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Use of Accelerated Hydrogen Peroxide Skin Cleanser to Reduce Methicillin-resistant *Staphylococcus aureus* Colonization in Clients of the Calgary Drop-In and Rehab Centre

by

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Abstract

The objectives of this study were to determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the population that accesses the Calgary Drop-In and Rehab Centre (CDIRC), and to evaluate the feasibility of providing Accelerated Hydrogen Peroxide (AHP) skin cleanser as a method of reducing MRSA carriage.

In March, 2008, the point prevalence of MRSA was 9.89% in the population that accesses CDIRC's services. Risk factors such as showering less than twice a week and a history of antibiotic use in the previous seven days were associated with a higher likelihood of testing positive for MRSA.

Low study recruitment and significant loss to follow-up limited the ability to assess the efficacy of the AHP skin cleanser as a means of reducing MRSA carriage in this population. Adherence to the study protocol by participants was poor.

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Finally, thanks to all the residents and users of CDIRC for sharing their time and stories with me. I got more out of this study than can ever be written in a paper.

Dedication

For Mitch -

You know why.

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List of Abbreviations

Abbreviation	Definition
AHP	Accelerated hydrogen peroxide
β-lactam	Beta-lactam antibiotic
BSI	Bloodstream infection
CA-MRSA	Community-associated MRSA
CDC	United States Center for Disease Control
CDIRC	Calgary Drop-In and Rehab Centre
CFU	Colony forming unit
CHG	Chlorhexidine gluconate
CNS	Coagulase-negative staphylococcus
DTES	Downtown Eastside (Vancouver)
ENT	Ears, Nose, and Throat
GI	Gastrointestinal
H_2O_2	Hydrogen peroxide
HA-MRSA	Healthcare-associated MRSA
mec	*Staphylococcus aureus* methicillin resistance gene
MIC	Minimum inhibitory concentration
MLST	Multi-locus sequence testing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
PBP	Penicillin-binding protein
PCR	Polymerase Chain Reaction
PFGE	Pulse-field Gel Electrophoresis
PVL	Panton-Valentine Leukocidin
Rodac	Replicate Organism Detection and Counting
S. aureus	*Staphylococcus aureus*

SCC	Staphylococcal cassette chromosome
SSTI	Skin or soft tissue infection
ST	Sequence type

CHAPTER ONE: INTRODUCTION

Staphylococcus aureus is part of the normal flora of humans and is frequently carried in the nose and on the skin. Carriage is usually asymptomatic in healthy people, but can result in severe infections if bacteria breach the skin's defences. *S. aureus* usually causes infections such as boils and abscesses, but in serious cases infections can lead to bacteremia, pneumonia, and in rare situations, death.

Methicillin was first introduced in the 1960s to treat *S. aureus* infections. Shortly after its introduction, strains of methicillin-resistant *S. aureus* (MRSA) began to appear. MRSA has since become resistant to a wide range of antimicrobials.

Although originally restricted to healthcare settings, MRSA has recently emerged as an important community pathogen. Community strains differ from nosocomial strains in terms of virulence, risk factors for infection, and susceptibility to antimicrobials. Risk factors identified for community-associated MRSA acquisition include crowding, poor hygiene, compromised skin, sharing contaminated personal items, and frequent skin-to-skin contact.

Homeless individuals experience many of the risk factors associated with community-associated MRSA carriage. In 2004, an outbreak of MRSA was reported in the marginalized population in Calgary, Alberta, Canada. The prevalence of MRSA in that population was found to be 7.4% (95% CI 4.6 - 11.2) (M. Gilbert, 2007). Although the prevalence of MRSA appears to remain low in the general population, rates seem to be increasing in this marginalized group.

Topical agents such as chlorhexidine gluconate have been used in conjunction with nasal mupirocin to attempt MRSA decolonization in healthcare settings. These attempts have met with limited success. There have been few attempts to reduce MRSA colonization in community settings.

The objectives of this study were to determine the prevalence of MRSA in the population that accesses the Calgary Drop-In and Rehab Centre, and to evaluate the feasibility of providing Accelerated Hydrogen Peroxide skin cleanser as a method of reducing MRSA carriage and thus, MRSA infections.

CHAPTER TWO: BACKGROUND

2.1 Introduction to Chapter Two:

This chapter presents the rationale on conducting research on methicillin-resistant *Staphylococcus aureus* (MRSA) in the population that uses the Calgary Drop-in and Rehab Centre (CDIRC). The key objectives of this study were to determine the prevalence of MRSA in the population that accesses CDIRC's services and to explore the feasibility of implementing an intervention using Accelerated Hydrogen Peroxide (AHP) skin cleanser to reduce MRSA skin carriage in this population.

Chapter Two is divided into six main sections: (1) *Staphylococcus aureus* (2) Methicillin-resistant *Staphylococcus aureus* (MRSA) (3) the Epidemiology of MRSA (4) the Epidemiology of Community-associated MRSA (5) Decolonization, prevention, and treatment of Community-associated MRSA and (6) Health and the homeless.

2.2 *Staphylococcus aureus*

People carry a mixture of microorganisms on their skin. One of the most common organisms isolated from body surfaces is a type of bacteria called *Staphylococcus aureus* (*S. aureus*). Although carriage of *S. aureus* is usually symptom-free, infections can result if bacteria get past the body's natural barriers. *S aureus* is the cause of many infections in both community and healthcare settings.

2.2.1 Staphylococcus aureus *carriage patterns*

Humans are a natural reservoir for *S. aureus* and an individual can carry bacteria for varying lengths of time. Although cross-sectional studies can only define a person as either a carrier or a non-carrier, longitudinal studies have demonstrated that there are actually three possible carriage patterns: persistent carriage when all isolates collected from an individual over the study period are positive for *S. aureus*; intermittent carriage when specimens collected at various points during the study period alternate between positive and negative; and non-carriage when no specimens collected from an individual during the study period are positive for *S. aureus* (Kluytmans et al., 1997).

A review of longitudinal studies investigating *S. aureus* carriage patterns in healthy adults was done by Vandenbergh in 1999 (VandenBergh et al., 1999). The review looked at seven different studies and found there were significant differences in the estimates of the three carriage patterns between the various studies. The estimates of persistent carriage of *S. aureus* ranged between 10% and 35%; estimates of intermittent *S. aureus* carriage ranged between 20% and 75%; and estimates of non-carriage of *S. aureus* ranged

between 5% and 70%. The variation in the estimates most likely derives from differences in 1) the characteristics of the study populations which included medical students, army recruits, and university personnel 2) follow-up periods which ranged from six weeks to 19 months 3) time between cultures which ranged from one week to three months and 4) criteria for the definitions of persistent and intermittent carriage.

S. aureus is frequently isolated from the nostrils (anterior nares) and nose picking has been associated with *S. aureus* nasal carriage. Wertheim investigated the possible association between nose picking and *S. aureus* nasal carriage in a study of 238 outpatients and 86 healthy hospital volunteers at a tertiary-care hospital's ear, nose, and throat (ENT) clinic between June 2001 and May 2003 (Wertheim et al., 2006). Outpatients and volunteers were given a questionnaire that asked questions regarding behaviour and symptoms related to the nose, and underwent *S. aureus* nasal screening. They were not informed that the investigation was looking at nose-picking behaviour as a primary determinant of *S. aureus* nasal carriage. Following the questionnaire and the collection of nasal samples, the ENT outpatient participants underwent a nasal examination by an ENT doctor. The results of the study showed that, for the ENT clinic outpatients, nose pickers were more likely than non-nose pickers to carry *S. aureus*. For the healthy hospital volunteers, there was a positive association between the self-reported frequency of nose picking and both the frequency of *S. aureus* nasal colonization as well as the *S. aureus* bacterial load in the nose. Hands appear to be major vehicles for transmitting staphylococci from the nose to the environment and vice versa. This is important as transmission of *S. aureus* tends to occur by direct contact with either

someone carrying the bacteria on their skin or by contact with a contaminated environment (Chambers, 2001).

S. aureus has been isolated from other anatomical sites including the axillae, perineum, groin, and throat. It can also be found in the gastrointestinal tract (Bhalla et al., 2007).

2.2.2 Prevalence of Staphylococcus aureus

Colonization is regularly defined as the asymptomatic carriage of bacteria - although bacteria are present, the individual carrying the bacteria has no symptoms. Infection occurs when bacteria breach the skin barrier and cause disease. Isolates of *S. aureus* are often described as either colonizing or infecting based on the anatomical site the culture was recovered from and whether or not there was evidence of infection such as redness of the skin or drainage of pustular material (Baggett et al., 2003). Isolates collected from normally sterile sites such as blood and bone are generally classified as infections while isolates recovered from sites such as skin are usually classified as colonizations.

Prevalence studies have frequently been used as a measure of the proportion of *S. aureus* colonization in specific groups of people at certain points in time. The prevalence of *S. aureus* colonization is defined as the number of individuals colonized with *S. aureus* in a population divided by the total number of individuals in that population. Prevalence estimates of *S. aureus* colonization fluctuate based on the population studied and the anatomical sites sampled.

A population-based study in the United States found a prevalence of nasal *S. aureus* colonization of 31.6% (Graham et al., 2006). This study was a secondary analysis of the

2001-02 National Health and Nutrition Examination Study (NHANES) survey. The NHANES database includes in-home interview data, examination data from a mobile examination center, and laboratory data. Nasal swabs were collected from 9,622 non-institutionalized participants that completed the survey and the swabs were tested for the presence of *S. aureus*. Of those participants, 3,040 (31.6%) had nasal swabs that tested positive for *S. aureus*. This was a secondary analysis of the NHANES data so the authors were limited to previously collected variables. *S. aureus* colonization was included for the first time in the 2001-02 iteration of the NHANES survey so comparison of prevalence rates cannot be made between years.

Klutymans *et al.* reviewed several cross-sectional studies on *S. aureus* nasal carriage published between 1934 and 1994 and reported an overall prevalence of 37.2% in the general population (Kluytmans et al., 1997). Estimates of prevalence ranged from 19.0% to 55.1% for the various studies examining nasal carriage in the general population. This was likely due to differences in the quality of sampling and the various culture techniques used. This lack of homogeneity between studies makes comparison difficult and limits the ability to present an accurate estimate of the prevalence of *S. aureus* nasal carriage.

A study published in 1991 looked at the prevalence of nasal, axillary, and perineal carriage of *S. aureus* in pregnant women attending an outpatient antenatal clinic in London, England (Dancer & Noble, 1991). The study reported a *S. aureus* prevalence of 20% when the nose was the only site screened. A prevalence of 0.6% was found when only the axillae were screened and a prevalence of 8.2% was found when only the perineum was screened. Screening at multiple sites resulted in an overall prevalence of

33%. The findings from Dancer's study showed that about one in three pregnant women were colonized with *S. aureus* and that screening should be done at multiple sites to obtain a more accurate estimate of the prevalence of *S. aureus* in a population.

2.2.3 Staphylococcus aureus *infections*

Asymptomatic colonization with *S. aureus* is more common than infection (Chambers, 2001) and the relationship between colonization and infection is not completely understood, although there is evidence to suggest that colonization with *S. aureus* often precedes infection (Graham et al., 2006).

A multicenter study was done in Germany between September 1993 and September 1994 to determine whether nasal carriage acted as a source of *S. aureus* bacteremia (von Eiff et al., 2001). The study involved collecting nasal samples from 219 hospitalized patients with laboratory-confirmed *S. aureus* bacteremias. After genotyping, 82% of the isolates from the blood samples were found to be identical to those from the nares in the same patients. The study was cross-sectional so it was not possible to determine whether *S. aureus* nasal colonization was followed by infection or if the reverse was true.

To explore the temporal relationship between colonization and infection, Von Eiff *et al.* performed a follow-up study between June 1994 and June 1999 (von Eiff et al., 2001). This study was limited to a single hospital and involved prospectively collecting nasal samples over the five year period and comparing them with blood samples from patients who went on to develop bacteremias. Of the 14 patients who developed an *S. aureus* bacteremia, 12 cases had clonally identical isolates collected from the blood and nares.

More than 80% of the bloodstream infections caused by *S. aureus* in these hospitalized adults were preceded by colonization of the nose with the same strain. Evidence from this study supports the hypothesis that *S. aureus* nasal colonization often precedes infection.

Colonization with *S. aureus* has also been identified as a risk factor for subsequent infection in the population that uses drugs, likely due to increased opportunities for the bacteria to be inoculated through injection or inhalation (Lowy & Miller, 2002).

S. aureus is a versatile pathogen and can cause a range of infections in humans from superficial skin lesions such as abscesses and wound infections to systemic infections including bacteremia and pneumonia. It can also cause toxemic syndromes such as toxic shock syndrome and staphylococcal scalded skin syndrome (Jarraud et al., 2002).

2.3 Methicillin-resistant *Staphylococcus aureus*

2.3.1 The emergence of methicillin-resistant Staphylococcus aureus

Penicillin was introduced in the 1940s and was used to treat *S. aureus* infections. Shortly after its introduction isolates of penicillin-resistant *S. aureus* were identified. Methicillin was first used in 1959 to treat these penicillin-resistant strains of *S. aureus* and by 1961 methicillin-resistant strains were also being reported (Deresinski, 2005). The first methicillin-resistant case in Canada was reported by Low *et al.* in 1981 (Simor et al., 2001).

The acronym MRSA originally stood for "Methicillin-resistant *Staphylococcus aureus*", however, the term has now evolved to include strains of *S. aureus* that are resistant to all β-lactam antimicrobials including penicillins, cephalosporins, and monobactams (Nicolle, 2006).

Initially, MRSA infections were almost always acquired in healthcare settings such as hospitals and long-term care facilities. The most common risk factors identified with healthcare-associated MRSA included prior antibiotic use, admission to an intensive care unit, surgery, and exposure to another patient colonized with MRSA (Chambers, 2001).

Although originally restricted to healthcare settings, MRSA has now emerged as a significant pathogen in the community. Molecular typing of over 9,000 MRSA strains collected through the Canadian Nosocomial Infections Surveillance Program (CNISP) since 1995 has identified 10 MRSA clones in Canada. Types 1 through 6, and Types 8

and 9 are identified as nosocomial strains while Types 7 and 10 are the community strains (Table 1) (Mulvey et al., 2005).

Table 1 Methicillin-resistant *Staphylococcus aureus* nomenclature and associated locations, Canada

Canadian Nomenclature	Other Names	Associated Location
CMRSA1	USA600	Nosocomial
CMRSA2	USA100/800/New York	Nosocomial
CMRSA3	USA700	Nosocomial
CMRSA4	USA200/EMRSA16	Nosocomial
CMRSA5	USA500	Nosocomial
CMRSA6	USA700	Nosocomial
CMRSA7	USA400/MW2	Community
CMRSA8	EMRSA	Nosocomial
CMRSA9		Nosocomial
CMRSA10	USA300	Community

2.3.2 Molecular Features of methicillin-resistant Staphylococcus aureus

MRSA is a highly adaptable organism. Its genome has evolved due to mutation of its own genes and acquisition of exogenous genes (Eguia & Chambers, 2003). The ability of *S. aureus* to acquire antibiotic resistance mechanisms has contributed to its emergence in both the community and nosocomial settings (Zetola et al., 2005).

2.3.2.1 Methicillin resistance

The methicillin resistance gene (*mec*A) encodes a methicillin-resistant penicillin-binding protein (PBP2a) that is not present in methicillin-susceptible *S. aureus* (MSSA) strains (Enright et al., 2002). *mec*A is carried on a mobile genetic element called a staphylococcal cassette chromosome (SCC). SCC*mec* can be thought of as an antibiotic

resistance island which carries the *mec* gene complex that encodes β-lactam resistance and also contains transposons and copies of plasmids that carry various resistance genes against other non-β-lactam antibiotics (Hiramatsu et al., 2001). At least five different SCC*mec* forms have been described which differ in size and genetic composition and are numbered from I to V (Zetola et al., 2005). Community-associated MRSA (CA-MRSA) usually carries the type IV or V SCC*mec*. The smaller type IV cassette usually only includes methicillin-resistance elements, which accounts for the increased susceptibility to antibiotics other than β-lactams in the community strains (Kowalski et al., 2005), (Said-Salim et al., 2005). The smaller size may also serve as an evolutionary advantage by making type IV more amenable to horizontal spread (Zetola et al., 2005). Antibiotic selective pressure is believed to be lower in the community than in the hospital, so the survival advantage of having multiple drug-resistance is also lower (Chambers, 2001). Since there are generally less antibiotics used in the community, community strains do not experience the same selective pressure to carry resistance to multiple antibiotics as hospital strains. There is no survival advantage in the community in carrying the larger SCC*mec* cassettes.

Although originally resistant only to β-lactams, many MRSA strains have become resistant to multiple antimicrobials and are currently usually susceptible only to glycopeptides such as vancomycin (Enright et al., 2002). However, in the past few years an increasing number of vancomycin-resistant MRSA isolates have been identified. At present, vancomycin resistance is not a concern for community strains.

2.3.2.2 Panton-Valentine Leukocidin

Panton-Valentine Leukocidin (PVL) is a leukotoxin which is lethal to white blood cells such as neutrophils and monocytes (Boyle-Vavra & Daum, 2007), (Kowalski et al., 2005),(Eguia & Chambers, 2003). PVL is known for causing pore formation on the polymorphonuclear cells of the host (Johnson et al., 2007) by producing cytotoxins capable of causing severe tissue necrosis and white blood cell destruction (Allen, 2006).

Many community MRSA strains harbour the genes that encode the PVL toxin (Tenover et al., 2006). It is the most consistently present transferable toxin locus among the CA-MRSA strains (Boyle-Vavra & Daum, 2007). PVL is almost never found in the hospital-associated MRSA strains.

A review of 14 CA-MRSA isolates responsible for causing infections were submitted to the French Reference Center for Staphylococcal Toxemia between January 1999 and December 2001 (Dufour et al., 2002). Results of genetic fingerprinting showed they all harboured the PVL genes. The study defined an infection as community-associated if the positive MRSA culture was isolated from an individual with no history of hospitalization, surgery, or outpatient care, as well as no family member employed at a healthcare facility. The study also examined hospital-associated MRSA infections and found that none of the isolates associated with hospital-associated infection had the PVL genes.

PVL has been associated with skin and soft tissue infections (SSTI) such as cellulitis, cutaneous abscesses, and furuncles. However, the actual role PVL plays in CA-MRSA infections is controversial. Although PVL may be associated with the high virulence

potential of CA-MRSA to cause SSTI, it is also possible that the leukotoxin is acting as an epidemiologic marker rather than a virulence factor.

In a prospective, observational study, Ellis *et al.* found that colonization with PVL-positive strains was associated with an increased risk for skin and soft tissue infections (Ellis et al., 2004). A cohort of 812 Army personnel enrolled in medic training at Fort Sam Houston, Texas in 2003 was eligible for participation. Recruits underwent a nasal swab and completed a basic questionnaire. Eight to ten weeks later the participants had an additional nasal specimen collected. Participants were also monitored regularly for SSTI. Nasal specimens positive for MRSA were genetically fingerprinted. PVL genes were detected in 66% of all the CA-MRSA isolates. There were also six patients hospitalized, of which five had PVL-positive infections. In this study, CA-MRSA colonization with PVL-positive strains was a risk factor for SSTI.

In mouse studies, Voyich found that the presence of PVL alone cannot account for this increased virulence – isogenic (genetically identical) strains of both CMRSA7 and CMRSA10 without the PVL genes were as lethal as the wild-type strains harbouring PVL and they caused similar skin abscesses (Voyich et al., 2006).

2.3.3 Testing for methicillin-resistant Staphylococcus aureus

2.3.3.1 Specimen collection

The method used to collect specimens depends on the purpose of the study and the sites being sampled. Colonization studies rely more on specimens collected from surfaces such as nares and skin, while studies looking at MRSA infections might use fluids from

purulent abscesses, respiratory secretions, or blood from patients with symptoms of bacteremia (Babel & Decker, 2008).

In colonization studies, MRSA specimen collection is usually done using a system including sterile swabs and transport medium. To collect a specimen, the swab is moistened with the transport medium and then the anatomical site selected for sampling is swabbed. The swab is then placed into the transport medium for conveyance to the laboratory. The time between specimen collection and laboratory testing is important in maintaining the viability of a sample (Smismans et al., 2009).

Recently, the use of contact plates has become more common in bacterial studies. The plates are designed in a manner that allows agar to be poured so that it extends about the rim of the plate. Since the agar is convex above the rim, it can be pressed against any surface believed to be contaminated with microorganisms. Contact plates can be prepared with a variety of media depending on the bacteria being tested for. These plates contain a grid, which makes it easier to count colonies. Also known by the trademark name, "Rodac" plates (Replicate Organism Detection and Counting) they are very useful in quantifying the amount of bacterial load a given surface has.

2.3.3.2 Laboratory methods for identifying methicillin-resistant *Staphylococcus aureus*

After a specimen has been collected, there are a variety of laboratory methods used to identify if the sample is positive for MRSA. The process can be indirect where the initial step is to determine whether a suspect colony is *S. aureus* followed by subsequent testing

of that *S. aureus* colony for methicillin-susceptibility. Other methods involve the use of selective media developed for the presumptive identification of MRSA colonies. There are also methods such as Polymerase Chain Reaction (PCR) where the sample can be tested directly for MRSA.

2.3.3.2.1 Methods to identify *S. aureus*

One of the methods used to determine if a bacterial colony belongs to the staphylococcus genus is catalase testing. In this method, hydrogen peroxide is used to distinguish between staphylococci and streptococci bacteria. Staphylococcal bacteria produce an enzyme called catalase that breaks down hydrogen into the components water and oxygen. When a sample from a catalase-positive bacterial colony is added to a drop of 3% hydrogen peroxide the catalase breaks down the H_2O_2 into water and oxygen. The production of oxygen causes white bubbles to form immediately. Streptococci and enterococci species are catalase-negative and so hydrogen peroxide will not be decomposed into the two components, oxygen will not be produced, and no bubbles will appear.

Colonies that are catalase-positive can be streaked on blood agar plates and incubated. Blood agar is a type of media used to differentially grow strains of bacteria. Certain bacteria produce a type of enzyme called hemolysin that breaks down red blood cells. If an organism produces hemolysin, the area surrounding the colony on a blood agar plate will be clear due to the lysis of the blood cells. *Staphylococcus aureus* is often beta-hemolytic.

Beta-hemolytic colonies can be further tested using a coagulase test to distinguish between the staphylococcal species. Coagulase is an enzyme produced by certain bacteria. *S. aureus* is the only species of staphylococcus that is coagulase-positive. To do the test, rabbit plasma is mixed with the colony and if the suspect colony is *S. aureus* the serum will clot. If no coagulation occurs, the colony is not *S. aureus*.

2.3.3.2.2 Methods for determining methicillin resistance based on minimum inhibitory concentrations of β-lactams

Once a sample has been identified as *S. aureus*, further testing can be done to determine if the bacteria is resistant to β-lactams and should be classified as MRSA. Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of an organism in a susceptibility test (Bou, 2007). The MIC for an antibiotic can be used to establish "break-points" for classification of an organism as resistant or susceptible. Some of the most common methods for determining MIC are disk diffusion testing, oxacillin E-testing, and broth microdilution testing.

Resistance to methicillin is carried on the bacterial *mecA* gene and the presence of the enzyme PBP2a (as described in 2.3.2.1 Methicillin Resistance) is what allows bacterial colonies to grow and divide even when β-lactams are present (Babel & Decker, 2008). The β-lactam oxacillin is often used for resistance testing instead of methicillin as it is more stable during storage (Babel & Decker, 2008). Although all strains of MRSA produce PBP2a, strains can exhibit heterogeneous resistance to β-lactams resulting in only a proportion of the colonies grown from a sample showing resistance in standard laboratory tests (Babel & Decker, 2008). There have been four classes of resistance

described with class I expressing considerable heterogeneity (as few as 1 cell in every 10^8-10^9 expressing resistance) up to class IV (every cell expressing resistance) (Hryniewicz, 1999). This variation in resistance expression makes MRSA differentiation solely by MIC difficult as strains with a high heterogeneity may be miss-classified as susceptible due to low expression of resistance.

Disk diffusion testing is done to measure the MIC of antibiotics for bacteria. For MRSA MIC testing, Mueller-Hinton agar plates are inoculated with *S. aureus* in a suspension adjusted to match the 0.5 McFarland standard - a reference standard that measures turbidity of a suspension to make sure the number of bacteria are within a given range. The 0.5 McFarland standard is equivalent to roughly 10^8 colony forming units (CFU)/mL (Bou, 2007). Disks containing the antibiotic are placed on the agar and plates are incubated at 35 degrees Celsius for 24 hours. The diameters of the clear areas surrounding the antibiotic disks are then measured. The size of the clear area (the zone of inhibition) is used to determine the level of antibiotic resistance the bacteria exhibits. The breakpoint frequently used to define MRSA using oxacillin disks is a zone of inhibition ≤10mm (Shariati et al., 2010). Although oxacillin was previously the antibiotic of choice for MRSA disk diffusion, the use of cefoxitin has recently been proposed as an alternative due to its higher sensitivity and ease of reading (Broekema et al., 2009, Velasco et al., 2005). For cefoxitin, MRSA is defined by a measured zone of inhibition ≤21mm (Broekema et al., 2009).

E-testing is also used to determine the MIC of an antibiotic. For MRSA oxacillin-susceptibility testing, *S. aureus* suspensions adjusted to the 0.5 McFarland standard are

inoculated onto a Mueller-Hinton plate supplemented with NaCl. Oxacillin E-strips are then placed on the plates. The E-strips are plastic strips that have a predefined gradient of antibiotic concentrations across a MIC range. When the strips are applied to a plate and the plate is incubated, an inhibition zone centered around the strip is visible. The MIC is read at the point where the growth of the bacteria intersects the strip. The MIC breakpoints are frequently defined as ≥ 4 indicating oxacillin resistance and ≤ 2 indicating oxacillin susceptibility (Shariati et al., 2010).

Broth microdilution is another method used to determine the MIC of an antibiotic. To perform oxacillin broth microdilution for MRSA, varying concentrations of oxacillin are added to set of tubes of Mueller-Hinton broth that have been supplemented with 2% NaCl (Bou, 2007). A suspension of *S. aureus* adjusted to the 0.5 McFarland standard is then inoculated into the tubes of broth containing the gradient of oxacillin concentrations. After incubation at 35 degrees Celsius for 24 hours, the MIC can be determined. MRSA is often defined as an oxacillin MIC ≥ 4 μg/mL (Babel & Decker, 2008).

Each type of testing has strengths and weaknesses and a balance between speed of result, sensitivity and specificity, and cost help determine the testing method chosen.

2.3.3.2.3 Methods to detect methicillin-resistant Staphylococcus aureus

In addition to methods that use MIC to determine the methicillin-susceptibility of an isolate, there are also methods used to identify MRSA positive strains. These techniques can either involve the testing of previously identified *S. aureus* colonies (as described in

2.3.3.2.1 Laboratory methods to identify *S. aureus*), or through direct inoculation of a sample onto selective media.

Oxacillin agar screen testing is done using a plate containing Mueller-Hinton agar supplemented with both salt (4% NaCl) and oxacillin (6.0 µg/mL) (Shariati et al., 2010; Velasco et al., 2005). Bacteria from discrete colonies identified as *S. aureus* are suspended in a broth medium that is adjusted to meet the 0.5 McFarland standard. The suspension of *S. aureus* is inoculated onto the plate and incubated at 35 degrees Celsius for 24 hours. Any growth on the plate is considered methicillin-resistant.

A serotype is a group of microorganisms that are classified together based on a variety of factors that allow for differentiation between two members of the same species. *S. aureus* can be serotyped based on the presence of PBP2a (von Eiff et al., 2007; Velasco et al., 2005). A slide agglutination test is often used for serotyping. In this testing method, a serum containing antibodies to the PBP2a protein is mixed with a *S. aureus* colony. If the colony is MRSA positive and thus producing PBP2a, the serum will clump.

The development of Oxoid's Denim-Blue Agar Plates allows for quick identification of presumptive MRSA directly from a specimen without first requiring the isolation of *S. aureus* (see Appendix A for Denim-Blue Technical Sheet). The agar contains a chromagen which yields a denim-blue color when MRSA is present. It is a selective medium for MRSA and it contains compounds that inhibit the growth of other microorganisms. Samples can be inoculated onto the plate directly from patients' swabs and are then incubated aerobically at 35-37 degrees Celsius for 18 to 24 hours. The

medium loses specificity if plates are incubated longer than 24 hours. MRSA appears as discrete, smooth-edged colonies that are solid denim-blue in colour. This test yields only presumptive MRSA so further confirmatory tests need to be done.

Polymerase Chain Reaction (PCR) was originally developed as a method of generating large amounts of DNA sequences from small samples. PCR is done by taking a small amount of DNA from the collected specimen and denaturing the DNA into two single strands. Then short strands of reference DNA (primers) unique to the microorganism of interest are used in conjunction with polymerases (DNA synthesizers) to synthesize large amounts of DNA. The results of the PCR test can be either positive (DNA can be synthesized from the sample DNA and the primer DNA, indicating they are the same) or negative (the sample DNA cannot be synthesized with the primer and polymerase indicating that the DNA is different). Using specific primers allows for identification of gene sequences unique to certain bacterial species and PCR can be used to differentiate between strains of the same species. For example, all MRSA have *mec*A (the gene encoding for methicillin resistance) so using a *mec*A-specific primer allows for differentiation between MRSA and non-MRSA strains. PCR can yield results in 2-3 hours and can be performed directly from clinical specimens which is useful when rapid MRSA screening is required (Boyce & Havill, 2008).

2.3.3.2.4 Molecular techniques for typing methicillin-resistant Staphylococcus aureus

Various molecular methods are used for typing MRSA strains. The aim of typing MRSA is to group related strains together and to discriminate between divergent strains. Being

able to differentiate between isolates allows for epidemiological comparison of types within and between individuals and populations.

Phage typing has been used to differentiate between strains of MRSA. Viruses that infect bacteria are called bacteriophages (phages), and some phages can only infect a single strain of MRSA. Phage typing relies on the use of bacteriophage typing sets (Tenover et al., 1994). If a phage specific to a strain of MRSA is able to infect a bacterium, the bacterium is considered phage positive and can be identified as that unique strain of MRSA. This method fell out of favour due to its poor reproducibility and the high demand on time and labour required, however, Wildemauwe argues that the revised standardization of experimental phages along with the development of new phages may improve the future applicability of the technique (Palavecino, 2008; Tenover et al., 1994; Wildemauwe et al., 2010).

MRSA isolates are often typed using Pulse-Field Gel Electrophoresis (PFGE), a technique used to identify the relatedness of bacterial samples (Enright et al., 2002). In this technique, MRSA DNA is digested with a restriction enzyme and then the fragments of DNA are separated by size using an electric field across a gel matrix. The pattern of the sample DNA fragments are compared to patterns from other specimens collected in the study, or to reference patterns (Trindade et al., 2003). Comparing PFGE patterns can be time-consuming and analysis often relies on qualitative interpretations of pattern similarities. However, these disadvantages are becoming less important as the use of bionumeric software increases. Bionumeric software measures the level of congruence between PFGE patterns from different samples. This means that the PFGE patterns can

be quickly compared using a computer, which eliminates the subjectiveness previously inherent in the method. PFGE may not be suitable for population-level studies as it is very discriminatory although it remains very useful in localized investigations (Lowy & Miller, 2002).

Another method used to differentiate between different strains of MRSA is repetitive sequence-PCR (rep-PCR). The technique targets the repetitive sequences in bacterial genomes using primers that are specific to those sequences. An advantage of rep-PCR is that the DNA does not need to be pure or abundant. Different laboratories can also use the same primers which allows for comparison of MRSA isolates between studies and geographical regions.

The *spa* typing method involves sequencing a short repeat region (x region) of the *S. aureus* protein A (*spa*) gene. The x region is highly polymorphic which allows for discrimination between samples (Palavecino, 2008). The x region is amplified using PCR and then sequences are obtained using a sequencer (von Eiff et al., 2007). The sequences can then be entered into a software package to perform comparisons between sequences and obtain the *spa* type (Deurenberg & Stobberingh, 2008). This method provides a high level of discrimination between samples.

Multi-locus sequence typing (MLST) has recently been used to characterize MRSA isolates. In *S. aureus* testing it involves sequencing the internal fragments of seven unrelated “housekeeping” genes - housekeeping genes are those genes that are always present in a given species, but that still have enough variation within the species to allow for numerous alleles (variants of the gene) at that locus (Trindade et al., 2003). The

seven housekeeping genes used for *S. aureus* are carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glpF), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi) and acetyl coenzyme A acetyltransferase (yqiL) (http://www.mlst.net). Different strains of bacteria will have different alleles at each of the housekeeping loci. The allele at each of the seven loci will determine the allelic profile for the isolate (Enright et al., 2002). The allelic profile defines the strain's sequence type (ST). For example, CMRSA10 is ST8-MRSA-IV in MLST nomenclature, and its' allelic profile is 3-3-1-1-4-4-3 (Enright et al., 2002).

This technique allows for sufficient variability to discriminate among the different strains (Lowy & Miller, 2002). For *S. aureus* there are seven different housekeeping loci and each locus can have up to 30 different alleles, resulting in the possibility of 30^7 different allelic profiles (Trindade et al., 2003). This variation gives MLST significant discriminatory power. There is an added advantage to characterizing MRSA isolates using MLST. The allelic profiles of the isolates can be entered in a web-based database so comparisons of isolates can be made to strains from laboratories in other countries. One of the drawbacks to using MLST is that the equipment necessary to use it is very expensive. It is not a technique used frequently at the community level.

2.4 Epidemiology of methicillin-resistant *Staphylococcus aureus*

2.4.1 Laboratory methods used to differentiate between healthcare- acquired and community- acquired methicillin-resistant Staphylococcus aureus

It is unclear why certain MRSA strains dominate in the hospital setting while others dominate in the community. The three main features used to distinguish between community-associated and healthcare-associated strains of MRSA in the laboratory are differences in the SCC*mec* type, differences in the antimicrobial susceptibility patterns, and the presence of Panton-Valentine Leukocidin (PVL). It is becoming more difficult to distinguish between community and healthcare strains as CMRSA10 is beginning to circulate in hospitals.

In 2000, Naimi *et al.* performed a prospective cohort study designed to compare CA-MRSA and HA-MRSA cases in patients with MRSA infections in Minnesota. They classified 1100 cases as either community-associated or healthcare-associated based on healthcare risk factors and then described the microbiological characteristics of the isolates (Naimi et al., 2003). They reported that 85% of the isolates classified as community-associated had the SCC*mec*IV as compared to 12% of the healthcare-associated isolates. A limitation in this study was the probability of misclassification of isolates as either CA or HA based on epidemiological definitions. However, this bias probably worked in both directions and wouldn't influence the overall results. Multiple studies since then have identified the SCC*mec*IV as characteristic of community-associated strains.

The smaller type IV cassette generally confers resistance only to methicillin. This accounts for the increased susceptibility to antimicrobials other than β-lactams in the community strains (Kowalski et al., 2005), (Said-Salim et al., 2005). CA-MRSA strains are generally susceptible to non-β-lactams while hospital strains are typically resistant to multiple antibiotics.

The presence or absence of Panton-Valentine Leukocidin (PVL) has also been used to characterize an isolate as either community-associated or healthcare-associated. CA-MRSA strains harbour the genes that encode the PVL toxin more frequently than HA-MRSA strains. Tenover *et al.* analyzed 187 CMRSA10 (USA300) isolates from the United States Centre for Disease Control (CDC) strain collection and found that all of the strains had the PVL genes (Tenover et al., 2006). The isolates underwent testing for antimicrobial resistance, SCC*mec* typing, and testing for various genes encoding for toxins and virulence factors. These isolates were compared to other strains of MRSA. It was found that the other CA-MRSA strain, CMRSA7 (USA400) also harboured the PVL genes, although in only 62.5% of the isolates. Neither of the HA-MRSA strains, CMRSA5 (USA500) or CMRSA2 (USA100) had the PVL genes.

Although laboratory methods can identify the bacterial characteristics that are most commonly associated with either community or healthcare strains of MRSA (ex. SCC*mec* type, antibiotic susceptibility pattern, presence or absence of PVL), they cannot distinguish where an infection was acquired.

2.4.2 Epidemiologic methods used to differentiate between healthcare-acquired and community-acquired methicillin-resistant Staphylococcus aureus

A standard case-definition for CA-MRSA does not exist and substantial heterogeneity is found among the definitions used to define CA-MRSA (Zetola et al., 2005). A review by Salgado in 2003 showed there were at least eight different definitions being used to classify MRSA as community-associated between 1966 and 2002 (Salgado et al., 2003). The majority of studies in the review involved hospitalized patients and the most common difference in community-associated classification was the timing of the isolation of MRSA in relation to the time of admission, either with or without the inclusion of healthcare-associated risk factors. The timing between hospital admission and subsequent isolation of MRSA used to define an isolate as community-associated ranged between 24 to 72 hours.

The CDC's criteria for distinguishing healthcare-associated MRSA from community-associated MRSA are based on risk factors. Community-associated MRSA is defined using the following criteria:

- Diagnosis of MRSA was made in the outpatient setting or by a culture positive for MRSA within 48 hours after admission to the hospital.
- No medical history of MRSA infection or colonization.
- No medical history in the past year of:
    - Hospitalization

  - Admission to a nursing home, skilled nursing facility, or hospice
  - Dialysis
  - Surgery
- No permanent indwelling catheters or medical devices that pass through the skin into the body.

2.4.3 Defining community-associated methicillin-resistant Staphylococcus aureus

CA-MRSA can be defined using either laboratory criteria such as antibiotic susceptibility patterns or molecular typing or by using epidemiological methods such as the presence or absence of risk factors.

The eight most commonly cited criteria used to identify CA-MRSA are (David et al., 2008):

1. Isolate is susceptible to clindamycin
2. Isolate is resistant to less than 3 non beta-lactam antibiotics
3. Isolate carries SCC*mec*IV
4. Isolate is recovered from SSTI patients
5. Isolate carries PVL genes
6. Isolate is classified as Sequence-type 8 (ST8) and presumptively CMRSA10
7. Isolate is obtained within 48h of hospital admission

8. All isolates from criterion 7 lack the CDC definition risk factors

Defining an isolate as community-associated or healthcare- associated based on only one criterion may result in misclassification of cases. For example, if CA-MRSA is defined solely by the absence of the CDC risk factors (criterion 8), there is a risk of underestimating the prevalence of MRSA in the community. MRSA can be carried in the nares for more than a year and one retrospective cohort study estimated the half-life of MRSA colonization in known carriers to be 40 months (Sanford et al., 1994). Given the duration for which MRSA colonization can persist, an infection may develop in a setting different from that where the organism was initially acquired (Salgado et al., 2003). If the CDC definition based on hospital exposure in the last twelve months is used, an infection may be considered community-associated when the initial acquisition came from healthcare contact outside the one year range allowed for by this definition. Epidemiologic definitions that are based on time after hospital admission and presence/absence of healthcare exposure may underestimate the extent of the community MRSA reservoir (Carleton et al., 2004).

Relying only on antibiotic susceptibility patterns may also lead to a misclassification of CA-MRSA as HA-MRSA. Community-associated strains of MRSA are generally more susceptible to erythromycin, clindamycin, ciprofloxacin, and levofloxacin. However, these differences aren't enough to use as a means of defining CA-MRSA as increasing antimicrobial resistance patterns have been observed among community-associated isolates (Kowalski et al., 2005).

Kowalski suggests using a combination of laboratory factors to determine whether to classify an isolate as community- associated: 1) Lack of the multi-drug resistant phenotype 2) The presence of virulence factors such as PVL and 3) Type IV SCC*mec* and molecular distinction from nosocomial strains (Kowalski et al., 2005).

2.5 Epidemiology of community-associated methicillin-resistant *Staphylococcus aureus*

2.5.1 Origins of community-associated methicillin-resistant Staphylococcus aureus

In the late 1990's reports of MRSA colonization and infection in people without a history of recent contact with healthcare settings began to increase (David et al., 2008). Sporadic reports of MRSA had been reported in community settings since 1980 but were associated with typical HA-MRSA risk factors such as hospitalization and IV drug use (Chambers, 2001). The current US epidemic gained attention following a series of fatal infections in native children living in Minnesota and North Dakota (Moellering, 2006). These children lacked the traditional risk factors for MRSA infections (CDC, 1999). Testing showed that the strains of USA400 (CMRSA7 in Canadian nomenclature) were related to one another, but differed from the isolates in local hospitals (Chambers, 2001). The CMRSA7 type went on to cause infections throughout the United States. CMRSA10 has since replaced CMRSA7 as the predominant CA-MRSA strain in the U.S. and is considered endemic in many communities (Wibbenmyer, 2008).

Community-associated MRSA (CA-MRSA) has been endemic in Canadian northern prairie aboriginal communities for almost 20 years (Nicolle, 2006). In 1997 a community-based cluster of MRSA isolates in rural Manitoba with a PFGE pattern not previously seen in that part of the province was observed (Kurbis & Wylie, 2001). A later molecular analysis of the Manitoba isolates has shown that the PFGE pattern seen in this cluster was indistinguishable from the PFGE pattern seen in the Minnesota cluster

(Wylie & Nowicki, 2005). The strain responsible for the Manitoba outbreak was CMRSA7. In Canada, the first report of CMRSA7 strains harbouring the PVL toxin genes came out of east-central Saskatchewan in 2000 (Mulvey et al., 2005).

The incidence of CA-MRSA infections increased among Canadian First Nations people in northern Manitoba and Saskatchewan between 2003 and 2006 (Larcombe et al., 2007). Isolates collected from individuals in northern First Nations communities were typed by PFGE and the majority were indistinguishable from strains responsible for outbreaks in Minnesota, North Dakota and Manitoba. Using current Canadian nomenclature, the strains were identified as CMRSA7.

The first outbreak associated with CMRSA10 in Canada was reported in a marginalized population in Calgary in 2004 and almost simultaneously in intravenous drug users (IDU) in Vancouver. In the spring of 2004, an increased number of MRSA-skin infections were noted by a physician working in a Calgary correctional facility (Gilbert et al., 2006). At the same time, physicians working in laboratories and hospitals in the Calgary Health Region noticed an increase in skin infections attributable to MRSA prompting an outbreak investigation to be initiated. In this investigation, 42 individuals were identified with infections caused by CMRSA10. Epidemiologic findings from this investigation showed that a history of illicit drug use, homelessness, or incarceration were risk factors for infection with CMRSA10.

In the spring of 2000, an MRSA prevalence study was done in Vancouver's Downtown East Side (DTES) (Daly et al., 2002). Nasal specimens were collected from 229 injection drug users and were typed by PCR. All of the MRSA isolates were identified as

CMRSA5 which is considered a hospital strain. However, a follow-up study of the same population done in 2006 revealed that CMRSA10 had become the predominant strain accounting for 75% of the isolates (Al-Rawahi et al., 2008). The laboratory methods as reported in 2000 were not consistent with most testing methodologies used at the time to identify MRSA strains and the prevalence of MRSA may have been underestimated.

The origins of the major MRSA strains are poorly understood (Enright et al., 2002). CA-MRSA strains were initially thought to be hospital strains that had spread to the community; however, based on molecular typing, it is more likely that horizontal transfer of SCC*mec*IV into MSSA clones of different lineages has occurred at various times (Boyle-Vavra & Daum, 2007). Enright *et al.* used MLST to type 359 MRSA isolates and 553 MSSA isolates collected between 1961 and 1999 from 20 different countries (Enright et al., 2002). After typing, there were 11 major MRSA clones within five groups of related genotypes identified. This study shows that the major MRSA clones have arisen from successful MSSA strains. There is evidence that methicillin-sensitive *S. aureus* (MSSA) clones have become MRSA on more than one occasion (Enright et al., 2002),(Boyle-Vavra & Daum, 2007).

Vandenesch *et al.* used MLST to show that within a continent, the genetic background of community-associated strains did not correspond to the genetic background of the healthcare-associated strains (Vandenesch et al., 2003). They examined 21 MRSA isolates from each of six distinct PFGE patterns. All of the distinct PFGE patterns matched perfectly with a unique MLST sequence type. All of the sequence types of the

CA-MRSA strains in a geographic region were different from the sequence types of the HA-MRSA strains, suggesting that the community strains did not arise out of hospitals.

An outbreak of CA-MRSA skin infections in Alaska Natives was noted in August of 2000 (Baggett et al., 2003). A retrospective cohort study was done looking at laboratory-confirmed MRSA infections at the regional medical centre. Infections were classified as CA-MRSA if the patient's infection was diagnosed in the outpatient setting or within 48 hours of hospitalization, as well, the patient had to have no history of hospitalization, surgery, dialysis, indwelling line or catheter, or admission to a long-term care facility in the year prior to MRSA diagnosis. Regional MRSA hospital rates were low and cases of nosocomial MRSA were very rare making it unlikely that the emergence of CA-MRSA in the region was due to the spread of a hospital strain. Additionally, the villages where most of the infected people lived were very remote and helped support the almost certainty of community-acquisition of MRSA. Cases were based on confirmed laboratory cultures and were not strain typed.

Molecular typing and antimicrobial susceptibility testing done on a random selection of 490 inpatient and outpatient isolates collected over a period of seven years (1996-2002) in San Francisco showed that although the majority of patients had previous exposures to healthcare settings, the SCC*mec*IV genotypes identified were associated primarily with community-onset disease rather than healthcare facility onset, suggesting they were not descendants of hospital isolates (Carleton et al., 2004).

The introduction of CA-MRSA into the hospital is an inevitable outcome of a wide spread community reservoir (Carleton et al., 2004). An investigation into MRSA

bloodstream infections (BSI) demonstrated infections due to USA300 (CMRSA10) have emerged as a major cause of healthcare-associated and nosocomial bloodstream infections (Seybold et al., 2006). MRSA isolates from 132 cases of MRSA BSI were collected over 7.5 months at an urban public hospital in Atlanta. A total of 39 (34%) of the isolates were identified as CMRSA10 suggesting that community-associated strains were responsible for over a third of the hospital's MRSA BSI.

As healthcare-associated strains move into the community and community-associated strains move into healthcare settings, it will become increasingly difficult to distinguish between the two types.

2.5.2 Prevalence of community-associated methicillin-resistant Staphylococcus aureus

2.5.2.1 Determining the prevalence of community-associated methicillin-resistant *Staphylococcus aureus*

Both method and setting influence CA-MRSA prevalence estimates and studies vary widely. Prevalence has been estimated for outbreak and non-outbreak situations; clinical or surveillance purposes; and healthcare or community settings. Studies are either retrospective or prospective and look at either infection or colonization, or both (Furuya et al., 2007). Differing definitions, methods, and study populations in published studies makes it difficult to state what the prevalence of CA-MRSA actually is. The significant heterogeneity in the studies also makes pooling data to get an overall prevalence rate inappropriate.

Folden *et al.* demonstrated that using different definitions to define CA-MRSA can result in widely varying estimates of the overall proportion of CA-MRSA in a population

(Folden et al., 2005). In this hospital-based study, two different definitions for CA-MRSA were used: 1) the hospital's classification system which was based on the general CDC guidelines for nosocomial infections (at the time of the study) in which an infection already present on admission was considered community-acquired and 2) a definition based on healthcare risk factors where HA-MRSA was defined as any cases identified in patients a) 48 hours or longer after admission b)with a history of hospitalization, surgery, dialysis, or residence in a long-term care facility c) a permanent indwelling device at time of culture or d) known positive culture for MRSA prior to the recent admission. Retrospectively applying both definitions to 100 MRSA isolates collected between 2001 and 2003 showed that there was a marked difference between the proportions of cases classified as CA-MRSA for the two definitions. Use of the hospital/CDC definition gave a proportion of CA-MRSA of 49% as compared to the estimate of 5% based on the risk factor definition. A serious limitation in this study is that it relied solely on retrospective chart review to classify cases. Since the risk factor definition was based on what was recorded in the patient's chart, it was not possible to verify any of the risk factors that might have been missing. If healthcare risk factors were missing from the charts, it would increase the proportion of CA-MRSA cases, making the difference between the estimates smaller. Although this study did not look at prevalence rates specifically, the findings from this study support the hypothesis that using different definitions can result in dramatically different estimates.

A study done in New York City demonstrated how varying methodology can result in varying prevalence estimates (Furuya et al., 2007). In this study, three different methods

were used to estimate the prevalence of CA-MRSA infection and/or colonization in northern Manhattan: 1) a non-outbreak retrospective review of clinical and surveillance isolates identified in a computerized hospital database 2) the prospective collection of surveillance cultures from the same facility's emergency department and 3) prospective collection of surveillance isolates from the community using random digit dialing to identify potential subjects. Varying the definition of CA-MRSA, the choice of denominator for prevalence calculations and the method and setting of sample collection resulted in varying prevalence estimates. The prevalence of nares colonization with CA-MRSA was compared across the three methods. When the denominator was all *S. aureus* cases, the prevalence estimates were 2.1% using the hospital database, 5.0% using the emergency department data, and 1.2% using the community method. Although the estimates varied, the p value comparing the prevalence across the three case-finding methodologies was 0.45 indicating no significant difference existed. When the denominator was the number of all MRSA isolates, the prevalence estimates were 5.5% from the hospital database, 33% from the emergency department, and 50% from the community. There was a significant difference in the three methodologies when the denominator was changed ($p<0.001$). This study shows that varying methodologies can result in different prevalence estimates within a distinct geographical region.

Studies reporting on the prevalence of CA-MRSA in the community have often occurred as a response to outbreaks of infection in specific populations. For example, an outbreak of CA-MRSA10 in 2004 led to a prevalence study looking at risk factors in Calgary's marginalized population (Gilbert, 2007). There has been an increase of SSTI associated

with MRSA in intravenous drug users (IDU) over the past few years, and a prevalence study was done in Vancouver's IDU population in response to this upsurge (Al-Rawahi et al., 2008). Prevalence studies have been done in the community on other target populations following MRSA outbreaks including day care attendees, military recruits, and intravenous drug users (Salgado et al., 2003).

A meta-analysis looking at CA-MRSA colonization in various communities demonstrated an overall prevalence rate of 1.3% (Salgado et al., 2003). However, with the varying definitions of CA-MRSA used in those studies, and the fact that the majority of studies focused on patients who were seeking healthcare in either a clinic or hospital setting, the usefulness of this prevalence estimate in determining the level of CA-MRSA in the community is limited (Ellis et al., 2004).

2.5.2.2 Population level estimates of community-associated methicillin-resistant *Staphylococcus aureus* prevalence

Few studies have been done to determine CA-MRSA prevalence in the general population outside of outbreak situations. A secondary analysis of NHANES data from 2001-02 (as described in Section 2.2) found the prevalence of colonization in the non-institutionalized United States population with MRSA to be 0.84% (95% CI 0.4- 1.2) (Graham et al., 2006). The NHANES study was not able to provide molecular fingerprinting of the isolates, so the proportion of those MRSA cases that were CA-MRSA cannot be determined. The data for this study were collected in 2001-02, and it is likely that the prevalence in the general population has changed since the study was completed. This study also included individuals with epidemiological risk factors for

HA-MRSA acquisition including hospitalization and residence in long term care facilities.

Another group also undertook an analysis of the NHANES data from 2001-02 (Kuehnert et al., 2006). This study was completely independent of the Graham study and found the MRSA prevalence of colonization in the non-institutionalized United States population to be 0.8% (95%CI 0.4- 1.4). This slight difference in prevalence was likely due to differences in analysis.

2.5.2.3 Methicillin-resistant *Staphylococcus aureus* prevalence estimates in select healthy populations

Ellis *et al.* performed a prospective, observational study on a cohort of 812 Army personnel at Fort Sam Houston, Texas in 2003 (Ellis et al., 2004). Eligible participants were soldiers that had recently completed basic training and were enrolled to begin medic training. Recruits provided a nasal swab upon arrival at the base and eight to ten weeks later had an additional nasal specimen collected. A prevalence of nasal colonization with MRSA of 3% (95% CI 2.0 – 4.7) was reported following the initial nasal swabs (Ellis et al., 2004). The prevalence dropped to 1.6% (95% CI 0.9 – 2.7) at the time of the second swab in the absence of MRSA eradication efforts. Basic training involved living in crowded barracks with inadequate hygiene and possible skin trauma which are all risk factors for acquiring CA-MRSA. The decrease in MRSA prevalence between the first and second nasal swabs likely corresponded to increased living space and improved hygiene during the medic training as compared to conditions during basic training.

A cross-sectional study was done on a population of predominantly healthy children and their guardians attending an outpatient clinic in New York City to determine the prevalence of MRSA (Shopsin et al., 2000). Nasal swabs were collected from 500 individuals (275 children and 225 guardians) and PFGE was used to type the isolates. Only one participant, a five year old girl, was identified as having MRSA colonization. The prevalence estimate from this study was 0.2%. The strain type was not reported and the child had a history of hospitalization.

Care should be taken when applying population level findings to localized geographical regions as local MRSA epidemiology varies between areas and groups.

2.5.2.4 Community-associated methicillin-resistant *Staphylococcus aureus* prevalence estimates in marginalized populations

There were 833 homeless and marginally housed adults recruited into an MRSA prevalence study done between August 1999 and April 2000 in San Francisco (Charlebois et al., 2002). Results from the nasal specimens collected from participants showed 23 individuals colonized with MRSA, resulting in an overall prevalence of MRSA in this population of 2.8% (95% CI 1.7 - 3.9). Although PFGE typing was done on the MRSA isolates, the results were not compared to PFGE patterns of the major healthcare-associated or community-associated strains found in San Francisco at the time so the proportion of MRSA colonization due to community strains cannot be determined.

In 2005 a study was done to determine the prevalence of MRSA colonization or infection in a marginalized population in Calgary (Gilbert, 2007). Nasal specimens were self-

collected from 274 individuals recruited from various locales including an outreach needle exchange van, homeless shelters, detoxification centres, an inner-city medical clinic, and new admissions to a local corrections facility. The prevalence of any strain of MRSA was 7.4% (95% CI 4.6 – 11.2) and the prevalence of infection or colonization with CMRSA10 was 5.5% (95%CI 3.1-9.0).

In 2006 a point prevalence survey was done looking at MRSA nasal carriage among 301 IDUs in Vancouver, Canada (Al-Rawahi et al., 2008). Nasal specimens were collected from participants with a history of injection drug use within the previous year who were recruited from various locations in the downtown east side including medical clinics, drop-in centres, and a safe-injection site. A prevalence of MRSA nasal carriage of 18.6% (95% CI 14.4 – 23.5) was reported and CMRSA10 was identified in 75% of the MRSA cases. The overall prevalence of CMRSA10 was 14% (95% CI 10.2 – 18.4). The prevalence of MRSA in this population is higher than the MRSA prevalence of 7.4% (95% CI 4.7 – 10.9) observed in 2000 (as described in Section 2.5.1). It was also interesting to note that in 2000 all of the isolates were identified as CMRSA5 as compared to only 25% of the isolates in 2006, indicating that not only had the prevalence rate increased, but that CRMSA10 was replacing CMRSA5 in this population. Although the Downtown East Side (DTES) has a high proportion of marginally housed individuals, it is unknown from this study the proportion of the IDUs sampled that was homeless or marginally housed.

A prevalence study done in Cleveland, Ohio in 2008 found a prevalence rate in the homeless population of 25.6% (95% CI 19.9-32.0) (Landers et al., 2009). The study

collected nasal swabs from 215 participants recruited from various sites including three shelters and an outreach event. This prevalence rate is higher than other rates reported for marginalized populations.

In April 2006, the prevalence of MRSA in 44 residents of three Ottawa, Ontario homeless shelters was reported as 4.5% (95% CI 0.6 - 15.5) (Szakacs et al., 2007). Participants in this study completed a questionnaire and then provided nasal swabs. A unique feature of this study was that participants also provided one of either rectal, anal, or groin swabs to assess gastrointestinal carriage. However, the anatomical sites where the positive MRSA cultures were collected from were not reported and the study population was too small to assess gastrointestinal carriage of MRSA in this group. After typing with PFGE all of the isolates were identified as CMRSA2, which is usually associated with hospital strains. The large confidence interval around the prevalence estimate is due to the small sample size.

2.5.3 Community-associated methicillin-resistant Staphylococcus aureus *infections*

The clinical syndromes associated with MRSA differ between the hospital-associated and community-associated strains. A six-month study comparing 127 CA-MRSA and 156 HA-MRSA infections identified by retrospective review showed that CA-MRSA infections were significantly more likely to be SSTI such as abscesses, furuncles (boils), and follicultis (H. Huang et al., 2006). HA-MRSA was more likely to cause bloodstream, respiratory tract or urinary tract infections.

Infections attributed to CA-MRSA appear to be increasing. In 2004, physicians in an Emergency Care Service in Baltimore noted an increase in SSTI due to MRSA (Johnson et al., 2007). A retrospective study was initiated and microbiology cases between October 1, 2000 and September 30, 2005 were reviewed. Researchers compared the number of SSTI visits due to MRSA per 1000 ER visits for each of the five years. The proportion of MRSA SSTI increased over the five years of the study from 4% to 42% ($p<0.01$). Molecular typing was done on 296 of the MRSA isolates. In the first year, 31% of the isolates causing SSTI were CMRSA10 which increased to 89% by the fifth year.

SSTI account for most of the morbidity attributable to CA-MRSA, and mortality is still uncommon (Kowalski et al., 2005). However, CA-MRSA infections have been reported with serious outcomes including endocarditis, necrotizing fasciitis, sepsis, and pneumonia (Zetola et al., 2005).

2.5.4 Risk Factors for community-associated methicillin-resistant Staphylococcus aureus

Traditional risk factors for healthcare-associated MRSA acquisition have been identified and include antimicrobial drug use, presence of an indwelling device, and longer length of stay in the hospital (Graffunder & Venezia, 2002). Any combination of these factors may increase the risk of MRSA acquisition in healthcare settings. The risk factors identified for community-associated MRSA acquisition are slightly different from those for HA-MRSA strains, although both share previous antimicrobial drug use as a risk factor for MRSA acquisition.

A study done to determine the prevalence and risk factors for nasal colonization of MRSA among the urban poor in San Francisco found that participants who were colonized with MRSA were 2.4 times more likely to have used antibiotics in the previous twelve months as compared to participants without MRSA colonization (Charlebois et al., 2002). The study involved collecting nasal samples and risk factor questionnaires from 833 homeless and marginally housed adults between August 1999 and April 2000. Although antibiotic use was identified as a risk factor for MRSA colonization, the authors reported that specific recollection of antibiotic use was generally poor.

A cohort of 812 Army personnel enrolled in medic training at Fort Sam Houston, Texas in 2003 was recruited into a study looking at CA-MRSA in soldiers (Ellis et al., 2004) Recruits underwent a nasal swab and completed a basic questionnaire examining risk factors for MRSA colonization. Eight to ten weeks after the initial visit, the participants had an additional nasal specimen collected. Antibiotic use in the six months prior to the initial nasal swab was found to be a risk factor for colonization (Ellis et al., 2004).

Although the risk for acquiring both CA-MRSA and HA-MRSA is higher with previous antimicrobial use, there are specific risk factors observed in the community that aren't an issue in the healthcare setting.

Populations that have been identified as "at risk" for CA-MRSA following outbreaks include: children (CDC, 1999), correctional facility inmates (David et al., 2008), homeless individuals (Gilbert et al., 2006), intravenous drug users (Al-Rawahi et al., 2008), and men who have sex with men (Diep et al., 2008). There have also been several

outbreaks reported among competitive athletes on fencing, football and wrestling teams (CDC, 2003).

These high-risk populations all share a high likelihood of person-to-person contact, which is important since transmission of CA-MRSA tends to occur through direct contact with individuals already colonized with MRSA (Hawkes et al., 2007). The CDC identified the five main risk factors for CA-MRSA transmission as: crowding, compromised skin, sharing contaminated personal items, lack of cleanliness, and frequent skin contact (Gorwitz et al., 2008).

Along with the frequent skin contact seen in high risk populations, personal hygiene has also been identified as a risk factor for CA-MRSA acquisition. An outbreak of MRSA at a women's corrections facility in Missouri in 2002-03 prompted the initiation of a case control study focusing on hygiene factors in inmates (Turabelidze et al., 2006). The study sample consisted of 30 cases with confirmed MRSA skin or wound infections and 80 controls without SSTI randomly selected from the prison population. The participants were administered a questionnaire by a trained interviewer that focused on specific personal hygiene factors including hand washing, shower and laundry practices, and the sharing of personal items. Analysis showed that inmates with MRSA were more likely to share personal items such as cosmetics and nail clippers than inmates without MRSA. Inmates with MRSA also tended to wash their hands and take showers less often than inmates without MRSA.

Once acquired, carriage of MRSA might be an indication of a higher risk for subsequent infection. Poor hygiene practices combined with staphylococcal colonization can create an ideal environment for infection to occur (Lowy & Miller, 2002).

Nasal colonization with CA-MRSA is a risk factor for subsequent MRSA infections (Ellis et al., 2004). In a prospective study by Ellis (described in Section 2.5.2.3) it was reported that 38% of the participants colonized with CA-MRSA at the initial sampling developed a soft-tissue infection within ten weeks as compared to only 2% in the group that was negative for MRSA colonization at the initial visit. The study did not report whether the infecting strains were the same as the colonizing strains.

Huang and Platt followed 209 patients discharged from the Brigham and Women's Hospital in Boston, Massachusetts that were newly identified as being colonized or infected with MRSA (Huang & Platt, 2003). The study followed the participants for 18 months. Of the 97 patients with MRSA colonization at discharge, 30 (31%) individuals developed a subsequent MRSA infection. The majority of those infections (80%) occurred at sites unrelated to the initial site where MRSA was identified. The study did not report whether the infecting strains were the same as the colonizing strains. In the absence of a control group, it is unknown what proportion of patients that were MRSA-negative at discharge developed infections.

Although there is evidence that carriage of *S. aureus* is a risk factor for subsequent infection with the same strain (as discussed in Section 2.2.3), longitudinal studies will need to be done to ascertain if individuals are being infected with their colonizing strains of CA-MRSA.

2.6 Decolonization, treatment, and prevention of community-associated methicillin-resistant *Staphylococcus aureus*

Multiple studies have explored the origins, prevalence, and risk factors for CA-MRSA. Although the epidemiology of CA-MRSA has been extensively described, there have been few studies on how to manage MRSA in the community (LaPlante et al., 2008). Management of CA-MRSA can involve decolonization of MRSA carriers, treatment of active infections, and prevention activities.

2.6.1 Decolonization

One option that can be used in the community for managing MRSA is the decolonization of MRSA carriers. Decolonization can be defined as the activity of removing bacteria from the body surfaces. Both topical agents and systemic antimicrobial agents have been used for MRSA decolonization, both alone and in combination.

Mupirocin is a topical antibiotic frequently used in nasal MRSA decolonization regimes. It is usually provided in the form of a cream in a 2% concentration and is applied to the anterior nares. There have not been many decolonization attempts made in the community using mupirocin alone.

Between January and December, 2005, Ellis *et al.* performed a double-blind, cluster randomized, placebo controlled study to determine if a decolonization regime of MRSA nasal carriers using mupirocin would reduce infection in those participants and prevent transmission in the treated individual's study group (Ellis et al., 2007). Overall, 66 out of the 1459 soldiers in the placebo group that completed the study were colonized with CA-

MRSA and 68 of the 1607 participants in the mupirocin group that completed the study were colonized with CA-MRSA. Participants with CA-MRSA colonization were given treatment (either mupirocin or placebo) twice daily for five days and were followed for 16 weeks. CA-MRSA was defined as colonization occurring in participants without known risk factors for MRSA. There was no decrease in the incidence of SSTI between the two study groups and mupirocin did not reduce new CA-MRSA colonization within the mupirocin study group.

Although the nares are the most common site of MRSA carriage, individuals may also be colonized at other anatomical locations. MRSA colonization at two or more body sites has been associated with persistent carriage (Popovich & Hota, 2008) and different MRSA strains can be isolated from different sites on the same person (O'Brien et al., 1999). Relying on nasal decolonization alone is not sufficient to prevent subsequent infections in MRSA carriers or prevent transmission to others.

Since MRSA can be isolated from body surfaces including the hands, axillae, and perineum, several antiseptic liquid soaps have been used in efforts to decolonize these sites. Although the effectiveness of these agents has been well documented in laboratory and hospital studies, community based studies of dermal decolonization with topical agents alone are lacking.

One of the most frequently used products in MRSA skin decolonization is chlorhexidine gluconate (CHG). In concentrations of two or four percent, CHG has been found to be very effective against a wide variety of bacteria including MRSA (Popovich & Hota, 2008). CHG has been reported to cause skin irritation in some individuals and it has a

moderate potential for inducing resistance in bacteria (Kampf & Kramer, 2004). It has been used for decolonization in healthcare settings, often combined with mupirocin administration. The role of CHG in an outpatient setting has not been established.

Accelerated Hydrogen Peroxide (AHP) foaming skin cleanser has been developed as a topical skin antiseptic. Hydrogen peroxide produces destructive free radicals that can disrupt cell membrane lipids, DNA, and other bacterial cell components (http://www.cdc.gov/hicpac/Disinfection_Sterilization/acknowledg.html). *S. aureus* produces the enzyme catalase that acts to protect bacterial cells by breaking down hydrogen peroxide into water and oxygen. The concentration and amount of H_2O_2 present in AHP overwhelms the bacteria's ability to decompose H_2O_2 into its' component parts using catalase and the cell dies. AHP is different from the hydrogen peroxide that can be purchased in drug stores as it contains less active ingredient than commercially available products, but has an accelerated germicidal performance. The mechanism of action of AHP does not promote bacterial resistance.

Antibiotics are used for the treatment of active infection (Deresinski, 2005), but in most situations oral antibiotics are not appropriate for decolonization due to the increased risk of resistance. Resistance to oral decolonization agents has evolved rapidly in settings where decolonization was attempted by this method (Naimi et al., 2003).

With little evidence to suggest that decolonization protocols for MRSA have long-term effectiveness, and a risk for increased resistance to decolonizing agents, the choice on whether to attempt decolonization in a group or population should be carefully considered.

2.6.2 Treatment of Infections

Although colonization of individuals with MRSA is often asymptomatic, infections can occur if the bacteria enter the body. Treatment options depend on both the site and the severity of the infection.

The most common clinical CA-MRSA infections are SSTI such as boils and abscesses. For lesions under 5cm, it is recommended that the initial treatment be incision and drainage. If medical follow-up is provided, drainage of small abscesses is sufficient (Kowalski et al., 2005). When the lesions are large, are surrounded by cellulitis, and occur in patients with fever or other systemic symptoms, treatment with antimicrobial drugs may be necessary (Moellering, 2008). Some older antimicrobials such as clindamycin, tetracycline, or trimethoprim/sulfamethoxazole (TMP/SMX) have been recommended for the treatment of less serious SSTI. However, their increased use may result in the emergence of increasing amounts of multidrug-resistant CA-MRSA (Diep et al., 2008).

In areas with known CA-MRSA, there needs to be careful thought regarding the management of infections. Antimicrobials should be reserved for severe infections and when CA-MRSA is suspected, β-lactams should not be prescribed (Baggett et al., 2003). When using oral antimicrobials, follow-up should be done within 48-72 hours as initial therapy may not be effective. Treatment failure may lead to increased severity of infection (Popovich & Hota, 2008). Vancomycin should not be used except in cases of seriously ill patients at risk of endocarditis or intravascular infection, or when MRSA is isolated from blood or bone (Baggett et al., 2003).

2.6.3 Prevention

Prevention of *S. aureus* infections is increasingly important as decolonization and treatment become more challenging.

Basic strategies for preventing transmission of MRSA include maintaining consistent and appropriate hand hygiene; not sharing personal items such as razors or towels; keeping draining wounds covered; and ensuring that communal bathing areas are clean (Nicolle, 2006). Since MRSA may survive on inanimate surfaces and cotton for up to 90 days (Neely & Maley, 2000), care should be taken to disinfect the environment regularly.

Prevention of transmission in the community requires that individuals take an active role to prevent the further spread of MRSA. Education measures need to be put in place to promote behaviours that lessen the risk of transmission.

2.6.4 Targeted interventions to reduce transmission and prevent community-associated methicillin-resistant Staphylococcus aureus *infections*

Interventions have been initiated in response to outbreaks in specific groups. These interventions often involve enhanced screening of the target population followed by decolonization attempts in conjunction with prevention efforts.

In August of 2001, a correctional facility in Georgia detected a cluster of MRSA skin infections among male inmates (Wootton et al., 2004). An intervention strategy was developed to prevent further infections. The intervention consisted of several components including 1) screening all inmates for SSTI at admission 2) providing liquid hand soap near all bathrooms 3) giving chlorhexidine gluconate body wash to all inmates

for the first five days of the intervention 4) providing education about skin and hand hygiene to all inmates and 5) waiving the mandatory $5 visit fee for any visit to the health clinic pertaining to skin infections. The intervention began on July 24, 2001 and the last day of intervention monitoring was December 31, 2001. The number of cases decreased from 11 at the start of the study to zero on the last day of the study. As this intervention involved multiple components, the effectiveness of any one intervention strategy cannot be measured. The success of this program may not be replicated in other settings due to the unique nature of this study population. The long term effectiveness of the intervention is also unknown.

An outbreak of CA-MRSA in a Pittsburgh high school football team in 2003 prompted the initiation of several interventions to prevent further infections on the team (Rihn et al., 2005). The interventions included 1) team education regarding personal hygiene behaviour such as not sharing equipment and frequent hand washing 2) weekly examination of team members for infectious lesions 3) referral of players with infection to their personal physician for wound culture and treatment 4) restriction of infected players from practice until treatment was initiated and coverage of lesions during practice 5) collection of nasal cultures from all players and staff to identify colonized individuals and 6) prescription of 2% mupirocin ointment (initially to MRSA positive individuals, but later in the season to the entire team). It was concluded that the interventions were ineffective at controlling MRSA infections in this group. There were 20 separate episodes of CA-MRSA infection reported for 13 out of the 90 players on the team and 3 players were colonized with MRSA. The compliance rate with mupirocin use was 39%

with the reasons for noncompliance being cost, inability or unwillingness to fill the prescription, and discomfort associated with administration of the ointment. The authors reported that the intervention was in response to an outbreak in week 4 of the season when four players were identified with abscesses. However, they do not report the timing of the subsequent infections, so it cannot be determined how long after the interventions were implemented that the infections occurred.

As these interventions had multiple components, the contribution from any one factor cannot be measured. Targeted interventions for specific populations are necessary.

2.7 Health and the homeless

2.7.1 Impact of homelessness on health

There is considerable variation in definitions applied to homelessness. Homelessness can be viewed as a continuum ranging from the absolute homeless to the marginally housed or "relative" homeless (Reid, 1998). The United Nations define the absolute homeless as individuals who are without shelter and who sleep in places not intended for human habitation; the relative homeless are defined as individuals who might have shelter but it doesn't meet the basic standards for health and safety (Hwang, 2001). The relative homeless group includes the "couch-surfers" who move between the houses of friends and relatives; the working poor staying at a shelter to save for a place of their own, and the un(der)employed who stay in shelters. The absolute homeless group is the population who "sleeps rough".

It is inappropriate to assume the homeless population is homogenous. The distinct homeless sub-populations have their own cultures, and sets of social practices and relationships in which they engage. They also occupy diverse physical environments that can affect health (Hodgetts et al., 2007). The length of time an individual is homeless can also vary and can be divided into three broad categories; the chronic homeless, the cyclical homeless, and the temporary homeless (Echenberg & Jensen, 2008).

Although people who are homeless live in a wide variety of conditions, the one unifying concept in defining homelessness is that housing is unstable and/or inadequate. However, homelessness is not simply a lack of shelter. It is a complex process reliant on

several determinants of health including income and social status, personal health practices and coping skills, social support networks, education and culture. Material hardships and economic and social exclusions are core determinants of health (Fox et al., 2003).

There are multiple pathways that result in homelessness (Susser et al., 1993). The reasons an individual becomes homeless can be structural such as the decrease in affordable housing and social housing supply or personal including catastrophic events, loss of employment, onset of mental illness, or substance abuse. The structural and personal factors can work in tandem as a pathway to homelessness.

The term “marginalized population” is often applied to homeless individuals. Marginalization is a social process of being relegated to a lower social standing. It can occur on the individual, community, or policy level. By definition, all homeless individuals are considered marginalized, however, not all marginalized people are homeless.

Homeless populations experience poor health compared to the general population; rates of respiratory illness, injuries and violence, and infectious diseases are significantly higher (Hwang, 2001). They also must contend with significant barriers to health care access. Although Canada has a universal health care plan, many homeless individuals cannot or do not access medical care. Disease is often more severe in homeless people, and that may be due to extreme poverty, delays in seeking health care, non-adherence to therapy, and cognitive impairment (Hwang, 2001). Homeless people deal with many

competing needs such as seeking food, shelter, and clothing as well as maintaining safety so adhering to prescribed diets or rest periods may not be possible (Plumb, 2000).

Income inequality is often associated with differences in health, and is also accompanied by differences in conditions of life at both the individual and population levels (Lynch et al., 2000). Although the effects of material deprivation on health are readily understood (e.g. limited money for prescription medications) they must be considered along with psychosocial effects. Homeless individuals often experience social exclusion and may also lack social support networks which also contribute to poor health status (Kawachi & Kennedy, 1997).

The urban poor and the homeless tend to have higher rates of hospitalization and a higher prevalence of injection drug use when compared to age, race, and sex-matched national averages in the United States (Charlebois et al., 2002). Crowded living conditions and limited access to sanitation facilities experienced by street and shelter dwellers place this population at risk for the acquisition and transmission of methicillin-resistant *S. aureus*.

2.7.2 Health interventions in homeless populations

Several factors need to be considered when attempting a health intervention in a homeless or marginalized population and methods used in the general population may not be applicable to this group.

Although a sampling frame (e.g. census data, telephone books, or membership in organizations) may be available for investigators to randomly select participants from known or well-defined populations, they do not generally exist for hidden populations

such as the homeless (Faugier & Sargeant, 1997). When the sampling frame is unknown, it may be possible to rely on a knowledgeable insider to provide initial access to individuals for participation in the study. If there is already an established relationship between the insider and members of the population, it can promote trust between potential study participants and the researchers.

Researchers often rely on non-random sampling methods including convenience and snowball sampling when recruiting homeless participants into research studies.

Convenience sampling involves recruiting a sample from the members of a population that are close by and readily accessible. This may involve recruiting from a specific area where the population of interest congregates. In studies of homeless populations, convenience sampling has been done in meal lines at food kitchens and in shelters. However, relying on shelters for recruitment will miss the proportion of the homeless population that does not access the shelter system (Toro et al., 1999).

Snowball sampling is a technique where the researcher recruits a study participant, and in turn, that participant recruits more subjects. Snowball sampling is a useful method for reaching hidden populations.

There have been attempts to randomly recruit homeless or marginally housed participants into health studies. In a 1995 study in Melbourne, Australia, researchers used a stratified systematic sampling method to study the health status of accommodated homeless individuals (Reid et al., 1998). The sampling frame consisted of a listing of 13,482 beds present in 808 establishments that provided accommodation for the homeless. The

researchers started at a randomly selected bed number and then sampled every 34th bed to ultimately obtain a sample size of 284 participants. The study had several limitations, with the most important being that the sample frame was only appropriate for the accommodated homeless and did not include the absolute homeless. It also may have been biased towards the unhealthy or unemployed, as researchers only went to a bed once, and the participants were included if they were present when the researcher was there. This may have increased the number of unemployed or ill people included in the study. However, with modifications, this method may prove viable as a method for randomly selecting participants that are shelter dwellers.

In addition to differences in homeless populations arising from the type of shelter being utilized, there are also significant variations in homeless subpopulations. Populations that are often included in research are families (especially homeless women with children) and runaway youth (Toro et al., 1999). Health intervention studies in homeless and marginalized populations have traditionally been focused on conditions such as mental illness or substance abuse and studies often compared interventions such as provision of housing or detoxification programs to usual standard of care (Hwang et al., 2005).

Although there have been infectious disease interventions reported, the majority have been on tuberculosis and HIV infections. Research gaps exist in the area of eradication of bacterial species including MRSA.

An intervention was done in France between 2001 and 2002 in an attempt to eradicate the bacteria *Bartonella quintana* in homeless people (Foucault et al., 2003). In this study,

homeless people with a positive blood culture for *Bartonella quintana* were randomized to receive either no treatment, or treatment with a combination of gentamicin and doxycycline. Participants were recruited from both emergency departments and shelters in Marseilles. Treatment lasted for 28 days, the first 14 days as inpatients and the second 14 days as either inpatients or in shelter medical facilities. There were 35 homeless individuals identified with *Bartonella quintana* bacteremia during the study period. Of those, 20 patients agreed to participate in the trial and 80% completed the study. The authors hypothesized that the 15 people with bacteremia that declined participation likely did so due to the two week hospitalization required at the beginning of the study.

A review of interventions to improve the health of the homeless reported that the majority of studies were plagued by small sample sizes (less than 50 people) and significant loss to follow-up (Hwang et al., 2005).

Adherence to study protocols has been an issue in interventions to improve the health of the homeless. Challenges to improving adherence include forgetfulness, drug-induced apathy, and a lack of interest in participating in time-consuming interventions that require extensive interactions with health care staff (Mills & Cooper, 2007).

2.7.3 Methicillin-resistant Staphylococcus aureus *in marginalized populations*

Risk factors for CA-MRSA include crowding, compromised skin, lack of cleanliness, frequent skin to skin contact, and the sharing of contaminated personal items. Individuals who are homeless may be at increased risk for CA-MRSA acquisition because they often

share crowded living conditions and may have limited access to showers and laundry facilities.

There have been several studies done to determine the prevalence of MRSA in marginalized populations (as described in Section 2.5.2.4). Estimates range from a low of 2.8% found in the urban poor in San Francisco in 1999-2000 up to a prevalence of 25.6% reported in a population of shelter users from Ohio in 2008. Canadian estimates have ranged from 4.5% in Ottawa in 2006 to 18.6% in IDU drug users in Vancouver in the same year. This considerable variation may not necessarily be due to increased rates of MRSA in certain areas, but rather variation in study design: study populations ranged from IDU to homeless individuals; sampling was either restricted to nares or expanded to include other sites; swabs were either collected by the individual or by study personnel; data collection was retrospective in response to outbreaks or prospective; and definitions of CA–MRSA differed. With this variation in study design comparing the prevalence of CA-MRSA in marginalized populations is challenging.

2.7.4 Homelessness in Calgary, Alberta

The City of Calgary conducts a one-night census of homeless persons every two years with the most recent census report available from May of 2008 (Stroick et al., 2008). The City defined a homeless individual as someone who was living on the streets, or was staying in an emergency shelter or facility that offered longer term shelter and support to people who would otherwise be living on the streets. The census included a count of individuals staying in 61 facilities, a count of individuals with no fixed address seen at 11 service agencies, and an estimate of individuals living on the streets.

The census from May 14, 2008 estimated there were 4,060 homeless individuals in Calgary. At the time of the 2008 census, 79% of the homeless were staying in shelters, 7% were seen at service agencies such as emergency rooms and police stations, and an estimated 14% were on the streets. Since this census was done during a period of dry weather, the proportion of the population that were shelter users may have been underestimated for the day. There were 4,060 homeless individuals counted in the census and 78% of the enumerated population staying at facilities and seen at service agencies were male. The majority (43%) of the homeless were working-age adults from 25 to 44 years old, and another 29% were middle-aged adults between 45 and 64 years old. Young adults between the ages of 18 and 24 comprised 9.4% of the homeless population. Approximately 2% of the homeless population were over the age of 65. Children under the age of 17 made up about 11.5% of the homeless population.

There were 3,195 individuals staying at a facility on the night of the census. A total of 77% of these individuals were male. The age group distribution was similar to the enumerated counts, which is expected as the majority of the enumerated group were staying in facilities. Of the individuals staying in facilities, 47% were using emergency beds and the other 53% were using transitional beds.

2.7.4.1 The Calgary Drop-In and Rehab Centre

The Calgary Drop-In and Rehab Centre (CDIRC) provides shelter and services to low-income and homeless individuals in Calgary. It has the capacity to house 1250 nightly and serves around 3500 meals daily. The three levels of housing available at the Riverfront site of CDIRC include intox beds on the first and second floors for individuals

who are under the influence of drugs or alcohol; emergency beds and day sleep on the third floor; and transitional housing on the fourth and fifth floors for people looking to move out of shelter living. Showers and laundry facilities are available free of charge, and there is also a hygiene office, a labour office, counselling, and a health centre on site. CDIRC also provides clean towels, soap and shampoo, and laundry detergent to its users. Individuals must be 18 years old or older to access CDIRC services, and the majority of users are male.

An in-house study of 214 individuals was done in 2007 to determine the employment status of CDIRC clients and reported that 60% of CDIRC clients at the time were employed and 75% of those employed were doing manual labour jobs (CDIRC, 2007). When the sub-population of clients between 46 and 55 years old was considered, half were working full or part time and 85% of the working individuals were doing manual labour.

The onsite health centre at CDIRC faces significant challenges in dealing with the complex medical problems faced by clients (Ries Ferrari et al., 2006). In November, 2005 151 CDIRC clients were surveyed to assess their perceived health and determine where medical attention was obtained, what medical conditions were present, the types of medications prescribed, and how clients paid for prescriptions. Results were stratified by intox clients, emergency bed users, and transitional bed users. Residents using intox and transitional beds were older and had been on the streets longer than residents using emergency beds. Clients tended to report their health as average, even in the presence of two or more medical conditions or concerns. The majority (71%) of the clients surveyed

did not have a family doctor and the primary service provided identified was the 8Th and 8th Medical Centre followed by CUPS.

2.7.5 Methicillin-resistant Staphylococcus aureus *in Calgary, Alberta*

In the spring of 2004, an increased number of MRSA-skin infections were noted by a physician working in a Calgary correctional facility (Gilbert et al., 2006). At the same time, physicians working in laboratories and hospitals in the Calgary Health Region also noticed an increase in skin infections attributable to MRSA prompting an outbreak investigation to be initiated. There were 42 individuals with MRSA isolates identified as CMRSA10. Epidemiologic findings from this investigation showed that a history of illicit drug use, homelessness, or incarceration were risk factors for infection with CMRSA10.

In 2005 a follow-up study was done to determine the prevalence of MRSA colonization or infection in the marginalized population in Calgary (Gilbert, 2007). There were 274 self-collected nasal specimens provided by individuals recruited from various locales including an outreach needle exchange van, homeless shelters, detoxification centres, an inner-city medical clinic, and new admissions to a local corrections facility. The prevalence of any strain of MRSA was 7.4% (95% CI 4.6 – 11.2) and the prevalence of CMRSA10 was 5.5% (95%CI 3.1-9.0).

The previous MRSA study in Calgary recruited from various sites, so the prevalence rate of MRSA colonization or infection is unknown for users of CDIRC. However, the prevalence rate of 7.4% observed by Gilbert *et al.* is likely similar to the prevalence in

clients of CDIRC. We know of no interventions done to reduce MRSA carriage in homeless populations. As colonization is a risk factor for subsequent infection, efforts should be made to reduce MRSA carriage in the homeless population that accesses CDIRC services.

CHAPTER THREE: METHODOLOGY

3.1 Introduction to Chapter Three: Methodology

This chapter presents the methodology used in this study. The chapter is separated into two main sections: (1) The prevalence study, and (2) the intervention study. Both of the sections are further divided to discuss: (a) Study design, (b) Study population, (c) Study procedures including recruitment and, testing, (d) Data collection and management including study sample, data handling, and laboratory procedures, (e) study definitions of MRSA colonization and infection, and (f) data analysis.

A section on the ethics approval process for this study is included at the end of the chapter.

3.2 Prevalence study

3.2.1 Study design

This was a descriptive cross-sectional study designed to estimate the point prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the population that accesses the Calgary Drop-In and Rehab Centre (CDIRC) and to examine risk factors for MRSA carriage in this group.

3.2.2 Study population

The study took place at the Calgary Drop-In and Rehab Centre, in downtown Calgary, Alberta, Canada on March 9 and March 11, 2008. All people accessing CDIRC services between 11:00 and 17:00 on March 9 and all individuals who were staying on the fourth or fifth floors on the night of March 11 were eligible for inclusion. Individuals unable to give verbal consent were excluded.

3.2.3 Study procedure

3.2.3.1 Recruitment

One week prior to the start of data collection, posters were put up at various places within CDIRC, including the health office and on the upper floors. The posters contained information on MRSA and some study details (Appendix B). Information sheets on MRSA were also available from the health office in the week prior to the study and at the study site on the testing day (Appendix C).

3.2.3.2 Testing

The study was conducted in the Intox Room at CDIRC. This is a large, auditorium-style room with a concrete dividing wall at the north end. Individuals entered through the main door and were directed to one of several volunteers sitting at tables throughout the room (Figure 1).

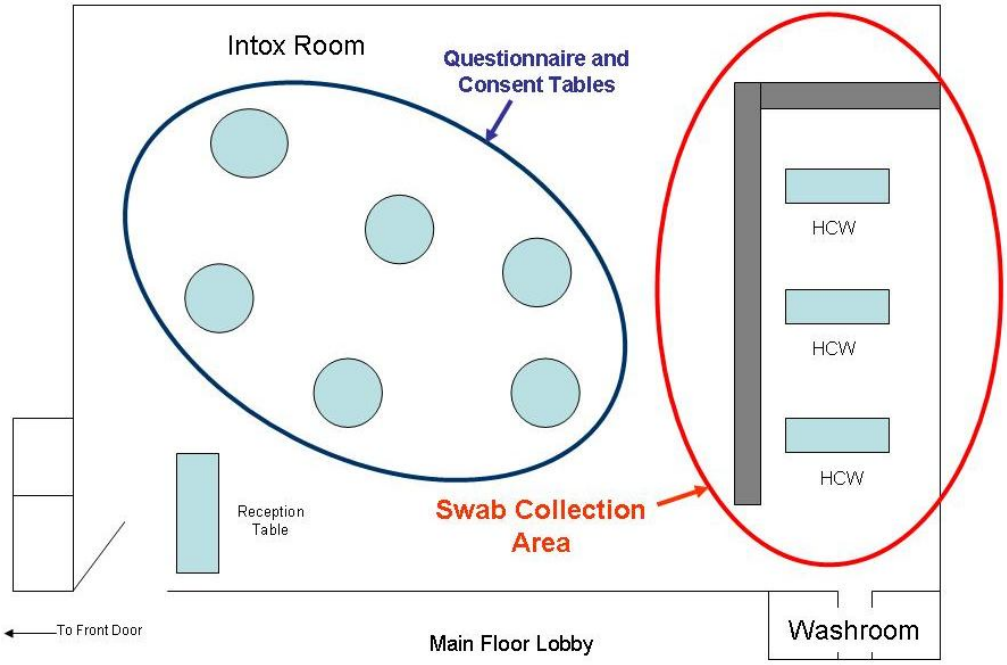

Figure 1 Schematic of the Intox Room at the Calgary Drop-In and Rehab Centre for the methicillin-resistant *Staphylococcus aureus* point prevalence study on March 9, 2008 (not to scale)

Study volunteers read the consent form out loud to all participants and were also encouraged to explain any parts of the form that were unclear (Appendix D). Copies of the consent form were offered to participants.

Once consent was obtained the volunteers led the participants through the questionnaire (Appendix E). Following the questionnaire, the volunteers escorted the participants to the testing stations in the swab collection area. Volunteer health care workers did the swab tests. Participants were swabbed in their anterior nares, across their torsos, and at a wound site if one was present.

To collect the nasal specimen, a sterile cotton-tipped swab was moistened with the solution in the transport container and then rotated for 10-15 seconds in the anterior nares (nostril). The swab was not inserted deeper than 1cm. The procedure was repeated with the same swab on the other nostril.

The torso specimen was collected using a Z-swab technique where the sterile swab was moistened and rubbed from one shoulder to the other, across and down the torso, and then from one side of the lower abdomen to the other side of the abdomen.

Participants were asked to identify if they had any wounds present. Wounds were defined as any skin or soft tissue infection such as a boil or furuncle that is pustular. The wound specimens were collected by using a dry sterile cotton-tipped swab and gently swabbing any exudate from the wound. If no pus was present the specimen was collected from the area around the wound. Health care workers did not attempt to collect pustular material from wounds that were scabbed over (i.e. they did not remove any scabs to collect pustular material).

Participants were instructed that their results would be available in one week at the health office on site at CDIRC. Results were placed in sealed envelopes and kept in a locked cupboard in the health office.

Data for the prevalence study was also collected on March 11, 2008 at CDIRC. Individuals were recruited on the fourth and fifth floors in the evening to ensure that both the intox and emergency-bed populations as well as the transitional populations were represented in the study. The same methods as above were used to obtain consent, complete the questionnaires, and collect the swab specimens.

3.2.4 Laboratory procedures

The swabs were placed in tubes of sterile culture medium and were then put in biohazard bags. At the end of the day, the bags were transported to Calgary Lab Services, a medical diagnostic laboratory that offers a full range of laboratory services to the Calgary Region and parts of Southern Alberta. The samples were stored overnight in the refrigerator and were plated the following morning.

The specimens were planted on Denim Blue agar (Oxoid chromogenic MRSA screening agar) plates and were incubated at 37 degrees centigrade for 24 hours (Figure 2). Denim blue colonies that appeared on the plates after the first 24 hours were presumptively considered MRSA. Plates that showed no growth were incubated for a further 24 hours. Plates that incubated for 48 hours had many bacterial colonies that could not be presumed as MRSA. Suspected MRSA colonies were “picked” and were tested using hydrogen

peroxide to identify catalase-positive species. Coagulase tests were done on the catalase-positive bacteria to determine if they were *Staphylococcus aureus*.

Isolates presumptively identified as MRSA were tested using a multiplex PCR assay for simultaneous identification of CMRSA7 and CRMSA10 (Zhang et al., 2008). The results from this assay can tell if an isolate is Staphylococci or not; if it is *S. aureus* or coagulase-negative staphylococcus; if it is MRSA or MSSA; and if it was CMRSA7, CMRSA10, or another MRSA strain.

3.2.4.1 Colonization and infection

Colonization is defined as the asymptomatic carriage of bacteria. In this study, MRSA colonization was determined by an MRSA positive swab test at the nares and/or the torso.

Infection occurs when bacteria breach the skin barrier and cause disease. In this study, participants were considered infected if they tested positive for MRSA at a wound site. If a participant tested positive for MRSA at a wound site and also at the nares and/or torso, they were considered infected.

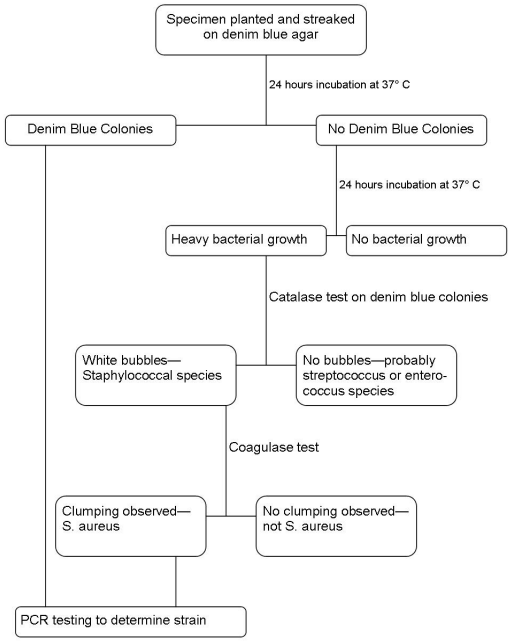

Figure 2 Microbiology testing done at Calgary Lab Services to identify methicillin-resistant *Staphylococcus aureus* positive isolates from the Calgary Drop-In and Rehab Centre prevalence study

3.2.5 Data collection and management

3.2.5.1 Study sample:

The sample size for the study was based on the estimated 7.4% (95% CI 4.6 -11.2) prevalence of MRSA colonization or infection seen in the homeless population in the 2004 study in Calgary's homeless population (Gilbert, 2007). Using the prevalence estimate from Gilbert's study, a sample size of 192 was required to detect a prevalence of 7.4% (with a range between 3.7% and 11.1%) with 95% confidence.

A non-probability convenience sampling method was used. In this type of sampling, all members of the population do not have equal chance of inclusion in the study and

participants are not recruited randomly. The size of the sample was dependent on how many individuals came into the centre on that day and also dependent on how many of those individuals were interested in participating.

3.2.5.2 Data handling:

Questionnaire answers were entered into a Microsoft Access password-protected database. Hard copies of the questionnaires were kept in a locked filing cabinet in a secure office.

3.2.6 Data analysis

Analysis was done using Stata version 9.0 (College Station, Texas). Frequencies on questionnaire variables were tabulated.

The prevalence of MRSA colonization/infection was calculated by dividing the number of MRSA cases by the total number of people tested.

Risk factor analysis was done by calculating Odds Ratios to determine if associations existed between MRSA status and the risk factor variables included in the study.

3.3 Intervention Study

The original intervention study was a non-random cohort design in which the positive cases identified in the prevalence study would be recruited into the intervention arm. However, there were delays between the initial sampling and the implementation of the intervention that made it not possible to contact more than one of those cases. Due to the nature of the population, this method proved to be not feasible and an amendment was made to the protocol. The rest of this section refers to the amended intervention.

3.3.1 Study design

This was a test-negative case-control study designed to explore the feasibility of providing Accelerated Hydrogen Peroxide (AHP) foaming skin cleanser to reduce skin-carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) in the population that accesses the Calgary Drop-In and Rehab Centre (CDIRC).

3.3.2 Study population

The study took place between November 23, 2008 and June 1, 2009. Any individual that accessed the Calgary Drop-In and Rehab Centre during the study period was eligible for inclusion. People unable to give verbal consent were not eligible to participate in the study.

3.3.3 Study procedure

3.3.3.1 Recruitment

A poster was displayed on the door to the Safeworks office whenever recruitment was occurring during the study period (Appendix F). Information sheets on MRSA were also available from the health office in the week prior to the study and during the period of data collection (Appendix C).

3.3.3.2 Testing

The study began on November 23, 2009 and five individuals were recruited. Later that week, Deb Canada contacted us with concerns about the AHP product becoming "cloudy". Undistributed product was returned to the company. The project started again on February 8, 2009. Due to the difficulty in recruiting, the original plan to recruit from CDIRC on Sunday afternoons was modified to include Tuesday afternoons as well. In mid-April, the decision was made to recruit participants from CDIRC on Monday and Wednesday as well. Data collection ended on June 1, 2009.

Individuals were read the consent script (Appendix G) and, after verbal consent was obtained, were led through the same questionnaire used in the prevalence study (Appendix E). Participants were swabbed in their anterior nares, across their torsos, and at a wound site if one was present. The same method of swabbing was used as previously described in Section 3.2.3.2. Wound sites were not swabbed if they were below the waist and above the knees.

In addition, two Rodac plates were used per participant, one on the upper chest and one in an axillae. The specimens were collected by removing the cover from the Rodac plate and pressing the exposed blood agar surface onto the skin. Care was taken not to move the plate from side-to-side during the collection to avoid obtaining smeared plates.

Participants were given a bottle of AHP skin cleanser and were directed to use it in the shower in place of their regular soap for the next seven days. Participants were given instructions to take with them (Appendix H). They were asked to return in one week for results and follow-up swabs.

On the participants' second visit they were re-swabbed as above (nares, torso, and wound if present) and Rodac plate specimen collection was repeated for the upper chest and one axillae. The participants were then led through the post-intervention questionnaire regarding use of AHP that included questions on frequency of use and participants opinion about the product (Appendix I).

Participants who failed to return after their initial swab for the seven-day follow-up visit were paged over the CDIRC intercom at subsequent study days. A record was maintained of the number of times a participant was paged prior to returning for the follow-up. Participants were paged until they returned for the follow-up visit; the study coordinator was informed they were no longer using CDIRC; or the study ended.

MRSA results were left in individual envelopes in the health office for all participants that didn't return for the follow-up visit.

3.3.4 Laboratory procedures

The swabs were placed in sterile culture medium, put in a biohazard bag and transported to the Infection Prevention and Control Laboratory. All swab specimens were planted within four hours of collection. The swabs were planted and streaked onto Denim Blue Agar and incubated overnight. The Rodac plates were incubated overnight.

The Foothills Medical Centre Infection Prevention and Control laboratory technician did a semi-quantitative bacterial colony count of the Rodac plates and identified the bacteria present. Normal flora bacterial density was categorized as trace, scant, light, moderate, or heavy. Plates that had less than 5 colonies on the entire plate were classified as scant; plates with more than five colonies over the entire plate were considered light, plates with colonies in three quadrants were considered moderate, and plates with multiple colonies in all four quadrants were considered heavy.

The presence of coagulase-negative Staphylococcal species, diphtheroids, and non-pathogenic Neisseria was recorded.

The denim-blue plates were read for presumptive MRSA as described previously in Section 3.2.4.

Participants were considered colonized if they tested positive at the nares and/or the torso from the swab tests or at the chest and/or axillae on the Rodac plate. Participants were considered infected if they tested positive at a wound site. If a participant tested positive at a wound site and any other site, they were considered infected.

3.3.5 Data collection and management

3.3.5.1 Sampling

An 80% reduction in MRSA was considered clinically significant in this study. A sample size of 8 individuals with MRSA would be needed to detect this 0.80 reduction in MRSA at 90% power. Since we had previously estimated the prevalence of MRSA colonization to be approximately 10% in the population that accesses CDIRC's services, a sample size of 80 would be required to assess the efficacy of AHP skin cleanser for MRSA positive individuals.

A non-probability convenience sampling method was used where individuals were recruited at CDIRC on various days of the week over a period of five months.

3.3.5.2 Data handling

Questionnaire answers were entered into a Microsoft Access password-protected database. Hard copies of the questionnaires were kept in a locked filing cabinet in a secure office.

3.3.6 Data analysis

Analysis was done using Stata version 9.0 (College Station, Texas). Questionnaire answers were tabulated to determine characteristics of the study population.

A census was taken of bacterial species present on the Rodac plates for each participant. The density of the bacterial load was recorded for each participant, and the change in bacterial density (if any) between the initial and follow-up visits was noted.

The prevalence of MRSA colonization/infection was calculated by dividing the number of MRSA cases by the total number of people tested.

Odds ratios were calculated to measure any possible associations between MRSA status and study variables. Odds ratios were also calculated to measure any reduction in the amounts of normal flora between the initial and follow-up visits relative to study variables such as number of times the AHP skin cleanser was used and length of time between initial visit and follow-up.

3.4 Ethics

The study was treated as two separate research projects. Both research proposals with all relevant documents including consent scripts and questionnaires were submitted to the University of Calgary Conjoint Health Research Ethics Board (CHREB) for approval before the study began. Amendments to the protocols were also submitted to CHREB and approval was received prior to continuing study activities (Appendix J, K, and L).

Other methods used to ensure ethical practice included:

- Assigning a unique study number to each participant so names were never used in the laboratory or in the database
- Using a computer protected by internet firewalls to minimize the risk of access by external intruders
- Storing data in a password protected database
- Keeping questionnaires in a secure location
- Presenting findings in aggregate

CHAPTER FOUR: RESULTS

4.1 Introduction to Chapter Four: Results

This chapter presents the results observed in this study. The first section of the chapter reports the results from the prevalence study and includes descriptive analysis of study participants' characteristics. A comparison is made between participants recruited on the two different days of the study. Results of the MRSA risk factor analysis are presented.

The second section of the chapter is focused on results from the intervention study. This section looks at participants' characteristics at the initial visit and presents laboratory results from the initial specimen collection. The intervention section also includes results from the participants' follow-up visits including participants' characteristics, responses to the intervention questionnaire, and laboratory results from follow-up specimen collection. The intervention section concludes with the results of the MRSA risk factor analysis for the initial visit, the results from the comparison between participants with respect to follow-up status, and the effect of AHP on bacterial load. Participants' adherence to the study protocol is also reported.

4.2 Prevalence study

4.2.1 Study participants' characteristics

There were a total of 182 individuals recruited into the prevalence study. On Day 1 (March 9, 2008) there were 152 participants enrolled and on Day 2 (March 11, 2008) there were 30 participants enrolled (Table 2).

Table 2 Characteristics of the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* point prevalence study participants (n=182)

	n	%
Participants recruited	182	
Day 1 – March 8, 2008	152	84
Day 2 – March 11, 2008	30	16
Male	154	85
Age in years at swab		
Mean (+/-sd)	42.7	(12.2)
Median (range)	44	(19,69)
Staying at shelter	152	84
Using Intox/emergency beds	82	54
Accommodations other than shelter		
Own apartment	15	8
Friend's home	5	3
Relative's home	2	1
On the street	3	2
Rooming house	1	1
Refused/Don't know	4	2

The average age of the participants was 42.7 years old (95% CI 40.9-44.5) and 154 (85%) were male (Table 2). A total of 152 (84%) of the participants were planning to sleep the night of the study at CDIRC, with 82 (54%) of those shelter dwellers using either the intox beds available to clients under the influence of drugs or alcohol, or emergency beds

available to clients that are not regular users of CDIRC (Table 2). Participants not staying in the shelter were planning to sleep at their own apartment (n=15), friends' or relatives' homes (n=7), on the street (n=3), or somewhere else (n=5) (Table 2).

The majority of the participants (n=124) reported taking five or more showers or baths in the previous week, with 81 (45%) taking more than seven or more showers or baths (Table 3). There were 20 (11%) participants who took 2 or fewer showers or baths in the week before the study (Table 3). A total of 151 (83%) of the participants used the showers at CDIRC (Table 3).

Table 3 Personal hygiene behaviour of participants in the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* point prevalence study (n=182)

	n	%
Number of baths or showers per week		
1-2	20	11
3-4	45	25
5-6	33	18
7 or more	81	45
Use showers at CDIRC	151	83
Frequency of clothes washing		
After wearing once	59	33
After wearing 2-3 times	98	54
After wearing 4-5 times	12	7
After wearing more than 6 times	10	6

Of the 182 participants in the study, 157 reported washing their clothes after wearing them three or less times (Table 3). The remaining participants either washed their clothes

after wearing them four or five times (n=12) or after wearing them more than six times (n=10) (Table 3).

Antibiotic use in the previous seven days was reported by 11 (6%) of participants, was denied by 107 (59%) participants and the remaining 64 (35%) individuals were not sure about previous antibiotic use or didn't answer the question (Table 4).

Table 4 Antibiotic use in the previous seven days reported by participants in the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* point prevalence study (n=182)

	n	%
Antibiotic use in past 7 days		
Yes	11	6
No	107	59
Don't know/Blank	64	35

Of the 182 people that provided specimens, 18 (10%) tested positive for MRSA. There were 13 (72%) individuals colonized with MRSA and 5 (28%) participants infected with MRSA (Table 5). Of the 18 individuals who tested positive, 10 (56%) had positive results from the nasal specimen only, 4 (22%) had positive results from a wound site only, 1 (6%) tested positive at both the nares and a wound site, and 3 (17%) tested positive at both the nares and the torso (Table 5). Overall, 13 participants reported a wound site which was subsequently tested for MRSA and 5 (38%) of the wound sites were MRSA positive.

Table 5 Frequency of participants in the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* point prevalence study that tested positive for colonization or infection with methicillin-resistant *Staphylococcus aureus* (n=18)

	n	%
Participants	182	
MRSA positive	18	10
Colonized	13	72
Infected	5	28
Site Positive		
Nares	10	56
Wound	4	22
Nares and torso	3	17
Nares and wound	1	6

Of the18 MRSA isolates collected, 12 (67%) were positive for the typical CMRSA10 strain, 4 (22%) were positive for a non-typical CMRSA10 strain, and 2 (11%) isolates were other MRSA strains not able to be typed using this PCR assay (Table 6).

Table 6 Strain types of methicillin-resistant *Staphylococcus aureus* isolates from the Calgary Drop-In and Rehab Centre point prevalence study (n=18)

Strain	**n**	**%**
CMRSA10	12	67
CMRSA10 (not typical clone)	4	22
MRSA strains other thanCMRSA7 or CMRSA10	2	11

4.2.2 Comparison of participants screened on Days 1 and 2

A total of 152 individuals were recruited into the study on Day 1 (March 9, 2008) and 30 were recruited on Day 2 (March 11, 2008).

There was no significant difference between participants recruited on the two days in terms of gender (p=0.73) or age (p=0.90) (Table 7). More participants on Day 2 reported taking between five and six baths or showers a week (33%) as compared to participants on Day 1 (15%). However, there was no significant difference found between the two days for participants that reported taking five or more showers a week as compared to participants that reported taking four or fewer showers a week (p=0.36). Participants on Day 2 were more likely to report washing clothes after wearing once (50%) as compared to participants recruited on Day 1 (29%) (p=0.02) (Table 7). There was no difference in antibiotic utilization between participants recruited on either day (Table 7).

Table 7 Comparison between individuals enrolled in Day 1 and Day 2 of the methicillin-resistant *Staphylococcus aureus* point prevalence study at the Calgary Drop-In and Rehab Centre with respect to risk factors for methicillin-resistant *Staphylococcus aureus* colonization or infection (n=182)

	Day 1		Day 2		p value*
	n	%	n	%	
Participants recruited	152		30		
Male	128	84	26	87	0.73
Age at swab					
Mean (+/-sd)	42.8	(12.2)	42.5	(12.8)	0.90
Median (range)	43	(19,69)	45.5	(25,60)	
Number of baths or showers per week					
1-2	19	13	1	3	0.14
3-4	37	24	8	27	0.79
5-6	23	15	10	33	0.02
7 or more	70	46	11	37	0.34
Use showers at CDIRC	123	81	28	93	0.10
Frequency of clothes washing					
After wearing once	44	29	15	50	0.02
After wearing 2-3 times	86	57	12	40	0.10
After wearing 4-5 times	10	7	2	7	0.99
After wearing more than 6 times	9	6	1	3	0.57
Antibiotic use in past 7 days					
Yes	9	6	2	7	0.89
No	92	61	15	50	0.28
Don’t know/Blank	51	34	13	43	0.31

* Two-sided

Of the 18 individuals that tested positive for MRSA colonization or infection, 16 (89%) were enrolled on Day 1 and 2 (11%) were enrolled on Day 2 (Table 8). There was no significant difference in the prevalence of MRSA colonization or infection among individuals tested on the two days (p=0.52) (Table 8). There was no significant

difference between the two groups in regards to whether the positive MRSA result represented an infection (p=0.35) or a colonization (p=0.35) (Table 8).

Table 8 Comparison between individuals enrolled in Day 1 and Day 2 of the methicillin-resistant *Staphylococcus aureus* point prevalence study at the Calgary Drop-In and Rehab Centre with respect to methicillin-resistant *Staphylococcus aureus* colonization or infection (n=18)

	Day 1		Day 2		p value*
	N	%	n	%	
	152		30		
MRSA positive	16	11	2	7	0.52
Colonized	11	69	2	100	0.35
Infected	5	31	0	0	0.35

*Two-sided

4.2.3 Methicillin-resistant Staphylococcus aureus risk factor analysis

There were 182 individuals recruited to the point prevalence study, and 18 of those tested positive for either colonization or infection with any strain of MRSA, giving a point prevalence of 9.89% (95% CI 5.51- 14.27).

Males were not more likely than females to test positive for MRSA (p=0.4) (Table 9). Those individuals who admitted to antibiotic use in the seven days prior to MRSA testing were 11.9 times more likely to have MRSA (p<0.001) (Table 9). Using an intox/emergency bed (n=8) was not associated with a higher likelihood of testing positive for MRSA as compared to sleeping in a room on the upper floors (n=6)(p=0.82) (Table 9). Individuals who took two or fewer showers or baths a week (n=5) were 4.08 times more likely to have MRSA than those participants who reported showering three or more

times a week (n=12) (p=0.01) (Table 9). Laundering clothes after wearing them once (n=6) as compared to laundering clothes after wearing them two or more times (n=12) was not associated with a higher likelihood of MRSA (p=0.97) (Table 9).

Table 9 Potential risk factors associated with methicillin-resistant *Staphylococcus aureus* infection or colonization in clients of the Calgary Drop-In and Rehab Centre participating in the point prevalence study (n=182)

Risk Factor	Cases	Controls	OR	95% CI	p value*
	n	n			
Gender					
Female	4	24	1.67	0.37- 5.91	0.4
Male	14	140			
Antibiotic use					
Yes	5	6	11.9	2.20 - 59.70	<0.001
No	7	100			
Shelter room type					
Intox/Emergency	8	74	1.14	0.33 - 4.19	0.82
Floor 4 or 5	6	63			
Frequency of showers or baths					
Two or less a week	5	15	4.08	0.98 - 14.58	0.01
Three or more a week	12	147			
Frequency of clothes laundering					
After wearing clothes once	6	53	1.02	0.30 – 3.13	0.97
After wearing clothes two or more times	12	108			

* Two-sided

A sub-analysis was done looking at participants recruited on Day 1 of the study. There were 152 individuals recruited to the point prevalence study on Day 1, and 16 of those tested positive for either colonization or infection with any strain of MRSA (Table 8).

Males were not more likely than females to test positive for MRSA (p=0.29) (Table 10). Those individuals who admitted to antibiotic use in the seven days prior to MRSA testing were 11.5 times more likely to have MRSA (p<0.001) (Table 10). Using an intox/emergency bed (n=8) was not associated with a higher likelihood of testing positive for MRSA as compared to sleeping in a room on the upper floors (n=4)(p=0.81) (Table 10). Individuals who took two or fewer showers or baths a week (n=5) were 4.3 times more likely to have MRSA than those participants who reported showering three or more times a week (n=10)(p=0.01) (Table 10). Laundering clothes after wearing them once (n=6) as compared to laundering clothes after wearing them two or more times (n=10) was not associated with a higher likelihood of MRSA (p=0.46) (Table 10).

Table 10 Potential risk factors associated with methicillin-resistant *Staphylococcus aureus* infection or colonization in clients of the Calgary Drop-In and Rehab Centre recruited on Day 1 of the point prevalence study, n=152

Risk Factor	Cases	Controls	OR	95% CI	p value*
	n	n			
Gender					
Female	4	20	1.93	0.41 – 7.21	0.29
Male	12	116			
Antibiotic use					
Yes	4	5	11.47	1.71 – 68.5	<0.001
No	6	86			
Shelter room type					
Intox/Emergency	8	70	1.17	0.29 – 5.64	0.81
Floor 4 or 5	4	41			
Frequency of showers or baths					
Two or less a week	5	14	4.29	0.99-16.15	0.01
Three or more a week	10	120			
Frequency of clothes laundering					
After wearing clothes once	6	38	1.5	0.42 – 4.92	0.46
After wearing clothes two or more times	10	95			

*Two-sided

Risk factor analysis could not be done for participants recruited on Day 2 as there were only two individuals out of the 30 who tested positive for MRSA colonization or infection.

4.3 Intervention study

4.3.1 Amendment to intervention protocol

The initial protocol was designed to recruit individuals who had tested positive for MRSA at the prevalence study to participate in the intervention arm of the study. However, of the eighteen individuals with MRSA, only one was available to participate in the intervention study. There were 7 (41%) individuals that were not recruited because they either had no interest in participating or did not return to the health office for their study results (Table 11). Other participants either moved out of CDIRC (n=2), were barred from entering CDIRC (n=2) or were not able to be found (n=3). Two of the participants identified as MRSA-positive in the prevalence study died before the intervention study began. However, their deaths were not attributed to their MRSA status. There was one individual who could not be identified due to missing information on the prevalence study questionnaire.

Table 11 Reasons why methicillin-resistant *Staphylococcus aureus* positive participants identified in the Calgary Drop-In and Rehab Centre prevalence study did not participate in the intervention study (n=17)

Reason for not participating	n	%
Deceased	2	12
Moved out of CDIRC	2	12
Barred from entering CDIRC	2	12
Unknown location	3	18
No interest in participating or did not return to health office for results	7	41
Unable to be identified due to missing information on prevalence study form	1	6

4.3.2 Initial visit results

4.3.2.1 Study participants' characteristics

There were 37 participants recruited into the intervention phase of the study between February 8, 2008 and June 1, 2009. A total of 27 (73%) of the participants were male (Table 12). The mean age at the time of the study enrollment was 39.7 and the median age was 40.

Table 12 Characteristics of the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* intervention study participants (n=37)

	n	%
Male	27	73
Age at swab		
Mean (+/-sd)	39.7	(8.6)
Median (min, max))	40	(24, 52)
Staying at shelter	35	95
Using Intox/emergency beds if staying in shelter	25	71

A total of 35 (95%) of the participants were planning to sleep at CDIRC on the night they were recruited into the study and 25 (71%) of those shelter dwellers reported they slept in either intox or emergency beds when they stayed in the shelter (Table 12).

Over half of the participants reported taking five or more showers in the week preceding the study and 9 (24%) participants took two or fewer showers or baths in the week before the study (Table 13). A total of 34 (92%) of the participants used the showers available at CDIRC (Table 13).

Table 13 Personal hygiene behaviour reported by participants at their initial visit for the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* intervention study (n=37)

	n	%
Number of baths or showers per week		
0	2	5
1-2	7	19
3-4	8	22
5-6	5	14
7 or more	15	41
Use showers at CDIRC	34	92
Frequency of clothes washing		
After wearing once	12	33
After wearing 2-3 times	23	64
After wearing 4-5 times	0	0
After wearing more than 6 times	1	3

Participants reported washing their clothes after wearing them three or fewer times in 35 (95%) instances (Table 13). One participant reported wearing clothes more than six times before laundering (Table 13).

Antibiotic use in the previous seven days was reported by 4 (11%) of participants and was denied by 33 (89%) participants (Table 14).

Table 14 Antibiotic use in the previous seven days reported by participants on their initial visit in the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* intervention study (n=37)

	n	**%**
Antibiotic use in past 7 days		
Yes	4	11
No	33	89

4.3.2.2 Laboratory results

Swab tests were done on both the nares and torso for 26 (70%) of the participants and on both the nares and wound for 2 (5%) participants (Table 15). The nares, torso and wound were swabbed for MRSA in 8 (22%) participants, and only the nares were swabbed for one (3%) participant (Table 15). Rodac plate samples were collected from the chest of 34 (92%) of the participants and from the axillae of 33 (89%) of the participants (Table 15).

Table 15 Swab site and Rodac plate specimen collection for methicillin-resistant *Staphylococcus aureus* at the initial visit in the Calgary Drop-In and Rehab Centre intervention study (n=37)

	Total	
	n	**%**
Site swabbed		
Nares	1	3
Nares and torso	26	70
Nares and wound	2	5
Nares, torso, and wound	8	22
Rodac plate sample		
Chest	34	92
Axillae	33	89

Out of the 37 participants recruited to the study, 4 (11%) tested positive for MRSA at one or more anatomical sites (Table 16). There were 3 (8%) participants with MRSA colonization as indicated by positive results from the nares and chest and 1 (3%) participant had an MRSA infection as indicated by a positive result from a wound site (Table 16).

Table 16 Anatomical sites testing positive for methicillin-resistant *Staphylococcus aureus* for either swab or Rodac plate collection methods in the Calgary Drop-In and Rehab Centre intervention study at the initial participant visit (n=4)

	MRSA Positive	
	n	**%**
Specimen collection site and method		
Nares (swab)	2	50
Wound (swab)	1	25
Nares (swab) & Chest (Rodac)	1	25

Trace amounts of normal flora were found on six (17%) of the Rodac plates collected from axillae (Table 17). There were 5 (14%) participants with scant normal flora on their axillae plates and 9 (26%) with light amounts of normal flora. Moderate amounts of normal flora were found on 6 (17%) of the axillae Rodac plates sampled from participants and 9 (26%) of the participants had heavy amounts of normal flora on their axillae Rodac plates (Table 17).

Table 17 Semi-quantitative bacterial load and bacterial species present on the chest and axillae Rodac plates collected at the initial visit from participants in the Calgary Drop-In and Rehab Centre intervention study

	Axillae (n=33)		Chest (n=34)	
	n	%	n	%
Amount of normal flora				
Trace	6	17	6	16
Scant	5	14	10	27
Light	9	26	9	24
Moderate	6	17	10	27
Heavy	9	26	2	5
Coagulase-negative staphylococcal species present	26	79	28	76
2 different species	13	50	12	43
3 different species	4	15	5	18
4 different species	0	0	1	4
Diphtheroids present	20	61	21	62
2 different species	3	15	2	10
3 different species	0	0	1	5

Coagulase-negative staphylococcal (CNS) species were identified on 26 (79%) of the axillae Rodac plates. Of those 26 plates, 13 (50%) had two species of CNS present, and 4 (15%) had three species of CNS (Table 17). Diphtheroids were found on 20 (61%) of the axillae Rodac plates with two different species of diphtheroids present on 3 (15%) plates (Table 17).

Trace amounts of normal flora were found on six (16%) of the Rodac plates collected from chest (Table 17). There were 10 (27%) participants with scant normal flora on their chest plates and 9 (24%) with light amounts of normal flora. Moderate amounts of normal flora were found on 10 (27%) of the chest Rodac plates sampled from participants

and 2 (5%) of the participants had heavy amounts of normal flora on their chest Rodac plates (Table 17).

Coagulase-negative staphylococcal species were identified on 28 (76%) of the chest Rodac plates. Of those 28 plates, 12 (43%) had two species of CNS present, 5 (18%) had three species of CNS, and 1 (4%) 4 species (Table 17). Diphtheroids were found on 21 (62%) of the chest Rodac plates with two different species of diphtheroids present on 2 (10%) plates and 1 (5%) plate had three different species of diphtheroids present (Table 17).

4.3.3 Follow-up visit results

4.3.3.1 Study participants' characteristics

Of the 37 participants recruited into the intervention study, 23 (62%) returned for a follow-up visit (Table 18). The mean age of the participants that returned for a follow-up visit was 37 years old (95% CI 40.7 - 47.3). The median age for the follow-up visit was 38 years old(range 24, 52). There were 18 (78%) males that returned for their follow-up visits.

Table 18 Frequency of seven-day follow-up visits by number of times paged over the intercom system for participants in the Calgary Drop-In and Rehab Centre intervention study (n=23)

	Returned for follow-up	
	n	%
Age in years		
Mean (sd)	37	(9.0)
Median (min/max)	38	(24/52)
Gender		
Male	18	67
Female	5	50
Number of Pages		
0	2	5
1-4	12	33
5-9	8	21
10 or more	1	3
Days between visits		
1-7	4	17
8-14	7	30
15-21	2	8
More than 21	10	43

Of those 23 that returned for a follow-up visit, 2 returned for the second visit without a reminder (Table 18). The remaining participants that had follow-up visits were paged between 1 and 11 times (Table 18). The mean number of pages made before a participant returned for a follow-up visit was 4.34 (95 % CI 2.57 - 6.13). The median number of pages made prior to the participant having a follow-up visit was 3 (min 0, max 11).

The average number of days between the initial swab and the follow-up visit was 23 (range 5, 63). A total of 4 (17%) participants returned for their follow-up visit within the recommended seven days and 7 (30%) returned for their second visit 8 – 14 days after the

initial visit (Table 18). Almost half of the patients returned three weeks or more after their initial swab.

4.3.3.2 Participant responses to follow-up questionnaire

Of the 23 participants that returned for a follow-visit, 17 (74%) reported using the AHP cleanser (Table 19). The soap was used 1-2 times by 3 (18%) individuals, 3-4 times by 5 (29%) individuals, and 5-6 times by 2 (12%) individuals (Table 19). A total of 7 (41%) of the participants reported using the AHP soap seven or more times (Table 19).

Table 19 Participant responses to the Calgary Drop-In and Rehab Centre intervention study follow-up questionnaire on accelerated hydrogen peroxide skin cleanser (n=17)

	N	%
Used AHP	17	74
Times AHP used		
1-2	3	18
3-4	5	29
5-6	2	12
7 or more	7	41
Perception of smell		
OK	4	25
Awful	1	6
Other	6	38
Can't remember	5	31
AHP rinsed off easily	16	94
AHP rinsed off completely	15	88
Liked skin after use of AHP		
Yes	12	52
No	3	13
Don't know	3	13
Other	5	22
Days between last shower and visit		
0	8	53
1	4	27
2	1	7
3	1	7
5	1	7

There were six participants that did not use the AHP and the following reasons were given:

- I'm positive I'm MRSA negative
- I broke up with my boyfriend who kept the product
- I'm very allergic and hypersensitive
- I lost the soap

The smell of the product was rated as OK by 4 (25%) of the participants (Table 18). There was one (6%) individual who thought it smelled awful and 5 (31%) participants couldn't remember the smell (Table 18). Comments made by the 6 (38%) individuals that answered "Other" were:

- Smelled strong and different
- Medicinal smell but only in shower
- Smells like it's cleaning
- Smells like medicine used for crabs. Smell didn't linger outside of shower
- Smelled like medicine
- I have no sense of smell

Of the participants that used the AHP, 16 (94%) reported that it rinsed off easily and 15 (88%) reported that the soap washed off completely (Table 19). A total of 12 (52%) of those that used the AHP reported that they liked the feel of their skin after using the product compared with 3 (13%) who didn't like the feel of their skin after using AHP (Table 19). There were 5 (22%) individuals that made comments on the condition of their skin following use of AHP. These comments were:

- Itching and red rash on legs about one hour after showering
- Skin felt nice
- Skin felt smooth and soft
- Skin dried up
- Cleared up wounds on legs
- Replenished and healed skin

There were 8 (53%) participants that reported having a shower on the same day of the second visit. Of the remaining participants, 4 (27%) showered the day before the follow-up visit and the other 3 (21%) participants reported showering two days , three days and five days before the second visit (Table 19).

4.3.3.3 Laboratory results

Of the 23 people that returned for follow-up visits, 17 (74%) were swabbed at the nares and torso, and 2 (8%) were swabbed at the nares and wound, 2 (8%) at the nares, wound and torso, and 2 (8%) at the nares only (Table 20). Rodac plate samples were collected from the chest of 22 (96%) of the participants and from the axillae of 20 (87%) of the participants (Table 20).

Table 20 Frequency of swab and Rodac plate specimen collection for methicillin-resistant *Staphylococcus aureus* at various anatomical sites at the follow-up visit for the Calgary Drop-In and Rehab Centre intervention study (n=23)

	n	%
Site swabbed		
Nares	2	9
Nares and torso	17	74
Nares and wound	2	9
Nares, torso, and wound	2	9
Rodac plate sample		
Chest	22	96
Axillae	20	87

Out of the 23 participants that returned for a follow-up visit, 4 (17%) tested positive for MRSA at one or more anatomical sites (Table 21). There were 2 participants with MRSA colonization as indicated by positive results from the nares, chest and axillae and

2 participants had MRSA infections as indicated by a positive result from a wound site (Table 21). Of the four participants that tested positive for MRSA at the follow-up visit, 3 (75%) had also tested positive for MRSA on the initial visit and 1 (25%) was a newly identified case.

Table 21 Anatomical sites testing positive for methicillin-resistant *Staphylococcus aureus* using either swab or Rodac plate collection methods in the Calgary Drop-In and Rehab Centre intervention study at the follow-up visit (n=4)

	MRSA Positive	
	n	**%**
Specimen collection site and method		
Nares (swab), Chest (Rodac), Axillae (Rodac)	1	50
Wound (swab)	2	25
Nares (swab) & Torso (swab)	1	25

Trace amounts of normal flora were found on 4 (20%) of the Rodac plates collected from axillae (Table 21). There were 5 (25%) participants with scant normal flora on their axillae plates and 7 (35%) with light amounts of normal flora (Table 22). Heavy amounts of normal flora were found on 4 (20%) of the axillae Rodac plates (Table 22).

Table 22 Semi-quantitative bacterial load and bacterial species present on the chest and axillae Rodac plates collected at the follow up visit from participants in the Calgary Drop-In and Rehab Centre intervention study

	Axillae (n=20)		Chest (n=22)	
	n	%	n	%
Amount of normal flora				
Trace	4	20	3	14
Scant	5	25	5	23
Light	7	35	5	23
Moderate	0	0	5	23
Heavy	4	20	4	18
Coagulase-negative staphylococcal species present	17	85	18	82
2 different species	7	41	10	56
3 different species	1	6	2	11
Diphtheroids present	11	55	13	59
2 different species	1	9	5	38
3 different species	2	18	1	8

Coagulase-negative staphylococcal species were identified on 17 (85%) of the axillae Rodac plates. Of those 17 plates, 7 (41%) had two species of CNS present, and 1 (6%) had three species of CNS (Table 22). Diphtheroids were found on 11 (55%) of the axillae Rodac plates with two different species of diphtheroids present on 1 (9%) plate and three different species of diphtheroid on 2 (18%) plates (Table 22).

Trace amounts of normal flora were found on 3 (14%) of the Rodac plates collected from chest (Table 22). There were 5 (23%) participants with scant normal flora on their chest plates and 5 (23%) with light amounts of normal flora. Moderate amounts of normal flora were found on 5 (23%) of the chest Rodac plates sampled from participants and 4

(18%) of the participants had heavy amounts of normal flora on their chest Rodac plates (Table 22).

Coagulase-negative staphylococcal species were identified on 18 (82%) of the chest Rodac plates (Table 22). Of those 18 plates, 10 (56%) had two species of CNS present and 2 (11%) had three different species of CNS identified on the plate (Table 22). Diphtheroids were found on 13 (59%) of the chest Rodac plates with two different species of diphtheroids present on 5 (38%) plates and 1 (8%) plate had three different species of diphtheroids present (Table 22).

4.3.4 Methicillin-resistant Staphylococcus aureus *risk factor analysis for initial visit*

There were 4 participants that tested positive for MRSA at the initial visit which gives a point prevalence of colonization or infection with MRSA of 10.8 % (95% CI 3.0 - 25.4). There were three colonizations and four infections giving a colonization prevalence of 8.1% (95% CI 1.7- 21.9) and an infection prevalence of 2.7% (95% CI 0.1 – 21.9).

Females were not more likely than males to have an MRSA infection or colonization (OR=3.2, p=0.27) (Table 23). There was no association between antibiotic use and colonization or infection with MRSA (OR=3.3, p=0.33) (Table 23). The use of an emergency bed at the shelter was not associated with a higher likelihood of testing positive for MRSA (OR=1.1, p=0.94) (Table 23). Participants that reported taking two or fewer showers or baths in a week were not more likely to test positive for MRSA than participants taking three or more showers or baths in a week (OR=1.0, p=0.97) (Table

23). A lower frequency of clothes laundering was not associated with a higher risk for MRSA colonization or infection (OR=0.64, p=0.71).

Table 23 Potential risk factors associated with methicillin-resistant *Staphylococcus aureus* infection or colonization in clients of the Calgary Drop-In and Rehab Centre at the initial visit of the intervention study (n=37)

Risk Factor	Cases	Controls	OR	95% CI	p value*
	n	n			
Gender					
Female	2	8	3.2	0.19- 48.03	0.27
Male	2	25			
Antibiotic use					
Yes	3	3	3.3	0.05- 59.88	0.33
No	3	30			
Using emergency bed					
Yes	3	22	1.1	0.07- 64.33	0.94
No	1	8			
Frequency of showers or baths					
Two or fewer a week	1	8	1.0	0.02-15.25	0.97
Three or more a week	3	25			
Frequency of clothes laundering					
After wearing clothes once	1	11	0.64	0.01-9.17	0.71
After wearing clothes two or more times	3	21			

*Two-sided

4.3.5 Comparison of participants with respect to follow-up status

There were 23 (62%) participants that returned for a follow-up visit. The participants that returned for a follow-up visit were younger than the participants that didn't return (p=0.02) (Table 24). There was no difference in return rates between males and females (p=0.35) (Table 24). There was no difference in return rates between shelter dwellers using emergency beds and shelter dwellers using transitional beds (p=0.72) (Table 24).

Participants with MRSA were equally as likely to return for a follow-up visit as participants without MRSA (p=0.58) (Table 24).

The mean age of participants who returned for their follow-up visits was also younger than the mean age of participants that did not return (p= 0.02) (Table 24). To further assess age this age difference between the two groups, age was collapsed into two categories - age in years <=39 and age in years >=40. Patients returning for the follow-up visit were 7 times more likely to be under 39 years old (p=0.01).

Table 24 Comparison of characteristics of participants that returned for a follow-up visit versus those that did not attend the follow-up visit for the Calgary Drop-In and Rehab Centre methicillin-resistant *Staphylococcus aureus* intervention study (n=37)

	Returned for follow-up (n=23)		Didn't return for follow-up (n=14)		p value*
	n	%	n	%	
Age in years					
Mean, sd	37	9.0	44	5.7	0.02
Median, min/max	38	24/52	46	32/51	
Gender					
Male	18	67	9	33	0.35
Female	5	50	5	50	
Using emergency bed					
Yes	15	71	10	77	0.72
No	6	29	3	23	
Frequency of shower					
Two or less a week	7	30	2	14	0.27
Three or more a week	16	70	12	86	
MRSA					
Positive	3	13	1	7	0.58
Negative	20	87	13	93	

*Two-sided

4.3.6 The effect of accelerated hydrogen peroxide skin cleanser on bacterial load

There were 19 participants that had an axillae Rodac plate collected at both their initial and follow up visits. No change in normal flora bacterial load was observed for 8 (42%) individuals as shown in the shaded cells in Table 25. A decrease in bacterial load was observed for 8 (42%) individuals as seen in the cells above the shaded cells (Table 25). There were 3 (16%) participants that experienced an increase in the amount of normal flora observed between the initial and follow up visits as seen in the cells below the shaded cells (Table 25).

Table 25 Frequency of changes in semi-quantitative normal flora bacterial load observed on axillae Rodac plates between the initial and follow-up visits of the Calgary Drop-In and Rehab Centre intervention study (n=19)

	Initial Visit				
	Trace	Scant	Light	Moderate	Heavy
Follow-up Visit					
Trace	1	0	1	0	2
Scant	0	2	0	1	1
Light	0	1	3	1	2
Moderate	0	0	0	0	0
Heavy	0	0	1	1	2

There were 20 participants that had a chest Rodac plate collected at both their initial and follow up visits. No change in normal flora bacterial load was observed for 4 (20%) individuals as shown in the shaded cells in Table 26. A decrease in bacterial load was observed for 7 (35%) individuals as seen in the cells above the shaded cells (Table 26). There were 9 (45%) participants that experienced an increase in the amount of normal flora observed between the initial and follow up visits as seen in the cells below the shaded cells (Table 26).

Table 26 Frequency of changes in semi-quantitative normal flora bacterial load observed on chest Rodac plates between the initial and follow-up visits of the Calgary Drop-In and Rehab Centre intervention study (n=20)

	Initial Visit				
	Trace	Scant	Light	Moderate	Heavy
Follow-up Visit					
Trace	0	1	1	1	0
Scant	1	0	1	1	0
Light	2	0	1	1	1
Moderate	1	1	0	3	0
Heavy	0	0	2	2	0

There was no difference in the reduction of bacterial load on the axillae Rodac plates between participants that used the AHP soap and those that did not (p=0.35) (Table 27). Using the product more frequently was not associated with a difference in the change of bacterial load on the axillae Rodac plates (p=0.21) (Table 27). Participants that returned for their follow-up visit within 7 days did not experience a greater reduction in bacterial load on the axillae Rodac plates than participants that returned for a later follow-up visit (p=0.74) (Table 27).

Table 27 Characteristics of participants with decrease in bacterial load of normal flora collected on the axillae Rodac plates between the initial and follow-up visits for the Calgary Drop-In and Rehab Centre intervention study (n=19)

Characteristic	Load decreased		OR	95% CI	p value*
	n	%			
Used soap					
Yes	5	36	0.37	0.02-4.68	0.35
No	3	60			
Frequency of AHP use					
Two or fewer	2	67	5.33	0.18-352.99	0.21
Three or more	3	27			
Returned for follow-up					
7 or fewer days	1	33	0.64	0.01-15.18	0.74
8 or more days	7	44			

*Two-sided

There was no difference in the reduction of bacterial load on the chest Rodac plates between participants that used the AHP soap and those that did not (p=0.48) (Table 28). Using the product more frequently was not associated with a difference in the change of bacterial load on the chest Rodac plates (p=0.93) (Table 28). Participants that returned for their follow-up visit within 7 days of the first visit did not experience a greater reduction in bacterial load on the chest Rodac plates than participants that returned for a later follow-up visit (p=0.06) (Table 28).

Table 28 Characteristics of participants with decrease in bacterial load of normal flora collected on the chest Rodac plates between the initial and follow-up visits for the Calgary Drop-In and Rehab Centre intervention study (n=20)

Characteristic	Load decreased		OR	95% CI	p value*
	n	%			
Used soap					
Yes	5	31	0.45	0.03-8.31	0.48
No	2	50			
Frequency of AHP use					
Two or less	1	33	0.89	0.04-65.39	0.93
Three or more	4	31			
Returned for follow-up					
7 or fewer days	3	75	0.11	0.001-2.07	0.06
8 or more days	4	25			

*Two-sided

4.3.7 Participants' adherence to intervention protocol

A participant was considered fully compliant with study protocol if they returned for their follow-up visit seven days after the initial visit; if they reported using the AHP skin cleanser; and if they consented to having all specimens collected (nasal and torso swabs collected, as well as Rodac sampling of axillae and chest).

Of the original 37 individuals recruited into the study, 4 (11%) returned for the follow-up visit in seven days. Of those that returned at the correct time, three reported using the AHP. There were only two individuals that used the AHP, returned when advised, and provided all the specimens required (one participant refused the torso swab at the initial and follow-up visits). Overall, 2 (5%) participants out of the original 37 recruited complied exactly to study protocol from start to finish.

There were 4 participants identified as MRSA positive following the initial sampling. Of those participants, 3 returned for follow-up visits and all three individuals returned more than 3 weeks after the initial visit. Only 1 of those participants used the AHP. None of the MRSA positive participants complied fully with the intervention study protocol.

CHAPTER FIVE: SUMMARY, DISCUSSION, AND CONCLUSIONS

5.1 Introduction to Chapter Five

In the preceding chapter, results from the prevalence study and the intervention study were presented and analyzed. In Chapter Five, the major findings from the two studies are summarized and discussed in the context of previous research. Strengths and limitations to the interpretation of the study findings due to study design are discussed. Recommendations for further research are presented.

Chapter Five is divided into two main sections focusing on the prevalence study and the intervention study separately. Each section is further divided into major findings, study limitations, strengths, and conclusions.

5.2 Prevalence Study

5.2.1 Summary of prevalence study

The objective of the prevalence study was to determine the prevalence of MRSA infection or colonization in the population that accesses the services provided by the Calgary Drop-In and Rehab Centre.

To achieve this objective, a cross-sectional survey was administered to users of CDIRC in conjunction with collection of nasal, torso, and wound site samples. Laboratory analysis was done on specimens to determine the presence and strain type of MRSA.

Prevalence was defined as the number of MRSA cases over the total number of individuals screened. A person was considered colonized if the nasal and/or torso specimen was positive for MRSA. A person was considered infected if the wound site yielded a positive result.

The questionnaire was used to provide basic demographics of the study population and risk factor analysis was done on survey variables including personal hygiene behaviours such as frequency of showering and clothes laundering.

5.2.2 Major findings

5.2.2.1 Prevalence of methicillin-resistant *Staphylococcus aureus* in the Calgary Drop-In and Rehab Centre

The prevalence of MRSA colonization or infection in the population that accessed the Calgary Drop-In and Rehab Centre was 9.89% (95% CI 5.51- 14.27) in March 2008. The prevalence attributed to CMRSA10 was 8.79% (95% CI 5.11 - 13.88).

An individual was considered colonized if they had MRSA isolated from the nasal specimen and/or the torso specimen. There were 14 individuals that had MRSA isolated from their nasal swabs, resulting in an MRSA nasal prevalence of 7.69% (95% CI 4.27 - 12.57). Of the 14 MRSA strains isolated from nasal swabs, 13 were identified as CMRSA10, giving a nasal carriage rate for CMRSA10 of 7.14% (95% CI 3.86 - 11.90). One of the participants with nasal carriage also screened positive for MRSA at a wound site and was classified as an infection using our study definition, thus the colonization prevalence of any strain of MRSA was 7.14% (95% CI 3.86 - 11.90).

There were five participants that were infected with MRSA at a wound site, resulting in a prevalence of infection in this study of 2.75% (95% CI 0.90 - 6.30). The prevalence of infection with CMRSA10 was 2.20% (95% CI 0.60 - 5.53).

An outbreak of MRSA in Calgary in 2004 identified the at-risk population to be individuals with a history of homelessness or incarceration (Gilbert et al., 2006). In response to that finding, a prevalence study was performed to determine the burden of MRSA in these high-risk groups (Gilbert, 2007). The study was designed to look at risk

factors for MRSA colonization or infection in the marginalized populations previously identified and recruitment occurred at a number of sites including an inner-city medical clinic and a local correctional facility. The Gilbert study included incarcerated individuals and IDUs in their study population and reported a prevalence of MRSA colonization or infection in Calgary's marginalized population of 7.4% (95% CI 4.6 – 11.2).

The overall MRSA prevalence rate of 9.89% we observed is higher than the prevalence of MRSA colonization or infection of 7.4% observed by Gilbert *et al.* However, the increase observed in our study was not significantly higher (p=0.17). CMRSA10 prevalence in our study was 8.79% (95% CI 5.11 - 13.88) as compared to the CMRSA10 prevalence of 5.5% (95% CI 3.1 - 9.0) in the Gilbert study. Although the contribution of CMRSA10 appeared greater in our study, the difference was not statistically significant (p=0.18). Our population was different from that of the Gilbert study and was restricted to individuals that accessed CDIRC at a specific point in time. We did not record information on previous incarceration or IDU. Thus, findings from our study should not be extrapolated to the overall marginalized population in Calgary.

The prevalence estimate of nasal colonization for the CDIRC population of 7.69% (95% CI 4.27 - 12.57) was less than the MRSA nasal carriage rate of 18.6% (95% CI 14.37 - 23.47) observed in Vancouver IDUs in 2006 (p=0.001)(Al-Rawahi et al., 2008). However, their study population was comprised solely of individuals that had used intravenous drugs in the past year. We don't know what proportion of our sample were

IDUs. As injecting drugs breaches the skin barrier, being an IDU may serve as an independent risk factor for MRSA infection.

In March of 2008 CDIRC had the capacity to house 1250 individuals nightly; we screened 182 people accounting for approximately 15% of the shelter's population. Males comprised about 84% of the study sample, which is a similar proportion to the prevalence study in Calgary's marginalized population done in 2006 (Gilbert, 2007). The 2008 Calgary homeless census also observed that 78% of the enumerated population staying at facilities or seen at service agencies were male (Stroick et al., 2008).

The median age of participants in our prevalence study was 44 years old and ranged from 19 to 69 years old. In the City of Calgary homeless census, the majority of the homeless enumerated at facilities were between the ages of 25-44 years (Stroick et al., 2008). Only 2% of the population were over the age of 65 years in the city census, and in our study 4 participants were over the age of 65 years, which was also 2%. The demographics of the participants, with respect to age and sex, sampled for the prevalence study were similar to the demographics of the homeless population as described by the City of Calgary in the 2008 homeless census.

5.2.2.2 Risk factors for methicillin-resistant *Staphylococcus aureus*

Several studies have described risk factors for MRSA acquisition in the community. Previous antibiotic use has been cited as a risk factor for MRSA as well as lack of personal cleanliness, compromised skin, crowding, frequent skin-to-skin contact, and sharing contaminated personal items.

We found an association between antibiotic use and MRSA colonization or infection. Participants that reported taking antibiotics in the previous seven days were almost 12 times as likely to have MRSA as patients who didn't report antibiotic use. However, around one third of the respondents either didn't know whether they had taken antibiotics recently or didn't answer the question, including 6 of the 18 MRSA cases (33%). A study done in San Francisco in 2002 also found that specific recollection of antibiotic use was generally poor in their study population of homeless and marginally housed adults (Charlebois et al., 2002).

Although previous antibiotic use has been established as a risk factor for MRSA acquisition, there is no consensus on what length of time should be considered when assessing the retrospective exposure risk. Many studies have looked at antibiotic use over longer periods of up to a year, however, we asked about antibiotic use within the past seven days to avoid potential recall bias. Even with this shorter period, the association between antibiotic use and MRSA was evident. This short time period did not improve the recollection of antibiotic use and our data was poorly collected for this variable.

Contributing to the poor quality of the data for the antibiotic variable was that the antibiotic question was not included in the questionnaire, but was asked by the health care worker during the course of taking the swab. It was reported by study volunteers that the question was not asked of all participants and may not have been recorded in all cases, thus accounting for a proportion of missing responses.

More research needs to be done to explore the relationship between antibiotic use and MRSA acquisition. A person can be colonized with MRSA for a long period of time which can make determining the temporal association between antibiotic use and acquisition of MRSA problematic. We did not ask why the participant was prescribed antibiotics. If the prescription was for a skin or soft tissue infection attributed to MRSA, this will bias the results towards a stronger association. Future risk factor assessments should include questions on the reason for antibiotic prescriptions and also explore adherence to the antibiotic regime as prescribed by a doctor. Questions should also be asked around the sharing of antibiotics in the event that an individual took antibiotics that were prescribed to another person. The location of where the antibiotics were used is also important, especially as we did not ask about previous hospitalizations. It is also possible that the prescription of antibiotics may serve as an indicator for poor health and increased risk for MRSA acquisition. A longitudinal study of MRSA incidence could assess the temporal relationship between antibiotic use and the subsequent acquisition of MRSA.

Colonization with MRSA has been identified as a risk factor for subsequent infection. In our study there were five participants that had wounds infected with MRSA, however only one was also MRSA positive at the nares and none of the individuals with MRSA infected wounds tested positive on their torso swabs. Since it is possible that individuals can be transiently colonized prior to identification of the infection, it can't be ascertained if infection was subsequent to colonization or not. It is also possible that colonization occurred at a site not sampled in the study. As this was a cross-sectional study done at a

single point in time, it is not possible to determine whether these infections were preceded by MRSA colonizations. We also cannot estimate how many of the colonizations observed will develop into MRSA infections. It is unknown whether the length of colonization impacts the likelihood of subsequent MRSA infection. A longitudinal study would need to be done in this population to determine the relationship between colonization and infection including whether individuals are being infected with the same strain they are colonized with.

It has been previously reported that personal hygiene -as defined by frequency of hand washing, frequency of showers per week, and number of personal items shared with others - was associated with increased risk for MRSA (Turabelidze et al., 2006). Our prevalence study confirmed that lack of cleanliness is a risk factor for MRSA. Participants who showered two or fewer times a week were at greater risk of having MRSA than participants who showered three or more times a week. The proportion of individuals in this study who reported showering two or fewer times a week was about 13%. If this proportion is extrapolated to the 1250 people who access CDIRC on a daily basis, an estimated 163 people might be at high risk for MRSA acquisition.

MRSA is usually transmitted through direct contact and crowding can increase the likelihood of skin-to-skin contact. Homeless individuals tend to live in circumstances that put them at a higher risk for MRSA acquisition than the general population. In our prevalence study, 45% of the participants reported staying in shelter beds classified as either Intox or emergency beds. The Intox facility is especially crowded and frequent contact occurs between clients. However, use of an Intox or emergency bed was not

associated with a higher likelihood of a MRSA test as compared to sleeping in a room on one of the upper floors.

The users of CDIRC are heterogeneous and may have differing levels of risk depending on what shelter services are used. For example, there may be differential access to showers between those using emergency or Intox beds and those staying in transitional beds on the fourth and fifth floors. The shower facilities available to emergency and Intox clients are available between 08:00 and 18:00 daily and require a reservation from the day office. The residents of the top floors have access to showers whenever the floor is open. To explore these potential differences we sampled from the general population on Sunday, March 9 and sampled from the transitional population residing on the fourth and fifth floors on Tuesday, March 11.

There was no difference in the prevalence of MRSA colonization or infection between the two days. Although there was no difference in the number of showers taken per week between residents of the upper floors and users of Intox and emergency beds, it was found that individuals taking two or fewer showers or baths a week were four times more likely to have MRSA than those participants who showered three or more times a week. This indicates that personal behaviour is more important than location when frequency of showers is considered.

Participants recruited on Day 1 were more likely to wear their clothes longer before laundering them. Half of the participants recruited on Day 2 reported laundering their clothes after wearing one time compared to 29% of the participants recruited on Day 1. The frequency of clothes washing might be a reflection of differing priorities between

individuals using Intox or emergency beds and those individuals using the transitional beds. Although differences in laundering practices exist, the risk of being colonized or infected with MRSA was not associated with the frequency of clothes laundering.

5.2.3 Limitations

5.2.3.1 Internal validity

5.2.3.1.1 Limitations due to study design

We performed a cross-sectional point prevalence study to determine the amount of MRSA in the population that uses CDIRC services and to explore risk factors. Cross-sectional studies have inherent limitations including the inability to assess temporal relationships such as causality and length-biased disease sampling.

In this study MRSA status was measured at the same time as the risk factor variables such as frequency of showering and antibiotic use. The prevalence of MRSA was then compared in different subpopulations of the risk factor variables. Since the estimate of prevalence and the recording of risk factors are limited to one point in time, the time order of events cannot be determined, *i.e.* it cannot be concluded that a specific risk factor preceded the acquisition of MRSA. For example, although an individual was staying at the shelter and was positive for MRSA on the day of the study, we don't know which came first – the shelter dwelling or the MRSA. Causality cannot be measured using a cross-sectional study design.

Another limitation that arises in some prevalence studies is length-biased sampling where diseases of long duration and mild severity may be over-represented in prevalence

estimates (Rothman, 2002). Some individuals are long-term carriers of MRSA and it has been known to persist on body surfaces for upwards of 40 months. So even if the incidence rate (the number of new cases in a given period of time) is low, the prevalence may still be high as prevalence reflects both the incidence rate and duration of disease. There is no way of determining how many of the MRSA cases identified in the prevalence study were long-term carriers of the bacteria, and how many were transiently colonized. A longitudinal cohort study would need to be performed to determine incidence rates.

5.2.3.1.2 Limitations due to methods used to conduct the study

There are several factors in the actual implementation of the study protocol that may bias either the prevalence estimate or the risk factor analysis.

A non-probability convenience sampling method was chosen which may have introduced bias into the study estimates. Selection bias occurs when there is a distortion in the study estimate resulting from errors in selecting participants (Rothman, 2002). Selection bias can happen if there are some members in a population that are either more or less likely to be included in the study.

One type of selection bias that may have occurred is self-selection bias in which an individual's reasons for choosing to participate in a study may be associated with the outcome under study (Rothman, 2002). Study posters were placed in CDIRC the week prior to the study clearly detailing the purpose of the study and a volunteer was present at the reception table on the day of the study to further explain the purpose of the research.

It is possible that people who had previous MRSA or were concerned about MRSA were more likely to participate in the study. There were 13 (7%) individuals that reported a skin or soft tissue infection on the day of the study, and 5 (38%) of those individuals that had a wound site tested were positive for MRSA while the overall prevalence of MRSA was about 10%, suggesting that individuals with MRSA skin infections were more likely to participate in the study. This may serve to over-estimate the prevalence of MRSA infection in the population that uses CDIRC's services.

Another selection bias that may have occurred is called the Healthy Volunteer Effect where volunteers in prevention and screening trials tend to be healthier than the general population (Pinsky et al., 2007). This effect can arise through self-selection of individuals that are healthier or self-exclusion by individuals that are not healthy. The true prevalence of MRSA would be underestimated if Healthy Volunteer Effect was present in this study.

There has been debate over what the best sites are to screen for MRSA. In our study testing was done at the nares, torso, and a wound site if present to determine the prevalence of colonization or infection with MRSA. Of the 182 individuals tested, there were 18 (9.9%) MRSA-positive individuals identified and 13 (7.1%) were considered colonized. Relying on nasal swabs alone to determine the colonization rate would have identified all 13 of the colonized cases. The three cases that were positive on the torso swab were also positive in their nasal swab, so adding the Z-swab did not pick up any additional cases that wouldn't have been identified by a nasal swab. In determining

MRSA colonization rates, this study has shown that relying on nasal samples alone is sufficient.

Although it is believed that MRSA rates are higher in marginalized populations, there have not been any published rates for the general Canadian population. However, the 2001-02 NHANES study in the United States found an overall prevalence rate of MRSA in the non-institutionalized population to be 0.84% (95% CI 0.4 - 1.2). This estimate is from 2001-02 and precedes the emergence of CMRSA10 as an important community strain. Canadian population level studies on MRSA colonization rates are needed to determine the amount of MRSA present in the non-marginalized population.

Studies done in select healthy populations such as military recruits and children attending outpatient clinics have also reported lower prevalence estimates than those observed in our prevalence study (Ellis et al., 2002 & Shopsin et al., 2000). As local MRSA epidemiology varies between geographical areas, findings from these select groups cannot be extrapolated to other groups or regions.

The infection prevalence was determined by the presence of MRSA in a sample collected from a wound site. There were 13 individuals that reported a wound and 5 of those individuals tested positive for MRSA, resulting in an MRSA infection prevalence of 2.7%. We did not record the location of the wound site. Participants were asked if they had a wound present and no attempt was made by the healthcare workers collecting the samples to actively look for wounds. It is possible that some individuals with wounds present chose not to disclose them or weren't even aware of them which would underestimate the infection prevalence of MRSA in this population.

MRSA can be carried in the gastrointestinal (GI) tract and to determine the prevalence of GI carriage, it is necessary to obtain either rectal, anal, or groin swabs. We decided not to collect swabs to assess GI colonization due to concerns with compliance. A prevalence study done in Ottawa in three homeless shelters required that participants provide a rectal, anal, or groin swab to assess gastrointestinal carriage of MRSA (Szakacs et al., 2007). However, the results from the study did not clearly identify the sites positive for MRSA, and the value of adding the swabs was unclear. The study population of 44 residents was also too small to assess GI carriage of MRSA.

5.2.3.1.3 Limitations due to data analysis

Specimens were swabbed onto Denim Blue Agar and were incubated overnight. The product manufacturer suggests incubating plates for 18-24 hours; however, some plates were incubated for up to 48 hours. This led to an increased number of false positives on those plates. This concern was nullified after further confirmatory analysis by catalase and coagulase testing. All presumed MRSA cases were sent for strain testing by PCR, and the final 18 cases were confirmed MRSA.

Odds ratios (OR) were used to measure the association between specific study variables and testing positive for MRSA. Our study was designed to determine the prevalence of MRSA colonization or infection in CDIRC's population and was under-powered when assessing risk factors for MRSA. The confidence intervals around some estimates, such as the OR for antibiotic use, were large indicating a lack of precision due to small sample size. Analysis was limited to univariate analysis due to the sample size.

5.2.3.2 External Validity

External validity assesses the extent to which a study's findings can be generalizable to relevant target populations (Rothman, 2002). As a cross-sectional study, our prevalence estimate is limited only to the study sample. It should not be extrapolated beyond the population that accessed CDIRC's services in March of 2008.

Local epidemiology of MRSA also varies between regions. Although CMRSA10 is the predominant strain in the marginalized populations of Calgary and Vancouver, the predominant strain identified in Ottawa's homeless population was CMRSA2. Not only are the strains different, but CMRSA2 is considered a nosocomial strain. Geographical differences make extrapolating prevalence estimates to other marginalized populations inappropriate.

There may be an association with weather and shelter use as well. Our study was done in the spring during a period of warm, dry weather. It is possible that the population using the shelter may be different in inclement weather. There may also be a seasonal aspect to the centre's users – for example, some people who choose to stay at the shelter in the winter months opt to sleep rough during warmer weather.

Given the transient nature of the homeless population, inferences to other populations should not be made.

5.2.4 Strengths and weaknesses of the prevalence study

This study yielded an up-to-date estimate of the prevalence of MRSA colonization or infection in the homeless population that uses CDIRC. Several lessons were learned about implementing a study in this population.

It is vital to have individuals involved in the study that know the population and can provide insights into access and barriers to participation. One of the strengths of this study was that several of the prevalence study volunteers also worked at CDIRC and were on site the day of the study to circulate in the building and recruit participants. It was also helpful to have access to the intercom system so regular announcements regarding the MRSA screening could be made.

Although results were made available to patients in the health office, the majority of participants did not retrieve their results envelope. There were five people with MRSA infections who could not be contacted due to this aspect of the study design. The location of their wound sites were unknown and the severity was not recorded. This was a missed opportunity to refer MRSA infected individuals to medical care.

Although the primary objective of the study was to determine the prevalence of MRSA colonization or infection, we also looked at risk factors identified in previous studies. In the decision to keep the questionnaire short, we omitted several variables that may have been important risk factors. We did not ask about previous incarceration or IDU which have been associated with increased risks for MRSA in other studies. We also did not ask about the length of time participants had been homeless. The sharing of

contaminated personal items such as nail clippers and cosmetics was previously identified as a risk factor for MRSA transmission. However, we did not include this in our questionnaire so it cannot be assessed in this study.

5.2.5 Conclusions

The prevalence of MRSA in the population that accesses CDIRC may be increasing and the strain most identified in this group is CMRSA10.

Our study shows that personal behaviours such as antibiotic use and frequency of showering have more of an impact on MRSA colonization or infection than the location where a person sleeps.

The prevalence of MRSA is unlikely to decrease until measures are taken to reduce the amount of MRSA in shelter-using populations, either through intervening to reduce risk factors or through reducing the MRSA bacterial load of colonized or infected individuals.

5.3 Intervention Study

5.3.1 Summary of intervention study

The objective of the intervention study was to explore the feasibility of providing AHP skin cleanser to reduce MRSA skin carriage in the population that accesses CDIRC. Feasibility was assessed by examining participant recruitment; adherence to study protocol; and effectiveness of the AHP skin cleanser.

The study was a test-negative case-control design. Participants had an initial visit in the study office where they gave verbal consent and were administered the same questionnaire used in the prevalence study. During this first visit, individuals provided a nasal and torso swab, as well as a wound swab if a wound was reported. Participants were also sampled at their chest and an axilla using Rodac plates. At the conclusion of the visit, the participants were given a bottle of AHP skin cleanser and were instructed on its use.

Participants were asked to return for a follow-up visit seven days following their initial visit. Participants that did not return for follow-up were paged over the intercom system and looked for in the lobby and parking lot. At the second visit, an additional set of specimens were obtained and participants were administered a questionnaire on the AHP skin cleanser.

Laboratory testing was done on both the swabs and Rodac plates to detect the presence of MRSA. A semi-quantitative bacterial count of normal flora was done for each Rodac plate.

The prevalence of MRSA was determined by dividing the number of MRSA cases detected by the total number of participants screened. A person was considered colonized if the nasal and/or torso specimen was positive for MRSA. A person was considered infected if the wound site yielded a positive result. The Rodac plates were compared both pre- and post-AHP use to explore the effect of AHP on bacterial loads.

The survey was used to provide basic demographics of the study population and univariate risk factor analysis was done on select survey variables including personal hygiene behaviours such as frequency of showering and clothes laundering.

5.3.2 Major findings

For the intervention to be feasible, it needed to be of low cost and easily applied to ensure future sustainability. The success of this intervention was dependent on:

- Recruitment of a sufficient number of participants
- Participant adherence to protocol which included returning for follow-up, use of AHP skin cleanser, and provision of study specimens
- Evidence that AHP skin cleanser reduced bacterial load; either of MRSA colonies or of normal flora

Findings from this study suggest that the methods chosen in this intervention are not feasible or sustainable in reducing MRSA colonization in this setting. Due to a limited sample size and poor adherence to protocol including a loss to follow-up of almost 30%

of the sample population, the effectiveness of AHP at reducing MRSA bacterial load was unable to be assessed.

5.3.2.1 Recruitment

A sample size of 8 individuals with MRSA was needed to detect an 80% reduction in bacterial load after using AHP skin cleanser. Since we had previously estimated the prevalence of MRSA colonization to be approximately 10% in the population that accesses CDIRC's services, a sample size of 80 was required to assess the efficacy of AHP skin cleanser for MRSA positive individuals.

The protocol initially involved having "MRSA Office Hours" every Sunday afternoon starting on November 23, 2008. On the first Sunday, five people were recruited into the study and given AHP skin cleanser. On November 24, 2008 we were advised by the product manufacturer that the batch of AHP held at in the warehouse had become cloudy and its efficacy was in question. On November 26, 2008 the undistributed bottles of AHP were collected from storage at the shelter and returned to the manufacturer. For the next three consecutive Sundays (November 30, December 7, and December 14), "MRSA Office Hours" were kept at CDIRC in the absence of product to attempt contact with the initial five individuals tested. Three of the five participants were eventually contacted and re-swabbed. None of the participants tested positive for MRSA on either the initial or the follow-up visit and as we did not know the efficacy of the spoiled AHP cleanser, they were excluded from further analysis.

We were provided with newly formulated AHP skin cleanser in late January, 2009, and "MRSA Office Hours" began again on February 8, 2009. The study period was extended to the end of March to attempt to recruit a sufficient sample size with adequate follow-up. However, the number of participants being recruited Sunday afternoons was not sufficient, and the decision was made in mid-March to add one additional evening a week in an attempt to reach more of the population. The study period was further extended to end mid-April.

After two weeks of "MRSA Office Hours" being held on Sunday afternoons and Tuesday evenings, recruitment was still low. The "MRSA Office Hours" were further expanded to four days a week. The decision was made to end recruitment on June 1, 2009.

5.3.2.1.1 The initial study visit

There were 37 people who had an initial visit in the intervention study. None of these individuals had participated in the previous prevalence study. Of those participants 73% were male. This is similar to the proportion of males found in both the earlier prevalence study and the City of Calgary homeless census (Stroick et al., 2008).

The median age of 40 years was slightly younger than median age of 44 years observed in the prevalence study and the age range was from 24-52 years. The older and the younger age groups were under-represented in the intervention study. As age in adults is not associated with MRSA carriage, it is not a limitation in this study.

Almost all of the participants (95%) recruited during the intervention study were planning to sleep at CDIRC on the night they were recruited and of those participants 71% were

using Intox or emergency beds. The intervention study was theoretically drawing from the same population as Day 1 of the prevalence study. In the prevalence study, on Day 1, 82% of the participants were planning to sleep in the shelter and 63% of those people were using Intox or emergency beds. The percentage of individuals staying in the shelter was significantly higher in the intervention study than the prevalence study ($p<0.001$). The prevalence study was conducted at a single point in time, so there may have been be fewer residents of CDIRC recruited and more users of CDIRC services passing through the building included. The intervention study had continuous recruitment over several months, so it may be more representative of the actual CDIRC population. The intervention study also recruited during periods of both inclement and fair weather and Day 1 of the prevalence study was dry and warm.

Out of the 37 individuals that came for an initial visit, four tested positive for MRSA, for a prevalence of infection or colonization with any strain of MRSA of 10.81% (95% CI 3.03 - 25.42). This estimate is not statistically different from the MRSA colonization or infection estimate of 9.89% measured in the prevalence study, indicating that the rates of MRSA did not change between the two study periods ($p=0.87$). Strain typing was not done for these isolates, so the contribution of CMRSA10 is not known.

At the initial visit, there were two individuals that had MRSA positive specimens collected from the nares swab, and one individual that had positive specimens collected from both the nasal and chest sites, resulting in a colonization prevalence estimate of 8.11% (95% CI 1.70 - 21.91) The colonization prevalence from the prevalence study was 7.14%. These two rates were not statistically different ($p=0.84$).

There was one individual that tested positive for MRSA at a wound site giving an infection prevalence of 2.70% (95% CI 0.07 - 14.16). This was comparable to the infection prevalence of 2.75% from the prevalence study. The two estimates of infection were not statistically different (p=0.99). The confidence intervals around the prevalence estimates are wide due to the small number of participants that were infected with MRSA.

It was observed in the prevalence study that showering two or fewer times week was associated with an increased likelihood of testing positive for MRSA. In the intervention study, this association was not seen. However, there were only four individuals identified at the initial visit as MRSA positive, which resulted in a large confidence interval around the odds ratio estimate. The estimate from the prevalence study was more precise.

A strong association between antibiotic use in the previous seven days and the presence of MRSA colonization or infection was measured in the prevalence study. Although MRSA-positive participants in the intervention study were three times more likely to have used antibiotics in the past seven days than MRSA-negative individuals, the association was not significant (p=0.33). The 95% confidence interval around the odds ratio estimate was 0.05 to 59.88. This large confidence interval indicates that our sample size was too small to adequately assess the possible association between previous antibiotic use and MRSA infection or colonization.

The information on antibiotic use was better captured in the intervention study than in the prevalence study. This might be attributed to the amount of time spent with each participant. On average, visits in the intervention study lasted about twenty minutes and

the interview and specimens were collected by the same individual. In the prevalence study, the antibiotic question was asked during specimen collection, which took about five minutes.

5.3.2.2 Adherence

An individual was considered fully compliant with study protocol if they returned for their follow-up visit within seven days after the initial visit; if they consented to having nasal and torso swabs collected, as well as Rodac plate sampling of the chest and axillae; and if they reported use of the AHP skin cleanser.

5.3.2.2.1 The follow-up visit

Between February 8 and June 1, 2009, there were 37 people recruited into the intervention study and of those 37 people, 23 (62%) came for their follow-up visit. This rate of follow-up is comparable to that achieved in other studies looking at health in homeless populations (Hwang, 2005). However, other studies on interventions into health of the homeless have been focused primarily on substance abusers and individuals with mental illness. We did not attempt to address these issues in our study.

Although studies with lower than sixty percent follow-up are often regarded as poor, a study with a follow-up rate of seventy or eighty percent might still have bias if the reason loss to follow-up occurred was associated with exposure and disease (Rothman, 2002).

An analysis was done to determine if the participants that returned for the follow-up visit were different from the participants that did not return with respect to both MRSA status and demographics.

The median age of participants that returned for the follow-up visit was 38 years and was younger than the median age of 46 for participants that did not return for the follow-up visit. The mean age of participants who returned for their follow-up visits (37 years) was also younger than the mean age of participants that did not return for the second visit (44 years)($p= 0.02$). Both the younger age group and the older age group were underrepresented in the intervention study and the distribution of ages was skewed. However, since there is no association between age and the risk for MRSA infection or colonization, this difference in ages between the two groups will not influence study results.

There was no difference in follow-up rates related to gender, MRSA status, or use of emergency beds versus transitional beds. There was no difference in follow-up visits between participants that reported showering more than three times a week as compared to participants that reported showering two or fewer times per week.

The loss to follow-up in the CDIRC intervention was not likely associated with any of the study variables examined in the questionnaire or in relation to MRSA status. It is more likely that the loss to follow-up was due to the nature of the population, and related to the reasons why the initial 18 MRSA positive participants from the prevalence study were not able to be traced; this is an extremely transient population with considerable movement between locations including other shelters, rehab programs, correctional services, and the street. There is also no way of knowing whether or not the participants that did not return used the AHP skin cleanser.

At the initial visit, participants were instructed to return in one week to obtain their MRSA test results and to have follow-up specimens collected. Attempts were made to track participants if they did not return in the week following their initial swab. Individuals were paged over the centre's intercom system on consecutive days during "MRSA Office Hours" until they returned for the follow-up visit or the study period ended. Participants were also actively looked for on the main floor lobby and in the parking lot in front of the building which resulted in a follow-up visits from two participants.

Of the participants who returned for the follow-up visit, an average of 23 days had elapsed between the initial visit and the follow-up visit (range 5, 63). Only 4 (17%) of the participants returned within the first week, and of those 4, only two returned without being paged first. The mean number of pages made prior to an individual having a follow-up visit was 3 with a range of 0 to 11 pages. An additional 7 (30%) participants returned for follow-up testing within two weeks.

5.3.2.2.2 Specimen collection

A full set of specimens for an individual consisted of nasal and torso swabs as well as Rodac plates of both chest and axillae. If participants reported a wound, a sample was collected from the wound site as well.

At the initial visit, 34 (92%) individuals consented to having both nares and torso swabs performed. There was one (3%) individual that only had a nasal swab collected, and two participants (5%) had only a nasal and wound swab collected. The three participants that

refused the torso swab were wearing multiple layers of clothing and did not want to remove them for the Z-swab.

Rodac plate sampling occurred for 34 (92%) of the individuals at the chest site. The three individuals that refused the chest swab were all swabbed at both the nares and the torso. There were four individuals that refused the axillae Rodac plates; two of those individuals provided both nasal and torso swabs, one provided a nasal swab only, and one provided both a nasal and a wound swab.

In the verbal consent form, participants were told they could refuse to provide any swab or plate they were not comfortable with. All participants readily provided nasal swabs, but the Z-swab and the Rodac plates required that participants remove or raise their clothing to provide access to the torso and axillae. This removal was difficult in some situations due to multiple layers of clothing, or the participant expressed they would not participate if the torso was included. In the interest of recruiting as many people as possible, and since the prevalence study showed nasal specimens were a reliable method of identifying MRSA colonization in this population, this was not considered a limitation to the study.

At the follow-up visit 4 (17%) of the 23 participants refused the torso swab. Of those 4 individuals, two had refused the torso swab at the initial visit as well. The other two participants had consented to providing a torso swab at the initial visit, but declined to provide the torso swab at the follow-up visit. Rodac plates were collected from the chest of 22 (96%) participants and from the axillae of 20 (87%) of participants. The participant that did not provide the chest Rodac plate had provided this sample at the initial visit. Of

the three participants that did not provide axillae Rodac plates at the follow-up visit, two had provided the sample at the initial visit.

5.3.2.2.3 Accelerated hydrogen peroxide skin cleanser use

To determine if AHP was effective at reducing the amount of MRSA colonization, a sample size of 80 was required. Our study recruited 37 people of which 23 (62%) completed the study. Of the individuals that completed the study, there were 17 (74%) that used the AHP skin cleanser and only one of those tested positive for MRSA at the initial visit. This sample size was not sufficient to determine the efficacy of AHP on reducing MRSA colonization.

5.3.2.3 Effectiveness of accelerated hydrogen peroxide skin cleanser

The primary objective of this study was to explore whether providing AHP skin cleanser to clients of CDIRC was a feasible method of reducing MRSA colonization in this population. Only 4 people initially recruited into the study were positive for MRSA; three of them returned for a follow-up visit, but only one of them reported using the soap. This was not a large enough sample to determine if using AHP in the shower was effective at reducing MRSA colonization.

A secondary objective of the study was to explore whether AHP skin cleanser was effective at reducing the bacterial load of normal skin flora including coagulase-negative staphylococcus species and diphtheroid species.

There were 19 participants in the intervention study that had Rodac plates collected from the axillae at both the initial and the follow-up visit. Of those individuals 8 (42%)

experienced a decrease in the normal flora bacterial load between the two visits. A further 8 (42%) individuals did not experience a change in bacterial load and 3 (16%) of the participants had an increase in the amount of normal flora observed on the axillae between the initial and follow-up visits.

There were 20 individuals that provided a chest Rodac sample at both the initial and the follow-up visit. A decrease in the amount of normal flora was observed for 7 (35%) of these participants. There were 4 (20%) individuals that did not experience any change in bacterial load between the two visits, and 9 (45%) people were observed to have a higher load of normal flora on the chest at the follow-up visit.

There was no association between the group that used the soap and experienced a reduction in bacterial load at the chest site and the group that didn't use the soap and also experienced a reduction in bacterial load. Higher frequency of AHP use among the participants that used the skin cleanser was not associated with a higher likelihood of reduction in bacterial flora.

Reduction in the bacterial load at the chest site between the individuals who returned within the first week was not significantly different than reduction in the bacterial load of participants who returned for a later follow-up visit.

The comparison of normal flora bacterial load between the initial and follow-up visits was similar for the axillae plates as well. There were 19 individuals that provided an axillae sample at both the initial and the follow-up visit. There was no difference in normal flora bacterial load between participants that used the AHP and participants that

didn't use the AHP. Greater frequency of AHP use was not associated with a greater reduction in bacterial load at the axillae. Returning for the follow-up visit within the first week was also not associated with a reduced load of normal flora.

During the initial visit, participants were given information on the prevention of MRSA which included the recommendations to wash their hands frequently and put on clean clothes after showering or bathing. These instructions may have influenced participants' behaviour for both the group that used the soap and the group that didn't.

5.3.3 Limitations

5.3.3.1 Internal validity

5.3.3.1.1 Limitations due to study design

This was a test-negative case-control study nested within a cohort. The cohort was composed of individuals who consented to participation in the study and were users of the services provided by CDIRC. Cases were defined as those participants that tested positive for MRSA and controls were individuals who tested negative for MRSA. Case-control studies are traditionally designed when people are chosen for a study based on the disease of interest and are compared retrospectively to a group of people chosen as controls who don't have the disease. This is a very effective method for exploring exposure risks for diseases that are relatively rare. However, it is also limited in that the study usually looks retrospectively at exposure.

A limitation inherent in retrospective case-control studies is recall bias where someone with the disease of interest may remember a specific exposure due to having the disease.

This intervention study avoids the problem of recall bias as the MRSA status of the individual was unknown at enrolment into the cohort. All subjects were given the questionnaire prior to disease status being known. Additionally, MRSA status was not revealed to individuals until the follow-up visit, so test results did not influence the number of times or whether the participant used the AHP. Sampling controls from the population that gave rise to the cases affords the efficiency gain of a case-control design over a cohort design (Rothman, 2002).

5.3.3.1.2 Limitations due to methods used to conduct the study

The intervention phase of the study was also subject to the same limitations in study conduct as the prevalence study including self-selection bias and healthy volunteer bias.

Follow-up of participants involved paging them to return to the study office and looking for them on the main floor and parking lot. This method was viable only for the participants that remained at CDIRC and were in the building the same day as the follow-up tracking. There is no way of ascertaining whether the people who didn't return for follow-up had moved out of the shelter, weren't present the days or times they were paged, or simply ignored the page.

Another potential limitation was that AHP skin cleanser was originally developed as a hand cleanser. Although it has been used in Europe for hand hygiene, this was the first study to look at it as a total body cleanser. Since this study was limited by a small sample size and a lack of adherence to the study protocol, the evaluation of the efficacy of AHP as a means of reducing bacterial load cannot be elucidated.

5.3.3.1.3 Limitations due to data analysis

The bacterial load on the Rodac plates was measured using a semi-quantitative ordinal measuring system. The categories had underlying colony counts, but were classified at level of bacterial load ranging from trace to heavy. Although the semi-quantitative method is somewhat subjective, all the plates were read by the same experienced laboratory technician so classification bias should not be present.

Some laboratory reports included only the amount of normal flora seen, and some reports were much more specific, counting the number of different coagulase-negative staphylococcal species and the number of different diphtheroid species present. Study estimates were limited only to the amount of normal flora seen to avoid the issue of the missing information in some reports. Use of semi-quantitative bacterial counts may not be the best method for measuring effectiveness of a decolonization regime because length of time, amount used, etc. However, this study illustrates the realities of an intervention in a real world setting.

Although we identified the nares as a common site of colonization in the prevalence study, we did not attempt nasal decolonization of MRSA using mupirocin. Mupirocin is a prescription drug and there are concerns about the emergence of bacterial resistance. There is no evidence to support giving nasal mupirocin to individuals not colonized with MRSA and it would not have been appropriate in this case-control study due to the test-negative design.

We did not obtain strain types for the intervention study so we cannot measure the proportion of MRSA colonizations or infections attributed to the CMRSA10 strain.

Odds ratios were used to measure the effect of specific study variables on the occurrence of MRSA. The confidence intervals around these estimates were large as a result of the small sample size. Inferences about the measure of association between risk factors and MRSA status should not be made based on this sample.

5.3.3.2 External Validity

The results of this intervention are not generalizable to an external population. The sample size was too small to confidently extrapolate these results even to the general population that uses CDIRC's services.

5.3.4 Strengths and Weaknesses of Intervention

We are aware of no other decolonization attempts implemented in a community-based marginalized population to reduce the rates of MRSA colonization. We explored two different means of intervening – providing AHP to individuals previously identified as MRSA positive, and providing AHP to individuals regardless of MRSA status.

The initial strategy in the intervention study was to recruit individuals that had been identified as MRSA positive in the prevalence study. However, there was a lag time of almost 8 months between the initial prevalence study and the intervention. This lag was due to delays in the production process of the AHP skin cleanser. The delay meant that of the 18 individuals identified as MRSA positive in the prevalence study, only one was available to participate in the intervention study. It is unknown if this method might have

been successful if the AHP skin cleanser had been available immediately after the MRSA positive individuals had been identified. However, since 7 (39%) people were not interested in participating, the sample size would probably still have been too small to yield viable results.

The second method of intervening was the implementation of the case-control study conducted over several months. One of the main strengths of this method was the implementation of "MRSA Office Hours". Having regularly scheduled hours in the Safeworks office made it convenient to schedule follow-up appointments with the participants. Some of the participants came by the office several times to discuss MRSA prior to consenting to participate in the study. Using the Safeworks office also allowed for recruitment of people who came to the office for reasons other than an interest in MRSA status. Although only 37 people participated in the study, information about MRSA was shared with many more people.

The intervention study had a small sample size and loss to follow-up of about 30% which meant that inferences could not be made about the effectiveness of AHP at reducing MRSA colonization. If the study had been extended for a longer period of time the sample size likely would have increased. However, with the advent of the warmer weather, the number of people in CDIRC during the day tends to decrease. By the end of the study period, there were several days when no new members were enrolled in the study and no participants returned for follow-up.

5.3.5 Conclusions

This study explored the feasibility of providing AHP as a means of reducing MRSA bacterial load in the population that accesses CDIRC. Two methods were attempted – intervening with previously identified MRSA cases and intervening with a representative sample of the population that uses CDIRC's services and both methods were not feasible.

The first attempt at reducing MRSA load focused on individuals identified as MRSA positive in the prevalence study. However, delays between the identification of MRSA status and the availability of the AHP skin cleanser meant that those participants were not able to be recruited into the intervention.

The second attempt was designed to reduce MRSA bacterial load in a representative sample of the CDIRC population. However, we were unable to recruit a large enough sample size to measure the effectiveness of AHP skin cleanser on MRSA bacterial load. This intervention also experienced a significant loss to follow-up of 38% despite repeated attempts to find study participants. Participant's adherence to study protocol was also poor. The number of participants identified with MRSA was too low to adequately assess the use of AHP and the reduction in normal bacteria load was not different between those that used the product and those that did not.

Although both attempts at reducing the MRSA bacterial load in the population that accesses CDIRC's services were unfeasible, the prevalence study showed that approximately 10% of that population are either colonized or infected with MRSA.

These rates are higher than those in the general population and are not likely to decrease without intervention.

5.3.6 Recommendations for future research

Health interventions in marginalized populations often involve the same challenges of small sample size, high loss to follow-up, and poor adherence to study protocols. Eliminating those challenges will be crucial in future attempts at reducing the MRSA bacterial load in the population that uses CDIRC.

The initial prevalence study was successful in obtaining a large sample size. This may be because the time commitment required of an individual was usually less than 15 minutes from start to finish. Future interventions implemented to reduce MRSA in marginalized communities can increase the chances of achieving an adequate sample size if they limit the amount of time required to participate in the study.

The prevalence study also did not require any further action from the participants other than picking up their results one week after the swab testing. However, there were very few individuals that picked up their results envelope from the health office, indicating that although individuals may be willing to participate at one point in time, they may not be interested in the future. We learned from both the prevalence and intervention studies that we cannot expect participants to return for follow-up, so future studies should be designed to either accept a large loss to follow-up or to design a study that does not require follow-up of individuals.

Adherence to study protocol by participants also proved to be a challenge in the intervention study. Adherence may have been poor as MRSA colonization is not a high priority issue for the population that accesses CDIRC. Studies in HIV and tuberculosis have also been plagued by poor adherence to study protocols and the outcomes from those diseases are arguably more severe than the SSTI most often seen in CA-MRSA cases. With competing interests, participation in an intervention to reduce MRSA may be limited. Interventions in CDIRC's population to reduce MRSA should require little or no effort by the participants.

Infection prevention and control measures can slow the spread of MRSA in the hospital; screening protocols can be implemented to identify colonized individuals and contact precautions can be used to reduce transmission once an individual is identified as being colonized or infected with MRSA. Measures used to manage outbreaks in controlled settings are not possible to implement in community settings. It is not feasible to screen all individuals that access CDIRC for MRSA and hospital contact precautions used to prevent transmission cannot be implemented in a shelter setting.

Infectious disease epidemiology often refers to the "Chain of Infection" when describing transmission of an infectious organism such as MRSA (Figure 4). Each link of the chain represents a component necessary for infection to occur. If the chain is broken at any link, transmission of infection can be stopped.

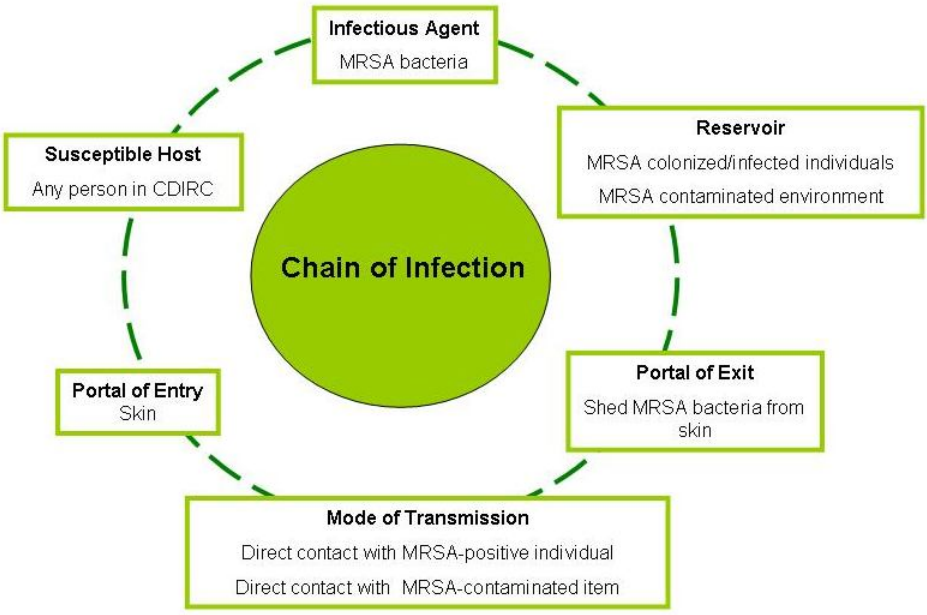

Figure 4 The methicillin-resistant *Staphylococcus aureus* chain of infection at the Calgary Drop in and Rehab Centre

It is unlikely that MRSA bacteria will ever be completely eliminated from the shelter so interventions will need to be focused on breaking the chain at links other than the infectious agent. It may prove beneficial to focus an intervention at several links in the MRSA chain of infection simultaneously.

Approximately 10% of the population that accesses CDIRC is colonized or infected and these individuals make up the MRSA reservoir (Figure 4). The objective of our intervention was to reduce the MRSA reservoir through providing accelerated hydrogen peroxide skin cleanser. By reducing the reservoir, the amount of bacteria shed would also be reduced and transmission by person-to-person contact could be decreased. However, there were significant challenges found in implementing an intervention that

relied on individuals to return for a follow-up visit as well as adhere to use of the skin cleanser. Instead of focusing on individuals, it may be feasible to provide a topical agent such as AHP for a period of time in the CDIRC showers and in small bottles from the hygiene office. MRSA prevalence can be measured before the provision of the product and after to measure if there were any changes in the prevalence. The provision of a topical skin cleanser would be just one component of a multi-faceted population level intervention.

The univariate risk factor analysis done in the prevalence study indicated that personal behaviours such as reduced frequency of showers place an individual at higher risk for MRSA acquisition. Providing a topical skin cleanser in the showers or through the hygiene office will not be effective for the high-risk individuals that are taking less than two showers a week. It might be appropriate to provide an incentive post-shower for the duration of the study, even if the individual declined use of the product. The frequency of showering may prove more important than the product used. The choice of incentive must be carefully considered to avoid issues of coercion.

For the study period, it is recommended that a weekly Skin and Wound Clinic be conducted at the shelter. This will allow clients to have their wounds assessed and proper wound care can be addressed. This may reduce the amount of bacteria shed in the environment and between shelter clients.

It is vital to include an educational component for both staff and clients in future interventions. Education should focus on reduction of risk factors and on modifiable behaviour such as hand hygiene and appropriate wound care.

Any further intervention done to reduce MRSA colonization in the population that accesses CDIRC should be multi-faceted and simple to ensure sustainability. Efforts should be made to focus on population-level interventions instead of requiring individual participation. The one day prevalence study shows that it is possible to recruit adequate participants in a study provided the time commitment is minimal, follow-up participation is not required, and participation in the intervention involves little or no effort.

The prevalence of MRSA colonization and infection is increasing in marginalized populations, and without intervention is likely to continue to increase. The risk factors for CA-MRSA acquisition and transmission are well understood, but interventions to modify acquisition and decrease transmission within the homeless are rare.

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APPENDIX A: OXOID DENIS BLUE AGAR TECHNICAL SHEET

OXOID

Denim Blue Agar (Chromogenic MRSA Screening Agar)

Product:

MP1571 Denim Blue Agar (Chromogenic MRSA Screening Agar)

pH 7.30 +/-0.2

Appearance of uninoculated medium:

Opaque pale off-white

Description:

Chromogenic MRSA Screening Agar is selective medium for the isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA). The medium uses a chromogen which yields a blue denim colour as a result of phosphatase activity. This enzyme is present in all MRSA. The antibiotic solution in the medium is selective for *Staphylococcus aureus*, containing compounds that inhibit the growth of competitor organisms while some encourage the production of MRSA markers.

Technique:

Bring medium to room temperature prior to inoculation. Inoculate plates directly from patient swabs, an isolated colony or broth suspension. If using direct colonly inoculation; it is advisable to make a 0.5 McFarland suspension, deliver a 50ul sample and streak for isolated colonies. Heavy inoculation may lead to a blue appearance (haze) in the main inoculum which should not be interpreted as a positive result. Incubate plates aerobically at 35-37°C for 18-24 hours. Some research indicates that larger colonies may be observed if incubated at 37 ° C.[1]

Interpretation:

Positive	Growth of denim blue colonies
Negative	Inhibition or Growth of white colonies

MRSA typically grow as discrete 0.5 to 1.0 mm smooth entire denim blue colonies after 18 to 24 hours. Incubation beyond 24 hours may affect the specificity of the medium. Atypical growth (eg.colonies with blue "halos") should not be interpreted as possible MRSA. Growth of white colonies are indicative of *Coagulase Negative Staphylococcus*; heavy growth typically represents *Staphylococcus hemolyticus*. The denim blue colonies are presumptive positive for MRSA. Confirmation tests include Staphytect Plus (DR0850M), Dryspot Staphytect (DR0100M) , Staphaureux Plus (RE30950102) and PBP2' Latex agglutination tests (DR0900A). Colonial morphology, gram stains and catalase tests will quickly rule out possible breakthrough bacteria.

Quality Control:

The Quality Assurance of this medium meets or exceeds all CLSI standards, according to CLSI document M22-A3. Testing of control organisms should be performed in accordance with established quality control procedures. A fresh culture should be used each time quality control testing is performed. The following is the minimum QC performance protocol used by Oxoid Co.

Organism tested	ATCC® #	Expected results
Staphylococcus aureus	33591	Growth; pinpoint to 1 mm blue colonies
Staphylococcus aureus	43300	Growth; pinpoint to 1 mm blue colonies
Pseudomonas aeruginosa	27853	Inhibition
Proteus mirabilis	12453	Inhibition
Staphylococcus epidermidis	12228	Inhibition
Staphylococcus aureus	25923	Inhibition
Enterococcus faecalis	29212	Inhibition

Storage Conditions:

Plates should be stored at 2 to 12°C away from direct light. Media should not be used if there is any sign of contamination, damage or deterioration such as shrinkage, cracking or discolouration of media. Discard expired plates.

References:

[1] Skulnick,M and Wiley,B "MRSA Results and Specificity 2005 Mt.Sinai", May 2005

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APPENDIX B: PREVALENCE STUDY RECRUITMENT POSTER

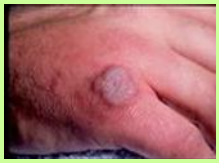
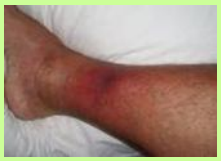
You could have

MRSA

And not even know it!

MRSA is a type of bacteria that has become resistant to certain drugs. It often lives on the skin and noses of healthy people without causing illness, but **MRSA** can sometimes cause serious infections.

MRSA infections can look like this….


A study is being done, at the Calgary Drop-In & Rehab Centre, to find out how many people have MRSA. This information will help find ways to prevent infection.

The test for MRSA is done by rubbing one Q-tip in your nose, a second one across your upper body, and another one on any wounds you have.

Test results will only be known to you.

Testing is on Sunday,
March 9, 11:00 a.m to 5:00 p.m.
At the CDIRC

If you have any questions about MRSA or this study, please contact Nurse James or the Safeworks nurses.

APPENDIX C: MRSA INFORMATION SHEET

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MRSA Information Sheet

What is MRSA?

Staphylococcus aureus is a bacteria that often lives on the skin, or in the noses of healthy people. MRSA is the term for *Staphylococcus aureus* bacteria that have become resistant to certain types of antibiotics. MRSA infections can be difficult to treat and drugs commonly used for treatment of other strains of *Staphylococcus aureus* are not always effective.

Traditionally, MRSA is seen in people who are taking antibiotics and those individuals who are receiving medical care. MRSA, like *S. aureus*, may also live on the skin or in the noses of people.

More recently, MRSA has been found in people who have no contact with the health care system. This is referred to as community-associated MRSA (CA MRSA). In the community, MRSA most commonly causes skin and soft tissue infections (e.g., boils or abscesses on arms, legs or elsewhere). These are treatable with drainage and antibiotics. Rarely, MRSA can cause severe invasive infections such as pneumonia and bloodstream infections. These severe infections require urgent medical treatment.

How is MRSA spread?

MRSA bacteria are spread through direct person-to-person contact with a colonized or infected person. It can be passed from hands that are not clean to any person, object or surface they touch. When hands are washed thoroughly or rubbed with alcohol-based hand products, MRSA will likely be removed.
However, if the immediate environment is not clean, hands can very quickly become soiled again. Frequent hand cleaning is necessary to either prevent spreading MRSA to others, or to prevent picking it up from others.

What do colonization and infection mean?

Colonization: Colonization occurs when bacteria are present on or in the body without causing illness. MRSA can colonize the nose, skin and moist areas of the body.
Infection: Infection occurs when bacteria get past the person's normal defences and cause disease (e.g., skin bacteria getting into the bloodstream via an intravenous catheter). Infections with MRSA may be minor, such as pimples and boils, but serious infections may also occur, such as blood stream infections and pneumonia.

APPENDIX D: PREVALENCE STUDY VERBAL CONSENT SCRIPT

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Verbal Consent Script

Methicillin resistant *Staphylococcus aureus* or MRSA, is a "super bug" that is resistant to many antibiotics and can cause serious infections in people. MRSA infections have been increasing in shelters in Calgary.

People with MRSA can carry it on their skin for a long time and not know it. Other people who come in contact with an infected person are at risk of getting infected as well. Infections can include boils and styes, or can be more serious and require hospitalization.

We would like to know how many people using the Calgary Drop-In and Rehab Centre have MRSA on their skin. We'd like to swab your nostrils, torso, and any open wounds you might have with a Q-tip and ask you a few questions. You will be able to learn the results of the test from Safeworks or the CDIRC nurse in about one week.

This study is confidential. No one in your group will be told your results.

There are no risks or side effects to taking part in this study. If we find MRSA on your skin, you can be referred to medical care to try and get rid of this bug. Your participation is voluntary and you will not be paid or reimbursed for participating. You'll be given a copy of this text and can refuse to participate at any point. The findings from this study will tell us the extent of MRSA in the shelter.

By being part of this study through giving your permission, you'll be helping doctors and scientists know more about this "super bug".

Thank you for helping us do this study.

APPENDIX E: PREVALENCE STUDY RISK FACTOR QUESTIONNAIRE

AFFIX CONSENT LABEL HERE

INTERVIEWER INITIALS

Prevalence Questionnaire

First Name:_____ Last Name: _____

1. When were you born?
   Year/month/day
   _____/_____/_____
   - O Don't know
   - O Refused

2. What is your sex?
   - O Male
   - O Female
   - O Transgender
   - O Don't know
   - O Refused

3. Where are you staying tonight?
   - O Own apartment or house
   - O Relative's house or place
   - O Friend's place
   - O Hotel/Motel room
   - O Rooming or boarding house
   - O Transition or halfway house
   - O On the street
   - O Jail
   - O Detox, Recovery house or treatment program
   - O Shelter
   - O Don't know
   - O Refused

3b. If shelter, do you usually stay in:
   - O A single room
   - O A shared room (<5 people)
   - O A large dorm room
   - O Don't know
   - O Refused

4. In the past seven days, how many times did you take a shower/bath?
   - O 0
   - O 1-2
   - O 3-4
   - O 5-6
   - O 7 or more

5. Where did you shower/bathe?
   - O Own apartment or house
   - O Relative's house or place
   - O Friend's place
   - O Hotel/Motel room
   - O Rooming or boarding house
   - O Transition or halfway house
   - O Jail
   - O Detox, Recovery house or treatment program
   - O Shelter
   - O Don't know
   - O Refused

6. How often do you wash your clothes?
   - O After wearing once
   - O After wearing 2-3 times
   - O After wearing 4-5 times
   - O After wearing more than 6 times

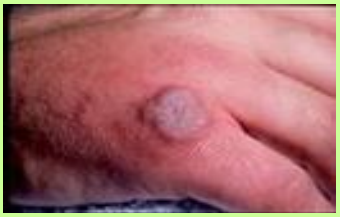
APPENDIX F: INTERVENTION STUDY RECRUITMENT POSTER

You could have

MRSA

And not even know it!

MRSA infections can look like this….



The test for MRSA is done by rubbing a Q-tip in your nose, across your upper body, and on any wounds you have.

MRSA Testing Today in the Safeworks Office!

APPENDIX G: INTERVENTION STUDY VERBAL CONSENT SCRIPT

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Verbal Consent Script

Methicillin resistant *Staphylococcus aureus* or MRSA, is a "super bug" that is resistant to many antibiotics and can cause serious infections in people. MRSA infections have been increasing in shelters in Calgary.

People with MRSA can carry it on their skin for a long time and not know it. Other people who come in contact with an infected person are at risk of getting infected as well. Infections can include boils and styes, or can be more serious and require hospitalization.

We are doing a study to see how well Accelerated Hydrogen Peroxide skin cleanser works at reducing the amount of MRSA on peoples' skin.

We would like to swab your nostrils, torso, and an open wound (if you have one) to see if you have MRSA there and ask you some questions. We'll give you a product called Accelerated Hydrogen Peroxide foaming skin cleanser to use in the shower for the next seven days, which may rid your skin of MRSA. After seven days, we'll swab your nostrils, torso, and open wound (if you have one) again to see if there is MRSA present. We'll also ask you some questions about your experience using the soap.

This study is confidential. No one in your group will be told your results.

Your skin carriage of MRSA may be improved during the study but there is no guarantee that this research will help you. There are no risks or side effects to taking part in this study.

Your participation is voluntary and you will not be paid or reimbursed for participating. You are free to withdraw from the study at any time. All you need to do is tell the study coordinator that you no longer wish to participate. You may refuse to answer any question or to complete any interview, even if you have started it. You may refuse to have a particular site swabbed. You understand that the study coordinator can withdraw you from the study if you do not meet some of the study requirements.

By being part of this study through giving your permission, you'll be helping doctors and scientists know more about this "super bug".

Thank you for helping us do this study.

APPENDIX H: INSTRUCTIONS ON ACCELERATED HYDROGEN PEROXIDE SKIN CLEANSER USE

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AHP Skin Cleanser

Use AHP Skin Cleanser every time you shower for one week. If you need more of the skin cleanser, please see Nurse James or the Safeworks nurses.

Try to put on clean clothes after you shower.

How to use:

Wet hands and skin

Stand outside spray of water.

Use one pump for each leg, one pump for your arms, one pump for your body, and one pump for your head. Work into your skin for 15 seconds.

Rinse well and dry thoroughly.

Caution:

Avoid contact with eyes. If splashed into eyes, wash immediately for at least 10 minutes with clean water. Seek medical advice if any irritation persists.

Avoid contact with open skin or wounds.

Frequent use may result in temporary whitening of the skin.

Do not consume this product.

This product does not contain any alcohol.

APPENDIX I: FOLLOW-UP VISIT QUESTIONNAIRE ON ACCELERATED HYDROGEN PEROXIDE USE

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AFFIX LABEL HERE

Post-Intervention Questionnaire

1. How many times did you use the product in the past seven days?
   - ☐ 0
   - ☐ 1-2
   - ☐ 3-4
   - ☐ 5-6
   - ☐ More than 7 times
2. How would you rate the smell of this product?
   - ☐ Smelled great
   - ☐ Smelled OK.
   - ☐ Smelled awful
   - ☐ N/A
3. Did the soap rinse off easily?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know
   - ☐ N/A
4. Did the soap rinse off completely?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know
   - ☐ N/A
5. Did you have any skin reaction? I.e. redness or itching?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know
   - ☐ N/A
6. Did you like the feel of your skin after using the product?
   - ☐ Yes
   - ☐ No
   - ☐ Don't know
   - ☐ N/A
7. When was your last shower? _____