) compared to a range of 6% (51/804) to 13% (127/974) for the pediatric patients in the Wolf *et al.* study (including both inpatients and outpatients). Therefore, MRSA was much less prevalent among pediatric than adult patients with *S. aureus* infections in Australia (Wolf *et al*., 2010). This trend has previously been described in other countries as well, including Switzerland (SEARCH, 2008) and the U.S.A. (Tillotson *et al*., 2008).

Clinical Characteristics and Outcomes

Summary: Clinical Characteristics and Outcomes

- The studies comparing MRSA clinical characteristics in adults and children were conducted in the United Kingdom, Australia, Taiwan, the United States, and Brazil
- Most included studies found differences in the clinical characteristics and outcomes of MRSA infections in adults compared to children
- Pediatric patients with MRSA bacteraemia were less likely to have an identifiable focus of the infection compared to adults, which rendered treatment more difficult
- Bone and joint infections were frequent sources of infection in children with *S. aureus* bacteraemias, whereas endocarditis was a frequent source of infection in adults.
- *S. aureus* bacteraemia mortality rates were higher in adults compared to pediatric patients
- One study of MRSA rhinosinusitis found pediatric patients were more likely to be infected by multiple organisms, to have fewer risk factors, to have repeated infections and to have higher antibiotic consumption
- Elderly MRSA patients were found to have a greater severity of infection relative to younger patients
- MRSA infection sites varied between adults and children (higher proportion of MRSA isolated in wounds / abscesses in children, higher proportion isolated in sputum, urine and blood in adults). As well, a higher proportion of adult isolates were collected in intensive care units.

Several of the studies included in the literature review discussed the similarities and differences in MRSA clinical characteristics between adult and pediatric patients. These characteristics included site or focus of infection, underlying conditions, multiple infections, and outcomes such as length of hospital stay and treatment success. Most of these studies found differences between adults and children in terms of the clinical characteristics of MRSA (Huang and Hung, 2006; Denniston *et al*., 2006; Suryati and Watson, 2002; Naimi *et al*., 2003; Warner *et al*., 2009; Sadoyama *et al*., 2000; Hudson *et al*., 2012).

Pediatric and neonatal cases of *S. aureus* bacteraemia (SAB) at Birminghams Heartlands Hospital in the UK were reviewed, as described above, and case characteristics and mortality were reported (Denniston *et al*., 2006). Generally, the authors made comparisons between pediatric cases of *S. aureus* bacteraemia with adult cases in the literature, but did not compare the characteristics specific to MRSA bacteraemia. The focus of infection, the treatment duration, the presence of comorbidities, the occurrence of complications, and the mortality rates for *S. aureus* bacteraemia were presented for neonates and children. However not all of these characteristics were presented separately for MRSA. As well, these characteristics were compared with the adult literature for *S. aureus* bacteraemias but not for MRSA bacteraemias specifically. There were 8 cases of neonatal MRSA bacteraemia in this study (Denniston *et al*., 2006). Central venous access was present in 88% (7 of 8 cases) and the treatment ranged from 5 to 28 days. Post-treatment complications included 2 abscesses and one death (mortality of 1/33 or 3%). There were 5 cases of pediatric MRSA bacteraemia. CVC (Central venous catheter) was present in 2 of 5 patients, one patient had Harlequin ichthyosis with MRSA colonization, and treatment ranged from 4 to 12 days with 2 patients receiving no treatment. One pediatric patient had recurrent fever and 4 of 5 recovered. Three of the cases were non-specific presentation whereas one was bacterial tracheitis and another was pneumonia. The pediatric patients ranged from 6 months to 15 years of age (Denniston *et al*., 2006).

For all SAB cases in this study the primary focus of infection was non-specific at presentation for 48% of cases (39 of 81 cases, 26 cases or 87% of neonatal and 13 cases or 25% of pediatric) (Denniston *et al*., 2006). The source of infection was presented separately for

pediatric MRSA only and 3 of 5 cases presented as primary bacteraemia. Adult studies suggested that finding a source of infection in *S. aureus* bacteraemia is important because those with an eradicable source are associated with lower morbidity and mortality (Jensen, 2002; Fowler *et al*., 1998). According to these studies, up to 93% of adult patients have an identifiable source whereas in this study, a focus of infection was found in only 15 (50%) neonatal patients and 41 (80%) of pediatric patients (Jensen, 2002; Fowler *et al*., 1998; Denniston *et al*., 2006). The authors concluded that it was more difficult to determine appropriate treatment for children as a result (Denniston *et al*., 2006). Pediatric and neonatal patients in this study had a lower SAB mortality rate compared to what was reported in the literature for adult patients (2% in children and 10% in neonates, 5% overall (Denniston *et al*., 2006), compared to 20-40% reported in the literature for adults (Fowler *et al*., 1998)). This difference is likely due to the fact that adults have more co-morbidities than children thus children. There were no deaths among the pediatric MRSA patients, and one death in the 8 MRSA infected neonates in the study, but adult rates of MRSA bacteraemia mortality were not presented (Denniston *et al*., 2006). They found that children but not neonates were less likely to have associated co-morbidity and that children were less likely to acquire SAB in the hospital, although 100% of the neonates in this study acquired the SAB in hospital (Denniston *et al*., 2006). As well both children and neonates in this study had lower mortality rates than what has been reported for *S. aureus* bacteraemia in adults but neonates had a higher mortality than children (Denniston *et al*., 2006). It appears that children have less severe clinical presentations of *S. aureus* bacteraemia whereas neonates tend to present with more severe and more similar clinical presentation to adults. This is likely

because the majority of hospitalized neonates are in the NICU and have more severe underlying health conditions. There was an insufficient number of MRSA cases and insufficient separate information provided for MRSA bacteraemia in the children in this study to determine whether the same trends are true for MRSA bacteraemia as all *S. aureus* bacteraemia.

As discussed above, the case characteristics of pediatric *S. aureus* bacteraemia in an Australian children's hospital were compared with published characteristics of adult *S. aureus* bacteraemia in Australia (Suryati and Watson, 2002). There were several differences between pediatric and adult patients in terms of the clinical presentation of *S. aureus* bacteraemia however the majority of their comparisons were based on all *S. aureus* bacteraemia cases (not specific to MRSA). The similarities included similar incidence rates of community-acquired *S. aureus* bacteraemia (0.56 per 1000 in this children's hospital compared to 0.84 to 2.43 cases per 1,000 discharges from 1980 to 1993 by Steinberg et al., 1996), and the importance of central venous access devices as a focus of infection (11% of CA-*S. aureus* bacteraemias and 26% of HA-*S. aureus* bacteraemias in this pediatric population compared to 19% and 23% respectively in an adult population). (Suryati and Watson, 2002; Steinberg *et al*., 1996). The differences between the pediatric patients in this study and adult patients reported in the literature included a much higher occurrence of bone and joint infections among pediatric CA *S. aureus* bacteraemia (20%) (Suryati and Watson, 2002), compared to 2% - 12% reported in adult studies (Gransden *et al*., 1984; Lautenschlager *et al*., 1993; Mylotte *et al*., 1987); endocarditis occurring infrequently in the pediatric patients (Suryati and Watson, 2002) whereas it has been an important focus of infection in adult bacteraemia cases (17% to 46%; Lautenschlager *et al.*, 1993; Libman and

Arbeti, 1984.) and a much lower case fatality rate in the pediatric patients (0.7%) (Suryati and Watson, 2002) compared to very high rates in adult patients ranging from 11% to 32% (Mylotte et al., 1987; Libman and Arbeit, 1984).

A population of adult and pediatric cases of CA-MRSA acute rhinosinusitis from a hospital in Taiwan were compared (Huang and Hang, 2006). They found differences in the clinical presentations between adult and pediatric patients including more repeated sinonasal infections (compared to MRSA identified as a single pathogen in adults) and more antibiotic consumption in pediatric patients, no predisposing risk factors in pediatric patients apart from Down syndrome and multiple infections compared to the adults who had previous nasal procedures, and a significant association between MRSA infection and age in the pediatric group but not in the adult group (Huang and Hang, 2006). In terms of the difference in multiple infection, the authors found that among patients with MRSA rhinosinusitis, a significantly higher proportion of the pediatric patients were infected with multiple organisms as compared to the adult patients (6 of 9 pediatric patients compared to 1 of 7 adult patients, p=0.054). (Huang and Hung, 2006).

All outpatient burn cases over a 3-year period were reviewed for the development of MRSA furunculitis and adult and pediatric MRSA furunculitis patients were compared on length of hospital stay, type of injury, cultures, antimicrobial sensitivity, and treatment (Warner *et al*. 2009). MRSA furunculitis occurs in burn patients when MRSA infects a hair follicle and the surrounding tissue (A.D.A.M. Medical Encyclopedia, 2010). A total of 28 MRSA furunculitis patients were identified (15 adults and 13 children). They found that adults had less extensive

burn injuries (total body surface area mean of 12% vs. 21%), shorter length of hospital stay (mean ± S.D., median, in days: 11 ± 15, 2; vs. 15 ± 20, 6) and shorter time post discharge to furuncle development (15 ±18 vs. 42 ± 50 days) than pediatric patients, although these differences were not statistically significant (Warner *et al*., 2009). The authors did find a significantly higher percentage of burns that originated outdoors in adult patients compared to pediatric patients (93% compared to 31%) (Warner *et al*., 2009). They proposed that the difference in the time from discharge to furuncle development between adults and children may have been due to the fact that the adult outpatient population was local whereas the pediatric population lived further from the hospital. The authors also proposed that the time difference could be attributable to the fact that the pediatric patients had less pain and wound care complications due to the fact that more pediatric patients sustained their injuries indoors whereas adults sustained injuries outdoors (Warner *et al*., 2009). Warner *et al*. (2009) recommended clindamycin for the treatment of MRSA furuncles in children due to the risk of arthropathy or risk of disruptions of growth cartilage development with ciprofloxacin. In adults they said that ciprofloxacin, clindamycin, and trimethoprim-sulfamethoxazole were all efficacious but recently ciprofloxacin has been believed to increase the emergence of resistant MRSA strains and disease recurrence.

In a 12-month-study of patients from a mixed (adult and pediatric) hospital in Minas Gerais, Brazil, the neonatal unit and pediatric units were associated with fewer nosocomial MRSA infections compared to other units (particularly surgical, and medical units) (Sadoyama *et al*., 2000). However, there were more cases of MSSA infections in the neonatal unit compared

to the pediatric medical, ICU and ambulatory units (Sadoyama *et al*., 2000). As well, the authors found that elderly patients tended to have higher rates of nosocomial MRSA colonization as well as greater severity of infection (Sadoyama *et al*., 2000).

Hudson et al. (2012) collected MRSA clinical isolates form 30 hospitals in California. They compared the characteristics of isolates from adult (≥18 years) and pediatric (<18 years) patients. The proportions of the isolation sites differed between adults and children (adult vs. pediatric %, respectively: wound/abscess 43% vs. 56%; sputum 29% vs. 19%; urine 10% vs. 3%; blood 9% vs.5%; other sites 8% vs. 18%). A higher proportion of adult isolates were collected from intensive care units (17% of adults vs. 12% of children), and were hospital onset (36% of adults vs. 25% of children) (Hudson et al., 2012). Overall, they found differences in the epidemiological characteristics of MRSA isolates collected from adults and children. The fact that a higher proportion of pediatric isolates were from wounds or abscesses is likely related to the fact that a higher proportion of pediatric MRSA was community-onset.

Risk Factors for MRSA Acquisition

Summary: Risk Factors for MRSA

- The studies that discussed differences in risk factors for MRSA in adults and children were conducted in the United States, the United Kingdom, Taiwan, and Brazil
- Adult patients were reported to have the traditional healthcare-associated MRSA risk factors, while these were less common in children
- One study found no characteristics associated with CA-MRSA in children, but found that hospitalization in the last year and age >60 years were associated with HA-MRSA colonization in adult patients
- Pediatric patients were found to have fewer co-morbidities than adult patients
- As well, the most common risk factors differed between adults and children
- One study found dermatological risk factors in children, but mainly tobacco use and diabetes as risk factors in adults
- Another study found that among MRSA rhinosinusitis patients, a history of nasal surgeries was the most common risk factor in adults, while previous antibiotic use was the more common risk factor in children

Several studies assessed the differences in risk factors for MRSA between adult and pediatric patients. Although most of these types of factors were not available for the current study, they were included as background information as a part of the differences found in the literature between the two populations of interest, and for consideration in future studies.

CA- and HA-MRSA isolates from inpatients and outpatients infected with MRSA between January 1st 2000 and December 31st 2000 at 12 surveillance sites in the Minneapolis-St Paul and Minnesota areas were compared for differences in risk factors as well as other characteristics. Based on medical records, the main underlying conditions in pediatric (age <18 years) CA-MRSA infected patients were dermatological (9%, n=5) whereas the main underlying conditions in CA-MRSA infected adult patients (≥18 years) were tobacco use (19%, n=15), and diabetes (17%,

n=13) (Naimi *et al*., 2003). As well, CA-MRSA patients were younger than HA-MRSA patients with a median age of 23 years vs. 68 years, respectively, $p<0.001$ (Naimi *et al*., 2003). This suggests that CA-MRSA tends to infect younger patients and that there are differences in the predisposing factors for infection in adult vs. pediatric patients.

As described above, *S. aureus* pediatric and neonatal bacteraemia cases at a hospital in the UK were characterized and compared with adult literature (Denniston *et al*., 2006). In the neonatal unit, MRSA was more closely associated with the presence of central venous line (88%) than with known MRSA colonization (38%) which suggests that central venous lines may be a risk factor for MRSA bactaraemia in neonatal patients. The authors also observed a lower percentage of infected pediatric patients with co-morbidities than adult patients with co-morbidities; however, this data was for *S. aureus* bacteraemias and MRSA combined therefore it is not possible to tell if the same would apply to the MRSA bacteraemia cases alone (Denniston *et al*., 2006).

Bacterial isolates cultured from 601 (292 adults, 309 children) acute rhinosinusitis patients were prospectively collected from October 2000 to March 2003 at Chia-yi Christian Hospital in south Taiwan in order to assess bacteriological and drug susceptibility data (Huang and Hung, 2006). Cultures were tested for antimicrobial sensitivity and patient data including medical history and previous therapy was also collected. Isolates were classified as community-acquired if they were obtained in an outpatient clinical or identified within 48 hours of hospitalization and the patient was not hospitalized in the 3 months prior to the culture. All of the isolates met the CA definition and at total of 16 out of the 53 *S. aureus* isolates were MRSA (7 adult, 9

children) (Huang and Hung, 2006). Huang and Hung (2006) found that the most important risk factor for MRSA acute rhinosinusitis was nasal surgeries for adults (5 out of 7 MRSA infected adults had a history of nasal surgery) and previous antibiotic use (8 out of 9 MRSA infected children had a history of antibiotic use) in children. In the 16 total MRSA rhinosinusitis patients, previous sinonasal surgery was significantly more common compared to non-MRSA-infected patients (31%, 5 of 16 in MRSA compared to 5%, 30 of 585 non-MRSA). Overall, previous antibiotic treatment was more common in MRSA-infected patients than in non-MRSA infected patients (75%, 12 of 16 in MRSA compared to 26%, 152 or 585 non-MRSA) and MRSA was significantly associated with previous antibiotic use (Fisher exact test, $p<0.01$). Huang and Hung (2006) suggest that the possible explanation for surgery being identified as a significant risk factor in CA-MRSA acute rhinosinusitis patients was that surgery changes the microenvironment in sinonasal tissue thus allowing *S. aureus* to survive. They hypothesize that the patients may have become long-term carriers after their surgery and acquired infection in the community from their carrier state (Huang and Hang, 2006).

The reasons for differences in the incidence of MRSA among children compared to adults were discussed in a commentary by Gray (2004), where it is thought that it is unlikely that children are less susceptible than adults to colonisation or infection with MRSA given that *S. aureus* is frequently found among pediatric patients (Gray, 2004). Gray suggested that the lower incidence of MRSA in children is due to demographic and epidemiological differences between these two subpopulations (Gray, 2004). The author noted that the most important risk factor for MRSA acquisition in adults is in-patient hospital care which is much less common in children

(Gray, 2004). As well, pediatric facilities tend to have better patient isolation and are generally independent of adult facilities. Finally, Gray's commentary (2004) suggested that the current epidemiology of MRSA in pediatrics is similar to that of adults in the mid-1990s, implying a potential time lag in MRSA trends.

A cohort of adult and pediatric patients was screened for MRSA at admission and during hospitalization in Brazil, as described in detail above (Santos *et al*., 2010). A total of 16 adult and 3 pediatric patients were colonized with MRSA at admission and 20 adult and 2 pediatric patients became colonized with MRSA during hospitalization (Santos *et al*., 2010). The authors conducted log-binomial regression and Cox regression survival analyses for the community-acquired and hospital acquired MRSA respectively. They did not find any characteristics associated with community-acquired colonization or infection in pediatric patients, however they did find a significant association between community-acquired MRSA colonization and hospitalization in the last year (PR = 5.3, 95%CI: 1.2 – 22.5) as well as age above 60 years for adult patients (PR=2.8, 95%CI 1.1 to 7.2, p=0.046) in univariate analyses (Santo *et al*., 2010). In the multivariate analyses, hospitalization in the last year and age above 60 years remained significant predictors (PR=3.0, 95%CI: 1.2- 7.7 and PR=5.6, 95%CI: 1.3 – 23.9; respectively) of MRSA colonization at admission among adult patients. The crude and multivariate Cox regression models did not find any significantly associated factors with infection or colonization during hospitalization in adult patients (Santo *et al*., 2010). It is important to note that these sample sizes were very small and therefore the regression analyses conducted by Santos *et al*. (2010) should be interpreted with caution. The small sample sizes may also explain the lack of

factors associated with MRSA colonization or infection for pediatric community-acquired MRSA and both adult and pediatric hospital-associated MRSA. The risk factors for community-acquired MRSA colonization in adults may serve a hypothesis generating purpose and should be further explored in larger studies.

Cases of Staphylococcal ocular infections were reviewed at an ophthalmic hospital in the UK, as described in further detail under the "Antimicrobial Resistance" section (Shanmuganathan *et al*., 2005). Overall there was a high prevalence of underlying medical conditions among the 17 cases caused by MRSA. The findings from this study were consistent with another study on MRSA conjunctivitis (Fukuda *et al*., 2002) and the authors proposed that the high prevalence of underlying medical conditions may be due to the fact that patients suffering from ocular infections are more likely to require long term care in facilities where MRSA colonization is endemic (Shanmuganathan *et al*., 2005). Only 5 of the 17 patients were swabbed at non-ocular sites and only 1 of these cultures was positive for MRSA. The authors therefore concluded that the relationship between colonization by MRSA and ocular infections remains unclear and that further studies are needed on this topic.

Molecular Characteristics of MRSA

Summary: Molecular Characteristics of MRSA

- The studies were conducted in the Korea, Japan, the United States, the United Kingdom, Australia, and South Africa
- Molecular characteristics assessed included: PFGE strain typing, SCC*mec* typing, the detection of toxin genes, and antimicrobial susceptibility testing

Among the articles retrieved in this literature review, many included an assessment of microbiological characteristics of MRSA in adult and pediatric patients. These characteristics included PFGE strain typing, SCC*mec* typing, the detection of toxin genes, and antimicrobial susceptibility testing. Overall, the two populations differed in the MRSA strains and microbiological characteristics that affected each population. Reported MRSA spa types, SCC*mec* types and multilocus sequence types in the two age groups were significantly different (Park et al., 2007; Saito *et al*., 1998; Hudson *et al*., 2012). Also, retained references (David *et al*., 2006; Park *et al*., 2007; Wolf *et al*., 2010; Nimmo *et al*., 2008) noted differences in the antimicrobial resistance patterns. Although some of the articles combined both epidemiological and microbiological characterization, none reported all of the characteristics that are presented in the current thesis. The findings of these studies will now be described in detail.

MRSA Strain Types

Summary: MRSA Strain Types

- Most studies found different MRSA strain types in adult and pediatric patients
- A study in a Korean hospital found that the most common SCC*mec* types and multilocus sequence types were SCC*mec* type II var-1 and ST5 in adults, compared to SCC*mec* type IVa and ST72 in children
- The PFGE patterns of isolates from a Japanese neonatal unit differed from those isolated in adults from the same hospital
- An American study compared spa types of adult and pediatric MRSA isolates and found significantly different distributions of the 3 most common types. Spa type 008 (corresponds to PFGE type USA300) was more common in children
- Another American study found that the predominant clone accounted for approximately the same proportion of adults and pediatric MRSA isolates

In a university hospital in Korea, 50 non-duplicate MRSA strains were isolated from hospitalized adult patients in 2003 and 88 strains were isolated from pediatric patients from 2003 to 2005 (Park *et al*., 2007). The adult and pediatric strains were compared using antimicrobial susceptibility tests, DNA isolation, *SCCmec* typing, multilocus sequence typing, detection of staphylococcal toxin genes, disk diffusion test (D-test), and detection of erythromycin resistance genes, and all statistical tests were done using Chi-Square or Fisher's Exact tests. There were statistically significant differences in the SCC*mec* types between adult and pediatric isolates (Park *et al*., 2007). The most common SCC*mec* type in adults was SCC*mec* type II var-1 (a type that varies from type II in the absence of the 381 base pair band corresponding to the pUB110 insertion) (22 out of 50 adult isolates (44%) vs. 0 out of 88 pediatric isolates). The most common SCC*mec* type in pediatric isolates was type IVA (60 out of 88 isolates (68%) vs. 9 out of 50 (18%). In terms of sequence types (ST) multilocus sequence

typing (MLST) were conducted on the isolates and found that ST5 was the most common in adults (29 (58.0%) vs. 6 (6.8%)) whereas ST72 was the most common in children (42 (48%) vs. 4 (8%)). As well, ST89 and ST30 were only detected in pediatric isolates. This study also tested for staphylococcal toxin genes and found that 41 (82%) of the adult isolates were toxin gene positive whereas only 18 (21%) of the pediatric isolates were toxin gene positive. (Park *et al*. (2007).

In summary, Park *et al*. (2007) found significant differences in the microbiological characteristics of MRSA between 50 adult and 88 pediatric MRSA isolates in a Korean hospital. They found statistically significant differences in the multilocus sequence types, SCCmec types, antimicrobial resistance, and staphylococcal toxin gene expression between pediatric and adult MRSA isolates which suggests that children and adults may acquire MRSA from different sources and may be subject to different drug selection pressures (Park *et al*., 2007). The adult isolates were generally more resistant to antibiotics than pediatric isolates as most adult isolates were resistant to up to 6 antibiotics whereas pediatric isolates were resistant to a maximum of 3 antibiotics. As well, the adult population had higher numbers of isolates resistant than pediatric patients for all of the antibiotics tested except for ampicillin, tobramycin, and trimethoprim-sulfamethoxazole (Park *et al*., 2007). However, the authors found a significantly higher rate of inducible clindamycin resistance in pediatric isolates (52 of the 88 (59%)). Park *et al*. (2007) proposed that this may indicate treatment failure, therefore suggesting that clindamycin should be used with caution in pediatric patients. Park *et al*. (2007) found different major MRSA clonal types among pediatric and adult isolates. The sequence types (in order of

decreasing prevalence) found primarily in adults were ST5 (CMRA2, USA100/800), ST239 (CMRSA3/6), ST254, and ST345, and in children were ST72 (USA700), ST1 (CMRSA7, USA400), and ST89. The ST5 clonal type were found to carry SCC*mec* type II or type II variant and ST72 carried SCC*mec* type IV or IVA, thus adults had predominantly SCC*mec* type II or II variant and pediatric isolates had SCC*mec* type IV or IVA. The clones that affected primarily adults (ST5, 239, 254, and 345) were associated with ermA gene whereas the clones affecting primarily children (ST72 and ST1) were primarily associated with the *ermC* gene, which is responsible for the inducible clindamycin resistance. Overall, adults isolates were more likely to be ST5 with SCC*mec* II or IIvar-1, were more likely to be multidrug resistant, to have the erythromycin resitance gene ermA and the staphyloccoal toxin gene sea/tsst-1. The pediatric isolates were more likely to be ST72 with SCC*mec* type IV or IVA with lower resistance to most antibiotics, and inducible clindamycin resistance due to the presence of the *ermC* gene.

233 MRSA isolates from the pharynx and skin of neonatal patients in the NICUs of 3 separate hospitals in Japan were collected from 1994 to 1996 in order to determine the mode of transmission of MRSA in the NICUs (Saito *et al*., 1998). As well, they collected 16 MRSA strains from adult patients admitted to one of the 3 hospitals and compared the adult and pediatric isolates on their PFGE patterns, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns, toxin production, and drug resistance (Saito *et al*., 1998).

According to PFGE pattern, the authors classified the strains isolated in neonatal patients as type A or B and subtypes A', A", and B' (Saito *et al*., 1998). The majority of isolates were of type A and subtype A' (38 of 40 at site JMSH in 1994 (95%), and 155 of 166 at sit JMSH

in 1995 (93%) (Saito *et al*., 1998). The authors found variation in the production of individual exoproteins within the same isolates using SDS-PAGE profiling but no change in PFGE patterns, coagulase types, enteroxin types or TSST-1 production (Saito *et al*., 1998). The neonatal isolates were also compared with 16 adult isolates from the same hospital site on PFGE pattern. No identical pattern to A', A", B or B' was seen in the adults and overall there were 14 different patterns in the adult isolates, only 2 of which were 2 were type A (Saito *et al*., 1998). Therefore different strains seemed to affect adult and neonatal patients within this Japanese hospital. Additionally, despite no contact between the 3 NICU personnel and the distant geographic locations of the hospitals, all isolates from the neonatal patients had similar or identical PFGE patterns (Saito *et al*., 1998). Saito *et al*. suggested that certain MRSA strains with the same genetic background may tend to be specific to NICUs and that colonization by these strains may be endemic in neonates. In contrast, the MRSA strains affecting adults tended to be much more variable (Saito *et al*., 1998).

A prospective cohort study of inpatients from 30 Orange County hospitals in California aimed to characterize MRSA strains isolated from adult (≥18 years) and pediatric (<18 years) patients (Hudson et al., 2012). 1124 adult and 159 pediatric isolates were collected between December 2008 and September 2009. The 3 predominant spa types were t008, t242, and t002. They observed significantly different distributions of these spa types among adults (t008, 41%; t242, 23%; t002, 19%) and children (t008, 69%; t242, 9%; t002, 6%; χ^2 $p<0.001$). Overall, the community-associated spa type (USA300) was significantly more common in pediatric patients (69% vs. 49%). Using BURP analysis of the *spa* types, the majority of adult isolates clustered into

3 spa clonal complexes (*spa*-CCs) while the majority of pediatric isolates clustered into two *spa*-CCs. They found significantly higher estimated genetic diversity (using Simpson's index of diversity) of MRSA isolates among adult compared to pediatric patients.

Hudson *et al*. (2012), also conducted analyses to assess the individual and hospital-level variables that were associated with the spa type t008 (USA300). They conducted bivariate and multivariate Generalized Linear Mixed Effects Models, clustered by hospital. In bivariate analyses, pediatric patients, wound specimens, isolation in a non-ICU ward, community onset, and isolation from a hospital with greater than 10,000 admissions were associated with *spa* type t008 (USA300). In multivariate analyses, isolates from pediatric patients, wounds, non-ICU wards, and hospitals with a high proportion of Medicaid-insured isolates remained significant predictors of *spa* type t008 (USA300). The authors propose that children are more likely to encounter CA-MRSA as a result of exposure to environments that facilitate the spread of MRSA such as schools, sport activities, daycares, and camps. The fact that wounds were associated with *spa* type t008 is likely due to the fact that wounds or SSTIs are the most common type of CA-MRSA infection. Hudson *et al*. proposed that the fact that the community strain USA300 (*spa* type t008) was associated with non-ICU wards might suggest that this community strain affects healthier patients or that it results in less severe infections than other HA-MRSA strains.

The epidemiological characteristics of community-associated and healthcare-associated MRSA were compared between 12 hospitals that were a part of a sentinel surveillance network established by the Minnesota Department of Health, as described above (Naimi *et al*., 2003). They found that one clone of MRSA, named "Clone A" 31 of the 49 pediatric isolates (63%) and

35 of the 57 adult patients (61%). This clone appeared to dominate in this particular population and interestingly comprised a similar proportion of isolates in both pediatric and adult patients. This is different from what has been found by several previous studies including that by Saito *et al*., 1998 and Park *et al*., 2007 who both found different strains affecting adult and pediatric MRSA patients and in different proportions.

Antimicrobial Resistance

Summary: Antimicrobial Resistance

- The majority of the included studies found higher resistance in adult compared to pediatric MRSA isolates
- There was significantly higher resistance in adult MRSA isolates from a Korean hospital to gentamicin, erythromycin, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and rifampin
- An American study found significantly higher resistance among adult compared to pediatric CA-MRSA isolates to erythromycin, clindamycin, ciprofloxacin, gentamicin, and tetracycline. They found similar percentages of antibiotic resistance between adult and pediatric HA-MRSA isolates.
- A study from South Africa also found significantly higher resistance to erythromycin and clindamycin in adults.
- In Australia the proportion of multi-resistance was higher in adults compared to children and pediatric isolates had higher susceptibility to most antibiotics
- Antibiotic susceptibilities varied by geographic region in Australian pediatric hospitals

A prospective study of inpatient and outpatient MRSA isolates identified at the University of Chicago Hospitals from November 2003 to November 2004 was conducted in order to compare the antimicrobial resistance patterns of pediatric and adult patients, stratified by site of onset (hospital vs. community) (David *et al*., 2006). There was a statistically significant

difference between 288 adult and 177 pediatric CA-MRSA isolates in the resistance to non-β-lactam antibiotics (David *et al*., 2006). Adult isolates were statistically significantly more likely to be resistant to non-β-lactam antimicrobials compared to pediatric isolates for erythromycin (93 vs. 87%, p = 0.03), clindamycin (52 vs. 7%, p<0.001), ciprofloxacin (62 vs. 11%, p<0.001), gentamicin (11 vs. 1%, p<0.001), and tetracycline (20 vs. 6%, p<0.001) (David *et al*., 2006).

In terms of antimicrobial susceptibility, Park *et al*. (2007), (methods described above) found that both adult and pediatric MRSA isolates had high resistance rate against ampicillin, tobramycin, and erythromycin (number. of resistant isolates in adults (%) vs. children (%), p-value, respectively: 50 (100) vs. 88 (100.0), p=*ns*.; 42 (96) vs. 41 (47), p=0.7046; and 46 (92) vs. 67 (76), p=0.02) but low resistance rates against trimethoprim, rifampin, chloramphenicol, teicoplanin and vancomycin (number of resistant strain in adults (%) vs. children (%), p-value, respectively: 8 (16) vs. 0, p<0.0001; 7 (14) vs. 1 (1), p=0.0035; 0 vs. 1 (1), p=*ns*; 0 vs. 0, p=*ns*; 0 vs. 0, p=*ns*). The adult strains had significantly higher resistance rates than the pediatric strains against gentamicin, erythromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, trimethoprim, and rifampin, (number of resistant isolates in adults (%) vs. children (%), p-value, respectively: 48 (96) vs. 41 (47), p<0.0001; 46 (92) vs. 67 (76), p=0.02; 41 (82) vs. 14 (16), p<0.0001; 41 (82) vs. 7 (8), p<0.0001; 12 (24) vs. 0, p<0.0001; 8 (16) vs. 0, p<0.0001; and 7 (14) vs. 1 (1), p=0.0035). Pediatric strains had significantly higher resistance to sulfamethoxazole (35 (40%) vs. 11 (22%), p=0.033). Fifty-two pediatric isolates and 5 adult isolates showed erythromycin-resistant and clindamycin-susceptible phenotype and all 57 scored positive for inducible clindamycin resistance on the D-test. The *ermA* resistance gene was detected in 38

(76%) adult isolates and 14 (16%) pediatric isolates whereas the *ermC* resistance gene was detected in 5 (10%) adult isolates and 52 (59%) pediatric isolates (Park *et al*., 2007).

Marais *et al*., 2009 compared 190 adult (≥18 years) and 44 pediatric (<18 years) MRSA isolates collected from 15 National Health Laboratory Services and 8 private diagnostic laboratories from all 9 provinces of South Africa from 2005 to 2006. The adults and children had similar resistance profiles to most of the antimicrobial agents but significantly lower resistance in pediatric isolates for Erythromycin (58% vs. 80%, p=0.0015), and Clindamycin (21% vs. 40%, p=0.018). However they found higher resistance in pediatric isolates to nitrofurantoin (38% vs. 18%, p=0.027) and Gentamicin (85% vs. 60%, p=0.001). They found no isolates resistant to Vancomycin. Overall, Marais and colleagues (2009) found significant differences between resistance profiles of adult and pediatric MRSA isolates, with generally higher resistance in adult isolates.

Patient case notes from 1997-2001 at an ophthalmic hospital in the UK were retrospectively reviewed for culture positive external ocular *Staphylococcus aureus* infections (Shanmuganathan *et al*., 2005). Seventeen of 548 (3%) cases were caused by MRSA (Shanmuganathan *et al*., 2005). There were observed differences in the antimicrobial resistance of MRSA strains affecting younger (younger than 25 years) and older (25 years and older) patients (Shanmuganathan *et al*., 2005). The strains affecting older patients were resistant to ofloxacin whereas 2 of 3 of the isolates from younger patients (ages 16 and 23 years; and 6 years; respectively) were sensitive to ofloxacin (Shanmuganathan *et al*., 2005). The results from this study are hypothesis generating since the sample size was too small for statistical analysis;

the study only suggests that it is possible that younger and older patients with MRSA ocular infections differ on strains' antimicrobial resistance patterns, and the authors were not aware of any reasons for these differences (Shanmuganathan *et al*., 2005). The results did suggest that MRSA was a rare cause of ocular infections at the time of the study in the UK (Shanmuganathan *et al*., 2005).

Pediatric *S. aureus* isolate data from 7 hospitals across Australia were compared to published data on adult patients from the same geographic regions of Australia, as described above (Wolf *et al.,* 2010). The study examined the pediatric data for the prevalence of multi-resistant (mMRSA) compared to non-multiresistant MRSA (nmMRSA) (Wolf *et al.,* 2010). Multi-resistant and non-multiresistant MRSA were defined as MRSA isolates that were non-susceptible to at least 3 antibiotics, and as MRSA isolates that were susceptible to at least 6 antibiotics, respectively (Wolf *et al.,* 2010). Multi-resistant MRSA isolates were present in all hospitals (where sufficient data was available) (Wolf *et al.,* 2010). The proportions of MRSA isolates that were multi-resistant varied between hospitals; however the proportion was low at all of the pediatric hospitals studied (number of mMRSA / number of MRSA isolates (%) by hospital: 0/72 (0), 1/67 (2), 4/60 (7), 27/370 (7), 5/49 (10) 17/122 (14)) (Wolf *et al.,* 2010). Compared to the overall low prevalence of multiresistant MRSA among the pediatric patients in the Wolf *et al*., 2010 study (total: 54/740 MRSA isolates, 7%), adult outpatients in the same areas of Australia had a higher prevalence of multiresistant MRSA (49% nationally in adults) (Nimmo *et al*., 2006; Wolf *et al*., 2010). Additionally, Wolf *et al.* (2010) reported that 96% of all pediatric MRSA isolates were susceptible to oral antibiotics appropriate for pediatric patients

thus suggesting that pediatric patients in Australia tend to harbour less resistant strains of MRSA than adult patients in the same areas. This trend has also been observed previously internationally in studies of CA-MRSA in children (although the authors in this study were unable to distinguish between community- and hospital-associated MRSA and the HA-MRSA were found in the international studies to have higher rates of mMRSA) (Wolf et al., 2010; Dietrich, 2004).

In terms of geographic patterns, Wolf *et al*. (2010) found variation in the rates of susceptibility to methicillin, erythromycin and cotrimoxazole between the 5 cities where the 7 pediatric hospitals were located (Wolf *et al*., 2010). However there was no difference in the rates of susceptibility to penicillin between the regions (Wolf *et al*., 2010). The epidemiology of MRSA in adult patients was found to vary between Australian cities due to outbreaks of distinct MRSA clones in different regions (Wolf *et al*., 2010; Nimmo *et al*., 2008). One possible explanation for the geographic trends in antimicrobial susceptibility rates among pediatric rates in the current study is that similarly to trends in adult hospitals, the strains that affect pediatric patients may vary by region. However, as discussed by David *et al*. (2006) it is also possible that these differences in susceptibilities could also be explained by different selective pressures due to varying antibiotic prescription practices in different hospitals or regions.

MRSA Acquisition Source (Healthcare-Associated vs. Community-Acquired)

Summary: Healthcare-Associated vs. Community-Acquired

- The included studies found that adults tend to acquire MRSA in health care settings, while children tend to acquire MRSA in the community
- Most studies supported the finding that healthcare associated MRSA strains are generally more resistant to antibiotics than community-associated strains
- Pediatric patients were found to acquire *S. aureus* bacteraemias in the community, however, similar to adult patients, neonatal patients were more likely to acquire *S. aureus* bacteraemias in a health care setting
- One study found significant differences in antibiotic resistance between adult and pediatric CA-MRSA cases (higher resistance in adults), but similar resistance profiles among adult and pediatric HA-MRSA
- The authors of a study of patients in an American hospital recommend clindamycin as a first-line antibiotic for pediatric CA-MRSA infections, but not for adults.

Several of the articles in the literature examined the differences between adults and children in relation to the source of MRSA (Denniston *et al*., 2006; David et al., 2006; Frank *et al*., 1999; Naimi *et al*., 2003). These studies suggest that pediatric patients are more likely to acquire MRSA in the community, while adults are more likely to acquire MRSA in a healthcare setting. As well, these studies examined the differences in antibiotic resistance between the two different sources of MRSA.

Incidence and Prevalence of HA and CA-MRSA

Pediatric and neonatal cases of *S.aureus* bacteraemia in a UK hospital were studied by Denniston *et al*. (2006), as described above. They compared their results with the literature on adult SAB and found differences in the source of acquisition for children, but similarities for neonates. The neonatal group (n=33) had 100% hospital-acquired infection while the paediatric group had 20% hospital-acquired infection, which was significantly different from an adult proportion reported in the literature as 58% ($p<0.0001$) (Denniston *et al.*, 2006). Although this data was for S. aureus, 27% of the neonatal cases (8 out of 33) and 6% of the pediatric cases (5 out of 51) were MRSA (Denniston *et al*., 2006). Therefore, all 8 neonatal MRSA cases were hospital-acquired but the number of hospital-acquired MRSA could not be determined from the published data. The authors did report that SAB due to MRSA is usually acquired in hospital (80%) (Denniston *et al*., 2006).

Antimicrobial Resistance Patterns for CA and HA MRSA

Differences in the antimicrobial resistance patterns between CA-MRSA and HA-MRSA have been noted in the literature, and these trends had been observed both in pediatric and adult patients (David *et al*., 2006; Kallen *et al*., 2000; Ellis *et al*., 2004; Dietrich *et al*., 2004; Buckingham *et al*., 2004; Frank *et al*., 2002; Naimi *et al*., 2003). In comparison to HA-MRSA strain, CA-MRSA strains were often found to have less resistance to multiple non-β-lactam antimicrobial drugs (David *et al*., 2006; Herold *et al*., 1998; Hussain *et al*., 2000).

In their current study, David *et al*., (2006) found that among community-acquired isolates, there were statistically significant differences in antimicrobial resistance between pediatric isolates compared to adult isolates, whereas in hospital-acquired isolates, pediatric and adult MRSA isolates had similar non-β-lactam antimicrobial drug resistance. The study was conducted at the University of Chicago Hospitals (a 577-bed tertiary care centre with 29,500 admissions and 379,000 outpatient visits annually) on both inpatient and outpatients prospectively identified from November 2003 to November 2004. A total of 1,149 MRSA-positive cultures were identified from 578 patients, 201 (35%) from children ≤18 years of age and 377 (65%) from adults ≥19 years of age. All of the isolates were classified as community-acquired or hospital acquired based on the 72-hour definition (isolates cultured from outpatients or cultured within 72-hours of admission from inpatients were considered CA) and then pediatric and adult isolates were compared within the community-associated and hospital associated isolates using the χ^2 test or the Fisher exact test (2-tailed, statistical significance considered if $p<0.05$). The majority of the adult and the pediatric inpatient isolates were CA-MRSA (64% and 72%, respectively). The authors tested the effects of changing the definition of community-associated MRSA from isolates identified ≤ 72 hours to ≤ 48 hours and ≤24 hours but did not find significant difference in the proportions of hospital and community associated isolates. (David *et al*., 2006)

Among the CA isolates, MRSA with resistance to only β-lactam antimicrobial drugs was more common in pediatric than adult patients (10% compared to 4%, $p=0.001$) and the adult isolates were more likely than pediatric isolates to be resistant to most of the non-β-lactam antimicrobial drugs (David *et al*., 2006). For the comparisons of adult and pediatric isolates

associated with community onset, the adult isolates had statistically significant higher percentage of resistance to ciprofloxacin ($p<0.001$), clindamycin ($p<0.001$), erythromycin ($p=0.03$), gentamicin ($p<0.001$), and tetracycline ($p<0.001$). The only significant differences in resistance between pediatric and adult hospital-associated isolates were in resistance to ciprofloxacin ($p=0.004$) and to gentamicin ($p=0.01$) (David *et al*., 2006). As well, among the CA-MRSA, a significantly higher percent of adult isolates were multi-drug resistant MRSA ($p<0.001$) (defined as resistance to 3 or more non-β-lactam antimicrobial drugs). A higher percentage of adult hospital-associated isolates were multi-drug resistant, however the difference was not significant ($p=0.35$). There was no significant difference between pediatric and adult isolates for rifampin, TMP-SMX, or vancomycin among the community or the hospital associated isolates (David *et al*., 2006).

Microbiology reports for MRSA isolates collected at the University of Illinois Hospital from 1987 to 1997 were reviewed (Frank *et al*., 1999). Around 1993, an increase in the number of pediatric MRSA isolates was observed. They also observed that clindamycin-susceptible isolates accounted for the majority of MRSA isolates in children in the mid-1990s, which was the opposite of what was observed in adult isolates (Frank *et al*., 1999). There was a significant association between clyndamycin susceptibility and community-acquired infection in children (based on χ^2 test) (Frank *et al*., 1999).

As described above, Naimi *et al*. (2003) compared CA- and HA-MRSA isolates from inpatients and outpatients infected with MRSA in the year 2000 at 12 surveillance sites in the Minneapolist-St Paul and Minnesota areas. There was a total of 1100 MRSA infections, 131

(12%) of which were community-associated and 937 (85%) were health cared-associated based on a risk factor definition (Healthcare-associated was defined as patients with: 1. an MRSA infection identified after 48 hours of admission to a hospital 2. a history of hospitalization, surgery, dialysis, or residence in a long-term care facility within 1 year of the MRSA culture date; 3. a permanent indwelling catheter or a percutaneous medical device present at the time of culture 4. a known positive culture for MRSA prior to the study period and community-associated was defined as any cases not meeting these criteria). Among the community-associated MRSA infections, 53 were pediatric (<18 years) and 78 were adult (≥18 years). Compared to healthcare-associated isolates, the community-associated MRSA were generally more susceptible to non- β-lactams antimicrobials and more likely to be susceptible to multiple classes of antimicrobials. In the 106 available CA isolates, there was no difference in susceptibility patterns between pediatric and adult isolates. These results are consistent with those found by David *et al*. (2006) with regards to the antimicrobial susceptibilities of community-associated isolates compared to healthcare-associated isolates however the results are contradictory to what David *et al*. (2006) and Frank *et al*. (1999) found with respect to the susceptibility profiles of pediatric compared to adult isolates.

The differences found by David *et al*., (2006), between adult and pediatric isolates associated with the community may suggest that different MRSA strains are colonizing pediatric and adult patients in the community. Although Frank *et al*. (1999) did not compare pediatric and adult isolates by community and hospital acquired isolates, they found large differences in clindamycin susceptibilities between adult and pediatric isolates, which is consistent with the

results of David *et al*. (2006). Some reasons for the differences in resistance in the community suggested by David *et al*. (2006) include the unique risk factors in children related to both their environments (schools, daycare centers, recreation facilities) and behaviours, differences in the host defences, or different selective pressures for antimicrobial resistance as a result of different prescribing practices among pediatric and adult healthcare. These results have important implications for therapy in treating MRSA, suggesting that pediatric and adult CA-MRSA require different first-line empiric antimicrobial drugs (David *et al*., 2006). However, the differences in resistance between adult and pediatric isolates may not be generalizable, given that the resistance patterns of MRSA can differ over time and by population as a result of differing antimicrobial prescribing practices and different host susceptibilities.

Although resistance patterns can vary in different populations, several studies conducted in other United States found that pediatric isolates have a low rate of clindamycin resistance (ranging from 67 to 100%) (David *et al*., 2006; Purcell and Fergie, 2002; Dietrich *et al*., 2004; Frank *et al*., 2002; Adcock *et al*., 1998; Shahin *et al*., 1999; Huang *et al*., 2004; Kaplan *et al*., 2005). Based on these results, David *et al*. recommend clindamycin as a first-line antimicrobial of choice for pediatric patients with a suspected CA-MRSA infection. Contrary to pediatric populations, studies on adult CA-MRSA have found inconsistent patterns of clindamycin resistance (David *et al*., 2006). Some studies, particularly in tertiary care medical centers in Chicago, have found high percentages of clindamycin resistance in adult isolates (David *et al*., 2006; Suntharam *et al*., 2001), Other studies, however, have found low clindamycin resistance in adult CA-MRSA isolates, particularly among young and urban poor adults. These studies

included a military beneficiary population in Texas, homeless and marginally housed adult population in San Francisco, and a healthy young adult population training at a military facility in Texas (Kallen *et al*., 2000; Charlebois *et al*., 2002; Ellis *et al*., 2004; respectively). The reasons for the differences are not entirely known, although they are possibly due to regional variations, differences in antimicrobial exposure, or differences in population characteristics (David et al., 2006). From these studies it appears that CA-MRSA isolates from adults in tertiary care centers show higher clindamycin resistance whereas isolates from surveys of adults in the community show lower clindamycin resistance (David *et al*., 2006). The more similar resistance patterns between adult and pediatric hospital associated MRSA isolates suggests that these adult and pediatric patients may have a more similar selective pressure, more similar host susceptibilities, and/or are affected by the same strains (David *et al*., 2006) than adult and pediatric patients with CA-MRSA.

Overall, it appears that adult and pediatric MRSA isolates may differ in antimicrobial resistance patterns when the isolates are community associated, whereas isolates that are hospital associated may be more similar. However, this appears to be dependent on the time, MRSA strains, and specific populations of study.

Spread from Adult to Pediatric Units via Healthcare Workers

Summary: Spread from Adult to Pediatric Units via Healthcare Workers

- One study from the USA reported an MRSA outbreak of MRSA in the NICU of a pediatric stand-alone facility
- The primary MRSA strain in the outbreak had previously been detected at a nearby adult facility
- The current study does not provide sufficient evidence of the transfer via healthcare workers; further research is needed

Saiman *et al*. (2003) reported a possible case of MRSA transmission from an adult facility to a pediatric facility via a rotating healthcare worker. The authors conducted a prospective epidemiologic analysis of an MRSA outbreak in a 45-bed level III-IV NICU in a stand-alone children's hospital in New York, USA from January to March 2001. Surveillance cultures of the anterior nares were obtained for all hospitalized infants and healthcare workers who had contact with the infants in the NICU 2 weeks prior to February 13th. Many of the healthcare workers were reported to rotate between the pediatric and adult hospitals (Saiman *et al*., 2003). Isolates were genotyped by pulsed-field gel electrophoresis, compared for genetic relatedness, and 13 representative samples were genotyped by comparative DNA sequencing of the *spa* gene (Saiman *et al*., 2003). Fourteen infants and 3 HCW were colonized with MRSA and 4 infants but no HCW were infected. Thirteen of 14 infants were colonized with the same outbreak strain referred to as "Clone B," a strain that had previously been detected in the adult hospital in 22 patients from January 1999 to November 2000. The 2 HCW who rotated between hospitals were colonized with "Clone C" and the non-rotating HCW was colonized with "Clone

B" (Saiman *et al*., 2003). This study did not provide firm evidence of transmission from the adult to the pediatric hospital but rather suggested the possibility of this type of transmission when HCW rotate between facilities (Saiman *et al*., 2003).

CHAPTER 3: Rationale

THESIS RATIONALE

The Healthcare-Associated Infections (HAI) section of the Public Health Agency of Canada in collaboration with the Canadian Hospital Epidemiology Committee (CHEC) from the Association of Medical Microbiology and Infectious Disease Canada (AMMI) felt there was no strong study directly comparing adult and pediatric MRSA patients on both epidemiological and microbiological characteristics. In addition, the CHEC members working in pediatric hospitals have long argued that MRSA prevention and control recommendations were based on adult protocols and were not specific enough to address the needs of pediatric patients.

A detailed literature review was needed to determine what studies existed that directly compared adult and pediatric patients. The results of this literature review confirmed that very few studies had directly compared these two populations and no study had been done at a national level for both epidemiological and microbiological characteristics. No study was found that compared adult and pediatric patients in Canada and the majority of the international studies on these two populations were either of poor quality (due to small sample size or methodology), or compared either laboratory or epidemiological characteristics of adult and pediatric MRSA but not both. A total of 18 articles were found that discussed differences between adult and pediatric MRSA. As described in the background section of this thesis, only

13 articles were found that directly compared the two populations, and only 5 were considered "acceptable quality" articles.

The Canadian Nosocomial Infection Surveillance Program's (CNISP) MRSA surveillance database provided the opportunity for an analysis of epidemiological and microbiological characteristics of MRSA of a nationally representative sample that included both adult and pediatric hospitalized patients. The unique qualities of the CNISP MRSA surveillance system are: 1. The surveillance data includes both adult and pediatric patients selected from the same population; 2. The surveillance system is representative of a national population; 3. There is a direct link between epidemiological and laboratory data; and 4. The surveillance data has been collected annually beginning in 1995 resulting in a large sample size over a long time span (13 years).

The importance of these analyses is supported by the lack of literature relating to the epidemiological and laboratory characteristics of MRSA in adult patients compared to pediatric patients, the increasing concern over the emergence of community-associated MRSA in young, previously healthy individuals without traditional risk factors for MRSA, and the increasing rates of MRSA among children. These results will provide evidence regarding differences in the epidemiology of MRSA that may exist between adult and pediatric patients.

CHAPTER 4: THESIS DESIGN

THESIS OBJECTIVES

The objectives of the current thesis work were twofold: to use pan-Canadian surveillance data collected by the CNISP[1] from 1995 to 2007

(i). to describe the epidemiology and the molecular characteristics of Methicillin-resistant *Staphylococcus aureus* (MRSA) in pediatric patients and compare them to those associated with MRSA in adult inpatient population and,

(ii). to explore the relationship between hospital characteristics and higher MRSA incidence rates.

RESEARCH QUESTIONS

MAIN RESEARCH QUESTION:

What is the clinical and molecular epidemiology of MRSA colonization and infection in pediatric and adult inpatients of Canadian acute care centers?

SECONDARY QUESTIONS:

1. Are there any differences between the MRSA colonization and infection rates in hospitalized pediatric and adult patients
2. Is there any difference in the way MRSA infection presents itself in pediatric patients in comparison to adult patients? (For example: Are there differences in the site of infections in these two populations? Are the infections equally severe in these populations?).
3. Are the pediatric and adult populations colonized / infected with same MRSA strain types?
4. Are pediatric patients more prone to HA- or CA-MRSA colonization and infection?
5. Are adult patients more prone to HA- or CA-MRSA colonization and infection?
6. What are the predominant strain types in either of the two populations?
7. What has been the trend of CA-MRSA and HA-MRSA in both populations over the study period?
8. Are antimicrobial resistance patterns similar in both populations?
9. What are the apparent differences between hospitals that reported high MRSA rates and those with lower rates?

STUDY DESIGN

There are three components to this thesis:

1. Detailed review of the literature for existing studies documenting any differences in the epidemiology of MRSA in adult and pediatric populations;

Use of the surveillance data collected by the Canadian Nosocomial Infection Surveillance Program (CNISP) of the Public Health Agency of Canada (PHAC) from 1995 to 2007 to:

2. Determine MRSA rates and for descriptive analyses;
3. Explore hospital characteristics associated with HA-MRSA rates

Chapter 5: Methods

DATA COLLECTION

- Literature review;
- Use of existing CNISP MRSA surveillance database.

DATA ANALYSIS

- Computation of annual incidence rates for both adult and pediatric hospitals using total number of patients admitted (or the total number of days spent by each patient during his/her hospitalization) during each surveillance year as denominators;
- Descriptive statistics of clinical characteristics of pediatric and adult MRSA patients, the molecular characteristics of MRSA strain types prevalent in pediatric and adult patients;
- Univariate logistic regression to identify patient characteristics associated with HA-MRSA vs. CA-MRSA (based on surveillance definition);
- Hospital-level models (repeated measures analysis): Univariate and multivariate Poisson regressions to identify hospital level characteristics associated with HA-MRSA rates over the 13 year surveillance period.

SOURCE OF DATA

MRSA surveillance data collected by the Canadian Nosocomial Infection Surveillance Program (CNISP) from sentinel hospitals across Canada from 1995-2007 was used in the current analyses. The CNISP MRSA Surveillance system is a prospective, laboratory-based, sentinel surveillance system (as described previously in CNISP, 2006 and Simor *et al*., 2010).

ETHICS

Patient confidentiality was maintained during data collection throughout the CNISP surveillance program by assigning a unique identifier to each patient in each participating hospital site prior to submission of the MRSA surveillance data to the PHAC. This link was kept at each site and was not shared, according to Good Clinical Practices, to the PHAC surveillance team.

Ethics board approval is not required for the secondary analysis of an existing database, which in this case was surveillance data;, its use is observational in nature, does not modify patient care, is considered to fall within regular procedures for hospital-based infection prevention and controls programs and is a routine component of quality assurance and patient care in Canadian health care institutions. For these reasons, research ethics board approval was only obtained at some of the participating CNISP hospitals – for the purpose of primary data collection. For the current analysis, no further consent or ethics approval was required given

that the analyses was within the scope of what has been done previously on this dataset and was considered to fall within regular PHAC surveillance activities. Permission to conduct the current analyses on the dataset was required and obtained from the Healthcare-Associated Infections Surveillance and Epidemiology Section within the Surveillance and Epidemiology Division of the PHAC and the CHEC members, who own the dataset.

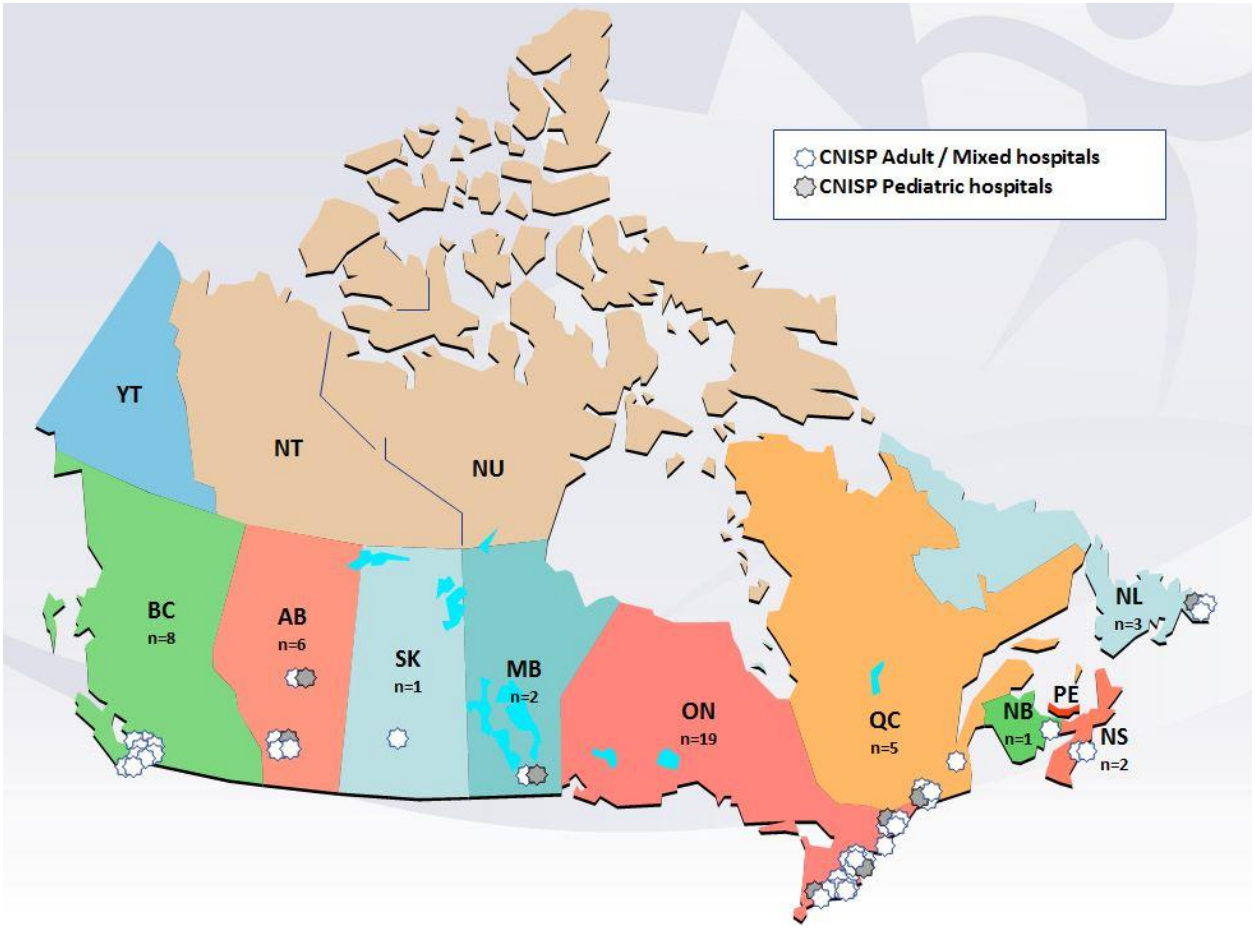
CANADIAN NOSOCOMIAL INFECTIONS SURVEILLANCE PROGRAM (CNISP)

CNISP Background

CNISP is a network of sentinel hospitals with representation from across Canada, and is a joint initiative between the Centre for Communicable Disease and Infection Control (CCDIC), the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC), and members of the Canadian Hospital Epidemiology Committee (CHEC) of AMMI Canada. The CNISP program was originally created in response to the absence of available information on the incidence and epidemiology of nosocomial infections in Canada prior to 1995. The objectives of the CNISP program are to provide rates and trends of healthcare-associated infections at Canadian healthcare facilities in order to provide scientific evidence to inform national clinical guidelines on nosocomial infections. As well, the monitoring of rates and trends allowed the creation of meaningful national "benchmarks" by comparing rates over time and between

facilities. In 1995, surveillance started in 22 sites in sentinel hospitals across Canada (some sites consist of a network of multiple hospitals) (detailed chronology in Appendix E, available from PHAC). Currently, the CNISP network comprises more than 50 hospital sites from 10 provinces. The majority of participating hospitals are tertiary care centres and there are three types of facilities: adult stand-alone facilities, pediatric stand-alone facilities, and mixed (adult/pediatric) facilities. Each site has a representative member of the Canadian Hospital Epidemiology Committee (CHEC), and these members work on sub-committees responsible for the various CNISP surveillance projects.

Figure 1: Map of CNISP pediatric and adult hospital sites (2007)



CNISP Data Collection

CNISP hospital sites collect data based on standardized protocols and submit anonymous patient data to the Center for Communicable Diseases and Infection Control (CCDIC) at the Public Health Agency of Canada (PHAC) where the information is compiled and analysed. A representative subset of laboratory isolates are collected from in-patients and sent to the NML for characterization. Laboratory and epidemiological data are merged resulting in the national dataset.

The CNISP MRSA surveillance collects data on newly identified MRSA cases at CHEC hospitals. This includes both MRSA-colonized and -infected patients and it is important to note that cases are only counted once. That is to say that repeat infections are not included in this surveillance unless the infection is caused by a different MRSA strain at another point in time. If a patient is initially colonized with MRSA but develops a MRSA infection within the same surveillance year, the patient is recorded as an infection and colonization record is removed. The MRSA surveillance project is laboratory-based therefore patients are identified based on laboratory confirmation of MRSA (detailed laboratory methods are described later). Only in-patients (and not out-patients) are included. Once a patient is confirmed as MRSA positive, the infection control professional conducts a chart review to collect demographic and clinical information.

A standardized MRSA surveillance definition is used to determine the presence of an MRSA infection. MRSA colonization is assumed if a positive MRSA culture was obtained from a

patient in the absence of clinical signs or symptoms of infection. As well, an attempt to identify the MRSA source (either healthcare-associated or community-associated) was made whenever possible based on the infection control professional's (ICP) clinical judgment. The following information from patients' charts helped in classifying the case as healthcare- or community-associated: length of stay in hospital prior to positive MRSA culture (generally a hospital stay of greater than 48 to 72 hours is considered as HA-MRSA - protocols changed slightly over time but mostly used "> 72 hours" as a criterion for HA-MRSA), previous MRSA status, date of admission, total length of hospital stay, prior hospitalization or admission to another healthcare facility (admission within the past 12 months), and where the patient was admitted from (e.g. long-term care facility). Community-associated MRSA was defined as MRSA cases where the patient had no established healthcare-associated risk factors and:

(i) was hospitalized < 72 hours (more recent protocols use < 48 hours);

(ii) had no previous history of MRSA;

(iii) had no medical devices such as urinary catheters, IV lines, feeding tubes, tracheostomy, dialysis access, etc.

(iv) had no history of hospitalization, surgery, or dialysis in the 1 year preceding the MRSA-positive culture;

(v) was not a resident of a long-term care facility in the 1 year preceding the MRSA-positive culture.

These surveillance definitions of healthcare- and community-associated MRSA were defined by the CHEC members in the surveillance protocol. The data collection protocols for MRSA surveillance varied slightly over the years but generally included: a unique patient identifier, date of birth, sex, ethnicity (First Nations or non-First Nations), date of positive culture, reason for the culture, where MRSA was acquired (healthcare- or community-

associated), and the anatomical site of MRSA isolation. (See Appendix F for a sample of a patient questionnaire and a CNISP surveillance protocol)

CNISP Hospital Profiles

As part of the CNISP surveillance program, individual hospital profiles are collected annually for each CHEC site. Denominator data collected includes number of patient admissions and patient-days and is used for rate calculations. Other information collected varies annually but generally includes province, size of the healthcare facility, teaching hospital status, type of healthcare facility (adult, pediatric, long-term care or other), total number of patient-days per year, total number of patient-admissions per year, number of full-time equivalent (FTE) ICPs, and total number of *S. aureus* isolates. (See Appendix for a sample hospital profile questionnaire).

CNISP MRSA Database

The CNISP MRSA database contains information collected from 1995-2007 for both adult and pediatric hospitalized patients. The table below lists all the variables available in this dataset.

Table 1. CNISP MRSA dataset variables, 1995-2007

Patient Characteristics
Unique Patient Identifier codes
Date of Birth
Age of Patient
Age groups (categorized)
Sex
Ethnicity (First Nations/ Non first nations)
Postal Code of patient's home address, (first 3 digits only) - if community-associated
Date of Admission
Date of positive culture
Reason for first culture
Where MRSA acquired (healthcare, your facility, community, etc.)
Epi Link – any epidemiological link with other patients – yes/no
Anatomical Site of MRSA isolation (yes/no) = Blood; Surgical Wound; Skin/soft tissue/burn; Respiratory/Sputum; Urine; Nose; Rectum; Other Site
Site of infection (Infected/unknown) = Blood; Surgical Wound; Other skin/soft tissue/burn; Respiratory/Sputum; Urine; Other Site
Site of colonization (colonized/unknown) = Blood; Surgical Wound; Skin/soft tissue/burn; Respiratory/Sputum; Urine; Nose; Rectum; Other Site
Number of infections (calculated from site of MRSA question)
Number of colonizations (calculated from site MRSA question)
Overall patient status (Infection vs. Colonization)
Type of Infection (available for 2006-2007, not prior): No infection; Urinary tract infection; Necrotizing fasciitis (surgical wound or other); Pneumonia = non-necrotizing/Necrotizing ; Soft tissue infection, non-necrotizing; Osteomyelitis; CVC-BSI; Bacteraemia = no source identified; Endocarditis; Meningitis; Conjunctivitis; Septic arthritis/bursitis; Other
Laboratory Characteristics
NML Identifier
NML date received
PFGE Smal Pattern
Epidemic Strain Type
Year first identified PFGE
PVL PCR (positive, negative)
Spa Type
Toxin Genotype
PhagetypeSCCmec
SCCmecSCCmec NML (II, II?, IV)
SCCmec NML (II, II?, IV)Antimicrobial resistance
Hospital level Characteristics
CHEC site ID
Region 1: Central, Eastern, Western
Region 2: AB, ATL, BC, ON, QC, SK/MB
Region 3: ATL, BC/AB, ON/QC, SK/MB
Province: AB, BC, ON, QC, MB, NB, NL, NS, SK
Hospital Profile (adult only, peds only, mixed adult & peds)

LABORATORY METHODS

MRSA positive isolates are identified at CNISP hospitals via routine screening procedures and clinical cultures and confirmed at each hospital's laboratory. The hospital laboratories then send isolates to the NML in Winnipeg, Manitoba for confirmation of MRSA by polymerase chain reaction (PCR) detection of *nuc* and *mecA* genes. From 1995-2005, all isolates were sent to the NML; however, from 2006-2007 only clinical isolates (non-screening isolates) were submitted (Simor *et al*., 2010). A duplicate set of strains was also sent to Sunnybrook Hospital (Toronto, Ontario) laboratory for storage and further testing, as described in Simor *et al*., 2010. A geographical representative subset of clinical MRSA isolates was selected each year for further characterization (with the exception of 1995-1996 when all isolates were selected). Further testing included molecular typing of the isolates by extracting and digesting the DNA with *SmaI* and using pulsed-field gel electrophoresis (PFGE). The DNA profiles generated by PFGE are analyzed using BioNumerics software, version 5.10 (Applied Maths) (Simor *et al*., 2010). In terms of PFGE typing, the strains are classified as CMRSA (Canadian MRSA) or other known types. Strains are further characterized by multi-locus sequence typing, staphylococcal chromosomal cassette *mec* (SCC*mec*) typing, and identification of the Panton-Valentine Leukocidin toxin using PCR and other methods that have been previously described (Simor *et al*., 2010 and Oliveira *et al*., 2002). All isolates submitted in 2006 and 2007 were tested by PCR for the presence of the Panton-Valentine Leukocidin (PVL) gene. Antimicrobial susceptibility testing was also done on a representative subset of isolates sent to the NML using MIC by microdilution.

STUDY PERIOD

For these analyses, the study period included the years 1995 to 2007. This time period was selected as this 13-year surveillance period allowed for the comparison of adult and pediatric patients over an extended period of time. As well, changes in the epidemiology of pediatric MRSA are suspected to have occurred during this time period. Surveillance data for 2008 and onward were not included in this study due to significant changes in the MRSA surveillance protocol.

SAMPLE SIZE AND STUDY POPULATION

A total of 37,169 MRSA positive patients from acute-care, tertiary, university-affiliated hospitals in Canada are included in this study.

There were 35,907 adults aged 18 years and older and 1,262 children (<18 years) identified with MRSA. Only a subset of all MRSA cases was sent to the NML for strain typing and further characterization resulting in strain type determination for 13,049 adult and 675 pediatric isolates. Both the laboratory strain typing and the surveillance definition of MRSA source of acquisition (CA- or HA-MRSA) were available for 10,916 adult patients and 465 pediatric patients.

STATISTICAL ANALYSES

Software: The following software programs were used to conduct the analyses:

SAS version 9.3, Microsoft Excel 2010, and MedCalc.

Rate Calculations

For the calculation of national MRSA rates, denominator data were extracted from the CNISP annual hospital profiles, including the number of patient hospital admissions and the number of patient-days spent in hospital. Annual hospital rates were calculated by summing all MRSA cases from the participating CNSIP sites, divided by the sum of all participating CNISP hospital denominators for that year. Overall, rates of MRSA colonization and infection over the 13-year surveillance period were calculated separately for adult hospitals and pediatric hospitals. Rates for adult and pediatric hospitals were calculated using data collected from a total of 24 adult-only hospitals and 8 pediatric-only hospitals. The number of hospitals included varied by year because hospitals joined or dropped out of the surveillance program over time. Mixed hospitals were excluded because separate adult and pediatric denominators could not be determined (adult and pediatric denominator data were combined). Rates were stratified by geographic regions. Separate healthcare- and community-associated MRSA (based on surveillance definition) rates were calculated by dividing the total number of health care associated cases by the total annual denominator. CA-MRSA rates were calculated in the same

way, by dividing the total number of community-associated cases by the total annual denominator. The CA-MRSA rates represent the rates of community-associated MRSA in CNISP hospitals each year. This dataset is unable to calculate the rate of MRSA outside of the health care setting.

Descriptive Analyses

Descriptive statistics were conducted by separating the pediatric and adult populations and assessing the MRSA characteristics for each. The clinical MRSA characteristics examined were sex, source of MRSA (healthcare-associated versus community-associated), status (colonization versus infection), site of MRSA infection, and reason for culture. Descriptive analyses were also conducted for the laboratory and molecular findings including the MRSA strain typing, the expression of the PVL gene, and antimicrobial resistance patterns. The two populations were also compared for strain types causing infection and strain types causing colonization. All variables were categorical and both the epidemiological and microbiological characteristics were calculated as proportions then compared using Chi-square tests or Fisher's Exact tests, where appropriate.

Further analysis was conducted in order to assess the correlation between the surveillance definitions of CA- and HA-MRSA and the strains that have been associated with health care and community settings in the literature. For the surveillance definition, we used the classification given by the reporting hospitals as health care associated or community-

associated MRSA, which were based on the definitions in the MRSA surveillance protocols (as described above). The microbiological definitions of HA- and CA-MRSA were based on the strains that have been associated with health care settings and community settings in the literature. For CA-MRSA, these were strain types CMRSA 7 and 10. HA-MRSA was defined as strain types CMRSA 1, 2, 3/6, 4, 5, 8, and 9 and all other strains (Danish CO-MRSA, European, ST398, ST88, ST97, USA1000-China/Taiwan, and USA1100-SWP/Oceania). Chi-Square tests were used to assess the correlation between the two different definitions. This analysis was conducted only for patients where both variables were defined and therefore reduced the sample size to adult n=10,916 and pediatric n=465.

The antibiotic susceptibility profiles of adult and pediatric MRSA isolates were compared by surveillance period as well as by source of MRSA (HA or CA). Antibiotic resistance testing was done at the NML and isolates were classified as “R” for resistant, “I” for intermediate, or “S” for susceptible. Based on expert opinion, isolates were classified into two groups: susceptible and non-susceptible; where an “S” result was considered susceptible and an “R” or “I” result was considered non-susceptible to the antibiotic tested. The adult and pediatric isolates were also compared on multi-resistance, defined as non-susceptibility to 3 or more non-β-lactam antibiotics.

CA versus HA-MRSA Univariate Analyses

Differences in the patient characteristics of healthcare- and community-associated MRSA were assessed for adult and pediatric patient populations separately using univariate

logistic regression analyses. No multivariate model was built as the surveillance program only collected a limited number of patient characteristics. It was decided that a multivariate model that excluded many known risk factors for MRSA (such as underlying medical conditions, history of antibiotic use, and invasive medical procedures) would be biased. Categorical variables were expressed as proportions and compared using the χ^2 test or fisher's exact tests as appropriate. The clinical characteristics that were assessed included age categories, sex, ethnicity, status (colonized vs. infected), reason for culture, site of infection, and PVL gene expression.

Hospital-Level Models

CNISP Hospital Dataset

Data used for the hospital level models were submitted annually by participating CNISP hospitals (hospital profiles) from 1995 to 2007. Hospital characteristics tested as predictor variables included: time (year), number of hospital beds, number of full-time equivalent (FTE) infection control professionals, geographic region (eastern, central, and western Canada), and the percentage of hospital occupancy. Other variables were derived from the hospital dataset and the MRSA surveillance dataset, including the ratio of the number of FTE to the number of beds and the percentage of high-risk age group patients. Based on the literature, the age group at high risk for MRSA in adult hospitals were patients aged 65 years and older (Denkinger *et al*., 2013; Friedmann *et al*., 2009; Gaszynska *et al*., 2011; Karas *et al*., 2009; Lucero *et al*., 2009;

Marchaim *et al*., 2010; Sader *et al*., 2012) and for children, patients aged 0 to 1 year old (Posfay-Barbe *et al*., 2008; Denniston *et al*., 2006; Babazono *et al*., 2008; Bizzarro and Gallagher, 2007; Burke *et al*., 2009; Carey *et al.*, 2008; Carey *et al.*, 2010; de Almeida Silva *et al*., 2003; Magara *et al*., 2011). Each MRSA patient was categorized as a 'high-risk age group' or 'not high risk' and then an annual proportion of high-risk patients was derived for each hospital. A variable on the number of patients who were aboriginal was available, but was not used because of the important proportion of missing data (data missing for at least 50% of MRSA cases) and consequently was determined to be potentially inaccurate.

All of the data from mixed hospitals (combined adult and pediatric facilities) were excluded as adult and pediatric denominators were not reported separately. There were 24 adult-only hospitals and 8 pediatric-only hospitals. Some adult hospitals sites (in the same geographic region) reported a single denominator data for multiple sites, therefore the number of cases were collapsed under a single rate. Two adult sites were excluded because there was only one data point (a single MRSA rate) and four were excluded because they had only 2 years of rate data, and in addition all 6 of these hospital sites had missing values for several of the predictor variables. For these reasons, the final models included only 18 adult and 8 pediatric hospitals. In addition to the hospitals with insufficient MRSA rate data, the variables collected in the hospital profile questionnaire varied over the surveillance period. For the first 3 years and last 2 years of surveillance several variables (percent occupancy, number of FTE ICP) were not collected. For the first three years, the values for the missing variables in 1998 were used to populate the years 1995, 1996 and 1997 and the values from 2005 were used to populate the

data for the years 2006 and 2007. For any other missing predictor values the last available value was used. When no value was available for that hospital in any other surveillance year, the value was left as missing (these were excluded by the statistical software). All of the predictor variables included in the models were time-varying; therefore the approach of using the values from previous or later years may have introduced some error. However the majority of the hospital characteristics vary only slightly between years (for example bed size typically does not change drastically from one year to the next), therefore we felt this approach was the most appropriate.

Hospital-Level Statistical Models

Hospital MRSA rates and hospital characteristics were collected annually over a 13-year surveillance period therefore the data are repeated measures of the same individuals (hospitals), over time. Therefore, the assumption of independence of the responses is violated. Statistical models that ignore the correlation between the measures taken on the same individuals (known as the "intracluster correlation" or "ICC") would lead to the underestimation of the standard errors (increased chance of a type I error) for time invariant predictors or to the overestimation of the standard error (increased chance of a type II error) for time-varying predictors (Ghisletta and Spini, 2004; Twisk, 2003). For these reasons, we selected a model that accounted for the intracluster correlation, where each hospital is treated as a cluster. The outcome we modeled was the MRSA rate, which is a count (discrete) variable, therefore this

variable is assumed to follow a Poisson distribution (Twisk, 2003). There are two longitudinal Poisson regression model (or log-linear regression model) options for this type of data: a Generalized Linear Mixed Effects Model (GLMEM) or a Marginal Model using Generalized Estimating Equations (GEE) (Fitzmaurice et al., 2004; Twisk, 2003). Both of these models are variations on the generalized linear model (GLM) but differ in that they account for correlated data (Ghisletta and Spini, 2004).

Generalized Linear Mixed Effects Models are referred to as "subject-specific models" because they add individual-specific random effects to the measurements in the model in order to account for the covariance among repeated measures (Fritzmaurice *et al*., 2004). The random effects are assumed to follow a normal distribution (Twisk, 2003). The parameters in these models estimate the change in the log odds of response per unit increase in the predictor variable, for any individual having unobservable or unmeasurable characteristics that determine the individual's response (individual variation) (Fritzmaurice *et al*., 2004). Thus the interpretation of the parameters is conditional on the underlying subject effect. The objective of this type of model is to make inferences about individuals rather than population averages. This type of model is ideal for time-varying covariates, that is to say covariates that vary within an individual over time (for example the percent occupancy of a hospital varies from year to year). For GLMEM, estimation is done by pseudo-likelihood estimation methods (Fritzmaurice *et al*., 2004).

Marginal Models are population-averaged models where the mean response is modeled based only on the covariates of interest, without random effects (Ghisletta and Spini, 2004;

Fritzmaurice *et al*., 2004). The mean response is modeled separately from the within-subject association among the repeated measures, adjusting for this association afterwards (Fritzmaurice *et al*., 2004). The method of estimation for this type of model is Generalized Estimating Equations (GEE) (as opposed to Maximum Likelihood Estimation, MLE) (Ghisletta and Spini, 2004). The parameters (β) for this type of model are computed using a working correlation matrix, which is based on an assumed structure for the variances and covariances (Fritzmaurice *et al*., 2004). There are two possible types of standard errors for the parameter estimates: robust standard errors (SE) and model-based standard errors. Robust SE are valid even when the working correlation matrix is misspecified and are best suited to longitudinal designs where the number of subjects is relatively large and the number of repeated measures is relatively small (rule of thumb: <4 predictors requires ~ 25 clusters, 5-12 predictors requires ≥ 100 clusters) (Fritzmaurice *et al*., 2004). Model-based standard errors are based on direct modeling of the association among the responses and are valid only provided that the correlation matrix is correctly specified. Model-based SE are best for data where there are fewer than 20 clusters (Fritzmaurice *et al*., 2004). The parameters in this model estimate the population-averaged log odds ratio of the response. Therefore, the target of inference for the parameters is the population of hospitals in Canada, rather than individual hospitals.

One of the advantages of Marginal Models is that they do not require distributional assumptions for observations because it only requires a model for the mean response. As well, this model works for data where the number of repeated measurements is not the same across all subjects. The GEE approach can be used for binary, ordinal, count or continuous responses,

and for continuous or categorical, time-variant or time-invariant covariates. This model uses all pairs of responses in the case of missing data, rather than case-wise deletion. Overall, the main advantage is that GEE Models provide unbiased estimation of population-averaged parameters even when the working correlation matrix is misspecified (Ghisletta and Spini, 2004).

Based on the different characteristics of MRSA in adult and pediatric hospitals, as well as the differences observed in the MRSA rates trends, it was determined that two separate models should be built for adult hospitals and pediatric hospitals. Diagnostic tests were run on the data in order to examine the data and to assess the model assumptions. This included histograms and frequency tables to assess whether the normality assumption was met, to assess the time trend for the response variable, and to assess the distribution of clusters and repeated measures. After evaluating the diagnostic tests it was determined that the normality assumption was questionably met. However, these types of models are able to handle non-normally distributed responses (Fitzmaurice *et al*., 2004; SAS Institute, 2013). Both types of models (Marginal Models using GEE and GLMEM) were run for the two populations in order to compare the two models. The Marginal Models (GEE) were run using the Proc Genmod procedure and were compared with Generalized Linear Mixed Models using the Proc Glimmix procedure in SAS 9.3 (SAS Institute, 2013).

Data for 24 adult hospitals and 8 pediatric hospitals were available. Since the number of clusters was relatively small (less than 20 for pediatric and a maximum of 24 for adult hospitals), model-based standard errors were determined to be the most appropriate for estimating the parameters in the Marginal (GEE) Models. The time variable (year from 1995 to 2007) was

modeled as a continuous variable. The response variable for both models was the number of MRSA cases (including both infections and colonizations) offset by the log of the hospital denominators in person-years. We ran bivariate Marginal (GEE) and GLMEM models for each of the covariates (models that included only year and one predictor variable), separately for adult and pediatric hospitals. Interaction terms with year were also tested. For pediatric hospitals, an exponential term for the time variable (year squared) was tested in both GEE and GLMEM models based on the exponential trend of rate over time that was observed in the diagnostic tests. After running the bivariate models (GEE and GLMEM for adult and pediatric hospitals), a p-value of less than or equal to 0.1 was considered for inclusion of predictor variables in the multivariate model. A cut off of $p<0.05$ was used for retention of variables in the final multivariate models.

CHAPTER 6: RESULTS

MRSA Rates

Overall, a total of 24 adult and 8 pediatric hospitals were included in the rate calculations, while 22 mixed hospitals were excluded because age-specific denominators were not available. In Canadian adult hospitals included in the study, only 71 MRSA cases of infection and 42 cases of colonization were reported during the first surveillance year (1995). The numbers of MRSA cases rose dramatically over the surveillance period (1995-2007), increasing to 1,161 MRSA infections and 2,701 cases of colonization in 2007, which translated to an increase in the infection rate from 0.38 (95%CI:0.30-0.48) to 3.17 (95%CI:2.99-3.36, p<0.001) infections per 1,000 patient admissions or 0.46 (95%CI:0.36-0.58) to 4.21 (95%CI: 3.97-4.46, p<0.001) infections per 10,000 patient-days. For the same hospitals, the MRSA colonization rates increased from 0.22 (95%CI: 0.16-0.30) to 7.37 (95%CI:7.10-7.66) per 1,000 patient admissions or 0.26 (95%CI:0.19-0.36) to 9.80 (95%CI:9.43-10.18) per 10,000 patient days over the surveillance period.

In Canadian pediatric hospitals, only one case of colonization and zero infections were reported in the first year of surveillance and this increased to 88 MRSA infections and 39 colonizations in the last surveillance year. Over the 13-year period, infection rates in pediatric hospitals increased from 0 (95%CI: 0-0.001) to 1.74 (95%CI: 1.40-2.15, p<0.001) infections per

1,000 patient admissions or 0 (95%CI:0-0) to 2.69 (95%CI: 2.15-3.31, p<0.001) infections per 10,000 patient-days. Rates of colonization increased from 0.04 (95%CI: 0.001-0.22) to 0.77 (95%CI: 0.55-1.06, p<0.001) / 1,000 patient admissions or 0.08 (95%CI: 0.0019-0.42) to 1.19 (95%CI: 0.85-1.63, p<0.001) /10,000 patient-days. On the whole, there was a marked increase in MRSA rates in Canada from 1995 to 2007: from 0.62 (95%CI: 0.50-0.72) to 10.49 (95%CI: 10.21-10.88, p<0.001) cases per 1000 patient admissions or 0.73 (95%CI: 0.60-0.87) to 14.01 (95%CI:13.57-14.46, p<0.001) per 10,000 patient-days in adult hospitals and from 0.04 (95%CI: 0.001-0.22) to 2.51 (95%CI: 2.10-2.99, p<0.001) /1000 admissions or 0.08 (95%CI: 0.0019-0.46) to 3.88 (95%CI: 3.23-4.61, p<0.001) /10,000 patient-days in pediatric hospitals (Fig. 2).

Generally, MRSA incidence rates in Canadian adult hospitals were significantly higher than rates in pediatric hospitals (average incidence rate ratio (AIRR) for adult vs. pediatric. MRSA (colonization and infection) per 1,000 patient admission 1995-2007: 13.9 (95%CI: 7.78 – 20.1); AIRR for adult vs. pediatric MRSA Infections: 11.4 (95%CI: 2.35-20.42). In adult hospitals, MRSA colonization rates were higher than MRSA infection rates (average rate ratio (ARR) for colonization versus infection per 1000 patient admissions from 1995-2007: 1.94 (95%CI: 1.27-2.62)), contrary to the situation in pediatric hospitals where MRSA infection rates were similar to or surpassed the corresponding colonization rates (ARR colonization vs. infection per 1000 patient admissions from 1995-2007): 1.24 (95%CI: 0.65-1.83) (Figure 2). Pediatric MRSA rates, specifically infection rates, started to increase more dramatically around the early 2000s (Figure 2B) when the rate went from 0.16 (95%CI: 0.056-0.35) infections per 1,000 patient admissions

or 0.29 (95%CI: 0.11-0.64) per 10,000 patient-days in 2002 to 1.74 (95%CI: 1.40-2.15) per 1,000 patient admissions or 2.69 (95%CI: 2.15-2.31) per 10,000 patient-days in 2007admissions.

Figure 2A: Overall MRSA incidence rates in Canadian hospitals for adult patients, CNISP 1995-2007

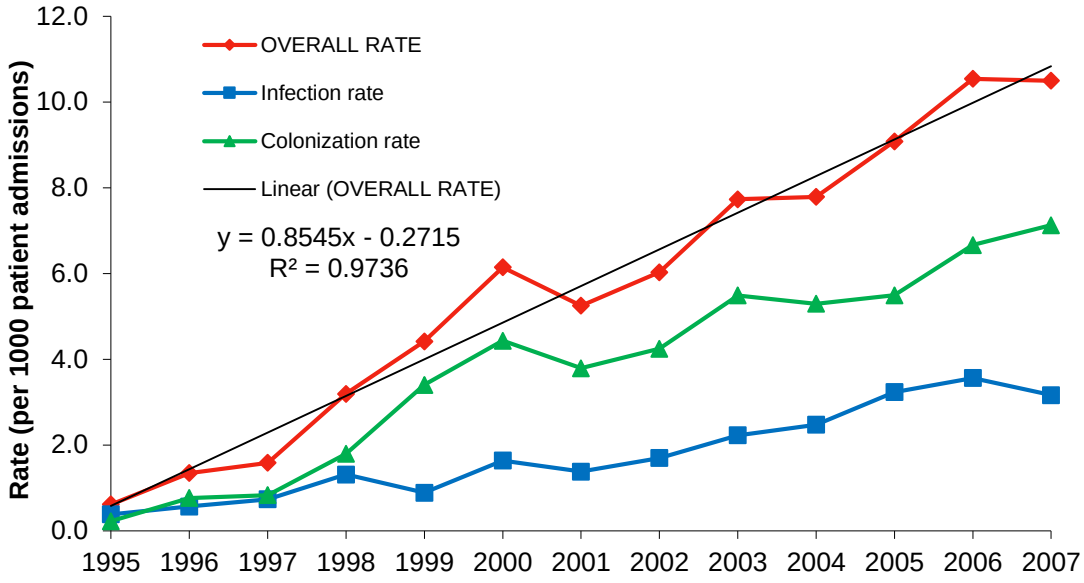
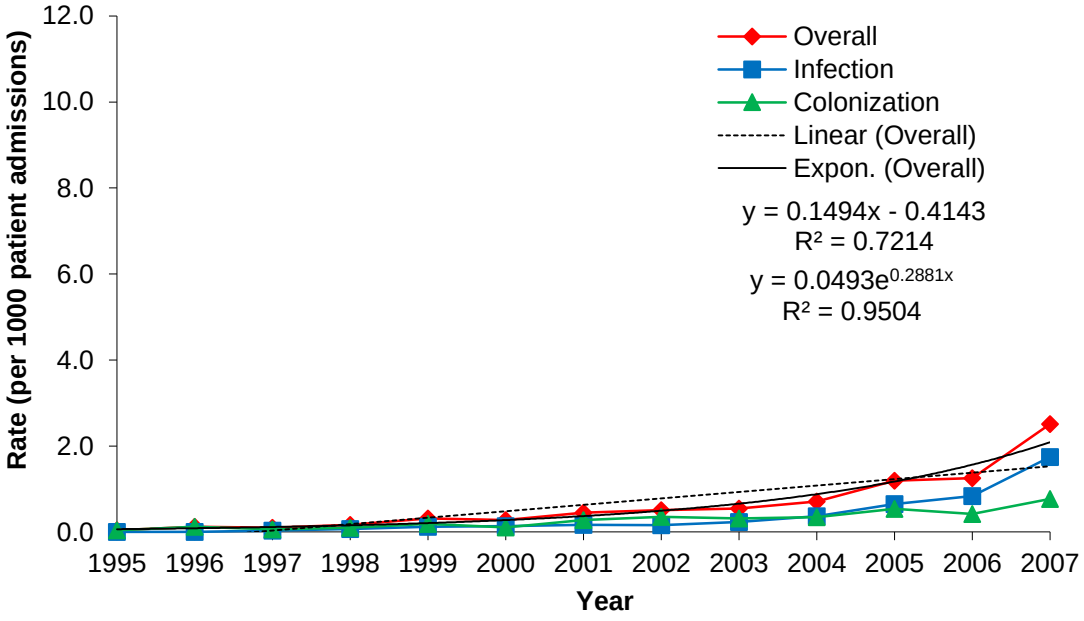


Figure 2B: Overall MRSA incidence rates in Canadian hospitals for pediatric patients, CNISP 1995-2007



Rates by Geographic Region

Regional pediatric rates were not calculated due to the small number of pediatric hospitals that were able to submit both numerator and denominator data. Overall MRSA rates for central Canadian adult hospitals were significantly higher than other regions, from 0.83 (95%CI: 0.65-1.05) per 1,000 patient admissions in 1995 to 12.88 (95%CI: 12.36-13.42) per 1,000 patient admissions in 2007 compared to 0.64 (95%CI: 0.45-0.89; C vs. W p=0.22) to 7.99 (95%CI: 7.52-8.47; C vs. W p<0.001) per 1,000 patient admission in Western hospitals and 0.15 (95%CI: 0.060-0.31; C vs. E p<0.001) to 9.26 (95%CI: 8.41-10.16; C vs. E p<0.001) per 1,000 patient admissions in Eastern hospitals (Figure 3); However, it is worth noting that the margin of difference in regional rates significantly decreased over time (Figure 3).

Further categorization revealed that MRSA infection rates were more similar between regions than the overall MRSA rates (infection and colonization combined; Figures 3 and 4). Western adult hospital infection rates overtook the rates of other regions in 2003 and increased continuously until the end of the surveillance period in 2007 (from 2.41 (95%CI: 2.11-2.75) to 4.20 (95%CI: 3.86-4.55) infections per 1,000 patient admissions, p<0.001, Fig. 4). At the same time in Central Canada, the infection rates in adult hospitals stabilized from 2003 onward (around 2.24 (95%CI: 2.02-2.47) infections per 1,000 patient admissions) to become the lowest in the country (Figure 4).

Figure 3. Overall regional adult MRSA incidence rates, CNISP 1995-2007

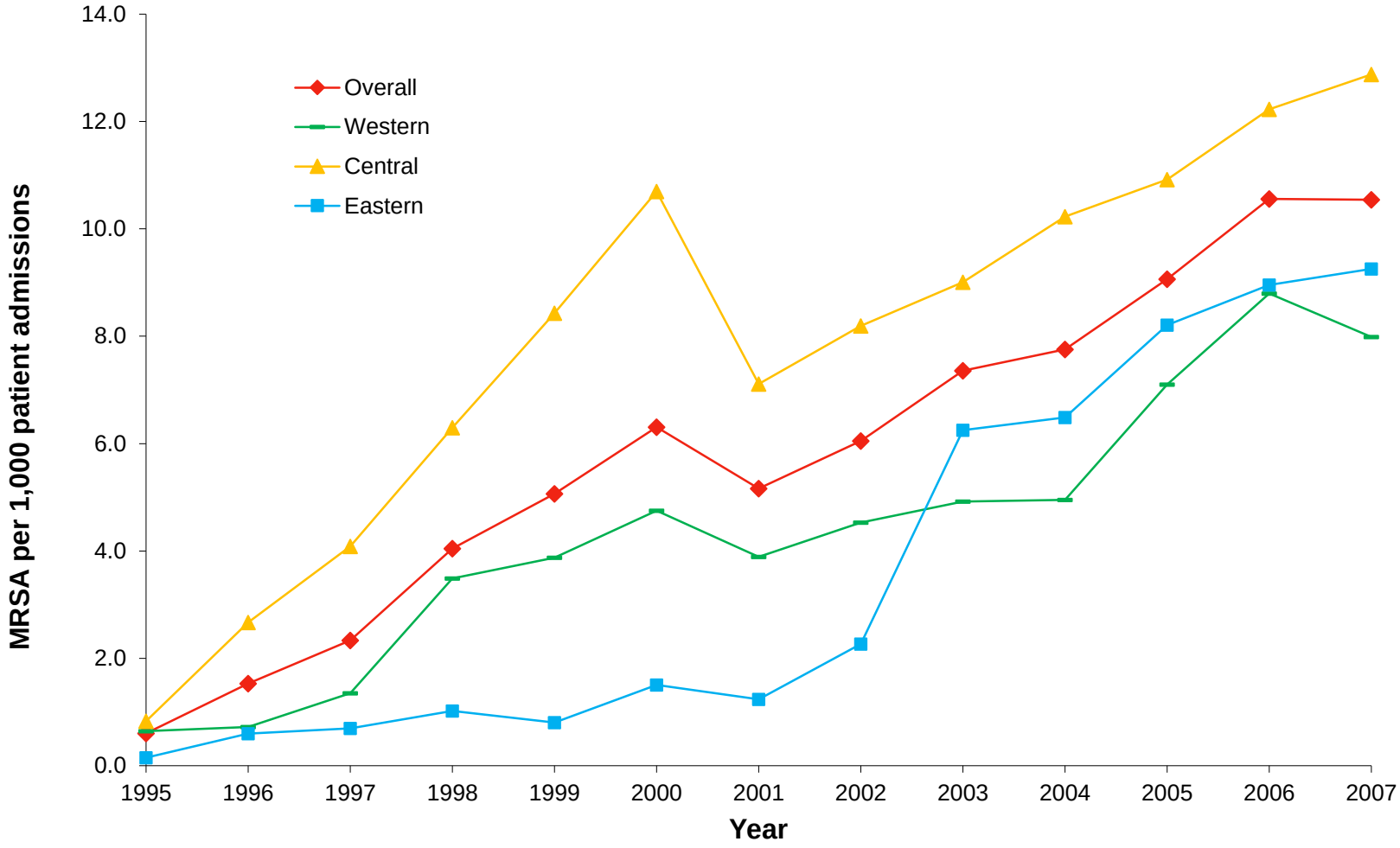


Figure 4. Regional adult MRSA infection rates, CNISP 1995-2007

HA vs. CA-MRSA Rates

Overall, HA-MRSA rates were much higher in adults than children, whereas CA-MRSA rates were more similar between the two populations until the end of the surveillance period (Figures. 5A & 5B). HA-MRSA rates in adult hospitals increased substantially over the study period (from 0.30 (95%CI: 0.23-0.39) to 4.25 (95%CI: 4.04-4.46) infections per 1,000 patient admissions ($p<0.001$), whereas pediatric HA-MRSA rates increased less dramatically, from 0 (95%CI: 0-0.0001) to 0.24 (95%CI: 0.12-0.41) infections per 1,000 patient admissions ($p=0.014$, Fig. 5A). The increase in HA-MRSA infection rates was linear in both populations (R^2= 0.88 for adults and 0.62 for children, Figure 5A). In terms of CA-MRSA, rates were generally higher in adult hospitals compared to pediatric hospitals for most of the surveillance period although the difference was much smaller than that for HA-MRSA. However, pediatric CA-MRSA infection rates approached adult rates in 2007 (1.21 (95%CI: 0.92-1.55) infections per 1,000 patient admissions in children versus 1.40 (95%CI: 1.27-1.52; $p=0.29$) infections per 1,000 patient admissions in adults; Figure 5B). Overall, both adult and pediatric CA-MRSA infection rates followed exponential trends over time (R^2=0.95 for adults and 0.67 for children; Figure 5B). Contrary to the trends in adult hospitals, colonization rates were very similar to infection rates in pediatric hospitals (for both CA-MRSA and HA-MRSA) and were surpassed by infection rates in the last surveillance period (2004-2007) (Fig 5).

Figure 5A. Healthcare-associated MRSA incidence rates, CNISP 1995-2007

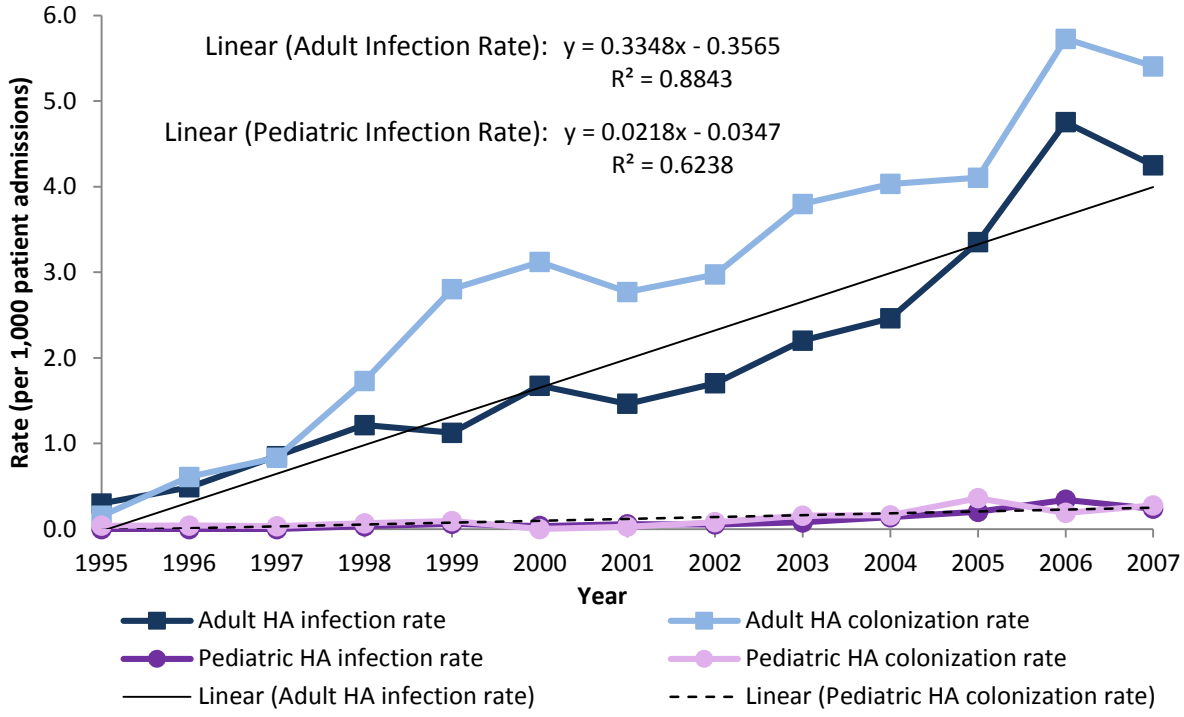
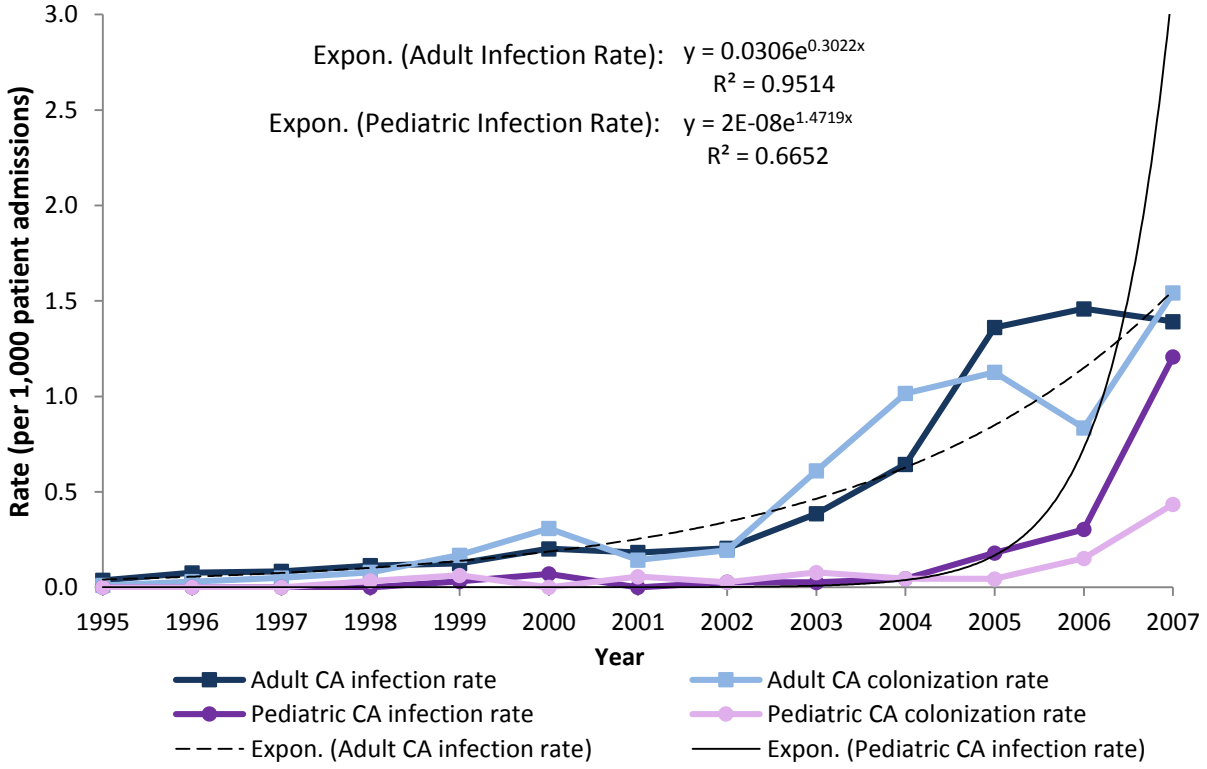


Figure 5B. Community-associated MRSA incidence rates, CNISP 1995-2007



Comparison of Adult and Pediatric Case Characteristics

Among the MRSA cases reported to CNISP, the proportion of cases that were colonizations (versus infections) was significantly higher in adults compared to pediatrics (68% of 35,907 vs. 51% of 1,262; $p<0.001$; Table 2). Reason for culture was also significantly different, a higher proportion of adult MRSA was detected through admission screening (34% in adults vs. 22% in children, $p<0.001$), while the proportion of MRSA identified in clinical isolates was greater in children (61% vs. 38%, $p<0.001$). The proportion of HA-MRSA cases identified was significantly greater in adults (72%) compared to children (42%, $p<0.001$), while CA-MRSA cases were significantly more prevalent in children (34% vs. 14%, $p<0.001$; Table 2). The infection sites differed significantly between adults and children. Skin and soft tissues were the most common site of infection for adult and children although the proportion was significantly higher in children (53% vs. 11%, $p<0.001$) (Table 2). Surgical wounds (19 vs. 10%), bloodstream infections (13 vs. 10%), respiratory infections (21 vs. 13%) and urinary tract infections (11 vs. 4%) were all significantly more common in adults ($p<0.001$). (Table 2).

Table 2. Descriptive characteristics of adult and pediatric MRSA inpatients in CNISP hospitals from 1995-2007.

	Adult No. (%)[1]	Pediatric No. (%)[1]	χ^2 *p*-value
Sex			
Male	20465 (57)	689 (55)	*ns*[2]
Female	15330 (43)	570 (45)	*ns*
Status			
Infected	11220 (32)	608 (49)	<0.001
Colonized	24361 (68)	642 (51)	<0.001
Reason for Culture			
Admission Screen	12105 (34)	274 (22)	<0.001
Clinical Isolate	13471 (38)	763 (61)	<0.001
Outbreak Investigation	3398 (10)	68 (5)	<0.001
Routine Screening	1606 (5)	28 (2)	<0.001
Source[3]			
Healthcare-associated	25845 (84)	532 (55)	<0.001
Community-associated	4878 (16)	434 (45)	<0.001
Site of Infection[4]			
Surgical Wound	2308 (19)	67 (10)	<0.001
Skin/Soft Tissues	3877 (32)	360 (56)	<0.001
Blood	1574 (13)	62 (10)	0.016
Respiratory	2596 (21)	81 (13)	<0.001
Urine	1329 (11)	24 (4)	<0.001
Other sites [5]	555 (5)	50 (8)	*N/A*[2]

1 Totals may not add up to 35,907 (Adult) or 1,292 (Pediatric) due to missing data

2 *ns*: Not significant, *N/A*: not applicable

3 Patients were the source was unknown were excluded

4 Site of infection: only includes infected MRSA cases where the site of infection was known. Adult n=12,239, Pediatric n=644

5 The category "other" was selected on the questionnaire as the site of infection. Therefore the types of infections included in the "other" category are unknown.

Epidemic Strain Types

PFGE results revealed that overall CMRSA 2 accounted for a significantly higher proportion of MRSA cases (infections and colonizations combined) both in adult (48%) and pediatric (34%) patients (Table 3). After CMRSA2, CMRSA1 (20%) and CMRSA3/6 (13%) accounted for most of the strain types detected in the adult population patients while CMRSA10 (23%) and CMRSA7 (18%) were the other predominant strains isolated from pediatric patients. In adult isolates, the predominant *SCCmec* type was type II (53% comparison with pediatric, $p<0.001$), followed by type IVa (26%) whereas in pediatric isolates, it was type IVa (70%, comparison with adult $p <0.001$) followed by type II (11%). Of the isolates tested for PVL, 59% of pediatric isolated tested positive for PVL compared to only 24% ($p<0.001$) of the adult isolates (Table 3).

Table 3. MRSA epidemic strains, SCC*mec*, and PVL gene expression in adult and pediatric isolates, CNISP 1995-2007

	Adult	Pediatric	Total	p-value
	No. (%)	No. (%)	No. (%)	
PFGE				
CMRSA 1	2613 (20)	50 (7)	2663 (19)	<0.001
CMRSA 2	6213 (48)	230 (34)	6443 (47)	<0.001
CMRSA 3/6	1684 (13)	32 (5)	1716 (13)	<0.001
CMRSA 7	218 (2)	124 (18)	342 (2)	<0.001
CMRSA 10	1027 (8)	153 (23)	1180 (9)	<0.001
Other[2]	1297 (10)	86 (13)	1383 (10)	0.018
Total Strain Typed[1]	13052 (36)	675 (54)	13727 (37)	
SCC*mec*				
SCC*mec* II	1606 (53)	29 (11)	1635 (50)	<0.001
SCC*mec* III	259 (9)	7 (3)	266 (8)	0.001
SCC*mec* IVa	775 (26)	177 (70)	952 (29)	<0.001
Other[3]	375 (12)	41 (16)	416 (13)	0.013
Total SCCmec Typed[1]	3015 (12)	254 (20)	3269 (9)	
PVL				
Positive	1176 (24)	277 (59)	1453 (27)	<0.001
Negative	3800 (76)	196 (41)	3996 (73)	<0.001
Total PVL Tested[1]	4976 (14)	473 (37)	5449 (15)	

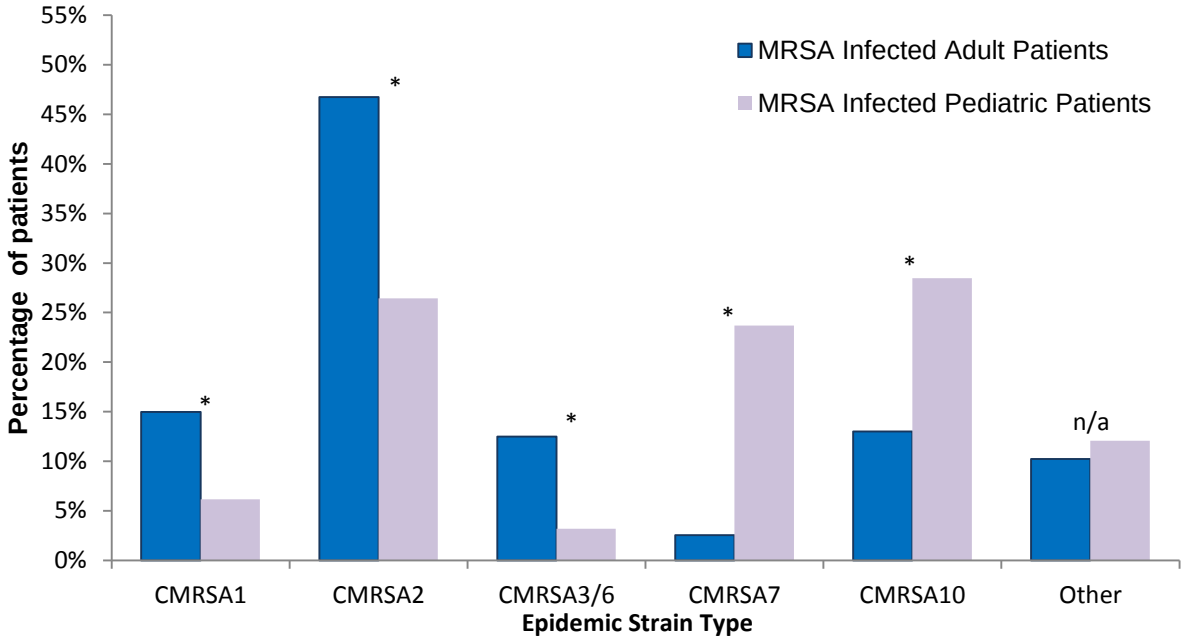
1 Total numbers & percentages of MRSA isolates that were tested for epidemic strain type, SCC*mec* type, and PVL, respectively.

2 Other PFGE strains included CMRSA4, CMRSA5, CMRSA8, CMRSA9, Danish CO-MRSA, European, ST398, ST88, ST97, USA1000-China/Taiwan, USA1100-SWP/Oceania, and USA700.

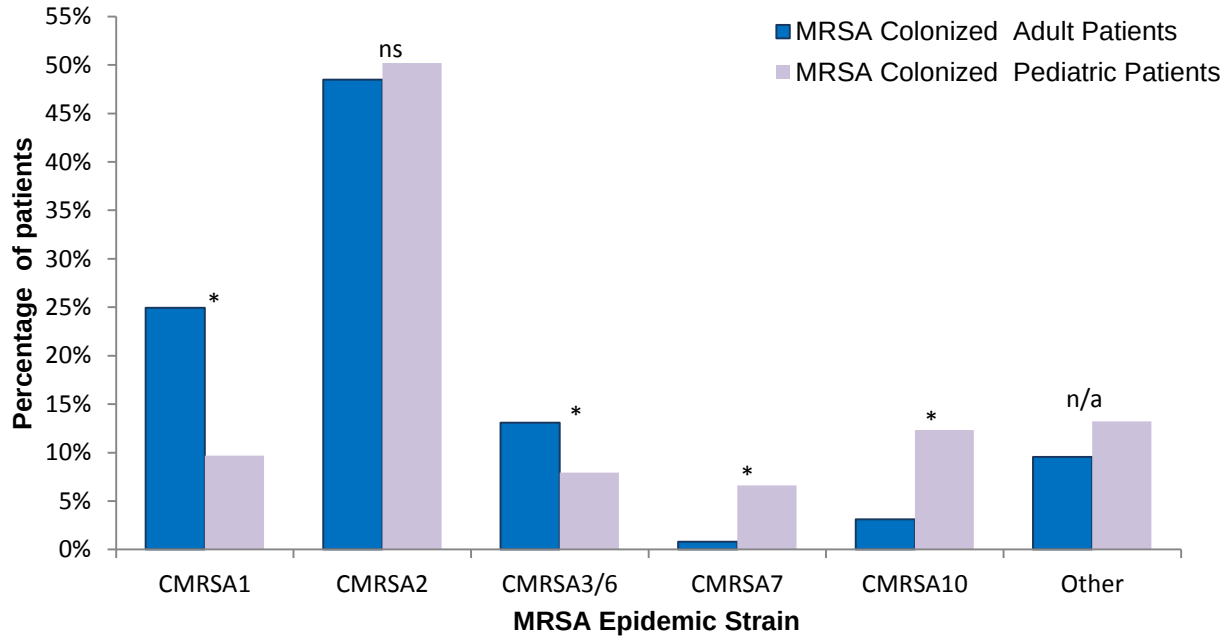
3 Other SCC*mec* types included I, I var, III var, IVa & IVd, IVb & IVd, IVb, IVc, IVd, IVg, V,VIII, ccr1 & 5; and mec tbd

Among adults, CMRSA1 (15%) and CMRSA2 (47%) were the major cause of infections while CMRSA2 (26%), CMRSA7 (24%), and CMRSA10 (29%) caused the majority of infections in children (Figure 6A). Strain types causing colonization were similar to those causing infection in adults (CMRSA1, 25% and CMRSA2, 49%), whereas in children, CMRSA2 was the predominant strain (50% of colonizations) and CMRSA7 and CMRSA10 only 7% and 12%, respectively (Figure 6b). The epidemic strain types were plotted for patients classified as HA-MRSA and CA-MRSA based on surveillance definition (Figure 7). The predominant strain in HA-MRSA cases was CMRSA2 for both adults (50%) and children (46%). However, CMRSA1 was the second most prevalent strain in adults with HA-MRSA (20%), whereas CMRSA7 (10%) and CMRSA10 (11%) were the next most prevalent in children (Figure 7A). In community-associated cases, CMRSA2 was still the predominant strain in adults (41%), followed by CMRSA10 (35%), while in children the predominant strains were CMRSA10 (44%), CMRSA7 (23%), and CMRSA2 (19%) (Figure 7B).

Figures 6A. Laboratory characterization of MRSA strain types in MRSA-infected patients, CNISP 1995-2007



Figures 6B. Laboratory characterization of MRSA strain types in MRSA-colonized patients, CNISP 1995-2007



* *p*-value for the Chi-Square test comparing adult and pediatric patients was statistically significant at an $\alpha = 0.05$ level; ns = non-significant; n/a = not applicable

Figure 7A. MRSA strains isolated from patients thought to have acquired MRSA in a healthcare setting, CNISP, 1995-2007)

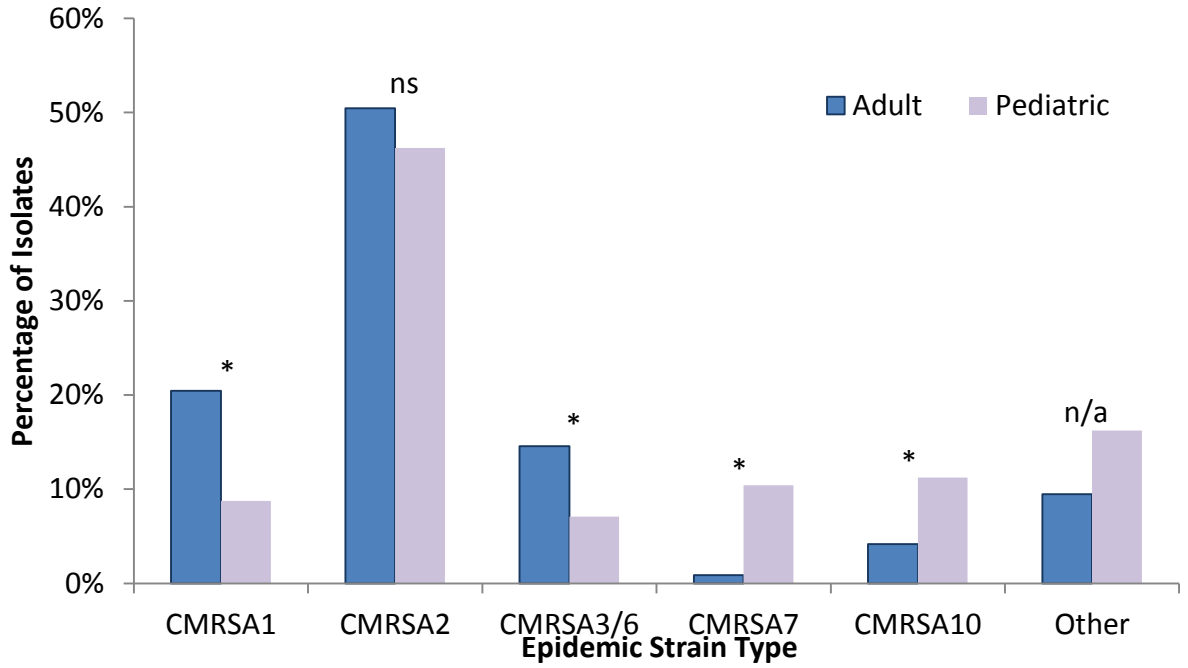
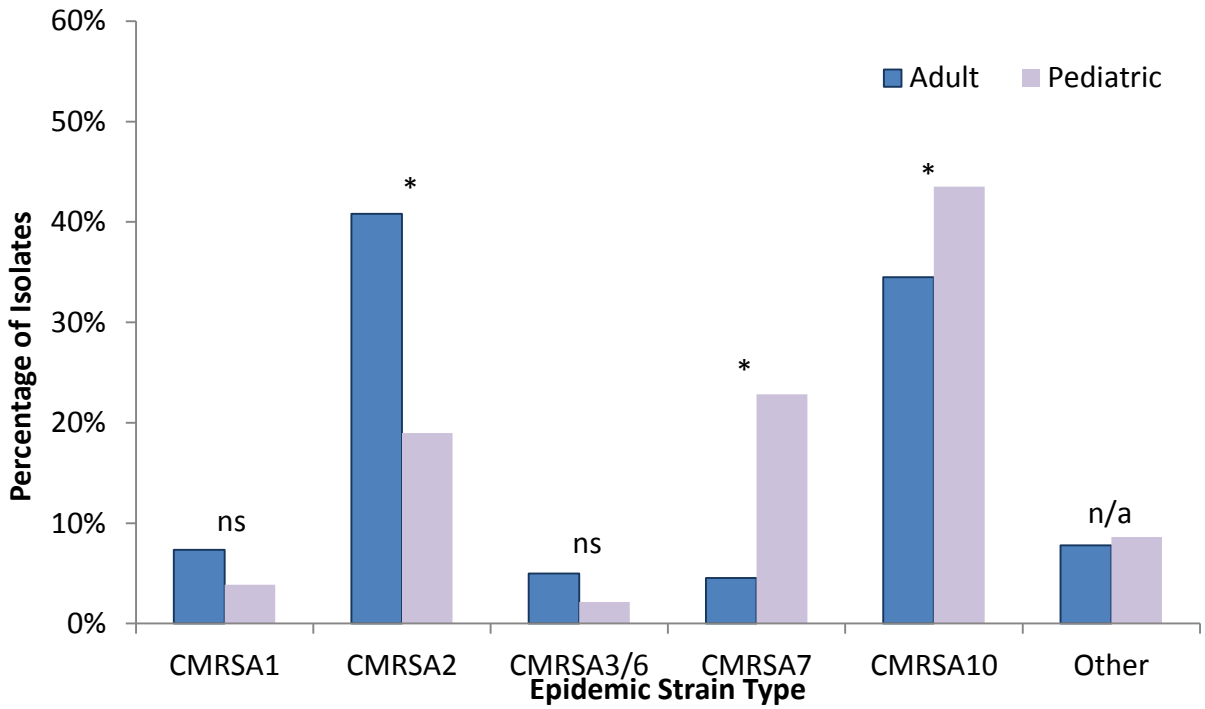


Figure 7B. MRSA strains isolated from patients classified as CA-MRSA by surveillance definition



* p-value for the Chi-Square test comparing adult and pediatric patients was statistically significant at an $\alpha = 0.05$ level; ns= non-significant; n/a= not applicable

Correlation between Surveillance and Microbiological Definitions

The surveillance and microbiological definitions of healthcare- and community-associated MRSA cases correlated well. Both definitions showed that adults and children differed significantly in the proportion of MRSA that were healthcare-associated vs. community-associated (for both infection and colonization, all $p<0.001$) (Figure. 8). A much higher proportion of infections and colonizations in adult patients were healthcare-associated based on both strain typing and surveillance definition. Conversely, pediatric colonizations were mostly caused by healthcare-associated MRSA (81.1% based on strain type and 61.5% based on surveillance definition), while pediatric infections were mostly community-associated (54.9% CA-MRSA based on strains and surveillance definition) (Figures 8A and 8B). Predictive values for the results of the surveillance definition of HA vs. CA MRSA were calculated using the strain definition as the reference. Predictive values were mostly higher for HA-MRSA (69-98%) but lower for CA-MRSA (20-75%; Figure 8C). The overall agreement between the two definitions was calculated and was 84% for infections and 89% for colonizations in adults, compared to 72% for pediatric infections, and 75% for pediatric colonizations (Figure 8C). The Kappa Coefficient for the agreement between the two definitions of HA and CA MRSA was 0.39 (95%CI: 0.37-0.42) for adult MRSA and 0.45 (95%CI: 0.37-0.52) for pediatric MRSA. The Chi-square test for equality of the adult and pediatric Kappa Coefficients was 1.77, $p=0.18$ (Figure 8D).

Figure 8. Correlation of laboratory (strain type) and surveillance definitions of MRSA.

Figures 8A. Healthcare- *versus* Community-Associated MRSA by surveillance definition, CNISP 1995-2007[2]**.**

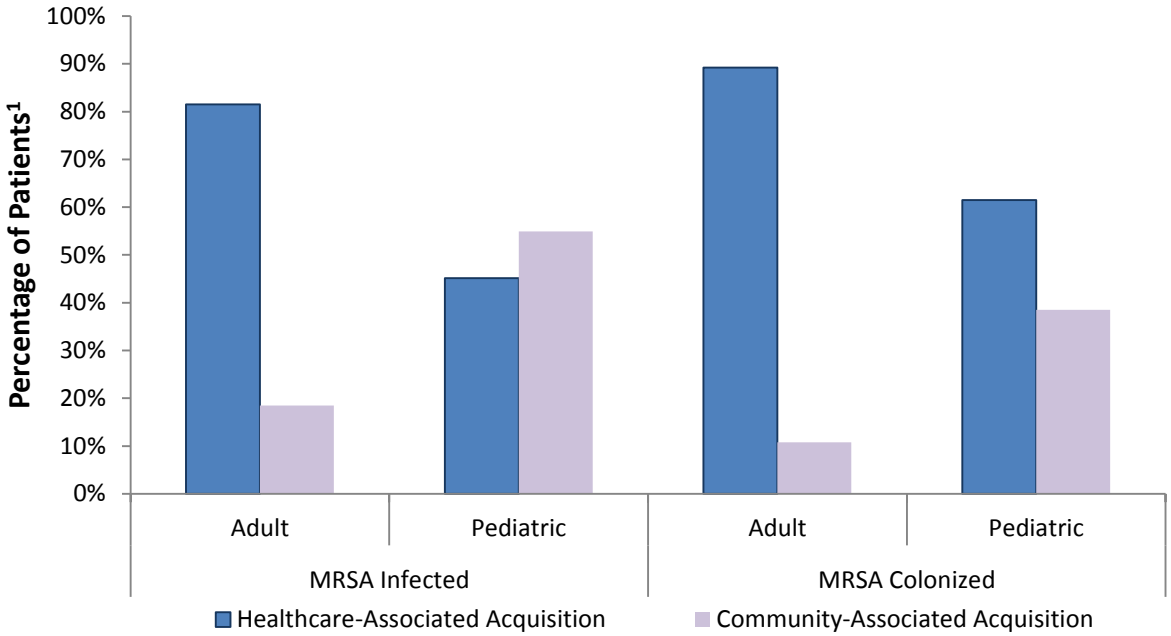


Figure 8B. Healthcare- *versus* Community-Associated MRSA based on the strain definition, CNISP 1995-2007[2]

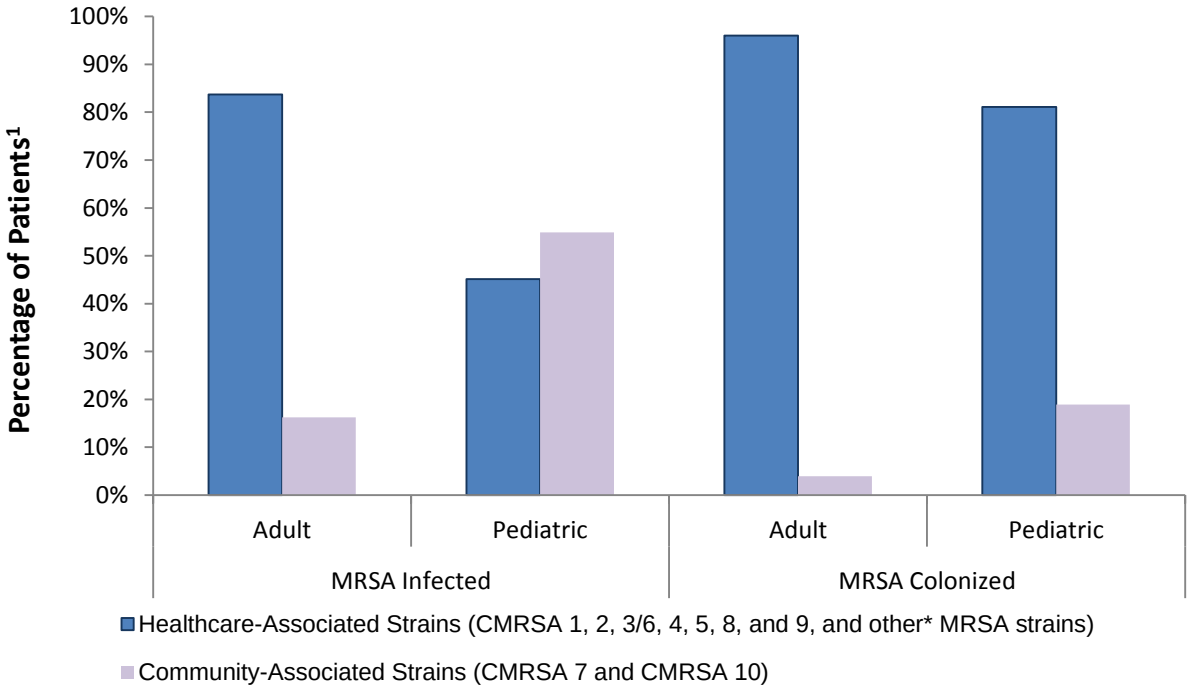


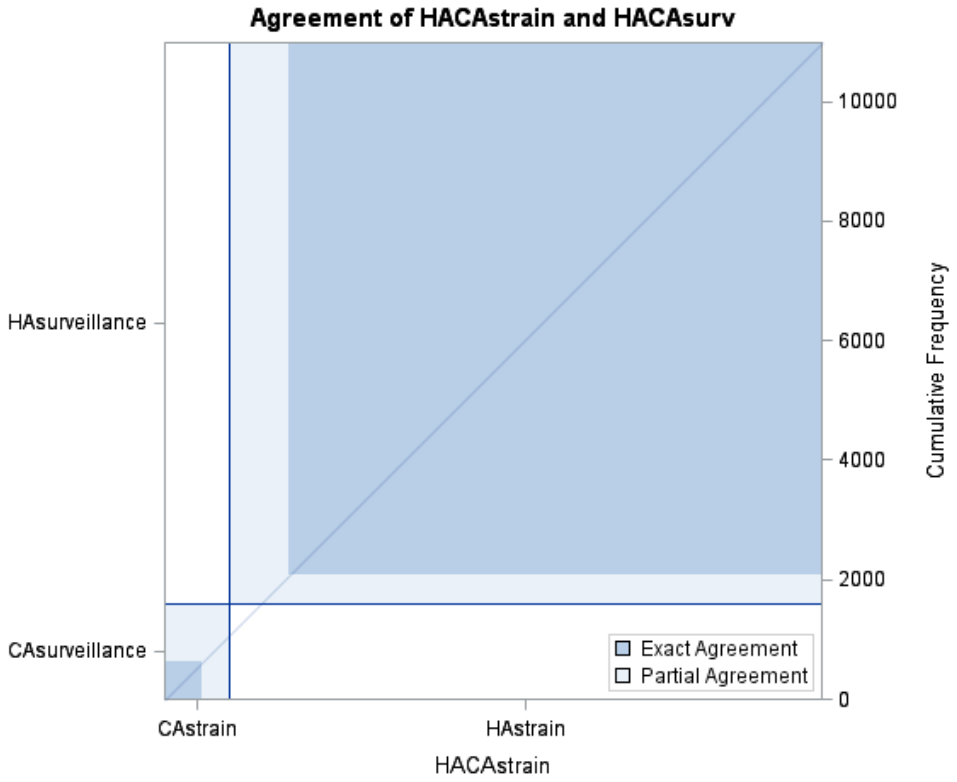
Figure 8C. Predictive values and agreement for the surveillance definition of CA and HA MRSA, using strain typing as the reference, CNISP 1995-2007

	Infection			Colonization		
	HA PV[3]	CA PV[3]	Agreement[4]	HA PV	CA PV	Agreement
Adult	92%	51%	84%	98%	20%	89%
Pediatric	69%	75%	72%	96%	42%	75%

Figure 8D. Kappa coefficients for the agreement between the surveillance and strain definitions of CA and HA MRSA.

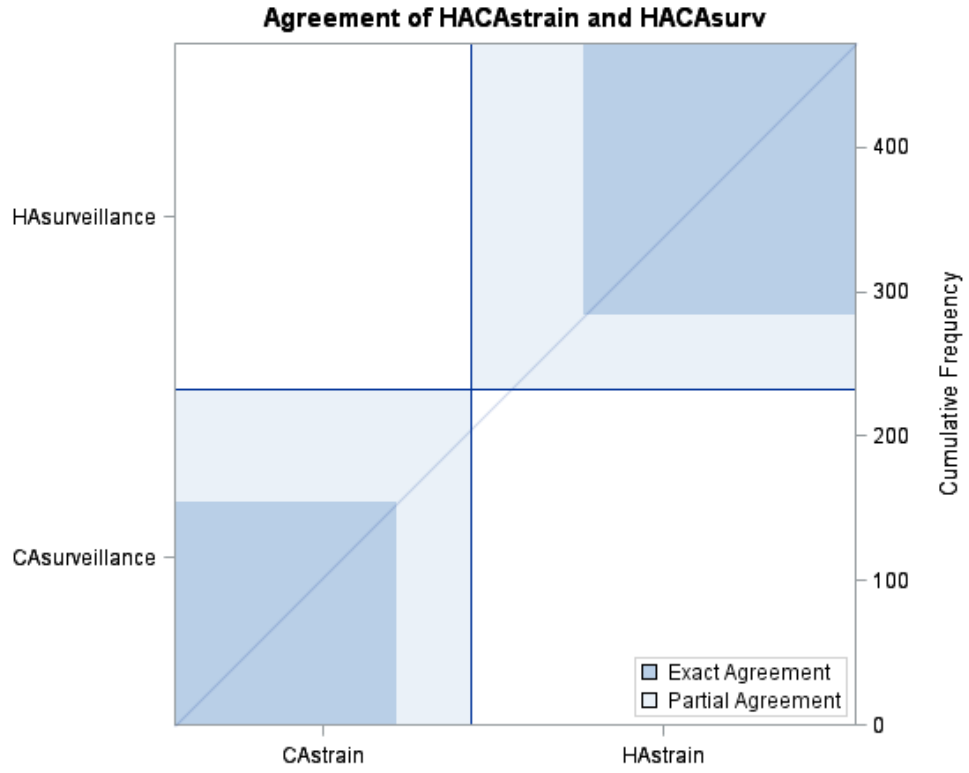
Correlation for Adult Isolates:

	Surveillance Definition			
Strain Definition	**HA**	**CA**	**Total**	**Kappa (95% CI)**
HA	626	474	1100	0.39 (0.37-0.42)
CA	977	8907	9884	P-value
Total	1603	9381	10984	<0.001



Correlation for Pediatric Isolates:

	Surveillance Definition			
Strain Definition	**HA**	**CA**	**Total**	**Kappa (95% CI)**
HA	154	52	206	0.45 (0.37-0.53)
CA	78	188	266	P-value
Total	232	240	472	0.023



1 Patients for whom strain typing was not done and the surveillance definition of source of acquisition (healthcare- or community-associated) was unknown were excluded (total included: Adult n=10,984; Pediatric n=472).

2 All of the comparisons of the proportions of HA vs. CA MRSA between adult and pediatric patients were statistically significant (p<0.001 for all 4 comparisons).

3 HA PV= HA-MRSA Predictive Value (Positive Predictive Value), CA PV= CA-MRSA Predictive Value (Negative Predictive Value). Calculated by doing positive and negative predictive value calculations, using the strain typing HA vs. CA as the reference definition.

4 Total proportion of agreement between the surveillance definition and the strain type definition (proportion of isolates that had the same classification as HA or CA based on both definitions)

*Other MRSA strains included: Danish CO-MRSA, European, ST398, ST88, ST97, USA1000-China/Taiwan, and USA1100-SWP/Oceania.

MRSA Stains Over Time

In the graphs of the PFGE strain types over time, we observed different trends in adult and pediatric patients. For adult isolates, CMRSA1 was the predominant strain causing both infections and colonization from 1995 to 2001 (Range: 25-57% of isolates). CMRSA1 was replaced by CMRSA2 around 2002 onward (2002-2007 Range: 51-70% of isolates), and the proportion of isolates that were CMRSA1 continued to decrease for the remainder of the surveillance period (1% of isolates in 2007) (Figures 9A). The predominant strain in pediatric infection and colonization isolates was CMRSA2 for the majority of the surveillance period (Figures 9B). However, CMRSA10 replaced CMRSA2 as the predominant pediatric strain in 2005 (CMRSA10: 38% vs. CMRSA2: 31% of isolates in 2005) and CMRSA7 became the second most prevalent strain in 2006 (25% of isolates) (Figure 9B).

For adults, the proportions of different strains over time were fairly similar between infection and colonization isolates (Figures 10A and 11A). For both, CMRSA1 was predominant until 2001 and was replaced by CMRSA2 for the remainder of the surveillance period. The community strain, CMRS10 was the second most prevalent strain from 2005-2007 (Figures 10A and 11A). The predominant strains causing infections in children differed from the strains causing colonizations in pediatric patients. For pediatric colonizations, CMRSA2 was the predominant strain for the majority of the surveillance period. CMRSA7 and 10 accounted for the next highest proportions of pediatric colonizations from 2003 to 2007 (Figure 10B). For pediatric infections, CMRSA10 replaced CMRSA2 as the predominant strain from 2005 to 2007

and CMRSA7 was the second most prevalent strain in pediatric infection isolates from 2006-2007 (Figure 11B).

In terms of the CA-MRSA strains, CMRSA7 (USA400) was present throughout the entire surveillance period in both populations. For adults, CMRSA7 accounted for a very small proportion of MRSA isolates, increasing very slightly from 2003-2007 but still accounting for less than 5% of all adult isolates. In pediatric isolates, the proportion of MRSA caused by CMRSA7 increased over the surveillance period to become the second most prevalent strain by 2006 (Figure 9B). Based on Figures 10 and 11, the majority of CMRSA7 were infections rather than colonizations. The other CA-MRSA strain, CMRSA10 (USA300) only appear in adults in 1999 (colonizations) and in pediatric in 2003 (infections).

Figure 9A: Proportions of MRSA PFGE strain types among adult isolates over time, CNISP 1995-2007

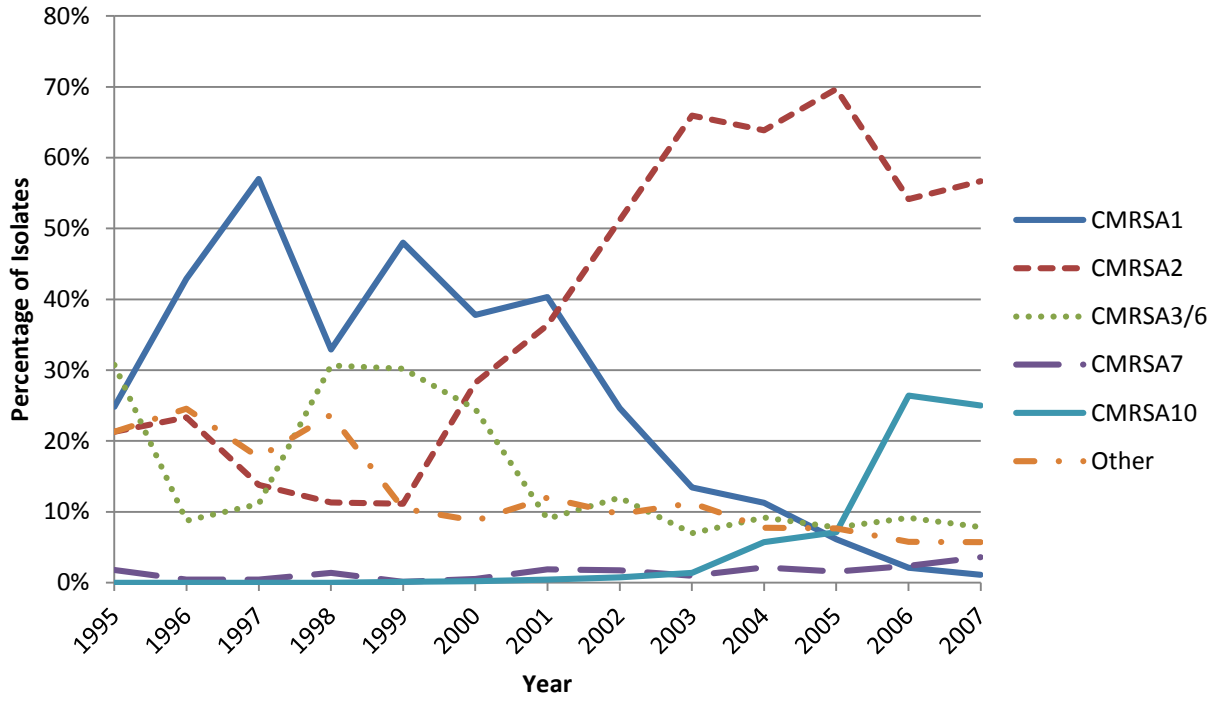


Figure 9B. Proportions of MRSA PFGE strain types among pediatric isolates over time, CNISP 1995-2007

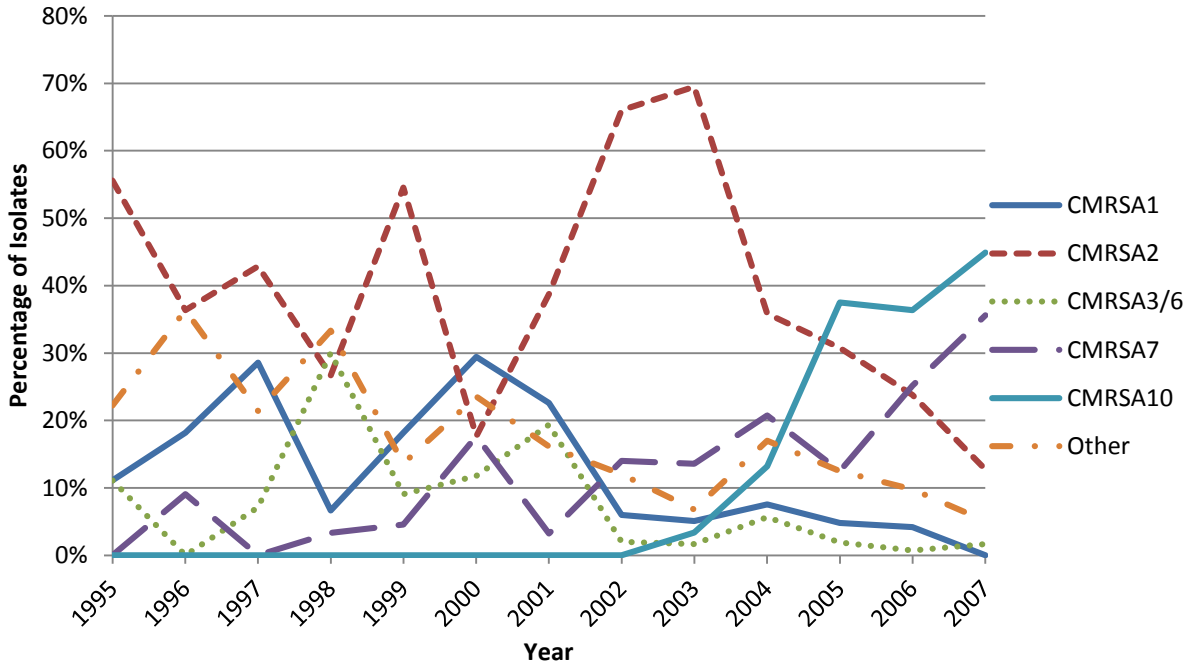


Figure 10A. Proportions of MRSA PFGE strain types causing colonization in adult isolates over time, CNISP 1995-2007

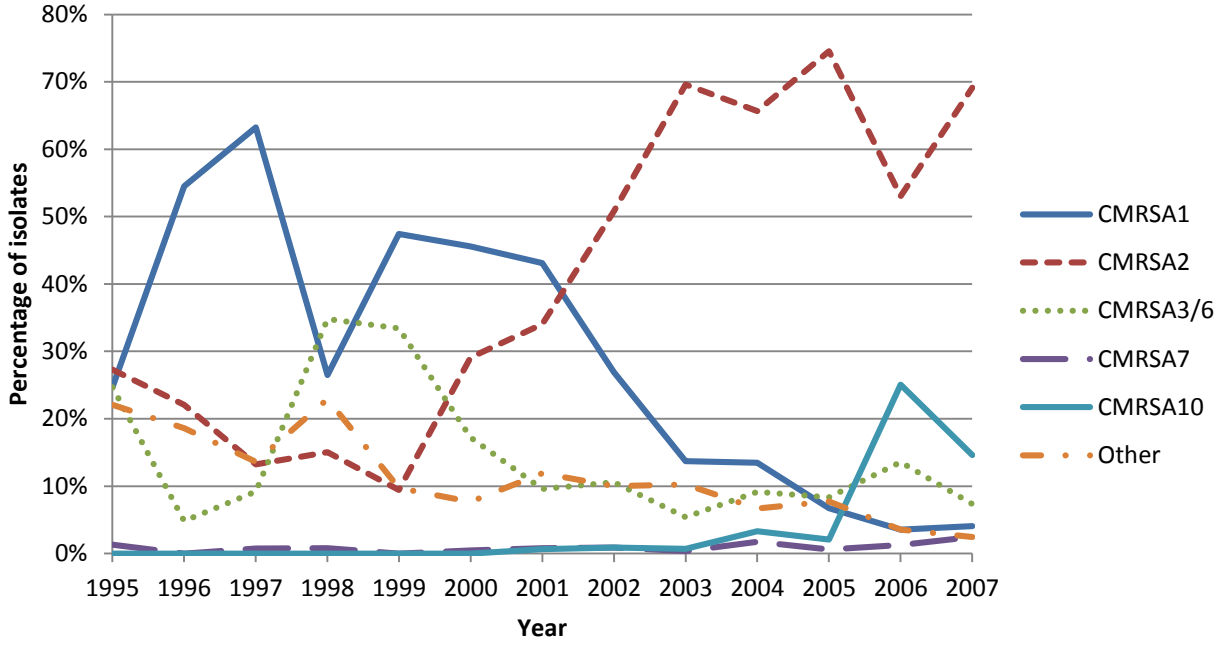


Figure 10B. Proportions of MRSA PFGE strain types causing colonization in pediatric isolates over time, CNISP 1995-2007

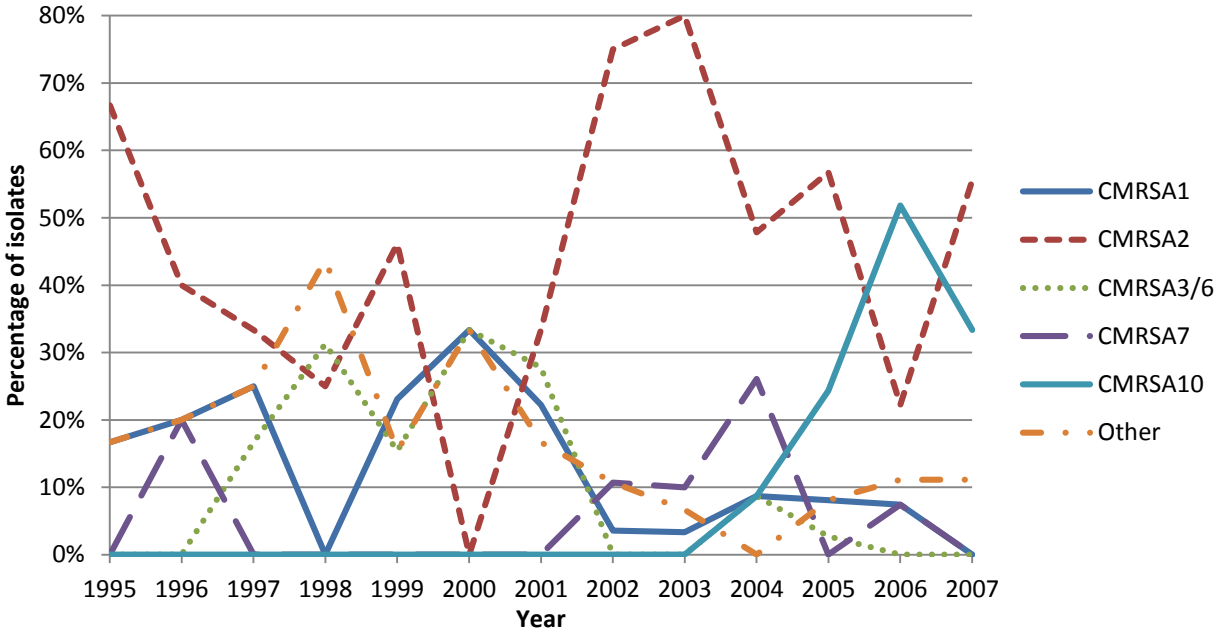


Figure 11A. Proportions of MRSA PFGE strain types causing infections in adult isolates over time, CNISP 1995-2007

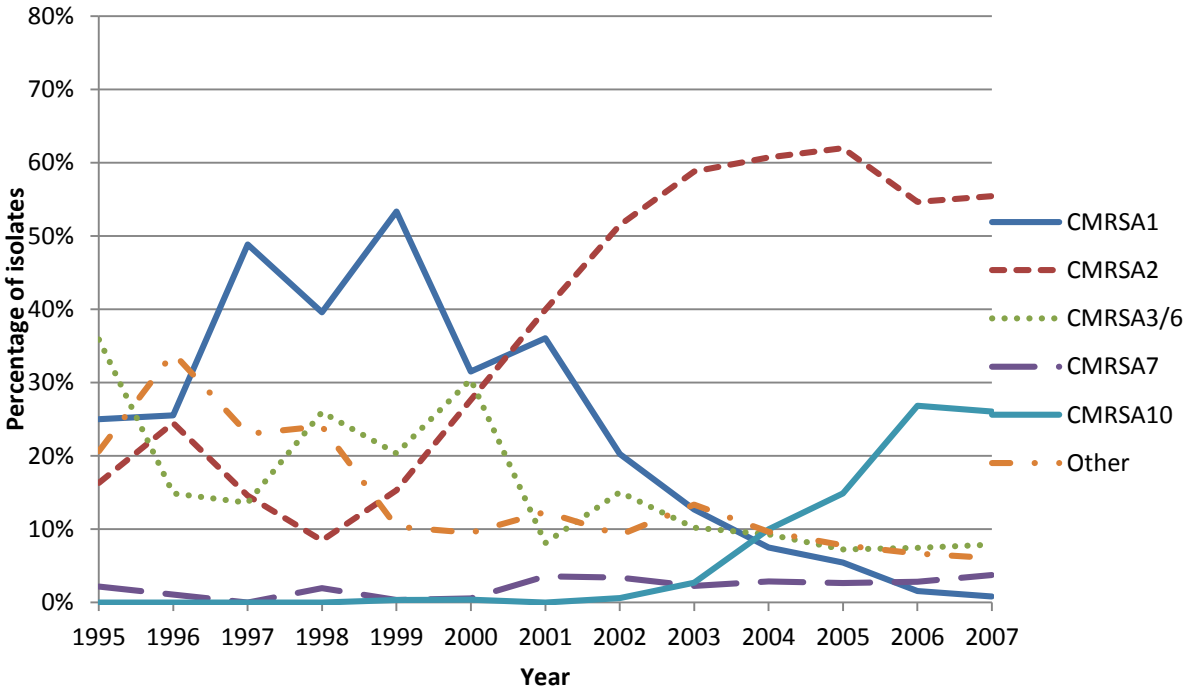
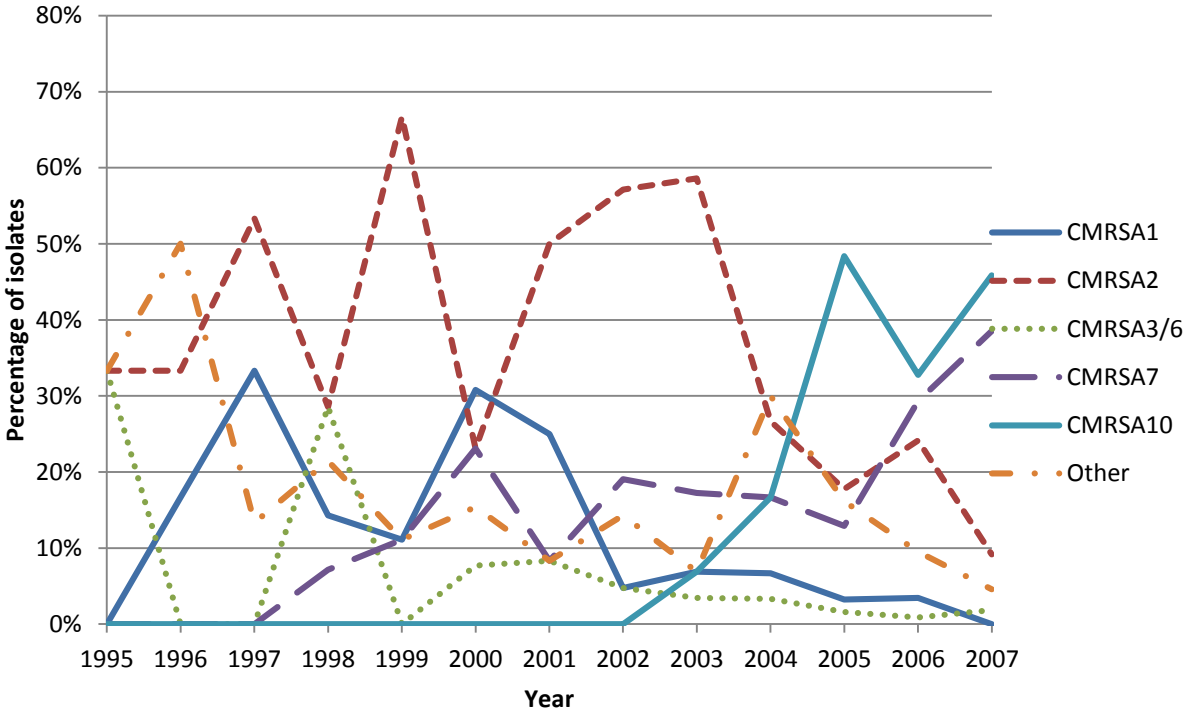


Figure 11B. Proportions of MRSA PFGE strain types causing infections in pediatric isolates over time, CNISP 1995-2007



PVL Expression and SCC*mec* Types

The proportions of PVL positive isolates were plotted against the PFGE epidemic strain types. For both adults and children, CMRSA7 (USA400) and CMRSA10 (USA300) both had the highest PVL expression. For adults, 61% of CMRSA7 and 97% of CMRSA10 isolates were PVL positive, and for children, 81% of CMRSA7 isolates and 100% of CMRSA10 isolates were PVL positive (Figure 12). PVL expression was also plotted against SCC*mec* types. The majority of SCC*mec* typeIV were PVL positive (91% and 87% of SCC*mec*IVa; 35% and 56% of SCC*mec*IV(nt) for adult and pediatric isolates, respectively; Figure 13). Almost 0% of SCC*mec* type II were PVL positive for both populations.

Figure 12. Proportions of PVL positive isolates by PFGE strain type, 1995-2007 CNISP

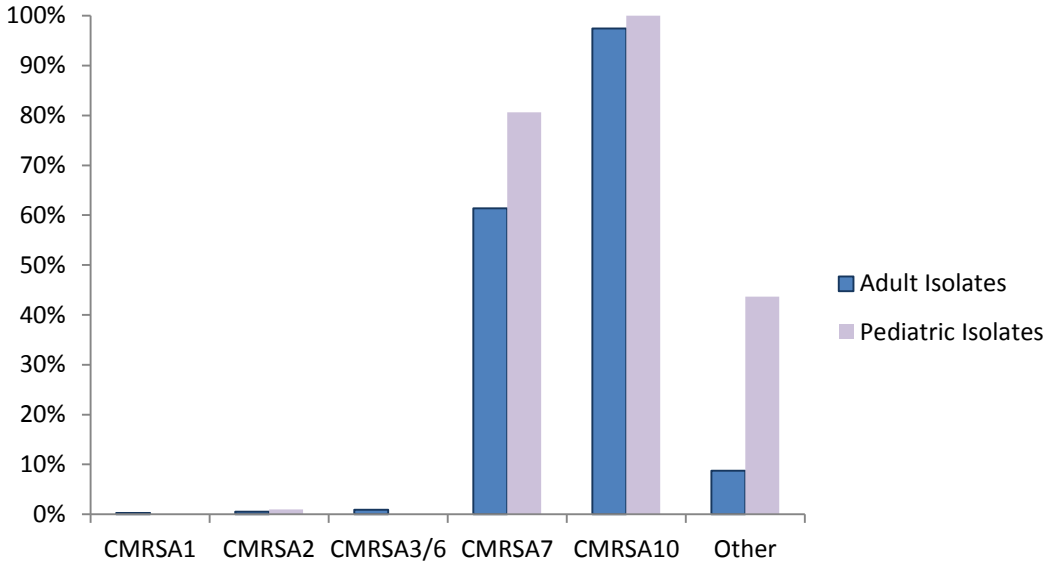
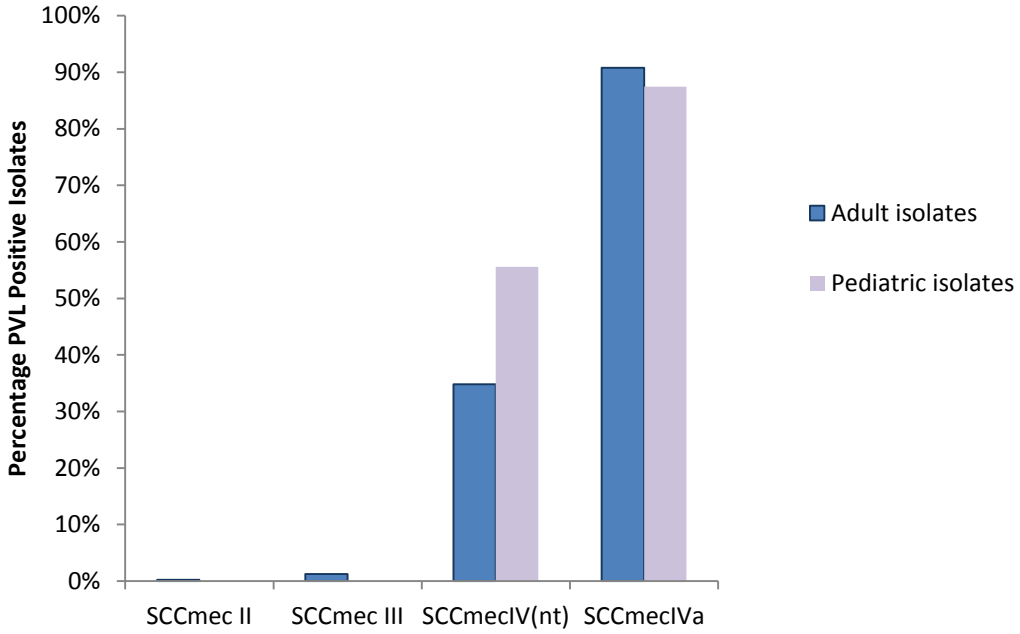


Figure 13. Proportions of PVL positive isolates by SCC*mec* typing, 1995-2007 CNISP



SCC*mec* Types by PFGE Strain

The proportions of SCC*mec* types were also examined by PFGE strain type (Figure 14). For adults, most CMRSA1 and 2 isolates were SCC*mec* type II (both 88%) and most CMRSA7 and 10 isolates were SCC*mec* type IVa (98 and 97%, respectively; Figure 14A). Other PFGE types were mostly associated with SCC*mec* types other than type II or IVa, for adults and children (Figure14A and B). For pediatric patients, the majority of CMRSA7 and CMRSA10 were SCC*mec* type IVa (100 and 95%, respectively). A smaller proportion of pediatric than adult CMRSA1 and 2 isolates were associated with SCC*mec* types II (71% of CMRSA1 and 33% of CMRSA2 were associated with SCC*mec* types other than II or IVa, Figure 14B).

Figure 14A. MRSA PFGE Strains by SCC*mec* types for adult isolates, 1995-2007 CNISP

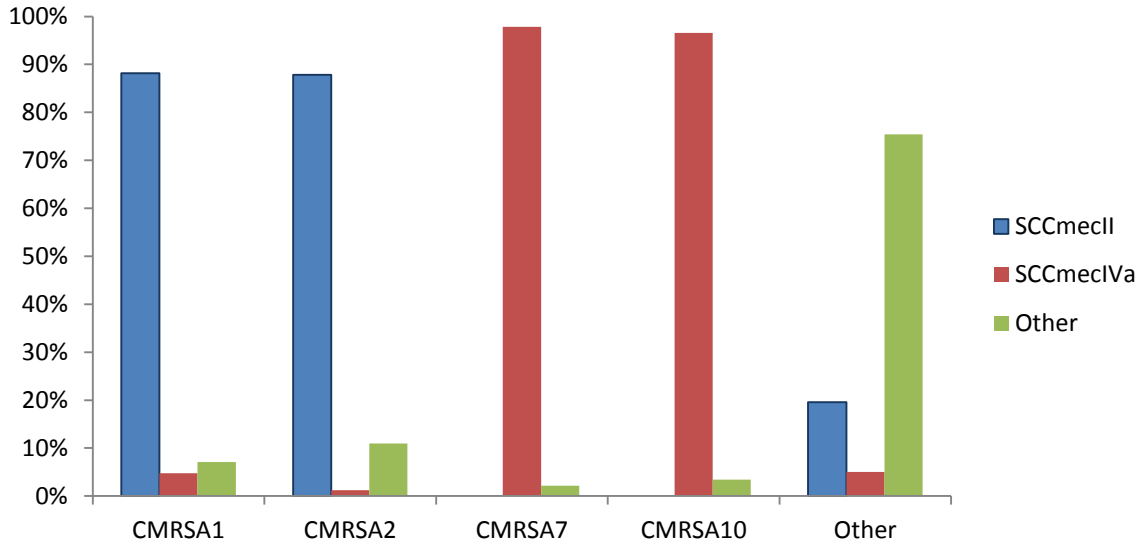
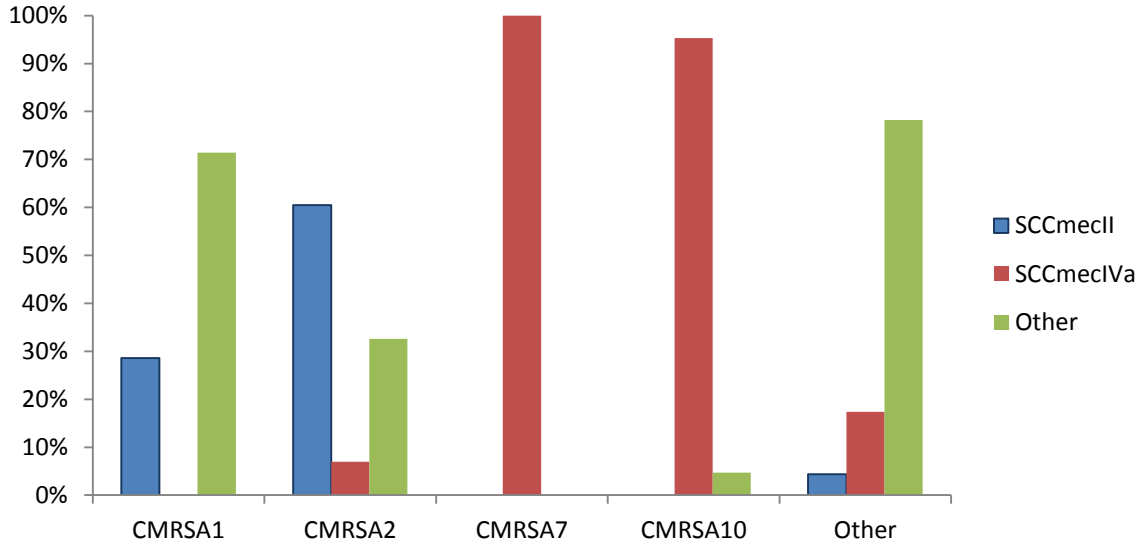


Figure 14B. MRSA PFGE Strains by SCC*mec* types for pediatric isolates, 1995-2007 CNISP



Strain Typing and PVL Expression by Infection Site

The proportions of different PFGE strain types were determined for each infection site (Table 4). CMRSA2 was the most common strain for all infection sites except for SSTIs in children. CMRSA10 accounted for the largest proportion of pediatric SSTIs (41%) and the second largest proportion of adult SSTIs (26%). The SCC*mec* typing was also established by infection site. In children, the predominant SCC*mec* type for most infection sites was type IVa, while SCC*mec* II was the predominant type in most adult infections (except for SSTIs where type IVa accounted for 49% of isolates). The majority of isolates were PVL negative. The only exceptions were pediatric SSTIs (76%) and pediatric blood infections (62%), where PVL expression was common.

Table 4. MRSA strains by infection site in adults and children from the 1995-2007 CNISP.

	Surgical Wound		SSTI		Respiratory		Urinary		Blood		Multiple Sites	
	Adult % (n)	**Ped % (n)**	**Adult % (n)**	**Ped % (n)**	**Adult % (n)**	**Ped % (n)**	**Adult % (n)**	**Ped % (n)**	**Adult % (n)**	**Ped % (n)**	**Adult % (n)**	**Ped % (n)**
PFGE Strain												
CMRSA 1	17 (197)	15 (6)	11 (213)	4 (10)	17 (216)	8 (4)	13 (93)	14 (2)	18 (95)	7 (2)	22 (122)	8 (2)
CMRSA 2	**45 (517)[1]**	**33 (13)**	**39 (736)**	17 (45)	**47 (580)**	**46 (22)**	**68 (494)**	**79 (11)**	**51 (272)**	**41 (12)**	**41 (228)**	**33 (8)**
CMRSA7	2 (23)	25 (10)	5 (87)	25 (65)	2 (22)	23 (11)	0 (3)	7 (1)	2 (10)	21 (6)	1 (5)	13 (3)
CMRSA10	8 (88)	8 (3)	26 (482)	**41 (106)**	7 (87)	8 (4)	2 (18)	0 (0)	10 (54)	17 (5)	10 (58)	17 (4)
Other	29 (331)	20 (8)	19 (349)	13 (33)	27 (339)	15 (7)	16 (114)	0 (0)	20 (106)	14 (4)	25 (140)	29 (7)
Total	(1156)	(40)	(1867)	(259)	(1244)	(48)	(722)	(14)	(537)	(29)	(553)	(24)
SCCmec												
SCCmecII	**54 (165)**	0 (0)	35 (285)	3 (4)	**57 (214)**	25 (4)	**76 (171)**	**75 (3)**	**51 (94)**	15 (2)	**55 (81)**	18 (2)
SCCmecIVa	23 (71)	**53 (8)**	**49 (393)**	**88 (120)**	19 (72)	**44 (7)**	7 (15)	0 (0)	29 (53)	**62 (8)**	26 (39)	**45 (5)**
Other	23 (72)	47 (7)	16 (127)	9 (13)	24 (92)	31 (5)	18 (40)	25 (1)	21 (38)	23 (3)	19 (28)	23 (4)
	(308)	(15)	(805)	(137)	(378)	(16)	(226)	(4)	(185)	(13)	(148)	(308)
PVL												
Positive	18 (107)	41 (12)	47 (550)	**76 (170)**	15 (98)	44 (15)	6 (24)	0 (0)	21 (60)	**62 (13)**	23 (62)	41 (7)
Negative	**82 (477)**	**59 (17)**	**53 (623)**	24 (54)	**85 (546)**	**56 (19)**	**94 (400)**	**100 (10)**	**79 (221)**	38 (8)	**77 (213)**	**59 (10)**
	(584)	(29)	(1173)	(224)	(644)	(34)	(424)	(10)	(281)	(21)	(275)	(17)

1 The strain accounting for the highest proportion for each infection site is bolded

Antimicrobial Susceptibility

Overall, adult and pediatric isolates differed significantly in their susceptibility patterns to a range of antimicrobial agents. Adults had significantly higher non-susceptibility to clindamycin (85%, n=5390/6366 vs. 56%, n=214/382, p<0.001), erythromycin (95%, n =6419/6781 vs. 73%, n=299/409, p<0.001), ciprofloxacin (92%, n=6216/6781 vs. 52%, n=211/409, p<0.001), tetracycline (19%, n=1257/6713 vs. 9%, n=38/405, p<0.001), and TMP-SMX (28%, n=1904/6781 vs. 11%, n=43/409, p<0.001) (Totals 1995-2007, Table 3). There was higher resistance to tetracycline in adult isolates but the difference was significant only for HA isolates (20%, n=946/4639 vs. 12%, n=14/113) and total isolates (HA, CA and source unknown, combined: 19%, n=1257/6713 vs. 9%, n=38/405, p<0.001) (Table 5). No significant differences were observed in the resistance to fusidic acid and rifampin between adult and pediatric isolates. Resistance to mupirocin was significantly higher in pediatric isolates compared to adult isolates for the total isolates (all isolates, including HA or CA source unknown): pediatric: 18%, n=73/405, vs. adult: 12%, n=791/6713, p=0.02) and for CA-MRSA isolates (pediatric: 19%, n=35/186, vs. adult: 10%, n=99/949). Overall, both adult and pediatric isolates had relatively low resistance to fusidic acid, tetracycline, mupirocin, and rifampin (range of 0 to 32% of isolates were non-susceptible to these antibiotics from 1995-2007) (Table5). On the other hand, resistance to clindamycin, erythromycin, and ciprofloxacin (range 62-96% in adults and 33-82% in children) was relatively high in both adult and pediatric patient groups from 1995-2007.

There was generally higher resistance in HA strains compared to CA strains for both adult and pediatric patients (Table 6). HA-MRSA strains had significantly ($0.001 \geq p \leq 0.05$)

higher proportions of non-susceptibility to clindamycin, erythromycin, ciprofloxacin, tetracycline, and TMP-SMX (Table 6). A higher proportion of pediatric CA-MRSA than HA-MRSA isolates were resistant to mupirocin (19%, n=35/186 vs. 11%, n=12/113, $p>0.05$), however this difference was not statistically significant. Overall, there was no isolate, adult or pediatric, that was non-susceptible to vancomycin (Tables 5 and 6).

To examine the antibiotic susceptibility patterns over time, the non-susceptibilities were divided into the 3 surveillance periods (1995-1999, 2000-2003, and 2004-2007). The non-susceptibility (or resistance) to tetracycline and TMP-SMX decreased over the 3 time periods in both populations and in HA-MRSA (1995-1999 to 2004-2007: tetracycline: adult: 32 to 10%; and ped.: 24 to 0%; TMP-SMX: adult:59 to 9% and ped.: 36 to 0%) and CA-MRSA (1995-1999 to 2004-2007: tetracycline: adult: 21 to 6% and ped.:14 to 6%; TMP-SMX: adult:33 to 4% and ped.:13 to 3%). The proportion of CA-MRSA isolates resistant to ciprofloxacin increased from the 1st to the 3rd surveillance period in both adults (from 68 to 83%) and children (from 33 to 53%), as did the resistance to erythromycin (from 82 to 91% in adults and from 67 to 73% in children). Although resistance was generally higher in adult isolates, changes in antibiotic resistance over time followed similar trends in both populations. In terms of mupirocin resistance, the difference between adult and pediatric isolates was non-significant for the first two surveillance periods ($p>0.05$). Resistance to mupirocin was greater in pediatric isolates only in the third surveillance period (2004-2007), and the difference was significant for HA, CA and overall isolates ($p<0.05$).

Proportions of isolates non-susceptible to three or more non-β-lactam antibiotics (multi-resistant isolates) were also assessed and compared between the two populations. Overall, the proportions of multi-resistant isolates were significantly higher in adults than children (83%, 5597/6781 vs. 41%, 166/409; $p<0.001$). As well, the proportion of multi-resistance was significantly higher in HA-MRSA isolates compared to CA-MRSA isolates for both populations, (Adults: 88%, 4124/4675 of HA-MRSA vs. 59%, 565/958 of CA-MRSA; $p<0.001$; and Children: 53%, 61/115 of HA-MRSA vs. 33%, 62/187 of CA-MRSA, $p<0.001$). The proportion of multi-resistance was fairly constant over time in both populations (Adult: HA-MRSA range: 87-90%, CA-MRSA range: 57-66%; Pediatric: HA-MRSA range: 50-78, CA-MRSA range: 27-34%). The proportion of multi-resistant isolates decreased slightly over the surveillance periods for the total isolates (all isolates, including HA, CA, and source unknown) in both adults (from 87% in 1995-1999 to 77% in 2004-2007) and children (from 47% in 1995-1999 to 38% in 2004-2007).

Table 5. Comparison of the proportion of non-susceptibility to antibiotics in adult versus pediatric MRSA isolates over time, and separated by community vs. healthcare associated strains.

Antibiotic[1] Agent	Source	Period 1 (1995-1999)			Period 2 (2000-2003)			Period 3 (2004-2007)			Total		
		Adult % (n/N)[2]	Ped. % (n/N)	p-value[4]	Adult % (n/N)	Ped. % (n/N)	p-value	Adult % (n/N)	Ped. % (n/N)	p-value	Adult % (n/N)	Peds % (n/N)	p-value
Clindamycin	HA	93 (1449/1562)	70 (28/40)	<0.001	89 (762/855)	67 (6/9)	ns	89 (1723/1946)	66 (33/50)	<0.001	90 (3934/4363)	68 (67/99)	<0.001
	CA	62 (59/95)	50 (5/10)	Ns	69 (77/111)	27 (3/11)	0.08	62 (460/738)	54 (87/161)	Ns	63 (596/944)	52 (95/182)	0.01
	Total[3]	91 (1889/2086)	59 (43/73)	<0.001	85 (1153/1353)	42 (21/50)	<0.001	80 (2348/2927)	58 (150/259)	<0.001	85 (5390/6366)	56 (214/382)	<0.001
Erythromycin	HA	97 (1811/1874)	86 (48/56)	<0.001	97 (829/855)	78 (7/9)	0.03	96 (1865/1946)	78 (39/50)	<0.001	96 (4505/4675)	82 (94/115)	<0.001
	CA	82 (89/109)	67 (10/15)	ns	90 (100/111)	64 (7/11)	0.03	91 (671/738)	73 (117/161)	<0.001	90 (860/958)	72 (134/187)	<0.001
	Total	95 (2388/2501)	79 (79/100)	<0.001	96 (1293/1353)	74 (37/50)	<0.001	94 (2738/2927)	71 (183/259)	<0.001	95 (6419/6781)	73 (299/409)	<0.001
Ciprofloxacin	HA	96 (1795/1874)	64 (36/56)	<0.001	96 (820/855)	78 (7/9)	ns	95 (1845/1946)	58 (29/50)	<0.001	95 (4460/4675)	63 (72/115)	<0.001
	CA	68 (74/109)	33 (5/15)	0.01	73 (81/111)	45 (5/11)	ns	83 (615/738)	53 (85/76)	<0.001	80 (770/958)	51 (95/187)	<0.001
	Total	93 (2331/2501)	54 (54/100)	<0.001	92 (1243/1353)	44 (22/50)	<0.001	90 (2642/2927)	52 (135/259)	<0.001	92 (6216/6781)	52 (211/409)	<0.001
Fusidic Acid	HA	5 (81/1607)	8 (4/49)	ns	8 (65/854)	11 (1/9)	ns	6 (111/1946)	10 (5/50)	ns	6 (257/4407)	9 (10/108)	Ns
	CA	8 (6/78)	8 (1/12)	ns	5 (6/111)	9 (1/11)	ns	4 (32/738)	4 (7/161)	ns	5 (44/927)	5 (9/184)	Ns
	Total	5 (109/2127)	7 (6/83)	ns	8 (103/1352)	10 (5/50)	ns	6 (164/2927)	5 (14/259)	ns	6 (376/6406)	6 (25/392)	Ns
Mupirocin	HA	9 (157/1838)	0 (0/54)	0.02	17 (142/855)	0 (0/9)	ns	13 (260/1946)	24 (12/50)	0.04	12 (559/4639)	11 (12/113)	Ns
	CA	7 (7/100)	14 (2/14)	ns	24 (27/111)	18 (2/11)	ns	9 (65/738)	19 (31/161)	<0.001	10 (99/949)	19 (35/186)	0.01
	Total	8 (205/2433)	5 (5/96)	ns	16 (218/1353)	22 (11/50)	ns	13 (368/2927)	22 (57/259)	<0.001	12 (791/6713)	18 (73/405)	0.02
Rifampin	HA	3 (55/1838)	0 (0/54)	ns	3 (28/855)	0 (0/9)	ns	1 (24/1946)	2 (1/50)	ns	2 (107/4639)	1 (1/113)	Ns
	CA	3 (3/100)	7 (1/14)	ns	2 (2/111)	0 (0/11)	ns	1 (7/738)	1 (2/161)	ns	1 (12/949)	2 (3/186)	Ns
	Total	3 (71/2433)	2 (2/96)	ns	3 (38/1353)	2 (1/50)	ns	1 (32/2927)	1 (3/259)	ns	2 (141/6713)	1 (6/405)	Ns
Tetracycline	HA	32 (597/1838)	24 (13/54)	ns	19 (161/855)	11 (1/9)	ns	10 (188/1946)	0 (0/50)	0.01	20 (946/4639)	12 (14/113)	0.04
	CA	21 (21/100)	14 (2/14)	ns	9 (10/111)	0 (0/11)	ns	6 (42/738)	6 (9/161)	ns	8 (73/949)	6 (11/186)	Ns
	Total	32 (784/2433)	22 (21/96)	0.03	17 (227/1353)	10 (5/50)	ns	8 (246/2927)	5 (12/259)	0.03	19 (1257/6713)	9 (38/405)	<0.001
TMP-SMX[5]	HA	59 (1097/1874)	36 (20/56)	<0.001	24 (209/855)	11 (1/9)	ns	9 (174/1946)	0 (0/50)	0.02	32 (1480/4675)	18 (21/115)	0.02
	CA	33 (36/109)	13 (2/15)	ns	12 (13/111)	0 (0/11)	ns	4 (26/738)	3 (5/161)	ns	8 (75/958)	4 (7/187)	0.05
	Total	57 (1420/2501)	30 (30/100)	<0.001	20 (274/1353)	10 (5/50)	ns	7 (210/2927)	3 (8/259)	0.01	28 (1904/6781)	11 (43/409)	<0.001
Vancomycin	HA	0 (0/1874)	0 (0/56)	—	0 (0/855)	0 (0/9)	—	0 (0/1946)	0 (0/50)	—	0 (0/4675)	0 (0/115)	—
	CA	0 (0/109)	0 (0/15)	—	0 (0/111)	0 (0/11)	—	0 (0/738)	0 (0/161)	—	0 (0/958)	0 (0/187)	—
	Total	0 (0/2501)	0 (0/100)	—	0(0/1353)	0 (0/50)	—	0 (0/2927)	0 (0/259)	—	0 (0/6781)	0 (0/409)	—
Multi-resistance[6]	HA	89 (1667/1874)	52 (29/56)	<0.001	89 (766/855)	78 (7/9)	ns	87 (1691/1946)	50 (25/50)	<0.001	88 (4124/4675)	53 (61/115)	<0.001
	CA	66 (72/109)	33 (5/15)	0.02	68 (75/111)	27 (3/11)	0.02	57 (418/738)	34 (54/161)	<0.001	59 (565/958)	33 (62/187)	<0.001
	Total	87 (2173/2501)	47 (47/100)	<0.001	86 (1160/1353)	40 (20/50)	<0.001	77 (2264/2927)	38 (99/259)	<0.001	83 (5597/6781)	41 (166/409)	<0.001

HA= Healthcare-Associated, CA=Community-Associated, ns= non-significant at an a = 0.05 level.

1 Linozolid Acid, Synercid, Ceptobiprole, Tigecycline, Daptomycin, and Teicoplanin were excluded due to insufficient data. Gentamicin was excluded based on the fact that it is not used in practice for the treatment of MRSA.

2 Non-susceptible isolates were defined as isolates that tested "resistant" or "intermediate" to a particular antibiotic.

3 Total includes isolates where the source of acquisition (HA vs. CA) was unknown, thus total not equal to HA plus CA.

4 Chi-Square tests of the equality of two proportions (Pearson Chi-Square Test) or Fisher's Exact Tests were used

5 (Trimethoprim-Sulfamethoxazole)

6 Multi-resistance was defined as non-susceptibility to 3 or more antibiotics

Table 6. Comparison of the proportion of non-susceptibility[2] to antibiotics in HA vs. CA MRSA isolates, by adult and pediatric patients

		Period 1 (1995-1999)			Period 2 (2000-2003)			Period 3 (2004-2007)			Total		
Antibiotic[1]		**HA % (n/N)[2]**	**CA % (n/N)‡**	**p-value[3]**	**HA % (n/N)**	**CA % (n/N)**	**p-value**	**HA % (n/N)**	**CA % (n/N)**	**p-value**	**HA % (n/N)**	**CA % (n/N)**	**p-value**
Clindamycin	Adult	93 (1449/1562)	62 (59/95)	<0.001	89 (762/855)	69 (77/111)	<0.001	89 (1723/1946)	62 (460/738)	<0.001	90 (3934/4363)	63 (596/944)	<0.001
	Ped.	70 (28/40)	50 (5/10)	ns	67 (6/9)	27 (3/11)	ns	66 (33/50)	54 (87/161)	ns	68 (67/99)	52 (95/182)	0.01
Erythromycin	Adult	97 (1811/1874)	82 (89/109)	<0.001	97 (829/855)	90 (100/111)	<0.001	96 (1865/1946)	91 (671/738)	<0.001	96 (4505/4675)	90 (860/958)	<0.001
	Ped.	86 (48/56)	67 (10/15)	ns	78 (7/9)	64 (7/11)	ns	78 (39/50)	73 (117/161)	ns	82 (94/115)	72 (134/187)	<0.05
Ciprofloxacin	Adult	94 (1762/1874)	76 (83/109)	<0.001	78 (671/855)	68 (75/111)	0.001	57 (608/1068)	24 (114/475)	<0.001	95 (4460/4675)	80 (770/958)	<0.001
	Ped.	73 (41/56)	67 (10/15)	ns	56 (5/9)	64 (7/11)	ns	54 (15/28)	21 (20/96)	<0.001	63 (72/115)	51 (95/187)	<0.05
Fusidic Acid	Adult	5 (81/1607)	8 (6/78)	ns	8 (65/854)	5 (6/111)	ns	6 (111/1946)	4 (32/738)	ns	6 (257/4407)	5 (44/927)	ns
	Ped.	8 (4/49)	8 (1/12)	ns	11 (1/9)	9 (1/11)	ns	10 (5/50)	4 (7/161)	ns	9 (10/108)	5 (9/184)	ns
Mupirocin	Adult	9 (157/1838)	7 (7/100)	ns	17 (142/855)	24 (27/111)	0.04	13 (260/1946)	9 (65/738)	<0.001	12 (559/4639)	10 (99/949)	ns
	Ped.	0 (0/54)	14 (2/14)	0.04	0 (0/9)	18 (2/11)	ns	24 (12/50)	19 (31/161)	ns	11 (12/113)	19 (35/186)	ns
Rifampin	Adult	3 (55/1838)	3 (3/100)	ns	3 (28/855)	2 (2/111)	ns	1 (24/1946)	1 (7/738)	ns	2 (107/4639)	1 (12/949)	0.04
	Ped.	0 (0/54)	7 (1/14)	ns	0 (0/9)	0 (0/11)	**n/a**	2 (1/50)	1 (2/161)	ns	1 (1/113)	2 (3/186)	ns
Tetracycline	Adult	32 (597/1838)	21 (21/100)	0.02	19 (161/855)	9 (10/111)	0.01	10 (188/1946)	6 (42/738)	0.001	20 (946/4639)	8 (73/949)	<0.001
	Ped.	24 (13/54)	14 (2/14)	ns	11 (1/9)	0 (0/11)	ns	0 (0/50)	6 (9/161)	ns	12 (14/113)	6 (11/186)	<0.05
TMP-SMX[4]	Adult	59 (1097/1874)	33 (36/109)	<0.001	24 (209/855)	12 (13/111)	<0.001	9 (174/1946)	4 (26/738)	<0.001	32 (1480/4675)	8 (75/958)	<0.001
	Ped.	36 (20/56)	13 (2/15	ns	11 (1/9)	0 (0/11)	ns	0 (0/50)	3 (5/161)	ns	18 (21/115)	4 (7/187)	<0.001
Vancomycin	Adult	0 (0/1874)	0 (0/109)	—	0 (0/855)	0 (0/111)	—	0 (0/1946)	0 (0/738)	—	0 (0/4675)	0 (0/958)	—
	Ped.	0 (0/56)	0 (0/15)	—	0 (0/9)	0 (0/11)	—	0 (0/50)	0 (0/161)	—	0 (0/115)	0 (0/187)	—
Multi-resistance[5]	Adult	89 (1667/1874)	66 (72/109)	<0.001	90 (766/855)	68 (75/111)	<0.001	87 (1691/1946)	57 (418/738)	<0.001	88 (4124/4675)	59 (565/958)	<0.001
	Ped.	52 (29/56)	33 (5/15)	ns	78 (7/9)	27 (3/11)	ns	50 (25/50)	34 (54/161)	0.04	53 (61/115)	33 (62/187)	<0.001

HA= Healthcare-Associated, CA=Community-Associated, ns= non-significant at an α= 0.05 level.

1 Linozolid Acid, Synercid, Ceptobiprole, Tigecycline, Daptomycin, and Teicoplanin were excluded due to insufficient data.

2 Non-susceptible isolates were defined as isolates that tested "resistant" or "intermediate" to a particular antibiotic.

3 Chi-Square tests of the equality of two proportions (Pearson Chi-Square Test) or Fisher's Exact Tests were used

4 Trimethoprim-Sulfamethoxazole

5 Multi-resistance was defined as non-susceptibility to 3 or more antibiotic

Epidemiological characteristics of HA versus CA MRSA

Univariate logistic regression analyses were performed to determine which factors were associated with (HA-MRSA) compared to community-associated MRSA (Table 7). Overall, age, and ethnicity, infection /colonization status, reason for culture, and site of infection differed significantly between HA-MRSA and CA-MRSA for both adults and children (all $p<0.001$). The only variable that did not differ between HA and CA-MRSA was sex ($p>0.05$ for adults and children). Adults aged older than 70 years made up the highest proportion (58%) of HA-MRSA cases, whereas 25 to 55-year-olds comprised the largest proportion (41%) of CA-MRSA cases (Table 7). For pediatric patients, the majority of HA-MRSA cases (60%) were found in infants aged less than 1 year, while CA-MRSA was more evenly spread across different age groups (although still highest in infants - 35%). The proportion of patients of First Nations origin was higher in CA-MRSA than HA-MRSA, and the difference was much more pronounced in children (37% of CA vs. 14% of HA-MRSA, $p<0.001$) than in adults (8% of CA vs. 2% of HA MRSA, $p<0.001$) (Table 7).

In adults, admission screening detected a higher proportion of CA-MRSA than HA-MRSA (52 vs. 36%) while in children a higher proportion of CA-MRSA were detected by clinical isolates (70 vs. 61%) and a greater proportion of HA-MRSA were detected for other reasons for culture including outbreak or cluster investigation, prevalence survey, other screening reasons or other indications (15 vs. 4%). In both populations, skin and soft tissue infections accounted for a greater percentage of infections in CA-MRSA (65 in adults and 77 in children), whereas a greater proportion of HA-MRSA was associated with surgical wounds, respiratory infections, urinary infections, and bloodstream infections (Table 7).

Table 7. Univariate logistic regression analyses of HA- and CA-MRSA characteristics among adult and pediatric patients, CNISP 1995-2007

		Adult			Pediatric		
		HA-MRSA, N (%)	CA-MRSA, N (%)	*p*-value[1]	HA-MRSA, N (%)	CA-MRSA, N (%)	*p*-value
Age Adult	≥18 to <25 yrs	475/25644 (2)	244/4832 (5)		N/A	N/A	
	≥25 to <55 yrs	4534/25644 (18)	1984/4832 (41)				
	≥55 to <70 yrs	5680/25644 (22)	820/4832 (17)				
	>70 yrs	14955/25644 (58)	1784/4832 (36)	<0.001			
Age Pediatric	≥0 to <1 yr	N/A	N/A		319/531 (60)	150/433 (35)	
	≥1 to <5 yrs				65/531 (12)	115/433 (27)	
	≥5 to <12 yrs				43/531 (8)	75/433 (17)	
	≥12 to <18 yrs				104/531 (20)	93/433 (21)	<0.001
Sex	Female	11059/25788 (43)	2077/4868 (43)		233/531 (44)	208/432 (48)	
	Male	14729/25788 (57)	2791/4868 (57)	ns	298/531 (56)	224/432 (52)	ns
Ethnicity	Non First Nations	14753/14999 (98)	1979/2147 (92)		258/301 (86)	102/163 (63)	
	First Nations	246/14999 (2)	168/2147 (8)	<0.001	43/301 (14)	61/163 (37)	<0.001
Status	Colonized	18045/25672 (70)	2829/4821(59)		317/526 (62)	170/432 (39)	
	Infected	7627/25672 (30)	1992/4821 (41)	<0.001	199/526 (38)	262/432 (61)	<0.001
Reason Cultured	Clinical Isolate	9516/20913 (46)	2078/4663 (45)		272/448 (61)	301/427 (70)	
	Admission Screen	7629/20913 (36)	2417/4663 (52)		111/448 (25)	110/427 (26)	
	Other[1]	3768/20913 (18)	168/4663 (4)	<0.001	65/448 (15)	16/427 (4)	<0.001
Site of Infection	Surgical Wound	1610/7294 (22)	113/1883 (6)		36/183 (20)	6/245 (2)	
	Skin/Soft Tissues	1754/7294 (24)	1220/1883 (65)		62/183 (34)	188/245 (77)	
	Respiratory	1694/7294 (23)	203/1883 (11)		33/183 (18)	20/245 (8)	
	Urine	883/7294 (12)	104/1883 (6)		11/183 (6)	4/245 (2)	
	Blood	707/7294 (10)	137/1883 (7)		23/183 (13)	18/245 (7)	
	Multiple	646/7294 (9)	106/1883 (6)	<0.001	18/183 (10)	9/245 (4)	<0.001

N/A = Not applicable;
ns = Not significant
p-values were calculated by univariate logistic regressions where HA-MRSA was modeled as the outcome (versus CA-MRSA)
1 Other reasons for culture included: outbreak or cluster investigation, prevalence survey, other screening reasons or other indications

Hospital Factor Longitudinal Models

A Marginal Model using Generalized Estimating Equations (GEE) or a Generalized Linear Mixed Effects Model (GLMEM) was used to model the outcome (hospital MRSA rates – including both infection and colonization). Separate models were built for adult and pediatric facilities. The final models for adult hospitals were done using Generalized Linear Mixed Effects Model (GLMEM) because the Marginal Model did not converge when the appropriate correlation matrix (autoregressive or m-dependent) were used. Marginal Models using Generalized Estimating Equations (GEE) were used for the final pediatric models as the GLMEM model parameters were not similar to the marginal model and the parameters were inconsistent with what was observed in the diagnostic graphs. Year was included in all models as a continuous variable. The model including both adult and pediatric facilities is not presented because the separate models were determined to be the most appropriate and the combined model did not add any value (the combined model reflected the adult characteristic due to the larger sample size of adult hospitals).

Hospital MRSA rates were plotted over time to assess time-trends. Adult hospital MRSA rates ranged from 0 to 32.9 per 10,000 patient-days, and the range for pediatric facilities was 0 to 14.6 per 10,000 patient-days. Adult hospital rates followed a strong linear trend (average MRSA rate: linear trend R^2=0.96) while pediatric hospital rates followed a strong exponential trend (average MRSA rate: exponential trend R^2=0.94). (Figure 15).

Figure 15A. MRSA rates (per 10,000 patient-years) over time for adult hospitals, 1995-2007

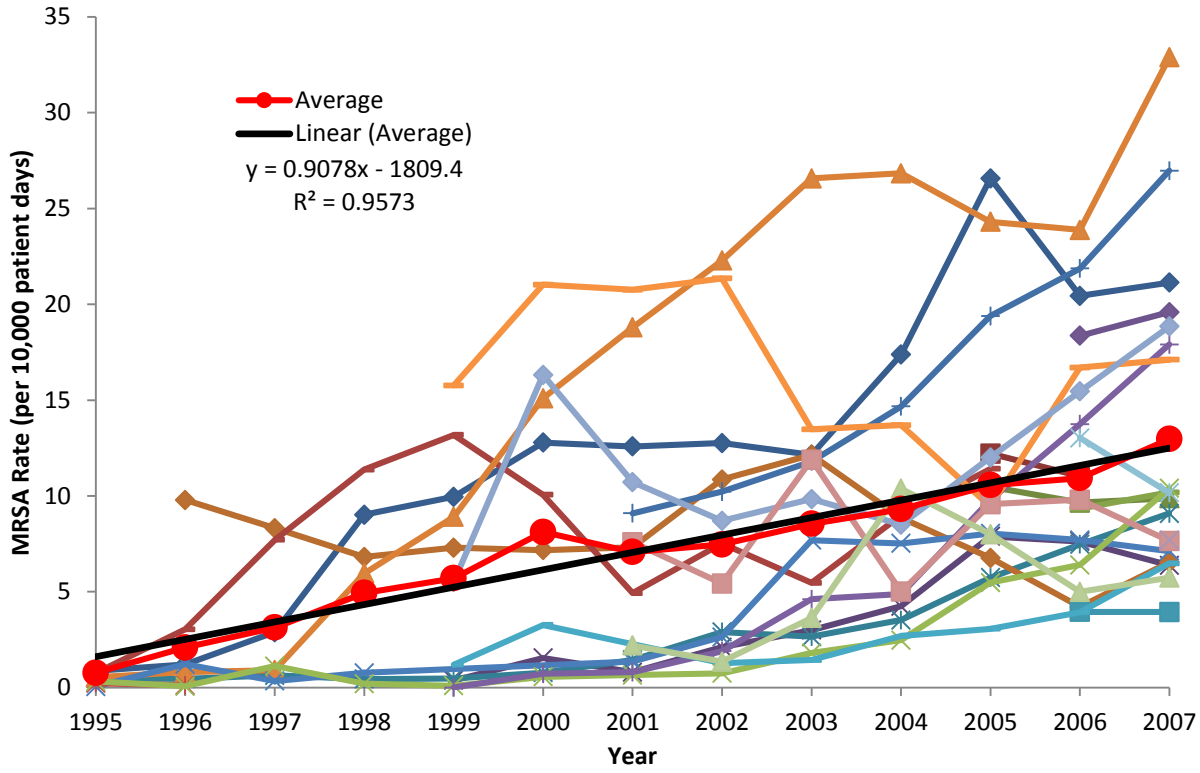
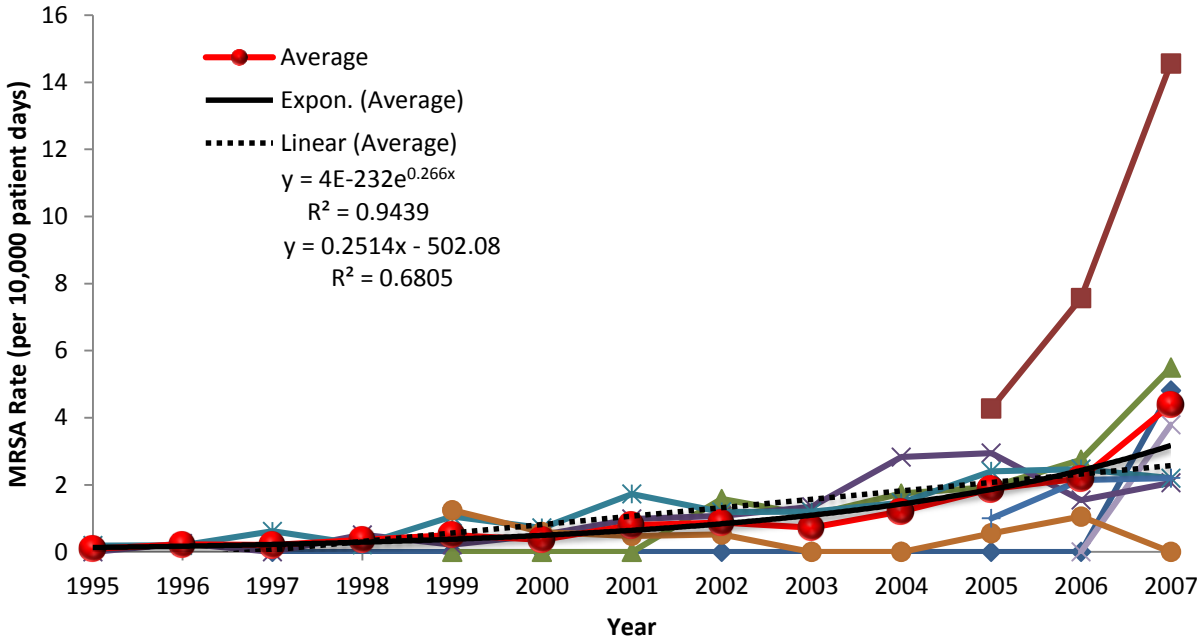


Figure 15B. MRSA rates (per 10,000 patient-years) over time for pediatric hospitals, 1995-2007



Characteristics significantly associated with adult hospital MRSA rates at an α=0.05 level were year, number of hospital beds, number of Full-time equivalent infection control professionals (FTE ICP), having greater than 100 beds per FTE infection control professional (ICP), having a higher proportion of patients age 65 and older, and having a higher hospital occupancy rate. Variables significant at a p-value of 0.1 or less were included in the multivariate model. Interaction terms with the surveillance year variable were also tested. The interaction between year and other predictor variables were significant in the bivariate analyses (Table 8). The measures of association for these models are odds ratios and can be calculated as e^{β} where β is the parameter estimate. Where there are significant interaction terms (Tables 8B and 9B), the effect measure for each predictor variable in the interaction cannot be calculated alone and instead must be calculated by adding the parameters for the individual variables and the interaction term.

Table 8A. Individual predictor variables for adult hospital MRSA rates, using generalized linear mixed effects models

Variables[1]	Parameter Estimate	Standard Error	p-value
Year			
Year	0.161	0.0339	<0.001
Bedsize			
Year	0.615	0.0342	<0.001
Bedsize	-0.0447	0.00866	<0.001
ICP[2]			
Year	0.943	0.0389	<0.001
Number of FTE ICP	-0.174	0.010	<0.001
Number of beds per ICP[3]			
Year	0.156	0.0434	0.002
>100 beds per FTE ICP	0.346	0.0368	<0.001
ICP and Bedsize[4]			
Year	0.188	0.0384	<0.001
Number of FTE ICP	-0.171	0.0102	<0.001
Bedsize	-0.0148	0.00886	0.097
Hospital Occupancy[5]			
Year	0.176	0.0368	<0.001
Percent Occupancy	0.0294	0.0150	0.052
Patient Age[6]			
Year	0.2201	0.0397	<0.001
Percent of patients age ≥65 years	0.0339	0.0117	0.0041
Region			
Year	0.1599	0.0338	<0.001
Central vs. Eastern Canada	0.1367	0.349	0.696
Western vs. Eastern Canada	0.2316	0.390	0.554

Table 8B. Interaction terms for predictors of adult hospital MRSA rates, using generalized linear mixed effects models.

Interaction Terms			
Variables	**Parameter Estimate**	**Standard Error**	**p-value**
Year and Number Beds per FTE ICP			
Year	0.1316	0.0483	0.0127
>100 beds per FTE ICP	0.07347	0.178	0.681
Year* >100 beds per FTE ICP	0.02595	0.0165	0.117
Year and Hospital Occupancy			
Year	-0.4670	0.0794	<0.001
Percent Occupancy	-0.4769	0.0547	<0.001
Year*Percent Occupancy	0.07224	0.00722	<0.001
Year and Region			
Year	0.3642	0.0466	<0.001
Central vs. Eastern Canada	3.5602	0.701	<0.001
Western vs. Eastern Canada	1.9573	0.859	0.024
Year*central vs. eastern Canada	-0.2811	0.0542	<0.001
Year*Western vs. Eastern Canada	-0.1436	0.666	0.033
Year and Patient Age			
Year	-0.009259	0.00562	0.1141
Percent of patients age ≥65 years	-0.32746	0.0276	<.0001
Year*Percent of patients age ≥65 years	0.04698	0.00334	<.0001

1 The predictor variables included in the bivariate (or multivariate models)
2 ICP = Infection Control Professionals, FTE ICP= Full-Time Equivalent Infection Control Professionals. Total number of FTE ICP per hospital.
3 Ratio of the number of beds at a hospital per FTE infection control professional. The variable was dichotomized to less than or equal to and greater than 100 beds per ICP based on the 2011 PIDAC Best Practices Guide. >100 beds per ICP was modeled.
4 Number of FTE and bedsize were modeled together for comparison with ratio variable "beds per FTE ICP"
5 The percentage of hospital occupancy was modeled as a continuous variable per 10% increase in percentage occupancy.
6 The proportion of MRSA patients aged 65 years and older at each hospital per year. The variable was modeled as continuous, per 10% increase in the proportion of patients aged 65 years and older.

Characteristics meeting the criteria for inclusion in the final model for pediatric hospitals ($p \leq 0.1$) were surveillance year, hospital occupancy rate, and the proportion of patients who were 0 to 1 year old. Number of hospital beds, number of FTE ICP, having greater than 100 beds per FTE ICP, and region did not meet the cut off for inclusion in the multivariate model. Given the strong exponential time trend observed in the graph of pediatric hospital rates over time (Figure 14B), year squared was tested in the

model; however, it was not significant (p > 0.1). The same year and predictor variable interactions as the adult models were also tested in the pediatric model. The only interaction that was significant was the interaction between year and region although the main year variable became non-significant (p>0.1).

Table 9A. Individual predictor variables of pediatric hospital MRSA rates, using marginal models

Variables[1]	Parameter Estimate	Standard Error	p-value
Year			
Year	0.2977	0.0629	<0.001
Year and Year Squared			
	0.0958	0.264	0.72
	0.0112	0.0171	0.51
Bedsize			
Year	0.2976	0.0646	<0.001
Bedsize	-0.1231	0.154	0.42
ICP[2]			
Year	0.2961	0.0616	<0.001
Number of FTE ICP	0.0534	0.105	0.61
Number of beds per ICP[3]			
Year	0.3038	0.0655	<0.001
>100 beds per FTE ICP	-0.1094	0.2437	0.65
ICP and Bedsize[4]			
Year	0.2915	0.0606	<0.001
Bedsize	-0.1808	0.152	0.24
Number of FTE ICP	0.1114	0.106	0.29
Hospital Occupancy[5]			
Year	0.2069	0.0358	<0.001
Percent Occupancy	0.3097	0.118	0.0089
Patient Age[6]			
Year	0.2873	0.0585	<0.001
Percent of patients age 0-1 year	0.0102	0.0049	0.037
Region			
Year	0.2812	0.0599	<0.001
Central vs. Eastern Canada	0.8951	1.0245	0.38
Western vs. Eastern Canada	1.3648	1.0340	0.19

Table 9B. Interaction terms for predictors of pediatric hospital MRSA rates, using marginal models

Interaction Terms			
Variable	**Parameter Estimate**	**Standard Estimate**	**p-value**
Year and Number Beds per FTE ICP			
Year	0.2994	0.0810	0.0002
>100 beds per FTE ICP	0.0073	1.1038	0.99
Year* >100 beds per FTE ICP	0.0093	0.0955	0.92
Year and Hospital Occupancy			
Year	0.0480	0.275	0.86
Percent Occupancy	0.1268	0.321	0.69
Year*Percent Occupancy	0.0204	0.0349	0.56
Year and Region			
Year	-0.2382	0.342	0.49
Central vs. Eastern Canada	-3.2128	2.513	0.20
Western vs. Eastern Canada	-12.3243	4.0089	0.0021
Year*central vs. eastern Canada	0.4567	0.347	0.19
Year*Western vs. Eastern Canada	1.2335	0.421	0.0034
Year and Patient Age			
Year	0.3110	0.0877	0.0004
Percent of patients age 0-1 year	0.0152	0.0145	0.29
Year*Percent of patients age 0-1 year	-0.0006	0.0014	0.69

1 The predictor variables included in the bivariate (or multivariate models)

2 ICP = Infection Control Professionals, FTE ICP= Full-Time Equivalent Infection Control Professionals. Total number of FTE ICP per hospital.

3 Ratio of the number of beds at a hospital per FTE infection control professional. The variable was dichotomized to less than or equal to and greater than 100 beds per ICP based on the 2011 PIDAC Best Practices Guide. >100 beds per ICP was modeled.

4 Number of FTE and bedsize were modeled together for comparison with ratio variable "beds per FTE ICP"

5 The percentage of hospital occupancy was modeled as a continuous variable per 10% increase in percentage occupancy.

6 The proportion of MRSA patients aged 0 to 1 year old at each hospital per year. The variable was modeled as continuous, per 10% increase in the proportion of patients aged 0-1 year.

Final Multivariate Hospital Level Models

Final multivariate models and are presented in tables 10 and 11. All of the predictor variables from the bivariate models that met the criteria for inclusion criteria ($p<0.1$) were tested in the multivariate model. A *p-value <0.05* was used as the criteria for retention in the final multivariate model,

therefore only 2 variables remained significant in each model. Factors that were significant predictors of hospital MRSA rates in adult facilities (using GLMEM) were the percent hospital occupancy and having greater than 100 beds per FTE Infection control professional. For every 10% increase in hospital occupancy, the odds of MRSA increased by 7% (OR=1.071, 95%CI: 1.054-1.010). For adult hospitals with a ratio of beds greater than 100 per ICP, the odds of MRSA were 1.5 times greater than those with a ratio equal to or less than 100 beds per ICP (OR=1.492, 95%CI: 1.435-1.551). For pediatric facilities, significant predictors for hospital MRSA rates in the multivariate Marginal Model were percent occupancy and the proportion of patients 0 to 1 year of age. For every 10% increase in the occupancy of pediatric hospitals, the odds of MRSA increased by 39% (OR=1.392, 95%CI: 1.106-1.751). For every 10% increase in the proportion of MRSA patients age 0 to 1 year old at a hospital, the odds of MRSA increased by 10%. There were no interaction terms that remained significant in the multivariate model.

Table 10. Final multivariate GLMEM[1] model of adult hospital characteristics predicting hospital MRSA rates, CNISP 1995-2007.

Variables	Odds Ratio	Odds Ratio 95% Confidence Interval		P-Value
Year	1.182	1.125	1.241	0.003
Percent occupancy[2]	1.071	1.054	1.010	<0.001
Number of beds per FTE ICP[3]	1.492	1.435	1.551	<0.001

1. Generalized Linear Mixed Effect Modeling
2. Per 10% increase in occupancy
3. FTE = full-time equivalent, ICP=Infection control professionals. Number of beds per FTE was calculated as a ratio. A cut off of 100 beds per FTE was used based on the 2011 PIDAC Best Practices Guide in order to create a dichotomous variable.

Table 11. Final multivariate marginal model (GEE)[1] of pediatric hospital characteristics predicting hospital MRSA rates, CNISP 1995-2007.

Variables	Odds Ratio	Odds Ratio 95% Confidence Interval		P-Value
Year	1.228	1.150	1.311	<0.001
Percent occupancy[2]	1.392	1.106	1.751	0.005
Proportion of patients 0-1 year of age[3]	1.102	1.017	1.194	0.017

1 Generalized Estimating Equations

2 Per 10% increase in occupancy

Percentage of all MRSA patients that were 0 to 1 year of age at a given hospital. OR per 10% increase.

CHAPTER 7: DISCUSSION AND CONCLUSIONS

DISCUSSION

This is the first study, to our knowledge, that compares adult and pediatric inpatients with respect to the epidemiological and laboratory characteristics of MRSA using a prospective, laboratory-based, national surveillance program. A review of the literature identified 18 studies that compared adult and pediatric MRSA; however, the majority of these studies had methodological limitations. Compared to the 18 studies identified through the literature, this study had more robust methodology, including duration of surveillance, sample size and combination of epidemiological, strain type and antibiotic resistance profiles.

Rates

Overall, steadily increasing rates of MRSA (both infections and colonizations) were observed in Canada from 1995 to 2007 in both adult and pediatric patients with higher rates seen in adults. Other studies comparing MRSA rates or prevalence in adult and pediatric patients have also reported higher rates in adults (Kairam 2011; Denniston *et al*., 2006; Huang and Hung, 2006; Khairulddin *et al.*, 2004; Santos *et al*., 2010; Wolf *et al*., 2010; Tillotson *et al*., 2008; Hudson *et al*., 2012). Possible reasons for higher rates in adults include: a significantly greater

proportion of adults is hospitalized or require health care compared to children (Gray, 2004), adult hospitals have a larger high risk population (greater number of immune-compromised patients, critically ill patients, invasive procedures, etc.) (CDC, 2011), typical healthcare-associated risk factors for MRSA are more common in adults (Gray, 2004), better patient isolation in pediatric facilities (Gray, 2004), and children are more likely to receive care from a parent reducing the contact with healthcare workers and thus minimizing spread between patients, and the majority of pediatric nosocomial infections are viral compared to adult nosocomial infections which tend to be bacterial (Raymond and Aujard, 2000). No evidence in the literature was found that suggested children were less susceptible to MRSA but rather that the difference in rates between adults and children was attributable to demographic, epidemiological and clinical factors that differed between the two populations (Gray 2004; Gorwitz, 2008).

The trend of MRSA rates over time (1995 to 2007) was linear in adult patients, while pediatric rates followed a more exponential trend. MRSA rates were much lower in both populations at the start of the surveillance period and increased over time. The increasing rates were likely a result of continued selective pressure from antibiotic use, the spread of the resistant *Staphylococcus aureus* bacteria within hospitals, and the emergence of MRSA in the community (PHAC, 2008). However, since the start of the surveillance period, screening techniques have improved in sensitivity, the number of hospitals actively screening for MRSA has increased, and laboratory tests for MRSA have allowed more rapid diagnosis; all of which may also have contributed in part to the increasing MRSA rates (PHAC, 2008; Jarvis *et al*., 2010).

The trend of increasing MRSA rates in hospitals has been reported in Canada (Simor *et al*., 2010) and the United States (Chambers, 2001) and many other countries worldwide (CDC, 2011).

In pediatric patients, the exponential trend may be attributable to the emergence of community-associated MRSA in the early 2000's. The MRSA rates in children were very low from 1995 until 2000 (0.04 to 0.28 cases of MRSA per 1000 patient admissions; Figure 1B) and started to increase more dramatically around 2001-2003 until 2007. This is consistent with other studies that reported an increase of pediatric MRSA in the early 1990s to early 2000s, mostly attributable to increasing rates of CA-MRSA (Hussain et al., 2000; Fergie and Purcell, 2001; Purcell and Fergie, 2002; Purcell and Fergie, 2005; Chen et al., 2006; Arnold et al., 2006; Frank, 1999; Bassetti et al., 2009; Hudson *et al*., 2012). Similar to our findings, several studies of children at the Driscoll Children's Hospital, South Texas, have reported exponential increases in the number of CA-MRSA cases between 1990 and 2003 (Fergie and Purcell, 2001; Purcell and Fergie, 2002; Purcell and Fergie, 2005). They reported an increase from 0 to 9 CA-MRSA cases per year between 1990 and 1999, followed by an exponential increase to 36 cases in 2000 to 459 cases in 2003. At the University of Chicago Children's Hospital (Illinois, USA), the prevalence of CA-MRSA was reported to have increased from 10 per 100,000 admissions from 1988-1990 to 259 per 100,000 admissions from 1993-1995 (Hussain *et al*., 2000). At the Johns Hopkins Children's Center in Baltimore, a retrospective review of pediatric SA cutaneous infections from 2002 to 2003 revealed that the proportion of SA cutaneous infections caused by MRSA had significantly increased from 15% to 45% between the first and the second 6-month period (Chen *et* al., 2006). In this same time frame, the proportion of MRSA among community-associated

Staphylococcus aureus isolates significantly increased from 33% to 65%; overall, 84% of the MRSA identified were community-associated (Chen *et al*., 2006). The incidence of CA-MRSA acute osteoarticular infections in children at Le Bonheur Children's Medical Center (Memphis, Tennessee, USA) increased following the emergence of CA-MRSA in the community (Arnold *et al*., 2006). They reported an increase in the number of *S. aureus* acute osteoarticular infections in children from 2.6 to 6.0 infections per 1000 admissions from 2000 to 2004 and the percent of these infections caused by MRSA rose from 4% to 40% during this same time period (Arnold *et al*., 2006). Finally, Frank (1999) reviewed computer records of all *S. aureus* isolates from 1989-1997 at the University of Illinois Hospital, USA, and observed the first CA-MRSA isolate from a pediatric patient in 1990. The number of CA-MRSA isolates in pediatric patients increased from 2 isolates in 1993 to 8 isolates in 1997 (Frank, 1999). Overall, these studies report a dramatic rise in the proportion of CA-MRSA as well as increases in the overall pediatric MRSA rates in a similar time frame as did our study.

In this study, rates of colonization were much higher than rates of infection among adult patients (Figure 1); while similar rates of infection and colonization were observed in pediatric patients. In fact, pediatric infection rates surpassed rates of colonization only in 2006 (Figure 1). There are many factors in adult hospitals such as poor hand hygiene compliance, high occupancy rates, and problems with environmental cleaning, which promote the transmission of MRSA. In addition, it is estimated that about one third of people are colonized with *Staphylococcus aureus* and, in a healthcare setting, a significant selective pressure associated with antimicrobial use will foster the development of MRSA. All of these factors may contribute

to the high rates of MRSA colonization in hospitalized adult patients (CDC, 2011). While people can be asymptomatically colonized with MRSA, infections usually occur when the skin integrity is lost and it was expected that colonization would be more common than infection in adults. Based on our observations, this is not the case for pediatric patients. One possible reason for the similar rates of infection and colonization in pediatric patients is that active MRSA surveillance may be less common in pediatric hospitals than adult hospitals; therefore, fewer pediatric MRSA colonizations are detected. Another possibility is that our surveillance system included only hospitalized pediatric inpatients therefore excluding many community-acquired pediatric cases. Our findings suggest that the major source of MRSA in children is the community, and this has been supported by other studies in the literature (Hudson *et al*., 2012; Gorwtiz, 2008). It is possible that only children with severe infections (mainly SSTIs) acquired in the community are admitted to hospital, whereas children colonized with CA-MRSA would not be captured by this surveillance system. Overall, we did not find any studies comparing rates of colonization and infection in adults and children.

Geographic Trends

MRSA incidence rates were divided into the three major geographic regions (central, eastern, and western Canada). Regional analysis was conducted only for adult patients, as only 2 to 5 pediatric hospitals reported rates for the first 10 years of surveillance (8 in total). As a result, there were too few pediatric hospitals per region. Regional differences in rates of MRSA among adult patients were observed with Central Canada reporting the highest rates. Potential explanations for the higher rates in central Canada include the region's population and urban

density (most populous region with the most urban centres), more accurate MRSA screening techniques, and/or central region hospitals performing better active surveillance. MRSA infection rates by region were calculated since infections pose a higher burden (Figure 4). Differences in infection rates between regions were much narrower than the overall MRSA rates. While central Canada had the highest overall rates for the entire surveillance period, central Canadian infection rates were surpassed by western rates in 2003 and eastern rates in 2005. This may suggest that the higher central Canadian MRSA rates were driven by higher rates of MRSA colonization, rather than infections, particularly in the later surveillance years. One possible explanation for this trend is that screening procedures may differ regionally. For example, it is possible that more central hospitals use active surveillance or use more sensitive laboratory techniques (e.g. PCR is a newer more sensitive method that was more likely to have first been used in the larger hospitals found in central Canada) and are therefore more likely to detect MRSA colonization. Alternatively central hospitals may have higher colonization rates for reasons that were not measured in this study. Further information on infection control programs and other hospital and patient characteristics would need to be assessed in order to better understand the reasons for differences in MRSA rates between geographic regions.

No population-based studies comparing the rates of adult and pediatric MRSA by region in other countries were found in the literature. However, a study of *S. aureus* isolates from pediatric hospitals in 5 cities across Australia found differences in the prevalence of MRSA and in the antimicrobial resistance patterns (Wolf *et al*., 2010). Nimmo *et al*., 2008 reported differences in the epidemiology of MRSA in adults across similar regions of Australia and found

that adult MRSA strains varied by region. Both of these studies suggested that differing circulating strains may be responsible for the differences in prevalence or rates of MRSA between regions (Wolf et al., 2010; Nimmo *et al*., 2008). This may also be one of the contributing factors in the regional differences observed in Canada.

HA vs. CA rates

Rates of healthcare-associated (HA) and community-associated (CA) MRSA were compared between adult and pediatric patients. Consistently higher rates of HA-MRSA (both infections and colonizations) in adult patients were observed compared to pediatric patients. As well, a much larger increase was observed in the rates of adult compared to pediatric HA-MRSA infections. This suggests that HA-MRSA largely affects older patients, which is consistent with the findings in study of a Brazilian mixed hospital (adult & pediatric patients) where elderly patients tended to have higher rates of HA- MRSA colonization than younger patients (Saydoyama *et al*., 2000). Although, there is the potential of reporting bias due to the improved screening, the large linear increases observed over the surveillance period suggests that adult HA-MRSA rates truly increased from 1995 to 2007.

A very different trend for CA-MRSA rates (compared to HA-MRSA rates) was observed among adult and pediatric patients. In adults, CA-MRSA rates were much lower than HA-MRSA rates, while in children CA-MRSA rates exceeded HA-MRSA rates after 2005. Although adult CA-MRSA rates were generally higher than pediatric CA-MRSA rates, the difference was small and the trends were similar in both populations. For the initial part of the surveillance period (1995-

2001) there were relatively low CA-MRSA rates (less than 0.25 cases per 1,000 patient admissions for both infections and colonizations) in both populations. We observed exponential trends for CA-MRSA infections and this increase started slightly earlier in adults (around 2002-2003), while pediatric CA-MRSA rates started to increase around 2004-2005. However, contrary to what was observed for HA-MRSA, the trends are relatively parallel between the two populations. This suggests that CA-MRSA affects both adult and pediatric populations and that its emergence in Canadian hospitals was similar in both populations.

Overall, the CA and HA-MRSA graphs suggest that HA-MRSA largely affects adult patients, while the community is the major source of MRSA in pediatric patients. We did not find any other studies which compared HA and CA- MRSA rates between adult and pediatric patients from the same population.

Epidemiological Characteristics

Several differences in the epidemiology of MRSA in adult and pediatric populations were observed. Firstly, a significantly higher proportion of adult MRSA cases were colonization (68%) and not infections; while colonizations accounted for approximately half (51%) of pediatric MRSA cases ($p < 0.001$). As discussed previously, the higher proportion of colonizations in adults may be due to a variety of factors such as differences in screening procedures, source of acquisition of MRSA (HA or CA), MRSA strain type, and differences in the prevalence of traditional MRSA risk factors. Our data suggests a higher proportion of MRSA infections in

children, although it not possible using this surveillance data to determine the extent to which this trend is attributable to the nature of participating hospital's screening programs or other factors. However, the lower proportion of MRSA colonization among pediatric patients may be a result of less active surveillance or the fact that CA-MRSA is less likely to be associated with a positive nasal screen and may require screening of the groin or rectal area (Fritz *et al.*, 2012). CA- MRSA accounted for a higher proportion of pediatric cases than adult cases (45% CA-MRSA in pediatric vs. 16% in adults, $p<0.001$). Other studies have also found higher proportions of HA-MRSA in adults and higher proportions of CA-MRSA in children (David *et al*., 2006; Hudson et al., 2012; Naimi *et al*., 2003). In 2006, at the University of Chicago Hospitals (a tertiary care hospital) David *et al*., found that 59% of adult and 70% of pediatric inpatient isolates were CA-MRSA ($p = 0.06$,). Among MRSA isolates collected from inpatients at 30 hospitals in Orange County, California between Dec. 2008 and Sept. 2009, 36% of adult isolates were HA-MRSA compared to 25% of pediatric isolates (Hudson et al., 2012). In comparison, the proportion of HA-MRSA for 2007 in our study was 79% HA-MRSA in adults vs. 40% in children ($p<0.001$). This is much higher than the proportions of HA-MRSA in Californian hospitals, and the University of Chicago Hospitals, particularly for adult patients. Naimi *et al*. (2003) reported that CA-MRSA patients tended to be younger than HA-MRSA patients (median ages 23 years vs. 68 years, respectively, $p<0.001$).

Other epidemiologic differences observed among adult and pediatric patients with MRSA included differences in the reasons for culture and the sites of infection. The proportion of cases detected by admission screen was higher in adults (34% of adults vs. 22% of children, $p<0.001$),

while the proportion of cases detected by clinical screening was significantly higher in pediatric patients (61% of children vs. 38% of adults, $p<0.001$). The fact that adults were more likely to be detected by admission screening, combined with the observation of a higher proportion of colonizations could indicate that admission screening was more frequent in adults. Pediatric patients were more likely to be infected with CA-MRSA and a higher proportion of pediatric cases were detected through clinical isolates which may indicate that children are more likely than adults to present to hospital with an MRSA infection. Adults and children differed significantly in the sites of MRSA infection. Skin and soft tissue infections are the most common sites in both populations, however they represent a significantly higher proportion of pediatric infections (56 vs. 32%, $p<0.001$). This is consistent with the literature suggesting that the majority of MRSA infections in children are skin and soft tissue infections acquired in the community (Fergie and Purcell, 2007; Gorwitz, 2008; Elliott, 2008; Hersh *et al*., 2009). We found significantly higher proportions of surgical wounds (19 vs. 10%, $p<0.001$), respiratory infections (21 vs. 13%, $p<0.001$), bloodstream infections (13 vs. 10%, $p<0.016$), and urinary tract infections (11 vs. 4%, $p<0.001$) in adults compared to children. These findings are consistent with a review of pediatric nosocomial infections which reported that children differ from adults in the sites of nosocomial infections (Posfay-Barbe *et al*., 2008). One possible explanation for the differences in sites of MRSA infections is the different sources of MRSA in the two populations (HA-MRSA in adults and CA-MRSA in children). In the literature, CA-MRSA has been shown to be associated with SSTIs, likely explaining the high proportion of skin and soft tissue infections in pediatric

patients (Crawford and Daum, 2005; Bassetti *et al*., 2009; Fergie and Purcell, 2007; Gorwitz, 2008).

Epidemic Strain Types

PFGE strain types, SCC*mec* types and PVL toxin expression were compared between adults and children. Overall, significant differences in the MRSA strains affecting Canadian adult and pediatric hospital inpatients from 1995 to 2007 were observed. In terms of PFGE typing, the predominant strain overall was CMRSA2 (USA100) in both populations, a strain that has been shown in the literature to be typically associated with health care settings (Golding, 2008; Christianson, 2007; Nichol *et al*., 2011). However, this strain accounted for a significantly higher proportion of adult cases compared to pediatric cases ($p<0.001$). The two strains that have been previously associated with community settings (CMRSA 7 and 10 or USA400 and USA300) (Golding, 2008; Christianson, 2007; Nichol *et al*., 2011) account for the largest proportion (41%) of pediatric MRSA isolates. As well, proportions of the typically community-associated strains were significantly higher in pediatric isolates than adult isolates (CMRSA7: 18% vs. 2% and CMRSA10: 23% vs. 8%). These findings suggest that strains typically associated with health care settings were more frequently isolated in adults (CMRSA1 and CMRSA2), while strains typically associated with community settings were more frequently isolated in children (CMRSA7 and CMRSA10) and are consistent with those observed in other studies (Hudson *et al*., 2012; David *et al*., 2006).

Epidemic strain types were examined separately for infections and colonizations (Figure 6). The majority of adult infections were caused by CMRSA1 and CMRSA2, (health care associated strains), whereas CMRSA7 and CMRSA10 (community associated strains) caused the majority of infections in children. Interestingly, the strain associated with the majority of colonizations was CMRSA2 (or USA100) in both populations. Although CMRSA7 (USA400) and CMRSA10 (USA300) accounted for the majority of pediatric infections, they accounted for fewer pediatric colonizations than CMRSA2. This may be in part due to the fact that this population included only hospitalized patients and CMRSA2 has traditionally been a healthcare-associated strain (Golding, 2008; Christianson, 2007; Nichol *et al*., 2011). It is possible that the current surveillance system does not capture many children who are colonized with CA-MRSA strains because they are not likely to present to hospital, whereas a community acquired MRSA infection may be the primary reason for hospitalization among children. Alternatively, it may be that CA-MRSA strains are less likely to colonize the nares, the main site tested in admission or universal screening (Fritz et al., 2012). One American study of MRSA isolates collected in the community found that 51% of MRSA colonizations would have been missed if only nares were cultured (Miller *et al*., 2012). Other studies have found evidence to suggest that extranasal colonization may serve as an important reservoir for community-associated MRSA, particularly for USA300 strain (CMRSA10) (Szumowski *et al*., 2009; Peters *et al*., 2011; Miko *et al*., 2012). Most of these studies examined populations at high risk for CA-MRSA (drug addicts, homeless people, and sex trade workers) and found the groin was significantly associated with CA-MRSA colonization. One study found the USA300 strain (CMRSA10) was associated with multiple body

site colonization (Miko *et al*., 2012). Thus a potential explanation for pediatric infection isolates being associated with CMRSA7 and 10 while colonization isolates were associated with CMRSA2 is that the majority of screening swabs were taken from the nares in these hospitalized patients, thereby more likely to miss patients colonized with CMRSA7 and 10 at extranasal sites.

In addition to PFGE typing, the subset of the surveillance isolates sent to the NML for further testing were SCC*mec* typed and tested for the expression of the Panton-Valentine Leukocidin (PVL) gene. Adult isolates were predominantly SCC*mec* type II, while pediatric isolates were predominantly SCC*mec* type IVa. As well, pediatric isolates in this study were significantly more likely to express the PVL toxin. SCC*mec* type IVa and PVL gene expression have been associated with community-associated MRSA (both strain and surveillance definitions) in the literature (Otto *et al.*, 2010; Fergie and Purcell, 2007; Vandenesch *et al.*, 2003; Crawford and Daum, 2005; Gorwitz, 2008; Cercenado and Ruiz de Gopegui, 2008; Nichol *et al*., 2011). As discussed, we observed higher proportion of the community associated strains, CMRSA7 and 10 in CNISP pediatric isolates, and a higher proportion of pediatric patients were classified as "CA-MRSA" based on the surveillance definition of community-associated MRSA, compared to adult patients.

Isolates were separated into those that were classified, based on the surveillance definitions (determined by the clinician examining the patient or by medical chart review), as "health care associated" and "community associated," and then the proportions of different PFGE strain types were plotted for each of the two categories (Figure 6.). In the isolates from adult patients believed to have acquired MRSA in a health care setting, the majority of isolates

were the typically "HA-MRSA strains" (CMRSA2 and CMRSA1: Golding, 2008; Christianson, 2007; Otto *et al.*, 2010; Nichol *et al*., 2011). Although CMRSA2 accounted for the majority of HA pediatric isolates, CMRSA7 and CMRSA10 HA-MRSA accounted for a total of 21% of pediatric HA isolates. The majority of isolates believed to be acquired in a health care setting were caused by the typically "HA MRSA strains;" however, there was some evidence to suggest the spread of community-associated strains within the hospitals (particularly pediatric hospitals). For the isolates classified as CA-MRSA (based on the surveillance definition), the majority of pediatric isolates were CMRSA7 or CMRSA10. However, CMRSA2 accounted for the largest proportion of adults classified as CA-MRSA, followed by CMRSA10. This seems to indicate that the "typically healthcare-associated strain", CMRSA2, has spread in the community among adults. Overall, we found a fair amount of crossover between strains that were typically "healthcare-associated" and "community-associated" for both populations. One possible factor in explaining this trend is that surveillance definitions are not always specific and easy to apply. The definitions are based on the time of positive culture relative to hospital admission or the presence of risk factors and these criteria can be complicated by factors such as delays in testing or hospital readmissions. However, given the amount of crossover between HA and CA strains, our findings strongly suggest that the typically healthcare associated strains (CMRSA1 and 2) are now circulating in the community and that the community strains (CMRSA7 and 10) are now circulating in the hospitals. This so called "mixing of MRSA reservoirs" has been reported in several other studies in the literature (Hudson *et al*., 2012; Kourbatova *et al*. 2005; Liu C, *et al*. 2008 Maree *et al*., 2007; Popovich *et al*., 2008; Seybold *et al*., 2006).

The surveillance definitions of HA and CA MRSA were also plotted in comparison to a "PFGE strain type definition" of CA-MRSA and HA-MRSA (Figure 7). The PFGE strain type definition of CA-MRSA included CMRSA strains 7 and 10 and the HA-MRSA definition included CMRSA strains 1, 2, 3/6, 4, 5, 8, 9 and other MRSA strains. The proportions of HA and CA-MRSA were fairly similar between the surveillance definitions and the strain definitions. However, when we calculated predictive values using the strain definition as the reference, the predictive values were fairly low for CA-MRSA. As well, we calculated Kappa coefficients for the overall agreement between the surveillance and strain definitions of HA vs. CA MRSA. The Kappa Coefficient for adults was 0.39 (95%CI: 0.37-0.42) and for children was 0.45 (95%CI: 0.37-0.52). Based on the Altman scale (1991), the agreement for adults is "fair" (K=0.21-0.40) and for children is "moderate" (K=0.41-0.60). This suggests that despite the similar proportions classified as HA and CA-MRSA, the agreement between the surveillance strain definitions was not very strong. In combination with the results from Figure 6, this provides further evidence of merging of the reservoirs of HA and CA-MRSA strains.

Epidemiology of Community and Health Care Associated MRSA

Univariate logistic regression models were used to assess the characteristics associated with HA-MRSA. In both populations there were significant differences observed in the age, ethnicity, infection versus colonization, reason for culture, site of infection and PVL between HA and CA-MRSA. The age groups at highest risk for HA-MRSA were: patients older than 70 years

(adults) and infants age <1 year (pediatric). This is consistent with what has been reported in the literature on age-related risk for MRSA (Khairulddin *et al.,* 2004; Denniston et al., 2006; Sadoyama *et al*., 2000). On the other hand, the age group at highest risk for CA-MRSA were adults aged 25 to 54 years. Naimi *et al*., 2003 compared CA-MRSA and HA-MRSA patients and found that CA-MRSA patients were younger, with a median age of 23 years (vs. 68 years for HA-MRSA, $p<0.001$), which is similar to our findings. In children, the age distribution of CA-MRSA cases was more evenly spread among the four age categories than for HA-MRSA, although infants still comprised the largest proportion of cases. For HA-MRSA, the majority of cases were colonizations rather than infections for both populations (70% of adults and 62% of children were colonizations). However, infections comprised the majority of pediatric CA-MRSA cases (61%). This is interesting as it suggests that the higher proportion of MRSA infections in children compared to adults is driven by CA-MRSA, providing further evidence for the hypotheses that either only CA-MRSA infected children are admitted to hospital or that children colonized with CA-MRSA are missed by current screening procedures. In terms of reason for culture, adult HA-MRSA was mostly detected through clinical isolates, whereas adult CA-MRSA isolates were mostly detected by admission screen. This is likely due to the fact that CA-MRSA is detected by screening upon admission while HA-MRSA is acquired after admission screening occurs. In pediatric patients, both HA and CA MRSA were more likely to be detected by clinical isolates. One potential explanation is that CA MRSA in children often presents as skin and soft tissue infections and therefore a clinical culture may often be taken from these wounds. Another possibility is that admission screening may be less common in pediatric hospitals in comparison

to adult facilities. In both adults and children, there was a fairly even distribution of the sites of infection among HA-MRSA (although SSTIs still accounted for the majority (34%) of pediatric HA-MRSA). In comparison, a large majority of both adult and pediatric CA-MRSA infections were skin and soft tissues infections (65% of adult and 77% of pediatric CA-MRSA). As previously discussed, this is consistent with the literature that suggests the majority of CA-MRSA infections are SSTIs (Crawford and Daum, 2005, Bassetti et al., 2009; Fergie and Purcell, 2007; Gorwitz, 2008).

MRSA Strains Over Time

Trends in the proportions of different PFGE strains were plotted over time for adult and pediatric isolates in order to examine the changes in predominant strain types over the surveillance period. Differences between the predominant strains in adult and pediatric isolates were observed. For adult isolates, CMRSA1 comprised the majority of isolates until 2001 and there was a distinct replacement of CMRSA1 by CMRSA2 for the remainder of the surveillance period. This was observed in both adult colonizations and infections which may indicate that CMRSA2 developed a selective advantage over other HA-MRSA isolates. Most of the other HA-MRSA strains decreased slightly over the surveillance period.

For the pediatric isolates, it is important to note that in the early surveillance years (up until about 2001), there were very few cases of MRSA, thus the strong fluctuations in the proportions of different PFGE types are due to the small numbers of cases (total number of

cases with strain data ranged from 9 in 1995 to 31 cases in 2001). The fact that CMRSA2 accounted for the largest proportion of isolates for the majority of the surveillance period, until 2004 when it was replaced by CMRSA10 further supports that CA-MRSA emerged in children in the early 2000s and has become the predominant source of MRSA in pediatric patients.

Another difference between the adult and pediatric isolates was that CMRSA7 (USA400) accounted for a very small proportion of adult isolates (less than 5% for the entire surveillance period compared to 0% to 36% in children). Conversely, CMRSA7 was the second most common infecting strain in pediatric isolates at the end of the surveillance period (2006-2007). CMRSA7 also accounted for a much smaller proportion of pediatric colonizations. This suggests that CMRSA7 (USA400) is largely a community associated strain and that it mostly causes infections in pediatric patients in the hospital setting.

The other community strain, CMRSA10 (USA300), first appeared in adults in 1999 and increased over the remainder of the surveillance period to become the second most prevalent strain in adults by 2006. When the isolates are divided into colonizations and infections, it appeared that this trend was more prominent for infections although CMRSA10 did emerge in colonizations as well. CMRSA10 emerged later in the pediatric population, first appearing in 2003 in infection isolates. Our findings are consistent with the literature that reports the emergence of CMRSA10 or USA300 in the community around 2000 and the rapidly replaced other community strains (Fergie and Purcell, 2007; Hudson *et al*., 2012; Gorwitz, 2008; Bassetti *et al*., 2009). Other studies have hypothesized that CMRSA10 possesses selective advantages over other MRSA clones, including increased fitness relative to health care strains as a result of

fewer antibiotic resistance genes, a smaller SCC*mec* element which is more readily transmissible (usually SCC*mec*IV), and a higher growth rate which may allow this strain to outcompete other healthcare strains for colonization (Hudson *et al*., 2012; Otto, 2010; Fergie and Purcell, 2007). Our findings appear to support these hypotheses. We observed increases in overall pediatric MRSA in the early 2000s (Figure 1B), and these increases corresponded with exponential increases in CA-MRSA (Figure4B) as well as the emergence of CMRSA10 around the same time (Figures 8-10). It appears that the emergence of CMRSA10 strain may have been largely responsible for the increases in pediatric MRSA rates observed in Canadian pediatric hospitals from around 2003 until 2007. Adult CA-MRSA rates also increased around 2000 onward (Figure 5A), which corresponded with the emergence of CMRSA10 (Figures 9-11).

There have been explanations proposed in the literature to explain the rapid spread and predominance of CA-MRSA, particularly the CMRSA10 (USA300) strain (Hudson *et al*., 2012; Crawford and Daum, 2005; Gorwitz, 2008; Fergie and Purcell, 2007; Okuma *et al*., 2002; D'Agata *et al*., 2009; Diep *et al*., 2006). Compared to other strains, CMRSA 10 has been found to contain a smaller SCC*mec* element (usually type IV) which is more readily transmissible and possibly offers an advantage in terms of DNA replication speed (Hudson *et al*., 2012; Okuma *et al*., 2002; D'Agata *et al*., 2009). The Staphylococcal chromosome cassette *mec* (SCC*mec*) is a mobile genetic element that contains the *mec*A gene which confers methicillin resistance. Due to genetic polymorphisms, there are several classes of SCCmec elements (types I-V) (Crawford and Daum, 2005). HA-MRSA strains have been found to contain SCCmec type I and II, while CA-MRSA strains have been found to contain the smaller types IV and V (Gorwitz, 2008; Crawford

and Daum, 2005; Bassetti *et al*., 2009; Fergie and Purcell, 2007; Nichol *et al*., 2011; Hudson *et al*., 2012; Okuma *et al*., 2002; D'Agata *et al*., 2009). Figure 13 illustrates the proportions of different SCC*mec* types by PFGE strains. For both adults and children, more than 90% of CMRSA10 (USA300) isolates were SCC*mec* type IVa. In adults, 61% of adult CMRSA7 isolates were SCC*mec*IVa while 80% of pediatric CMRSA7 isolates were SCC*mec*IVa. These findings are consistent with the literature on CA-MRSA strains. Other advantages of CA-MRSA strains include the carriage of smaller or fewer antibiotic resistance genes, resulting in higher fitness compared to HA-MRSA strains, and a higher growth rate, favouring colonization (Hudson *et al*., 2012; Okuma *et al*., 2002; D'Agata *et al*., 2009; Crawford and Daum, 2005). As well, a recent finding of a novel mobile genetic element known as the "arginine catabolic mobile element (ACME) has been found in the CMRSA 10 (USA300) strain, enhancing the ability to survive within the host and likely conferring increased fitness or pathogenicity (Fergie and Purcell, 2007; Hudson *et al*., 2012; Diep *et al*., 2006). Overall, these molecular characteristics of CA-MRSA strains, particularly CMRSA10 may explain the emergence and replacement of other strains by CMRSA10 for both adult and pediatric populations in Canada.

In addition to the apparent selective advantages of CMRSA10 (USA300), this strain is also more likely to express regulatory genes for the toxins Panton-Valentine Leukocidin (PVL) and alpha-toxin which may confer increased virulence and increased invasiveness of infections caused by CMRSA10 relative to CMRSA7 and other strains (Hudson *et al*., 2012; Otto, 2010; Bassetti *et al*., 2009; Crawford and Daum, 2005; Fergie and Purcell, 2007; Montgomery *et al*., 2008). In Figure 11 we plotted the proportions of PVL gene expression among the different PFGE

strain types in order to examine whether this trend held true for our population. Consistent with previous studies, a high proportion of CMRSA10 (USA300) isolates were PVL positive, for both adult (97%) and pediatric (100%) isolates. As well, several studies have reported that community strains are associated with PVL expression, including CMRSA7, so it was not surprising that a high proportion of adult and pediatric CMRSA7 isolates were PVL positive (61% and 81%, respectively). A relatively high proportion (44%) of pediatric "other strains" (this included CMRSA 4, 5, 8, and 9 and other non-CMRSA strains) were PVL positive. A closer examination revealed this was due to PVL expression in 8 of 8 European, 9 of 9 USA1100, SWP/Oceania, 4 of 8 USA1000, China/Taiwan, 1 of 1 Danish MRSA strains; all of which accounted for a small proportion of the total pediatric isolates but had high PVL expression. Since *SCCmec* type IV has been associated with community strains of MRSA the proportion of PVL positive isolates by *SCCmec* type was examined (Figure 12). The majority of *SCCmec* type IVa were PVL positive in both adults and children, which further demonstrates the association between community strains (CMRSA7 and 10), *SCCmec*IVa, and PVL gene expression.

In the literature, there have been reports of increasing numbers of MRSA skin and soft tissue infections admitted to hospitals as a result of increasing CA-MRSA infections (Crawford and Daum, 2005; Gorwitz, 2008; Fergie and Purcell, 2007; Bassetti et al., 2009; NeVille-Swensen and Clayton, 2011). As well, increasing virulence associated with CA-MRSA strains expressing the PVL gene has also been reported (Montgomery et al., 2008; Otto, 2010; Fergie and Purcell, 2007; Bassetti et al., 2009). For this reason, the strains and PVL expression by infection site were examined. CMRSA2 was responsible for the largest proportion of all infection sites with the

exception of pediatric SSTIs. CMRSA10 accounted for the largest proportion of pediatric SSTIs (41%) and the second largest proportion of adult SSTIs (26%). Although CMRSA2 accounted for a larger proportion of the other infection sites in pediatric patients, SSTIs were the most common infection site in children. The fact that a typically health care associated strain (CMRSA2) accounted for larger proportions of other infection sites is not surprising given that this is a hospital setting, therefore other types of infections like surgical wound infections, respiratory, urinary and blood infections are more likely to occur in hospitalized patients. It is interesting to note that adult SSTIs were still mostly associated with CMRSA2, suggesting a difference in the epidemiology of MRSA in adults and children. MRSA SSTIs acquired in the community followed by presentation to a hospital appear to be more common in children, whereas hospitalized adults appear to acquire infections mainly through health care contact.

The *SCCmec*IVa was the predominant *SCCmec* type in most pediatric infection sites, while *SCCmec*II was the predominant type in adult infections (except for SSTIs). PVL negative was more common for most infection sites with the exception of pediatric SSTIs (76%) and pediatric bloodstream infections (62%). Since more than half of isolates from pediatric bloodstream infection were PVL positive, this may support the role of PVL toxin expression in the invasiveness of CA-MRSA strains, but our study is cross-sectional and does not provide sufficient evidence to examine this hypothesis.

Antibiotic Resistance

Given that previous studies reported differences in antibiotic resistance patterns between the HA & CA- MRSA, we examined differences in antibiotic resistance between the adult and pediatric isolates over 3 surveillance periods (selected based on trends in adult MRSA rates). (Cernado and Ruiz de Gopegui, 2008; David *et al*., 2006; Herold *et al*., 1998; Hussain *et al*., 2000; Bassetti et al., 2009; David *et al*., 2006; Kallen et al., 2000; Ellis *et al*., 2004; Dietrich *et al*., 2004; Buckingham *et al*., 2004; Frank *et al*., 2002; Naimi *et al*., 2003).

Throughout the entire surveillance period, adult isolates were significantly more likely than pediatric isolates to be non-susceptible (resistant or intermediate) to most antibiotics (Table 5). For a few antibiotics (mainly fusidic acid, rifampin), resistance patterns were similarly low in both populations, indicating similar selective pressures for these antibiotics. Overall, resistance among MRSA isolates was fairly high to clindamycin, erythromycin, and ciprofloxacin, while relatively lower to TMP-SMX, fusidic acid, tetracycline, mupirocin, and rifampin. Resistance to clindamycin and erythromycin is associated to the same gene mutation, whereas resistance to ciprofloxacin only requires a single gene mutation – both mainly associated with HA-MRSA strains; this may explain why rates of resistance were high in both adult and pediatric populations (Gorwitz, 2008; Crawford and Daum, 2005). Mupirocin was the only antibiotic where pediatric isolates showed significantly higher resistance than adult isolates. Looking at the time periods, it is apparent that this trend occurred in the third surveillance period (2004-2007) and in this period, both pediatric CA and HA-MRSA isolates were significantly more

resistant than adult isolates. One likely explanation for this may be the selective pressure caused by an increased use of mupirocin in pediatric patients for the treatment of MRSA. There has also been a study conducted on Canadian MRSA isolates, which found evidence of increasing mupirocin resistance, particularly in CA-MRSA isolates (Simor et al., 2007). This study examined 4,980 MRSA isolates obtained between 1995 and 2004 from 32 Canadian hospitals, and found increases in the proportion of MRSA isolates with high-level mupirocin resistance. They found that patients with high level mupirocin resistance were significantly more likely to have CA-MRSA and be aboriginal (Simor et al., 2007). Thus another possible reason that we observed higher mupirocin resistance in pediatric patients is that a higher proportion of pediatric MRSA was community-associated.

The generally higher resistance in adult MRSA isolates in our study population is consistent with several studies in the literature which also found higher resistance to most antibiotics in adult compared to pediatric MRSA isolates (Marais *et al*., 2009; Park *et al*., 2007; David et al., 2006; Wolf *et al*., 2010). As discussed in the literature review, Marais *et al*. (2009) compared 190 adult (≥18 years) and 44 pediatric (<18 years) MRSA isolates collected from 15 National Health Laboratory Services and 8 private diagnostic laboratories from all 9 provinces of South Africa from 2005 to 2006. The adults and children had similar resistance profiles to most of the antimicrobial agents but significantly lower resistance in pediatric isolates for erythromycin and clindamycin. There were no isolates resistant to vancomycin in the study by Marais and colleagues (2009), consistent with the CNISP population. Although the resistance profiles of the South African population differed from those of the current Canadian population, they also

found significant differences between adults and children and generally higher resistance in adults. Park *et al*. (2007) compared 50 adult and 88 pediatric MRSA isolates from a hospital in Korea. They found significantly higher resistance in adult strains compared to the pediatric strains against gentamicin, erythromycin, clindamycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and rifampin. This was consistent with what was found in this study of Canadian isolates. However, while there was higher resistance to mupirocin in Canadian pediatric isolates, the pediatric strains in Korea had significantly higher resistance to trimethroprim-sulfamethoxazole. In an in-patient population from the University of Chicago Hospitals, there were significant differences between 288 adult and 177 pediatric CA-MRSA isolates (David *et al*., 2006). Adult isolates had statistically significantly greater non-β-lactam resistance compared to pediatric isolates for erythromycin, clindamycin, ciprofloxacin, and tetracycline. These findings are similar to this CNISP population although the resistance to clindamycin and ciprofloxacin were lower than what was found in this study.

Adult isolates are generally more resistant than pediatric isolates for a number of reasons. MRSA first emerged in adults in health care settings as a result of the selective pressure resulting from antibiotic use (Elliott, 2008; Bassetti et al., 2009; CDC, 2011). In addition to the increased antibiotic exposure, there are many more hospitalized adult patients, and therefore more susceptible patients. There are larger numbers of adults who are chronically ill, undergo invasive medical procedures, have compromised immune systems, and are prescribed antibiotics (Elliott, 2008; Lo and Wang, 2011; Coello *et al*., 1997; Laupland, 2008; Fukuta *et al*., 2012; Herold *et al*., 1998). Studies have also found that elderly patients harbour MRSA with

higher resistance (or resistance to a greater number of antibiotics), thus elderly patients (≥65 years old) may serve as a reservoir for resistant MRSA (Sader *et al*., 2012; Denkinger *et al*., 2013). The combination of the selective pressure caused by increased antibiotic exposure and the large number of susceptible patients may explain the higher proportions of resistance developing in MRSA affecting adults, and spreading among this population. As well, adult MRSA was more likely to be caused by the typical "health care associated," CMRSA1 and 2 and more likely to be classified as "HA-MRSA" based on the surveillance definition. We found consistently higher resistance in HA-MRSA compared to CA-MRSA (Table 6), and observed higher proportions of the typically HA-MRSA strains in adults. HA-MRSA strains have been found to be more resistant to antibiotics than CA-MRSA strains, which may explain why adult isolates were more resistant to antibiotics than pediatric isolates. As well, the differences in antibiotic resistance profiles between adults and children may be due to different prescribing practices in the two populations. The higher resistance to mupirocin in pediatric isolates provides evidence for this. Further, there have been other studies in the literature that found significant differences in adult and pediatric antibiotic susceptibility profiles, and proposed different prescribing practices as a potential explanation (David *et al*., 2006; Wolf *et al.,* 2010).

Overall, we found that MRSA isolated from patients classified as HA-MRSA (based on the surveillance definition) had higher resistance to the majority of antibiotics in comparison with patients classified as CA-MRSA. This trend held true for both adult and pediatric isolates throughout the 13-year surveillance period. As mentioned, there were several other studies reporting higher resistance in HA-MRSA in comparison to CA-MRSA (Kallen *et al*., 2000; Ellis *et*

al., 2004; Dietrich *et al*., 2004; Buckingham *et al*., 2004; Frank *et al*., 2002; Naimi *et al*., 2003; Cercenado and Ruiz de Gopegui, 2008; Herold *et al*., 1998; Hussain *et al*., 2000; Bassetti *et al*., 2009). One study reported similar rates of non-β-lactam resistance between pediatric and adult HA-MRSA isolates; however, they did find significantly less resistance in pediatric CA-MRSA isolates compared to adults (David *et al*., 2006).

This trend of higher resistance in HA-MRSA compared to CA-MRSA is not a new one. The same trend was observed in the emergence of penicillin-resistant *Staphylococcus aureus* first in hospitals, followed by the emergence in the community starting in the 1940s (Bassetti et al., 2009). There are several proposed reasons for which HA-MRSA is generally more resistant to antibiotics than CA-MRSA. One reason is the increased selective pressure for the development of resistance as a result of the much more frequent use of antibiotics in health care settings. As well, health care settings have more susceptible patients with typical risk factors for acquiring Staphylococcus – including MRSA, such as invasive procedures, prolonged hospital stays, chronic medical conditions, and antibiotic use (Lo and Wang, 2011, Coello *et al*., 1997, Laupland, 2008, Fukuta *et al*., 2012, Herold *et al*., 1998). Specifically, the fact that hospitalized patients are more likely to have previous, recent, and/or frequent antibiotic use explains the higher antibiotic resistance in health care associated strains. As well, hospitalized patients have compromised immune systems and undergo invasive medical procedures, which increases the population at risk for pathogens such as MRSA. The differences in antibiotic resistance between HA and CA-MRSA may also relate to the molecular characteristics of the MRSA strains. As previously discussed , the *SCCmec*IV elements associated with CA-MRSA strains (CMRSA7 and CMRSA10)

are smaller and may contain fewer resistance genes, possibly explaining the lower antibiotic resistance found in CA-MRSA isolates relative to HA-MRSA isolates (Hudson et al., 2012; Fergie and Purcell, 2007; Bassetti et al., 2009).

In addition to the higher proportions of isolates that were found to be resistant to the majority of antibiotics, adult isolates and HA-MRSA isolates were significantly more likely to be resistant to 3 or more non-β-lactam antibiotics (multi-resistant). The proportions of multi-resistant isolates were significantly higher in adults compared to children for the entire surveillance period. Moreover, in both populations, there was higher multi-resistance in HA-MRSA isolates compared to CA-MRSA isolates. This finding is consistent with that reported by other studies (Wolf *et al*., 2010 and Naimi *et al*., 2003; Bassetti et al., 2009). The higher proportions of multi-resistance are likely due to the reasons described previously. Given that the majority of adult isolates were HA-MRSA and a higher proportion of pediatric isolates were CA-MRSA, it is not surprising that adult isolates were more likely to be multi-resistant to non-β-lactams antibiotics.

In terms of resistance trends over time, resistance to most antibiotics was relatively constant over time in both adult and pediatric isolates. However, the resistance to TMP-SMX and tetracycline decreased quite markedly over the three time periods. This was in contrast to what was expected given the continuous selective pressure from antibiotic use in hospitals and the tendency for resistance to increase over time. One possible explanation is that CA-MRSA is less resistant to antibiotics and the proportions of CA-MRSA strains as well as the proportion of cases classified as CA-MRSA based on the surveillance definition increased over time in both

adults and children. Although the antibiotic susceptibility tables were stratified into HA and CA, this was based on the surveillance definition, and as shown in figure 6, community strains have been found among patients classified as HA-MRSA. Since no data on antibiotic usage or antimicrobial stewardship programs were collected, this study was not able to determine the reason for these trends.

Hospital Factor Models

In order to determine whether there may be any hospital level characteristics that could help to predict hospitals with higher rates of MRSA, we modeled hospital MRSA rates using Marginal Models (Generalized Estimating Equations) and Generalized Linear Mixed Effects Models (GLMEM). We built two separate models for adult and pediatric facilities, based on evidence from the literature review and from this study that the epidemiology of MRSA is different in these two populations. Given the small sample sizes (only 8 pediatric hospitals and 24 adult hospitals) and the fact that some models did not converge, we also constructed a model combining adult and pediatric hospitals that included a dichotomous predictor variable of adult vs. pediatric hospital. However, when we combined the two hospital types, we obtained the same convergence issues as with the adult only model (the Marginal model would not converge using an appropriate correlation matrix). This suggested that the combined sample size was not sufficient to overcome the sample size issue. Therefore, we determined that a combined model did not add any value, and that the effect of adult hospitals was dominating the combined model. We decided it was best to present two separate models, supported by the differing epidemiology observed between the two populations as well as the

fact that it allowed for a comparison of the different predictors associated with MRSA rates in adult versus pediatric hospitals.

Both Marginal Models and GLM Mixed Effect Models were modeled for adult and pediatric hospitals. This was done because we had to rely on model-based parameter estimates for the marginal models (due to the small number of clusters), which are less robust and susceptible to mis-specification of the correlation matrix (Fitzmaurice et al., 2004). Therefore we wanted to ensure that we obtained similar results in the two models, even though the parameters would not be exactly the same.

For the adult models an autoregressive correlation matrix or a m-dependent (where m is a number of time points) correlation matrix were the most appropriate for the data given that adult MRSA rates increased linearly over time. Autoregressive assumes a decaying correlation over time so that rates closer together in time are more strongly correlated than rates further apart in time. The m-dependent correlation matrix (mdep(3)) assumes that all measurements 1 time point apart are equal, and the same for measurements 2 and 3 time points apart and that time points more than 3 measurements apart have zero correlation. When the adult hospital rates were modeled, the marginal models would not converge using an autoregressive or an m-dependent correlation matrix. An exchangeable correlation matrix was deemed inappropriate as this type of correlation matrix assumes equal correlation between all time points (ex. that the rates in 1995 and 1996 were as equally correlated as the rates in 1995 and 2007). Therefore, only the GLMEM model could be used for adult hospitals.

For pediatric models, we fit the marginal models using an autoregressive correlation matrix and this seemed to be the most appropriate correlation structure for the data. However, when the GLMEM model was fit to the pediatric data the parameters were not similar to the marginal model and the parameters flipped when multiple variables were added in the same model (for example the parameter for year became negative which is opposite what was observed in the graph of the rates over time). We determined that the marginal model using GEE was most appropriate for the pediatric data.

In examining the pediatric MRSA hospital rate trends, it appears that a single hospital that joined the CNISP program in 2005 may have driven the exponential trend. This hospital appears to be an outlier with much higher rates (range between 4.3 and 14.6 MRSA cases per 10,000 patient days) than the other pediatric hospitals (the highest rate in the other pediatric facilities was 5.5 per 10,000 patient days). As a sensitivity analysis, the pediatric average rate was recalculated excluding this hospital. The average pediatric hospital rate excluding this hospital still followed a strong exponential trend over time (R^2=0.93 versus R^2=0.94 for the average including the outlier). While the higher rates at that particular CNISP pediatric facility may represent an MRSA outbreak or higher rates that could be due to a variety of reasons (higher community rates, active surveillance, better detection methods, etc.), this sensitivity analysis supports the observation of an exponential increase in the pediatric hospital rates across Canada during this surveillance period (1995-2007). This hospital was kept in the pediatric model because it did not alter the trend of pediatric hospital rates over time.

We tested the hospital number of beds and the number of FTE infection control professionals separately in the models and we tested the ratio of beds per FTE ICP in order to assess which was best to model the MRSA rates. Both options were not significant predictors of pediatric hospital MRSA rates. However, in the adult model, the number of beds and the number of ICP were significant predictors, whether these were modeled separately or as a ratio. Based on the hypothesis that the true "infection control effect" of the number of infection control professionals per hospital would be dependent on the workload, we decided to include the variables as a ratio in the final model. This was supported by the 2011 PIDAC recommendations which stated that hospitals should have no more than 100 beds per infection control professional (PIDAC 2011) and by a systematic review that found both bed occupancy and understaffing to be associated with higher MRSA rates (Kaier *et al*., 2012). Based on the PIDAC recommendation we also dichotomized the variable to >100 beds or ≤100 beds per ICP.

Interaction terms between year and hospital characteristics were tested in the bivariate models. This was done in order to assess whether the effect of any of the predictors varied over time. For pediatric hospitals, the interaction terms did not meet the criteria for inclusion in the multivariate model ($p>0.1$). For adult hospitals, the interaction between year and hospital occupancy rate, and the interaction between year and region met the criteria for inclusion in the multivariate model. Both interaction terms met the criteria for retention in the multivariate model ($p<0.05$), however the estimate for the year became negative. Based on the graphs that show strong linear trend of MRSA rates over time, this estimate was judged to be incorrect. This result is possibly due to the small number of clusters (18 hospitals) relative to the number of

repeated measures (13) and relative to the number of parameter estimates (8) in the model including interaction terms. For these reasons, we decided that the best final multivariate model was the model that excluded the interaction terms.

In the final multivariate models, hospital occupancy was predictive of both adult and pediatric hospital MRSA rates. However, the ratio of beds per FTE ICP was also a significant predictor of adult hospital MRSA rates but not pediatric rates, and the proportion of high-risk age group was significant only in the pediatric model. It was not surprising that the models differed, firstly because different types of models (Marginal and GLMEM) were used, but also given the differences in the epidemiology of MRSA in the two populations. It is important to note that the interpretations of the parameter estimates differ in the two different models.

It was not surprising to find that hospital occupancy (or bed occupancy) was a significant predictor of hospital MRSA rates in both populations, given that overcrowding in hospitals has been associated with higher rates of nosocomial infections, particularly MRSA (Borg et al., 2009; Conrad et al., 2010; Kaier et al., 2010; Kaier et al, 2012). A recent systematic review examined 22 articles on MRSA and 22 on other HAI and found increasing rates of MRSA with increasing bed occupancy and understaffing (Kaier et al., 2012). For the adult model, having greater than 100 beds per FTE ICP was a significant predictor of MRSA rates which supports the 2011 PIDAC recommendation stating that hospitals should have no more than 100 beds per infection control professional (PIDAC 2011). In addition, it could be suggested that excess workload on ICPs result in higher rates of MRSA because there is less time for teaching, auditing, surveillance and cohorting. However, the design of this study does not allow us to assess this causal relationship.

It is also possible that larger hospitals have a larger ratio of beds per FTE ICP and part of this association may be due to other unmeasured factors in this analysis such as the proportion of severely ill patients, types of medical procedures, differences in antibiotic prescribing practices or differences in screening programs for MRSA at different hospitals. Interestingly, a study comparing infection control programs in Canadian acute care hospitals found that the number of hospitals with one FTE ICP per 100 beds increased from 1999 to 2005, but this increase was not associated with a decrease in healthcare-associated infection rates, as of yet (Zoutman *et al*., 2008).

On the other hand, neither the number of FTE ICP, the number of hospital beds, nor the ratio of beds per ICP were not found to be significant predictors of increased MRSA rates in pediatric hospitals. This may be due to the small sample size (8 pediatric facilities) or that the number of beds and number of ICPs does not vary greatly between these pediatric facilities. Alternatively, the number of beds and ICP may not affect the rates of MRSA in pediatric facilities given that the main source of MRSA is the community, thus patients are admitted with MRSA and infection control measures by ICP do not impact overall hospital rates of MRSA. It may be that the ratio of beds per ICP would be predictive of HA-MRSA rates in pediatric facilities if we were able to model HA and CA MRSA rates separately. As suggested earlier, another reason for which the ratio of beds per ICP was not a predictor of pediatric hospital MRSA rates may be that infection control in pediatric facilities may be different compared to adult facilities. Pediatric hospitals may have better patient isolation and parents who actively participate in the patient care which increases one-to-one care for patients.

In pediatric hospitals, the proportion of patients aged 0 to 1 year was a significant predictor of MRSA rates. This is consistent with several other studies in the literature which found that neonates and patients in neonatal intensive care units (NICUs) are at increased risk relative to other pediatric patients for most healthcare acquired infections, including MRSA, as a result of their immature immune systems, prolonged hospital stay, and medical conditions requiring invasive procedures (Posfay-Barbe *et al*., 2008; Denniston *et al*., 2006; Babazono *et al*., 2008; Bizzarro and Gallagher, 2007; Burke *et al*., 2009; Carey *et al*, 2008; Carey *et al*, 2010; de Almeida Silva *et al*., 2003; Magara *et al.*, 2011). Although several studies have found that elderly patients are at higher risk for MRSA, patient age was not significant in the adult model (Denkinger *et al*., 2013; Fascia *et al*., 2009; Gaszynska *et al*., 2011; Hautala et al., 2008; Marchaim et al., 2010; Martin, 2009). One study found that elderly patients (≥65 years old) were associated with a substantial influx of multi-drug resistant organisms into the hospital and had significantly higher rates of MRSA compared to patients less than 65 years old) over a 12-year surveillance period (1998 to 2009) (Denkinger *et al*., 2013). Patient age (65 years or more) was not a significant predictor in our adult model and may be due to the small sample size, the fact that age is based on MRSA patients and not the hospital denominator (age of patient admissions or patient days), and/or the proportion of adult patients over 65 years of age is high in the majority of CNISP adult facilities.

To our knowledge, this is the first study to model both adult and pediatric hospital characteristics to predict MRSA hospital rates. We found only one article comparing adult and pediatric MRSA that assessed hospital-level characteristics in association with MRSA. In 2012

Hudson *et al*., modeled the individual (adult/pediatric, specimen source, community/hospital onset, and ward – ICU/non-ICU) and hospital factors (annual admissions greater or less than 10,000, LTAC facility, percentage of Hispanic patients, and percentage of Medicaid-insured patients) that were predictive of spa type t008 (USA300 or CMRSA10) using GLMEM models. This study predicted hospital characteristics associated with the community strain, USA300, but not overall MRSA rates. In multivariate analyses, they found that isolates from pediatric patients, wounds, non-ICU wards, and hospitals with a high proportion of Medicaid-insured patients were significantly associated with USA300 (CMRSA 10). This model predicted hospitals that are at risk for higher CA-MRA rates, while our model was aimed at determining the characteristics that predict overall MRSA burden and thus provide additional information on which to tailor hospital infection control programs.

STRENGTHS AND LIMITATIONS

The data analyzed for this thesis is subject to a number of limitations specifically given the inherent limitations of surveillance data (Brookmeyer and Stroup, 2004). Surveillance is conducted in order to establish baseline rates, detect outbreaks, reduce healthcare-associated infections, compare hospitals, guide treatment or prevention strategies, assess the impact of infection control measures, or to generate hypotheses concerning risk factors (Lautenbach and Woeltje, 2006). The data collected in surveillance programs are guided by these objectives and therefore will often not include all of the variables that may be necessary to adequately answer

research questions. Surveillance studies can therefore only be considered hypothesis generating as they are not sufficient to establish causation. For this thesis, the main research questions were determined retrospectively and the surveillance dataset was not designed for research purposes. We aimed to compare the epidemiology of MRSA in adults and children; however, we were not able to compare the individual risk factors for MRSA between these two populations because the risk factors that have been associated with MRSA in the literature were not captured by the CNISP surveillance system. In order to assess individual patient risk factors, information on underlying medical conditions, length of hospital stay, history of previous hospitalizations, medical procedures, and history of patient antimicrobial use would have been required (Lo and Wang, 2011; Coello *et al*., 1997; Laupland, 2008; Fukuta *et al.* 2012; Herold *et al*., 1998). As well, data on potential confounders such as socioeconomic status or race are not collected as part of the surveillance program and consequently any model of MRSA risk factors using this surveillance system would have been biased.

Another limitation of routine surveillance data is that it is often incomplete and tends to underestimate the true number of cases of the disease (Brookmeyer and Stroup, 2004). Some of the CNISP surveillance data was incomplete as many variables had missing data. Furthermore, for the current surveillance program, both the individual patient and hospital questionnaires were modified annually as a result certain variables were only collected for a subset of the surveillance period under study. In terms of the accuracy of the case estimates of this surveillance system, data is limited to inpatients from participating CNISP hospitals and therefore is systematically missing all MRSA cases that are not identified in a participating CNISP

hospital. Missed cases could include those in non-CNISP facilities, those in the community, and those that went undetected in a CNISP hospital (such as patients who acquired MRSA in hospital but were missed by screening.). The number of hospitals participating in the CNISP MRSA surveillance program increased over the surveillance period from 22 hospitals in 1995 to 48 hospitals in 2007. Therefore there was likely a differential under representation of the true healthcare associated MRSA rate and a differential underestimation of the true number of MRSA cases in Canada from the earlier part of the surveillance period to the later part of the surveillance period. A recent study was done to assess the representativeness of the CNISP program by comparing CNISP and non CNISP acute care hospitals which used 2006 Canadian census data and geospatial mapping to determine the proportion of the Canadian population served by CNISP hospitals (Rutledge-Taylor, 2012, ahead of publication). This study found that approximately 78% of the Canadian population lives within a 100-kilometre radius of a CNISP hospital; however there are no hospitals participating from the Yukon, Northwest Territories, or Nunavut. As well, non-CNISP hospitals had a larger proportion of small hospitals (less than 100 beds) and a much smaller proportion of non-CNISP hospitals had intensive care beds. Therefore the CNISP program is representative of a majority of the Canadian population; however, it lacks representativeness in the Canadian territories (regions which include large aboriginal populations who are at high risk for MRSA (Embil et al., 1994; Mitchell et al., 1996). In addition, CNISP hospital MRSA rates are not representative of smaller, rural, and non-acute care facilities. (Rutledge-Taylor, 2012, ahead of publication). A recent study of 3 northern remote communities in Saskatchewan, Canada (Golding *et al*., 2011), found very high rates of MRSA (146-482 per

10,000 population) and 98% of the strains were CMRSA7 (USA400), suggesting the epidemiology of MRSA is likely different in rural northern Canadian communities, than what has been reported in CNISP hospitals. Finally, given this is a sentinel-hospital based surveillance system that was designed to monitor the number of MRSA cases in Canadian healthcare facilities, the rate estimates only apply to MRSA in healthcare settings. The system was not designed to capture the total number of community-associated MRSA cases and the estimates of community MRSA rates apply only to the cases that present to hospital.

For MRSA rate calculations, all mixed hospitals were excluded because separate adult and pediatric denominator data were not available. Therefore rates were determined using the 24 adult and 8 pediatric stand-alone facilities. When examining the geographic trend in MRSA rates, only the adult facilities could be divided by geographic region given the small number of pediatric facilities. In terms of geographic representativeness, the 3 regions were fairly equally represented among adult facilities, with more hospitals from the central region from 1999 onwards but this is representative of the Canadian population distribution. For pediatric facilities, there was no representation of the eastern or western regions until 1997, and as with adult facilities, central Canada had the largest representation. The northern territory regions were not represented among adult or pediatric facilities.

We did several analyses comparing MRSA based on the source (HA and CA-MRSA). This included rate calculations, comparisons of the epidemiological characteristics, comparisons of antibiotic resistance profiles as well as comparing the surveillance definition with the strain definition of HA and CA-MRSA. One limitation that applies to all of these analyses was the fact

that the surveillance definition was determined based on clinical judgement of the examining physician, applying pre-established criteria (based on the literature and published in the CNISP surveillance protocols). As mentioned previously, the criteria depend on the time of positive culture relative to hospital admission which can be difficult to determine if there are delays in testing, hospital readmissions, healthcare facility transfers, or any other complicating factors. As a result, the source of MRSA (based on surveillance definition) was unknown for around 15% of adults and around 25% of children and there is potential for misclassification bias. The cases where the source was unknown were excluded for all analyses comparing HA and CA MRSA.

In terms of the laboratory component of the CNISP program, only a subset of the MRSA isolates are sent to the National Microbiology Laboratory for further characterization. As a result, molecular characterization data for all patients identified in this surveillance dataset does not exist. However, the isolates sent for further characterization were a representative subset and therefore should represent the strains circulating in CNISP hospitals. Among the subset sent to the NML, not all were SCC*mec* typed (3269 of the 13727 that were PFGE typed) or tested for the PVL gene (5449 of the 13727 that were PFGE typed).

Limitations of Hospital Factor Models

The hospital-level factor analysis had several limitations. First, sample sizes were small, with only 18 adult facilities and 8 pediatric facilities included in the final models. Based on the rule of thumb for GEE marginal models with fewer than 25 clusters, we had to use model-based

parameter estimates, which are less robust and more susceptible to mis-specification of the correlation matrix (Fitzmaurice *et al*., 2004). As well, several other problems with the sample may have introduced error or contributed to the problems with model convergence. This included: the small number of clusters relative to the number of repeated measures (8 pediatric and 18 adult hospitals with 13 repeated measures), unequal repeated measures between the different hospitals (range of 2 to 13 repeated measures) and there were multiple hospitals with only 2 or 3 years of data.

There were several limitations to the hospital profiles collected as a part of this surveillance program. The data collected varied yearly and therefore there were missing values for several predictor variables. Consequently, the data were mostly missing not at random (MNAR), but were systematically missing for all hospitals from 1995-1997 and 2006-2007. This may have introduced a source of error in the parameter estimates. As a result there is a possibility of differential under-estimation of the MRSA rates in earlier years relative to later years due to diagnostic or detection bias (improved detection methods, increasing number of hospitals using active surveillance over time). This may have contributed resulted in overestimating the effect of time and some hospital characteristics on hospital MRSA rates.

Another major limitation to the hospital profile dataset is that it is a part of a surveillance program and therefore limited data was collected. There are several factors that have been found to be predictive of MRSA in the literature that we would have wanted to include in the model but the data were not collected as part of this surveillance. For example more accurate measures of infection control programs would have improved the model but we were limited to

just a few measures such as number of beds, number of FTE ICP, occupancy rates which are more proxy measures of infection control implementations. In addition, including data on hand washing rates, antimicrobial stewardship programs, environmental cleaning, isolation facilities or practices, and surveillance methods would also have contributed to the model as these factors have been associated with MRSA rate reduction in the literature (Duerden, 2012; Borg et al., 2009; Teltsch et al., 2011; Simmons, 2011; Dancer, 2009; Graf et al., 2009; Pofahl et al., 2009). Further studies would be needed to assess the impact of infection control program implementations (such as antimicrobial stewardship implementation, staff training, hand washing, active screening, patient cohorting, etc.) by comparing MRSA rates before and after. In addition, more information relating to patient demographics of the CNISP hospitals, given that several studies have found marginalized and low socio-economic status populations to be at increased risk for MRSA (Dailey *et al*., 2005; Bratu *et al*., 2006). In Canada, aboriginal peoples have been found to be at increased risk for MRSA (Embil *et al*., 1994; Mitchell *et al*., 1996). Although an ethnicity variable (aboriginal/non-aboriginal) was collected in the hospital profile dataset, this variable was missing for more than 50% of patients and is suspected to have been poorly collected. For these reasons it was judged to be too inaccurate for inclusion in the model. As discussed, the CNISP program does not adequately represent northern geographic regions where a large proportion of these high-risk populations reside.

The only patient demographic variable included was the proportion of high-risk age group patients (age 0 to 1 year for pediatric and 65 and older for adults). This age variable is further limited by the fact that the proportions were based on MRSA patients and not on the overall

hospital populations. Given that these populations are at highest risk for MRSA, the proportions likely overestimate the true proportion of the hospital population that are in these age groups, however we would expect the proportions to be collinear with a measure based on the entire hospital populations (hospitals with larger proportion of patients age 65 years and older would be more likely to have a higher proportion of MRSA patients age 65 and older). Nonetheless, this is a potential source of error in our data.

Based on evidence from the literature, some of the other factors that should be considered in prediction models for hospital MRSA rates include: the procedures and services performed at the hospitals (Sadoyama et al., 2000; Coello et al., 1997; Bereket *et al*., 2012), hospital antibiotic usage or stewardship (Aldeyab et al., 2008; Kaier 2012; Lee et al., 2010; Cook et al., 2011), the movement between hospitals and long-term care facilities (Barnes et al., 2011; Lee et al., 2013), healthcare worker MRSA carriage (Boisseau et al., 2012), and the virulence of circulating strains in different regions (Bratu *et al*., 2006). In pediatric facilities specifically, NICU patients have been reported to be at increased risk for HAI including MRSA and for this reason, a variable on the number of NICU beds may be included in the pediatric model (Posfay-Barbe *et al*., 2008; Cipolla et al., 2011). Given that this study was not able to include many of the hospital characteristics that have been associated with MRSA rates or are suspected to be associated with MRSA rates in the literature, our models are therefore limited and should be considered as hypothesis generating.

For our study, we modeled all MRSA cases, including both infections and colonization. This method may be helpful for predicting factors that increase overall MRSA rates, although ideally

we would have liked to have built other models using MRSA infections alone as the outcome. One potential bias of modeling both infections and colonization is that hospitals with better detection methods and/or active screening may have had higher MRSA colonization rates (and infection rates to some extent) as a result of detection bias. Generally speaking, the larger hospitals would be more likely to have started using better detection methods (like PCR) and active screening before smaller hospitals, thus the effect of some hospital predictor variables (number of beds, hospital occupancy, number of ICPs, proportion of high-risk age group patients) that are associated with hospital size may have been exaggerated. Given more data, we would also like to have modeled HA-MRSA and CA-MRSA outcomes separately It may be that the characteristics that predict rates of HA and CA-MRSA differ, which could have implications for infection control strategies. For example, hospitals with higher rates of CA-MRSA may need different strategies for infection prevention and control than those with higher rates of HA-MRSA.

Although our study had several limitations and the conclusions are largely hypothesis generating, the study also had a number of advantages. The dataset was collected over 13 years with a very large sample size of 37,907 adults and 1,292 children. As well this national surveillance program has relatively good geographic representativeness (with the exception of northern areas). In the literature we found only 2 other studies comparing adult and pediatric MRSA with national data. The first examined only MRSA in Australian pediatric hospitals and compared the findings with adult data found in the literature and only looked at data from 2006 (Wolf et al., 2010). The second study collected MRSA samples from adults and children across

the 9 provinces of South Africa, however the sample size was small; only 46 children and 190 adult isolates, with limited comparisons of the two populations (some comparison of the proportions of antibiotic resistance) (Marais *et al*., 2009). This study was able to directly compare adults and children from the same population and during the same time period. 14 of the 18 articles comparing adult and pediatric MRSA directly compared adult and pediatric isolates or patients. The majority of these articles had small sample sizes, only looked at one specific type of MRSA infection, or only looked at strain types, resistance profiles, or epidemiological information, but not the combination of all three. In this surveillance dataset, the epidemiological data is linked with the laboratory data. This study was able to assess national MRSA rates, patient characteristics (including sex, MRSA status, reason for culture, and site of infection), MRSA molecular characteristics (PFGE typing, SCCmec typing, and PVL gene expression) and antibiotic resistance profiles. No study was found in the literature that compared adults and children on all of these characteristics of MRSA.

FUTURE RESEARCH AND IMPLICATIONS

Overall, the results from this surveillance program indicate differences in the epidemiological and laboratory characteristics of MRSA among adult and pediatric Canadian inpatients. MRSA rates were much lower in pediatric patients, likely as a result of parental involvement in patient care and better infection control practices in general. The major source of MRSA in pediatric patients was the community, which underlines the importance of

admission screening in pediatric facilities and the culture of any child presenting with a skin and soft tissues infection. Given the evidence in the literature suggesting the possibility of missing colonization with CA-MRSA strains by swabbing only nares, screening isolates may need to be taken from multiple sites in pediatric patients. Based on a rapid literature review this question does not appear to have been addressed in a health care context, and should be examined in future studies. More research is needed to examine the infection control practices in adult and pediatric facilities, particularly to assess whether there are any control measures that may be more effective in adults compared to children.

Adult isolates had higher resistance to all antibiotics than pediatric isolates and this suggests the need for a different treatment protocol in the two populations. The only oral antibiotic that maintains low resistance in both populations was trimethoprim-sulfamethoxazole and this is currently the drug of choice for the treatment of pediatric infections. For both populations, CA-MRSA had much lower resistance to most antibiotics and is therefore easier to treat. However, we found relatively high resistance to clindamycin, erythromycin, and ciprofloxacin in both populations (as high as 96% in adults and 83% in pediatric isolates), supporting the importing of cultures with susceptibility testing.

This study was done using surveillance data, future prospective studies are needed that are designed to answer research questions. More information is required on risk factors for MRSA in adults and children, using individual-level information. The CNISP MRSA surveillance program does not include any hospitals from the Canadian territories or from small, rural, or non-acute care facilities therefore more studies are required in order to determine the epidemiology of

MRSA in these settings. The target population of the CNISP surveillance programs is hospitalized patients in Canada; therefore this program is not able to assess the epidemiology of MRSA in the community setting. Surveillance in the community is lacking and this may have implications for hospital MRSA rates since the community serves as a reservoir for MRSA. Future studies should examine the epidemiology of MRSA in the community.

The hospital questionnaires for this surveillance program were limited in the information collected. Further studies could include antibiotic use, more accurate measures of infection control programs, hospital patient demographics (such as race, age, socioeconomic status) to predict hospital MRSA rates. Finally, our analysis (especially the hospital level analysis) was limited in the number of hospitals included; therefore, a larger sample size of hospitals is required in order to assess the hospital factors that predict MRSA rates.

CONCLUSIONS

Canadian MRSA rates have increased steadily since 1995 in both adult and pediatric patients although the rates were much higher in adults. Rates in adults increased linearly and rates in children increased exponentially from 1995 to 2007. There were significant differences in the clinical presentation as well as the molecular characteristics of MRSA in adults and children. While children are more likely to have community-acquired MRSA, adults are more likely to acquire MRSA in a health care setting. MRSA in children was significantly more likely to be PFGE strain type CMRSA7 or 10, SCC*mec* type IVa, and PVL positive. Conversely, MRSA in

adults was more likely to be PFGE strain type CMRSA2, SCC*mec* type II and PVL negative. Adult isolates and health care associated isolates were significantly more resistant to the majority of antibiotics than pediatric and community-associated isolates. These differences are likely due to the source of MRSA (health care associated in adults) and different prescribing practises. Overall, our findings may support the need for different infection control measures (routine practices and additional precautions) and different treatment protocols in pediatric and adult patients.

The hospital characteristics that predict higher rates of MRSA differ in adult and pediatric facilities. The factors that may predict higher rates of MRSA in adult hospitals are high occupancy rates and having a ratio of more than 100 beds per infection control professional. On the other hand the factors that may be predictive of high MRSA rates in pediatric facilities include high occupancy rates and a high proportion of patients ages 0 to 1 year old.

Further research is needed to establish more accurate models for the prediction of MRSA hospital rates. As well, future studies should examine the differences in adult and pediatric MRSA risk factors, and whether admission screening should include extranasal swabs for the detection of CA-MRSA.

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APPENDICES

Appendix A: Thesis Committee Biographies

Aboubakar Mounchili is a Doctor of Veterinary Medicine (DVM) and Master's of Science graduate from the Kharkov State Zooveterinary Academy in Ukraine. He also holds a PhD in Epidemiology from the University of Prince Edward Island (UPEI) and currently works for the Public Health Agency of Canada as a Senior Epidemiologist with the Healthcare-Associated Infections Surveillance and Epidemiology Section. From 2007 to 2009, Dr. Mounchili was an Assistant Professor with the Department of Health Management of the UPEI where he taught Epidemiology courses at both undergraduate and graduate levels. During the same period, he also served as Scientific Advisor within the Animal Health Risk Assessment Unit at the Canadian Food Inspection Agency (CFIA).

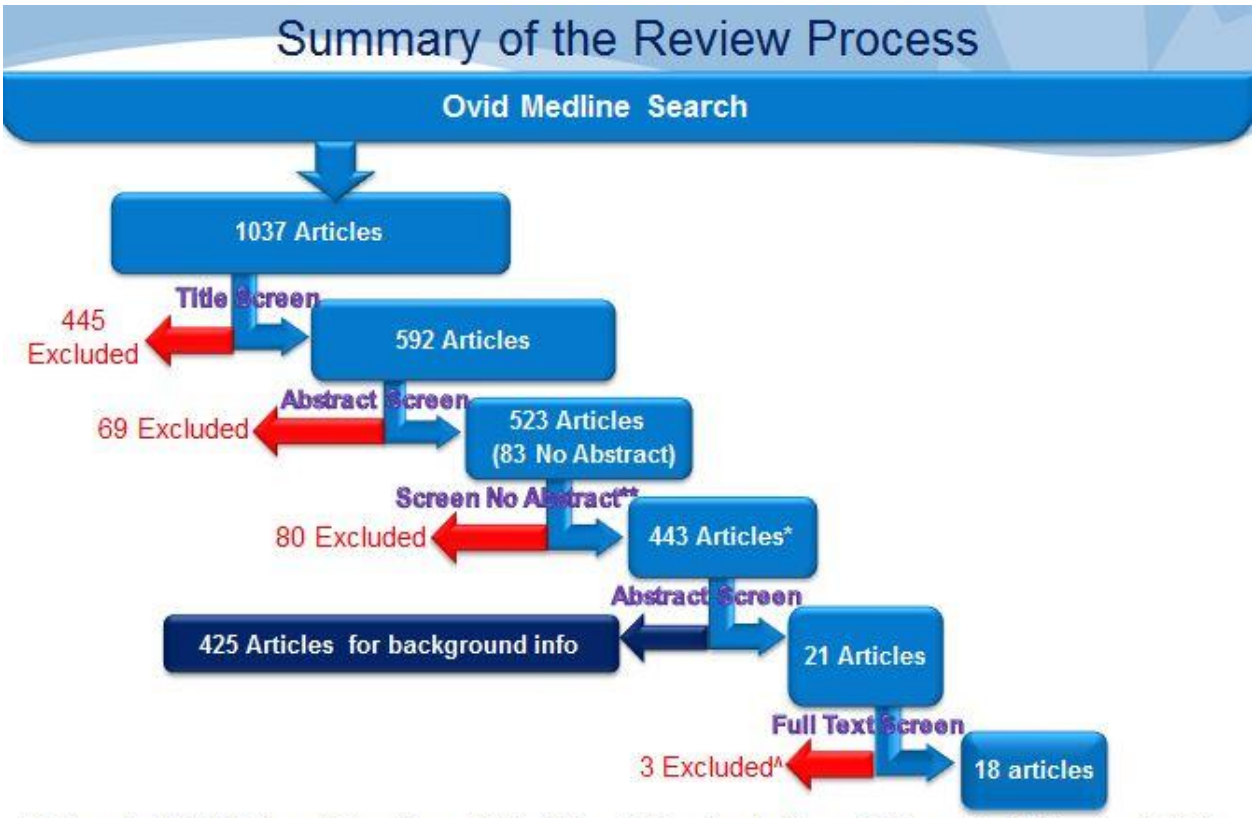
Linda Pelude has a Master's of Science in Epidemiology from the University of Ottawa. She is currently a Senior Epidemiologist with the Healthcare Acquired Infections Surveillance and Epidemiology (HAISE) Section at the Public Health Agency of Canada. Through HAISE national surveillance is conducted and rates are produced on various healthcare acquired infections including MRSA, VRE, CDI, CVC-BSI. Currently 54 hospitals in 10 provinces across Canada participate in this surveillance through HAISE's Canadian Nosocomial Infection Surveillance Program (CNISP). In addition, Linda works on social network studies that relate to the transmission of disease in hospitals and among injection drug users. She has extensive clinical background working in intensive care units of acute care hospitals and with injection drug users and sex trade workers though mobile needle exchange programs.

Dr. Caroline Quach is an Associate Professor of Pediatrics and an associate member of the Department of Epidemiology and Biostatistics at McGill University. She is the co-director of the McGill University Health Center Vaccine Study Center, is the Associate Infection Control Physician for Child and Adolescent Services of the McGill University Health Centre, and chairs the Montreal Children's Hospital Infection Control Committee. She also works as a pediatric infectious diseases consultant and a medical microbiologist at The Montreal Children's Hospital. She holds a cross-appointment at the Quebec Institute of Public Health (INSPQ) where she works in the Healthcare-Associated Infections and Immunization branches.

Dr. Quach graduated from the Université de Montréal Medical School, completed her pediatric residency training at the CHU Sainte-Justine, and her post-graduate Infectious Diseases and Medical Microbiology training at McGill University where she also obtained a Masters of Sciences in Epidemiology. She is a clinician scientist and holds a salary award from the Fonds de

recherche en santé du Québec (FRSQ – chercheur clinicien junior 2). Her research interests are focused on the prevention of infections – both healthcare-associated infections and vaccine-preventable diseases. She currently serves on the Quebec Immunization Committee (CIQ), the National Advisory Committee on Immunization (NACI) and chairs two provincial bloodstream infections surveillance programs (central line associated bloodstream infections in ICUs and all healthcare-associated blood stream infections) at the INSPQ.

Appendix B: Literature Review Process Chart



*37 General, 190 Risk Factors, 13 Surveillance, 50 Microbiology, 26 Prevalence/Incidence, 19 HA vs. CA, 148 International; **Full Text screen for articles where no abstract was available ^3 articles excluded

Appendix C: Literature Review Inclusion / Exclusion Criteria

Inclusion Criteria:

Population:

- Articles that compare pediatric and adult MRSA patients

Outcomes: Articles that include the following outcomes will be included:

- MRSA patient demographics
- MRSA case characteristics
- Hospital characteristics that are associated with MRSA
- MRSA infection and colonization rates
- MRSA infection and colonization prevalence
- Risk factors for risk factors and MRSA infection or colonization
- MRSA laboratory characteristics (including SCCmec, Strain types, PVL gene expression, antimicrobial resistance profiles)

Study Design:

- Epidemiological studies
- Surveillance studies, data, and reports
- Laboratory studies

Language:

- English or French

Publication:

- Articles published from 1990 onward

<u>Exclusion Criteria</u>:

Population:

- Articles that present data on adult MRSA patients only
- Articles that do not separate data by age
- Articles that present data on MSSA (methicillin-susceptible *S. aureus*) patients only

Outcomes:

- Demographics, rates, case characteristics for colonized patients only (articles that exclude outcomes on infected patients)
- Infection control program outcomes
- Articles that do not include separate data on MRSA (ie. Articles that combine other hospital-acquired infections with MRSA)
- Comparison of MSSA and MRSA
- therapy strategies for MRSA
- decolonization or clearance of MRSA as the main outcome
- risk factors for re-occurrence of MRSA
- Cost analysis as the main outcome

Study Design:

- Case studies
- Intervention studies
- Non-human studies (animal studies)

Language:

- Articles in any language other than English or French

Publication:

- Articles published prior to 1990
- Duplicate articles to 1990

Appendix D: Example of a completed SIGN checklist

(SIGN and Sleith, 2012)
Produced by: Carolyn Sleith
Version 3.0
20/11/2012

Methodology Checklist 3: Cohort studies

SIGN

Study identification (*Include author, title, year of publication, journal title, pages*)

Naimi, T. S., LeDell, K. H., Como-Sabetti, K., Borchardt, S. M., Boxrud, D. J., Etienne, J., Lynfield, R. (2003). Comparison of community- and health care-associated methicillin-resistant staphylococcus aureus infection. JAMA : The Journal of the American Medical Association, 290(22), 2976-2984. doi: 10.1001/jama.290.22.2976

Guideline topic: Adult vs. pediatric MRSA – HA vs. CA-MRSA	Key Question No:1	Reviewer:TL

Before completing this checklist, consider:

1. Is the paper really a cohort study? If in doubt, check the study design algorithm available from SIGN and make sure you have the correct checklist.
2. Is the paper relevant to key question? Analyse using PICO (Patient or Population Intervention Comparison Outcome). IF NO REJECT (give reason below). IF YES complete the checklist..

Reason for rejection: 1. Paper not relevant to key question ☐ 2. Other reason ☐ (please specify):

Please note that a retrospective study (ie a database or chart study) cannot be rated higher than +.

SECTION 1: INTERNAL VALIDITY

In a well conducted cohort study:		***Does this study do it?***
1.1	The study addresses an appropriate and clearly focused question.[i]	Yes ☑ No ☐ Can't say ☐
SELECTION OF SUBJECTS		
1.2	The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation.[ii]	Yes ☑ No ☐ Can't say ☐ Does not apply ☐

1.3	The study indicates how many of the people asked to take part did so, in each of the groups being studied.[iii]	Yes ☑	No □ Does not apply □
1.4	The likelihood that some eligible subjects might have the outcome at the time of enrolment is assessed and taken into account in the analysis.[iv]	Yes □ Can't say □	No □ Does not apply ☑
1.5	What percentage of individuals or clusters recruited into each arm of the study dropped out before the study was completed.[v]	Can't say	
1.6	Comparison is made between full participants and those lost to follow up, by exposure status.[vi]	Yes □ Can't say □	No ☑ Does not apply □

ASSESSMENT			
1.7	The outcomes are clearly defined.[vii]	Yes ☑ Can't say □	No □
1.8	The assessment of outcome is made blind to exposure status. If the study is retrospective this may not be applicable.[viii]	Yes ☑ Can't say □	No □ Does not apply ☑
1.9	Where blinding was not possible, there is some recognition that knowledge of exposure status could have influenced the assessment of outcome.[ix]	Yes □ Can't say □	No ☑ □
1.10	The method of assessment of exposure is reliable.[x]	Yes ☑ Can't say □	No □

1.11	Evidence from other sources is used to demonstrate that the method of outcome assessment is valid and reliable.[xi]	Yes □ No ☑ Can't say □ Does not apply□
1.12	Exposure level or prognostic factor is assessed more than once.[xii]	Yes □ No □ Can't say □ Does not apply ☑
CONFOUNDING		
1.13	The main potential confounders are identified and taken into account in the design and analysis.[xiii]	Yes □ No □ Can't say □
STATISTICAL ANALYSIS		
1.14	Have confidence intervals been provided?[xiv]	Yes □ No □
SECTION 2: OVERALL ASSESSMENT OF THE STUDY		
2.1	How well was the study done to minimise the risk of bias or confounding?[xv]	High quality (++) □ Acceptable (+)☑ Unacceptable – reject 0
2.2	Taking into account clinical considerations, your evaluation of the methodology used, and the statistical power of the study, how strong do you think the association between exposure and outcome is?	
2.3	Are the results of this study directly applicable to the patient group targeted in this guideline?	Yes ☑ No □
2.4	**Notes.** Summarise the authors conclusions. Add any comments on your own assessment of the study, and the extent to which it answers your question and mention any areas of uncertainty raised above. -High quality evidence for the main research question of this paper (differences between community and healthcare associated MRSA) however the quality of the evidence for our research question (differences between adult and pediatric patients) is acceptable due to limited information on methods and limited number of analyses. Overall quality assessment of "acceptable"	

[i] Unless a clear and well defined question is specified in the report of the review, it will be difficult to assess how well it has met its objectives or how relevant it is to the question you are trying to answer on the basis of the conclusions.

[ii] This relates to **selection bias**.* It is important that the two groups selected for comparison are as similar as possible in all characteristics except for their exposure status, or the presence of specific prognostic factors or prognostic markers relevant to the study in question.

[iii] This relates to **selection bias**.* The participation rate is defined as the number of study participants divided by the number of eligible subjects, and should be calculated separately for each branch of the study. A large difference in participation rate between the two arms of the study indicates that a significant degree of **selection bias*** may be present, and the study results should be treated with considerable caution.

[iv] If some of the eligible subjects, particularly those in the unexposed group, already have the outcome at the start of the trial the final result will be subject to **performance bias.*** A well conducted study will attempt to estimate the likelihood of this occurring, and take it into account in the analysis through the use of sensitivity studies or other methods.

[v] This question relates to the risk of **attrition bias**.*The number of patients that drop out of a study should give concern if the number is very high. Conventionally, a 20% drop out rate is regarded as acceptable, but in observational studies conducted over a lengthy period of time a higher drop out rate is to be expected. A decision on whether to downgrade or reject a study because of a high drop out rate is a matter of judgement based on the reasons why people dropped out, and whether drop out rates were comparable in the exposed and unexposed groups. Reporting of efforts to follow up participants that dropped out may be regarded as an indicator of a well conducted study.

[vi] For valid study results, it is essential that the study participants are truly representative of the source population. It is always possible that participants who dropped out of the study will differ in some significant way from those who remained part of the study throughout. A well conducted study will attempt to identify any such differences between full and partial participants in both the exposed and unexposed groups. This relates to the risk of **attrition bias**.* Any unexplained differences should lead to the study results being treated with caution.

[vii] This relates to the risk of **detection bias**.* Once enrolled in the study, participants should be followed until specified end points or outcomes are reached. In a study of the effect of exercise on the death rates from heart disease in middle aged men, for example, participants might be followed up until death, or until reaching a predefined age. **If outcomes and the criteria used for measuring them are not clearly defined, the study should be rejected.**

[viii]This relates to the risk of **detection bias.*** If the assessor is blinded to which participants received the exposure, and which did not, the prospects of unbiased results are significantly increased. Studies in which this is done should be rated more highly than those where it is not done, or not done adequately.

[ix] This relates to the risk of **detection bias.*** Blinding is not possible in many cohort studies. In order to assess the extent of any bias that may be present, it may be helpful to compare process measures used on the participant groups - e.g. frequency of observations, who carried out the observations, the degree of detail and completeness of observations. If these process measures are comparable between the groups, the results may be regarded with more confidence.

[x] This relates to the risk of **detection bias.*** A well conducted study should indicate how the degree of exposure or presence of prognostic factors or markers was assessed. Whatever measures are used must be sufficient to establish clearly that participants have or have not received the exposure under investigation and the extent of such exposure, or that they do or do not possess a particular prognostic marker or factor. Clearly described, reliable measures should increase the confidence in the quality of the study

[xi] This relates to the risk of **detection bias.*** The primary outcome measures used should be clearly stated in the study. **If the outcome measures are not stated, or the study bases its main conclusions on secondary outcomes, the study should be rejected.** Where outcome measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study.

[xii] This relates to the risk of **detection bias.*** Confidence in data quality should be increased if exposure level is measured more than once in the course of the study. Independent assessment by more than one investigator is preferable.

[xiii] Confounding is the distortion of a link between exposure and outcome by another factor that is associated with both exposure and outcome. The possible presence of confounding factors is one of the principal reasons why observational studies are not more highly rated as a source of evidence. The report of the study should indicate which potential confounders have been considered, and how they have been assessed or allowed for in the analysis. Clinical judgement should be applied to consider whether all likely confounders have been considered. If the measures used to address confounding are considered inadequate, the study should be downgraded or rejected, depending on how serious the risk of confounding is considered to be. **A study that does not address the possibility of confounding should be rejected.**

[xiv] Confidence limits are the preferred method for indicating the precision of statistical results, and can be used to differentiate between an inconclusive study and a study that shows no effect. Studies that report a single value with no assessment of precision should be treated with extreme caution.

[xv] Rate the overall methodological quality of the study, using the following as a guide: **High quality** (++): Majority of criteria met. Little or no risk of bias. Results unlikely to be changed by further research. **Acceptable** (+): Most criteria met. Some flaws in the study with an associated risk of bias, Conclusions may change in the light of further studies. **Low quality** (0): Either most criteria not met, or significant flaws relating to key aspects of study design. Conclusions likely to change in the light of further studies.

Appendix E: CNISP Chronology

In 1995 CNISP surveillance included:

1A, 02A, 03A, 04A, 05A, 06A, 07A, 08A, 08B, 08C, 09A, 10A, 11A, 12A, 13A, 14A, 15A, 15B, 16A, 17A, 18A, 20A

Total : 22 sites

In 1996 the following occurred:

Addition:

19A

Total : 23 sites

In 1997 the following occurred:

No new additions or deletions

In 1998 the following occurred:

Addition:

15C and 15D

Deletion:

12A

Total : 24 sites

In 1999 the following occurred:

Addition:

02B, 09B, 20J, 20G, 20S, 21A, 21B, 21C, 21D, 21E

Changes:

Site 08B amalgamated with site 07A

Total : 33 sites

In 2000 the following occurred:

Deletion:

13A

Total : 32 sites

In 2001 the following occurred:

Additions:

06B, 06C, 23A, 23B

Changes:

site amalgamation (site 09A and 09B)

Total: 35 sites

In 2002 the following occurred:

Addition:

25A

Total : 36 sites

In 2003 the following occurred:

Addition:

26A

Total : 37 sites

In 2004 the following occurred:

No changes noted in 2004

Total: 37 sites

In 2005 the following occurred:

Addition:

28A

24A

6 sites: 01B-G

Deletions:

(site 15C and 15D report with 15A)

Total: 43 sites

In 2006 the following occurred:

Addition:

07B, 07C, 07D, 23C, 27A, 29A, 30A

05C starts reporting separate from 05A

Deletion: 07A

Changes:

Site 07A retired

Represents data from UHN to end of 2005

07A replaced by 07B, 07C & 07D beginning in 2006

part of site 23A, as of 2006 reporting separately as 23C

Total: 50 sites

In 2007 the following occurred:

Addition:

Site 04B

Total: 51

Deletion: Site 01C reporting with 01A

Total:50

In 2008 the following occurred:

Deletion: 30a **Total: 49**

Appendix F: Sample Surveillance Protocol and Patient Questionnaire (2007)

Canadian Nosocomial Infection Surveillance Program

MRSA Surveillance Protocol

Surveillance for
Methicillin-resistant *Staphylococcus aureus* (MRSA)
In CNISP health care facilities

Version December, 2006

INTRODUCTION

Prior to 1995, national data describing the incidence and epidemiology of MRSA in Canada were not available. In 1995, national surveillance for MRSA was started in sentinel hospitals participating in the Canadian Nosocomial Infection Surveillance Program (CNISP) and has been ongoing.

The Canadian Nosocomial Infection Surveillance Program (CNISP) is a collaborative effort of the Canadian Hospital Epidemiology Committee (CHEC), a subcommittee of the Association of Medical Microbiologists and Infectious Disease (AMMI) and the Centre for Infectious Disease Prevention and Control (CIDPC) of the Public Health Agency of Canada.

Established in 1994, the objectives of CNISP are to provide rates and trends on nosocomial infections at Canadian health care facilities thus enabling comparison of rates (benchmarks), and providing evidence-based data that can be used in the development of national guidelines on clinical issues related to nosocomial infections. As of the year 2006, 48 sentinel CHEC sites (which may be networks of more than one hospital), with 8 paediatric stand alone sites and 29 CHEC members from 9 provinces participate in the CNISP network.

CHEC members participate in CNISP by working on sub-committees that direct the development, implementation and analysis of surveillance projects in CNISP. CHEC hospitals will often have ICP representative sitting on these committees. CHEC members participate voluntarily in CNISP projects by collecting standardized, case-by-case, non-nominal data on hospitalized patients. The data is submitted to CNISP for compilation and analysis.

MRSA data collected reflects all "newly-identified" MRSA cases from the CHEC hospitals. Colonized MRSA cases who are identified in prevalence surveys or during outbreak investigations are included in the data. If these cases should become infections this will not be picked up since cases are only included once unless reinfected at another time with a different strain. The rates provided therefore reflect these limitations (CHEC site only and only "newly identified" cases) and should be interpreted with this in mind.

GOALS AND OBJECTIVES

The objectives of this surveillance project are as follows:

1. To determine the incidence and burden of illness associated with MRSA in CNISP hospitals.
2. To describe the epidemiology of MRSA in Canada.
3. To characterize the molecular strains of MRSA in Canada.

METHODOLOGY

MRSA surveillance inclusion criteria

MRSA case definition:

- isolation of *Staphylococcus aureus* from any body site

AND

- resistance of isolate to oxacillin

AND

- patient must be admitted to the hospital.

AND

- is a "newly identified MRSA cases" at a CHEC facility
  - This includes:
    - MRSA cases identified for the first time;
    - Cases that have been previously identified at other non-CHEC sites (since we want newly identified MRSA cases at CHEC sites)
    - Cases that have already been identified at your site but are new cases. This can only be identified if the previously identified case has another strain. This means the person was exposed again to MRSA and acquired another strain of it from another source (a new Patient identifier should be assigned).

This DOES NOT include:

- MRSA cases previously identified at other CHEC sites
- Emergency, clinic or other outpatient cases
- Cases re-admitted with MRSA (unless it is a different strain)

Healthcare-associated definition:

Once the patient has been identified with MRSA, they will be classified as healthcare-associated based on the "best judgment" of the practitioner. This judgment should include review of:

- length of time in hospital prior to MRSA identification (generally >72 hours)
- knowledge of previous MRSA status
- date of admission
- length of stay in hospital
- prior hospitalization or other healthcare facility history (previously admitted in past 12 months)
- where patient admitted from (e.g., long-term care)

Newborn healthcare-associated case definition:

A MRSA case in a newborn may be considered as healthcare associated if the mother was not known to be a case on admission and where there is no epidemiological reason to suspect that the mother was colonized prior to admission, even if the newborn is < 48 hours of age.

In the case of a newborn transferred from another institution, MRSA may be classified as healthcare-associated if the organism was not known to be present and there is no epidemiological reason to suspect that acquisition occurred prior to transfer.

Community case definition:

No established health-care associated risk factors, and:

(i) hospitalized < 72 hours;
(ii) no previous history of MRSA;
(iii) no medical devices such as urinary catheters, IV lines, feeding tubes, tracheostomy, dialysis access, etc.
(iv) no history of hospitalization, surgery, or dialysis within 1 year of MRSA culture;
(v) not in residence at a long term care facility within 1 year of MRSA culture.

Data collection

Epidemiological data

Surveillance for MRSA is laboratory-based. Upon laboratory identification of MRSA from an in-patient for the first time, the infection control professional (ICP) is to be notified. A chart review to collect the patient's demographic and clinical information will then be conducted and data recorded.

Data elements will include:

- Unique identifier
- Date of birth
- Sex
- Ethnicity
- Culture date
- Reason for the culture
- Where MRSA was acquired (nosocomial or community)
- Anatomical site of MRSA isolation

Data will be collected using standardized data extraction forms that are revised at the beginning of every year (Appendix B). Any revisions to the form are to be made by the MRSA working group. Suggestions for revisions to the data extraction forms are collected throughout the surveillance year and then discussed with the working group at the end of the year. Those collecting the information are encouraged to make comments to improve the surveillance mechanism. Definitions and additional instructions for the completion of this form are provided in Appendices C and D.

WEBBS data entry

All MRSA cases are to be entered into the WEBBS internet surveillance data entry screens. Data can be changed in the case of errors in data, however data should not be updated if status changes from colonized to infected if patient was initially identified as colonized.

Denominator data - Rates

To obtain the necessary denominator information for the calculation of national MRSA rates, each participating health care facility has been completing a hospital profile on an annual basis. Data collected on this profile includes the number of *Staphylococcus aureus* isolates tested each year, the annual number of patient hospital admissions and the annual number of patient days spent in-hospital.

Laboratory data

MRSA isolates will be sent to the National Microbiology Laboratory (NML) in Winnipeg. Upon arrival, the cultures will be streaked for purity and stored. A duplicate set of strains will be sent to Sunnybrook lab for storage and additional testing. The strains will be confirmed as being MRSA using PCR to detect the *mecA* gene. Susceptibility testing and molecular typing using pulsed-field gel electrophoresis (PFGE) will also be conducted on submitted isolates. In certain cases, some strains will be further characterized using multi-locus sequence typing, identification of the Panton-Valentine Leukocydin (PVL) toxin, and *Staphylococcal* chromosomal cassette *mec* (SCCmec) typing.

Year 2007 Criteria for isolates:

Only clinical isolates that result in infection are to be sent to the lab. Therefore any isolates that are only colonized or clinical isolates that do not result in an infection do not need to be sent to the National Microbiology Lab (NML). It is therefore essential that ICP communicate to their laboratories which isolates (infected cases only) need to be saved and sent to the NML since the lab will not be able to determine this. The present criterion is different from past years' requirements. This new criterion for sending only infected clinical isolates will significantly decrease the number of isolates previously sent. Large and small number (MRSA) sites are to send in ALL of their infected clinical isolates.

Isolates should be sent to the following address:

National Microbiology Laboratory

Surveillance Period

Surveillance for MRSA was initiated in January 1995 and will continue to be an ongoing CNISP surveillance project.

ANALYSIS AND EVALUATION

Patient data collection forms will be completed at the CHEC sites and entered into the CNISP WEBBS surveillance site via the internet or batched and sent monthly to the Nosocomial and Occupational Infections Section (NOI) of the Public Health Agency of Canada for data entry.

National rates of MRSA will be calculated by patient days, *Staphylococcus aureus* isolates tested, and per patient admissions. Regional and national rates will be published. The incidence of MRSA among hospitalized patients, geographic trends and descriptive epidemiology of MRSA will be reported via CNISP reports, presentations and publications.

ETHICS

While this surveillance project is observational and does not involve any alteration in patient care, ethics approval may be sought at some hospital sites. Surveillance for nosocomial infections is a routine component of quality assurance and patient care in Canadian health care institutions and therefore informed consent is not required. A unique identifier linked to patient name will only identify patients at the local CHEC site and is not transmitted to the Public Health Agency of Canada. All data submitted is kept strictly confidential.

Attached Appendices:

Appendix A: MRSA patient questionnaire
Appendix B: Case definitions and data dictionary

Appendix A:

Surveillance of Methicillin Resistant *Staphylococcus aureus* (MRSA) A Newly Diagnosed Patient Questionnaire 2007

1.	CHEC Site # (see attached detail notes for explanation)	
2.	Unique Identifier Code (must include site #, year and three digit consecutive code e.g. 07A-07-001)	
3.	Date of Birth	____/____/____ DD MMM YYYY
4.	Date of Admission	____/____/____ DD MMM YYYY
5.	Sex	☐ Male ☐ Female
6.	Ethnicity	☐ Not First Nations ☐ First Nations ☐ Unknown
7.	What was the date of this patient's newly identified MRSA culture?	____/____/____ DD MMM YYYY
8.	Why was the first culture done? (Check one answer only)	☐ Admission screen ☐ Other screening ☐ Clinical Isolate ☐ Other Indication (please specify): _____
9.	Where was the MRSA acquired? (Check one answer only)	Health-care associated ☐ your facility ☐ another acute-care facility ☐ a long-term care facility ☐ another healthcare exposure ☐ Community-associated *(hospitalized < 72 hours, no previous history of MRSA, no hospital or long-term care admission in previous 12 months, no medical devices)* *If community, give the postal code of the patients' home address (first 3 digits only)* __ __ __ ☐ Unknown

10.	Is this patient epidemiologically linked to others within your institution?	☐ Yes ☐ No	
11.	At which site(s) has MRSA been isolated (positive culture obtained)?		
	Site of positive culture [1] (check each positive site)	Infected or Colonized	
	☐ Blood	☐ Infected	
	☐ Surgical Wound	☐ Infected	☐ Colonized
	☐ Other skin or soft tissue/burn	☐ Infected	☐ Colonized
	☐ Sputum / Respiratory	☐ Infected	☐ Colonized
	☐ Urine	☐ Infected	☐ Colonized
	☐ Nose		☐ Colonized
	☐ Rectum / Peri-anal / Perineum	☐ Infected	☐ Colonized
	☐ Other (please specify):_____ _____	☐ Infected	☐ Colonized
12.	What type of infection? (At the time patient was initially identified, within 72 hours of initial culture or associated with this episode by the best judgement of the ICP)	Check all that apply ☐ no infection ☐ urinary tract infection ☐ necrotizing fasciitis ☐ surgical wound ☐ other (please specify): _____ _____ ☐ pneumonia, non-necrotizing ☐ necrotizing pneumonia ☐ soft tissue infection, non-necrotizing ☐ surgical wound, non-necrotizing ☐ osteomyelitis ☐ catheter-related blood stream infection ☐ bacteremia, no source identified ☐ endocarditis ☐ meningitis ☐ conjunctivitis ☐ septic arthritis/bursitis ☐ other (please specify)_____ _____	

APPENDIX B:

MRSA Questionnaire Data Dictionary- definitions and notes

The numbers in these instructions correspond with the numbers of the questions on the surveillance form.

1. **CHEC Site #:**
This will be the 3-character alphanumeric number assigned to your institution. It will always begin with the two digit number assigned to your CHEC member e.g., 07, 15, and a letter assigned by the CHEC member for that specific institution e.g., A, B, C, etc. The CHEC Site # for each institution should always be the same for all the CHEC/CNISP surveillance projects and will always have all three alphanumeric digits reported as the CHEC Site #, e.g., 07A, 15A.

2. **Unique identifier:**
This number should never be longer than 8 characters. The 8 characters should consist of the 3-character CHEC site # (e.g., 09A), the Year the MRSA case occurred in (e.g., 05), and a consecutive number starting at 001 and continuing on with each additional case. An example of the first case in an Institution would be 09A05001. An example of the thirty-fifth case would be 09A05035, and so on. Include this ID on the lab isolate. If this is a re-infection please assign a new patient identifier.

3. **Date of Birth:**
Please enter Day (##), Month (e.g., May) and Year (2006) in this order

4. **Date of Admission:**
Please enter Day (##), Month (e.g., May) and Year (2006) in this order of the date of admission to hospital as an inpatient.

5. **Sex:**
Check male or female gender as appropriate

6. **Ethnicity:**
Ethnicity will be defined by the patient. When completing the questionnaire ask the patient, "Do you consider yourself a member of Canada's First Nation peoples, for example, Inuit, Aboriginal, Indian, Metis etc." If the patient answers 'yes' to the above question please check 'First Nations'. If the patient does not answer 'yes', check 'not-First Nations'.

7. **What was the date of this patients' newly diagnosed positive MRSA culture:**
MRSA culture defined as a *S. aureus* with oxacillin MIC >=4 mg/ml, growing on oxacillin screen plate and the presence of PBP2a detected by latex agglutination test. Enter Day (##) Month (e.g., May) and Year (2006) for only the newly diagnosed (Incident) MRSA cases.

8. **Why was the culture done: Check the appropriate response**

Admission Screening - This culture was done as part of a protocol on admission that requires patients to submit to a series of tests to determine the presence or absence of MRSA.

Clinical Isolate - These cultures were obtained because a physician ordered the culture as a result of some clinical indication or suspicion of infection.

Other screen- These cultures were taken in the course of working-up an outbreak or cluster, contact screen, transfer screen, prevalence screen or other screening for MRSA. These cultures would not have been taken routinely nor would they have been taken as a clinical isolate.

Other indication- this includes any other indication not listed above.

9. **Where was the MRSA acquired?**
Healthcare-associated:
Once the patient has been identified with MRSA, they will be classified as healthcare-associated based on the "best judgment" of the practitioner. This judgment should include review of:

- length of time in hospital prior to MRSA identification (> 72 hours)
- knowledge of previous MRSA status
- date of admission
- length of stay in hospital
- prior hospitalization history (previously admitted in past 12 months)
- where patient admitted from

Newborn nosocomial case definition:
A MRSA case in a newborn may be considered as healthcare-associated if the mother was not known to be a case on admission and where there is no epidemiological reason to suspect that the mother was colonized prior to admission, even if the newborn is < 48 hours of age. In the case of a newborn

transferred from another institution, MRSA may be classified as healthcare-associated if the organism was not known to be present and there is no epidemiological reason to suspect that acquisition occurred prior to transfer.

Community case definition:
No established health-care associated risk factors, and:

(vi) hospitalized < 72 hours;
(vii) no previous history of MRSA;
(viii) no medical devices such as urinary catheters, IV lines, feeding tubes, tracheostomy, dialysis access, etc.
(ix) no history of hospitalization, surgery, or dialysis within 1 year of MRSA culture;
(x) not in residence at a long term care facility within 1 year of MRSA culture.

10. **Epidemiological link:**
This refers to MRSA thought to be epidemiologically linked to another person with MRSA in your facility through (e.g., common exposures, shared rooms, contact with implicated health care worker, contact with another patient with MRSA). Using your best judgement identify whether an epidemiological link has been established between this patient and any other known MRSA person in your facility. Check yes or no.

11. **At which site(s) has MRSA been isolated (positive culture obtained)?**
For this questions please complete the table:

1. Check the box(es) in the 'Culture positives' column for each site that MRSA has been isolated.
2. In the second column identify whether the positive culture represented an infection or colonization. MRSA infection is the presence of an infection, determined by the manifestations of signs and symptoms associated with MRSA infections. MRSA colonization is the presence of MRSA on skin, surgical wounds, skin, soft tissue, nose, sputum, urine or other which are <u>not</u> manifesting clinical signs and symptoms of infection.

[i] Unless a clear and well defined question is specified in the report of the review, it will be difficult to assess how well it has met its objectives or how relevant it is to the question you are trying to answer on the basis of the conclusions.

[ii] This relates to **selection bias**.* It is important that the two groups selected for comparison are as similar as possible in all characteristics except for their exposure status, or the presence of specific prognostic factors or prognostic markers relevant to the study in question.

[iii] This relates to **selection bias**.* The participation rate is defined as the number of study participants divided by the number of eligible subjects, and should be calculated separately for each branch of the study. A large difference in participation rate between the two arms of the study indicates that a significant degree of **selection bias*** may be present, and the study results should be treated with considerable caution.

[iv] If some of the eligible subjects, particularly those in the unexposed group, already have the outcome at the start of the trial the final result will be subject to **performance bias.*** A well conducted study will attempt to estimate the likelihood of this occurring, and take it into account in the analysis through the use of sensitivity studies or other methods.

[v] This question relates to the risk of **attrition bias**.*The number of patients that drop out of a study should give concern if the number is very high. Conventionally, a 20% drop out rate is regarded as acceptable, but in observational studies conducted over a lengthy period of time a higher drop out rate is to be expected. A decision on whether to downgrade or reject a study because of a high drop out rate is a matter of judgement based on the reasons why people dropped out, and whether drop out rates were comparable in the exposed and unexposed groups. Reporting of efforts to follow up participants that dropped out may be regarded as an indicator of a well conducted study.

[vi] For valid study results, it is essential that the study participants are truly representative of the source population. It is always possible that participants who dropped out of the study will differ in some significant way from those who remained part of the study throughout. A well conducted study will attempt to identify any such differences between full and partial participants in both the exposed and unexposed groups. This relates to the risk of **attrition bias**.* Any unexplained differences should lead to the study results being treated with caution.

[vii] This relates to the risk of **detection bias**.* Once enrolled in the study, participants should be followed until specified end points or outcomes are reached. In a study of the effect of exercise on the death rates from heart disease in middle aged men, for example, participants might be followed up until death, or until reaching a predefined age. **If outcomes and the criteria used for measuring them are not clearly defined, the study should be rejected.**

[viii] This relates to the risk of **detection bias.*** If the assessor is blinded to which participants received the exposure, and which did not, the prospects of unbiased results are significantly increased. Studies in which this is done should be rated more highly than those where it is not done, or not done adequately.

[ix] This relates to the risk of **detection bias.*** Blinding is not possible in many cohort studies. In order to asses the extent of any bias that may be present, it may be helpful to compare process measures used on the participant groups - e.g. frequency of observations, who carried out the observations, the degree of detail and completeness of observations. If these process measures are comparable between the groups, the results may be regarded with more confidence.

[x] This relates to the risk of **detection bias.*** A well conducted study should indicate how the degree of exposure or presence of prognostic factors or markers was assessed. Whatever measures are used must

be sufficient to establish clearly that participants have or have not received the exposure under investigation and the extent of such exposure, or that they do or do not possess a particular prognostic marker or factor. Clearly described, reliable measures should increase the confidence in the quality of the study

[xi] This relates to the risk of **detection bias.*** The primary outcome measures used should be clearly stated in the study. **If the outcome measures are not stated, or the study bases its main conclusions on secondary outcomes, the study should be rejected.** Where outcome measures require any degree of subjectivity, some evidence should be provided that the measures used are reliable and have been validated prior to their use in the study.

[xii] This relates to the risk of **detection bias.*** Confidence in data quality should be increased if exposure level is measured more than once in the course of the study. Independent assessment by more than one investigator is preferable.

[xiii] Confounding is the distortion of a link between exposure and outcome by another factor that is associated with both exposure and outcome. The possible presence of confounding factors is one of the principal reasons why observational studies are not more highly rated as a source of evidence. The report of the study should indicate which potential confounders have been considered, and how they have been assessed or allowed for in the analysis. Clinical judgement should be applied to consider whether all likely confounders have been considered. If the measures used to address confounding are considered inadequate, the study should be downgraded or rejected, depending on how serious the risk of confounding is considered to be. **A study that does not address the possibility of confounding should be rejected.**

[xiv] Confidence limits are the preferred method for indicating the precision of statistical results, and can be used to differentiate between an inconclusive study and a study that shows no effect. Studies that report a single value with no assessment of precision should be treated with extreme caution.

[xv] Rate the overall methodological quality of the study, using the following as a guide: **High quality** (++): Majority of criteria met. Little or no risk of bias. Results unlikely to be changed by further research. **Acceptable** (+): Most criteria met. Some flaws in the study with an associated risk of bias, Conclusions may change in the light of further studies. **Low quality** (0): Either most criteria not met, or significant flaws relating to key aspects of study design. Conclusions likely to change in the light of further studies.