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Concordance of Genotyping and Phenotyping in the Classification of Methicillin-Resistant Staphylococcus Aureus

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Abstract

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MSc, Arabian Gulf University, 2006

BSc, Lebanese University, 1993

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have spread in Saudi Arabia, increasing morbidity, mortality, and financial burdens. Recent studies have suggested the phenotyping methods typically used to classify MRSA as either health care MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA) cases are unreliable, because they lack concordance with the results of genotyping. Yet the expense associated with genotyping precludes its use in the Saudi Aramco population in Saudi Arabia. The absence of a standardized and affordable method to classify MRSA into CA-MRSA and HA-MRSA has been a challenge for infection control programs in Saudi Arabia. The objective of this quantitative, secondary data analysis was to determine the most reliable phenotyping approach to strain identification using stored samples from John Hopkins Aramco Hospital. The ecological and antibiotics selection pressure theories framed this research. The results of concordance, and sensitivity and specificity tests, suggested hospital admission profiles and susceptibility pattern were the most reliable phenotypic predictors of genotype-based classifications. Multiple logistic regression for susceptibility pattern ($OR = 15.47$, $p < .001$) and hospital admission profile ($OR = 2.87$, $p = .008$) confirmed those results, whereas all other variables were not found to be statistically significant. These results can be used to clarify the epidemiological and molecular factors that affect the transition of MRSA from health care facilities to the Saudi Aramco community. Implications for positive social change include faster and more reliable classification of MRSA to aid in disease surveillance and the selection of appropriate treatments to reduce MRSA-related morbidity and mortality.

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Dedication

I dedicate this dissertation to my father and mother who would have been happy to see me holding PhD degree, to my wife and children for their endless support and patience during the process of completing this degree, and to Professor Giuseppe Botta and Doctor Khaled Tabbara who encouraged me to pursue my interests in health care sciences.

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Chapter 1: Introduction to the Study

In the early 1960s, Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were first isolated from patients exposed to health care risk factors such as hospitalization, surgery, dialysis, and indwelling devices (David et al., 2006). Like infection by other multidrug resistant organisms, MRSA infection increases patients' morbidity and mortality risk and health care costs (Centers for Disease Control and Prevention, 2013a). From the mid-1970s through the 1990s, the incidence of MRSA in health care settings dramatically increased (Panlilio et al., 1992). MRSA cases that were isolated within health care settings or from those who received recent care from such settings are referred to as health-care-associated MRSA (HA-MRSA). Then, in the late 1990s, a new strain of MRSA without health care risk factors was isolated. This new MRSA strain shares genetic background with *Staphylococcus aureus*. However, it has a distinct genetic code that had not previously existed (David et al., 2006). The new MRSA strains identified among individuals outside of health care settings and those who have not received recent care from such settings is referred to as community-associated MRSA (CA-MRSA).

CA-MRSA has been a growing problem with increasing incidence and prevalence in Saudi Arabian communities, and it has migrated into health care settings (Bukharie, 2010). Besides having resistant genes, CA-MRSA is known to acquire toxin-producing genes such as Panton-Valentine leukocidin (PVL). PVL is lethal to white blood cells and can cause tissue necrosis including necrotizing pneumonia in young patients (Gillet et al., 2002). Phenotyping and genotyping methods have been used to classify strains.

Phenotyping methods classify strains as HA or CA based the organism's observed characteristics, such as health care risk factor classification, infection type classification, and susceptibility pattern classification. Genotyping methods that classify strains using the organism's genetic code include pulsed field gel electrophoresis (PFGE) and polymerase chain reaction (PCR). However, phenotyping methods have become less reliable due to the increased incidence of CA-MRSA in health care settings and the continuous rise in antibiotic resistance among CA-MRSA strains (McCarthy et al., 2010).

Several studies have demonstrated that CA-MRSA strains originating from patients with no antecedent hospital exposure were clonally distinct from hospital endemic MRSA strains (Naimi et al., 2013; Vandenesch et al., 2003). The apparent phenotypic and genotypic differences between CA-MRSA and HA-MRSA were noted in anecdotal reports, case series, and outbreak studies, which often compared few CA-MRSA strains to historical HA-MRSA control isolates from worldwide collections (Enright et al., 2002; Vandenesch et al., 2003). Such comparison of contemporary cases to historical controls is flawed, as it does not allow for elimination of potential biases due to other factors that may have changed over time (e.g., clonal shifts). Thus, concurrent molecular genetic characterization of MRSA strains combined with well-designed epidemiologic studies will enable the identification of the transmission dynamics of CA-MRSA and HA-MRSA.

The aim of this study was to assess the molecular characteristics of the MRSA strains that cause infection within Saudi Aramco community and to compare and contrast the concordance/discordance of genotyping methods with the three common phenotyping

classifications. An assessment of each phenotyping method's validity in the identification and management of MRSA was needed to develop a potent infection control strategy that can address the needs of a community.

The findings of this study offer new insights into the epidemiological and molecular factors that affect the transition of MRSA from health care facilities to Saudi Aramco community settings. This study may make significant contributions to the international community by in elucidating the epidemiological and molecular forces affecting variations in MRSA disease frequency and disease severity (Chambers, 2005; Enright et al., 2002; Oliveira, Tomasz, & deLencastre, 2002).

Background

The number of cases of multidrug-resistant *Staphylococcus aureus* continues to increase, particularly MRSA, which is now a leading cause of nosocomial infection worldwide. A nosocomial infection is defined as any infection that develops during, or as a result of, an admission to an acute care facility (hospital) and which was not incubating at the time of admission (Siegel et al., 2006). MRSA is responsible for up to 60% of nosocomial infections in intensive care units (ICUs), likely due to carrying exogenous mobile genetic elements, inadequate antibiotic therapy, and contaminated hands (Inweregbu, Dave, & Pittard, 2005).

Treatment of MRSA infection has been challenging due to the inefficiency of first and second line antibiotics, making the only treatment choice the use of more toxic, expensive, and less effective antibiotics (Centers for Disease Control and Prevention (CDC), 2013a). The CDC has reported an encouraging decrease in the rate of HA-MRSA

in the United States with approximately 31,000 fewer cases and 9,000 fewer deaths between 2005 and 2013 (CDC, 2013b).However, in Saudi Arabia, the MRSA prevalence in hospitals increased from 5% in 1995 to 35% in 2013(Al Yousef, Mahmoud, & Taha, 2013).

Molecular epidemiology studies indicate that the massive geographic spread of MRSA resulted from the dissemination of relatively few epidemic clones. Five major lineages have been defined, which were mainly disseminated in southern and eastern Europe, Latin America, and the United States (Oliveira et al., 2002). The five major MRSA lineages are Iberian, Brazilian, Hungarian, New York/Japan, and pediatric (Stefani & Varaldo, 2003). The continuing dissemination of these lineages indicates that they are successful in terms of ability to cause infection, to persist, and to spread from one geographic site to another, including across continents (Oliveira et al., 2002). As mentioned earlier, the increased rate of MRSA infections outside hospital setting led to categorization of MRSA into two distinct groups, HA-MRSA and CA-MRSA (CDC, 2005). Nevertheless, several molecular and biological parameters have been involved in classifying MRSA.

HA- MRSA has been reported among patients with certain risk factors including recent hospitalization, dialysis, residence in a long-term care facility, presence of invasive devices, and history of MRSA infection and colonization (Klevens et al., 2007). HA-MRSA can cause a variety of diseases from noninvasive infection such as mild intermittent abscesses, to life threatening invasive systemic diseases such as necrotizing pneumonia, kidney infection, joint infection, and blood stream infection. Based on their

site of infection, HA-MRSA strains are classified into noninvasive and invasive types. In addition, the HA-MRSA strains, in particular the invasive form, are also typically resistant to multiple, non-beta-lactam antibiotics (Fey et al., 2003). The HA-MRSA strain types identified by PFGE are USA100, USA200, USA600, USA700, USA800, and less often USA500 (Klevens et al., 2007; McDougal et al., 2003). Other markers such as the presence of SCCmec I, II, and III, agr group II, and low PVL carriage have been used to distinguish HA-MRSA from CA-MRSA. PFGE genotyping aids in determining bacterial isolate identification by acting as DNA "fingerprinting." In epidemiology, genotyping techniques can help track the spread of infections, monitor trends in types, and track seasonal outbreaks (Healy et al., 2005). With regard to HA-MRSA infections, genotyping techniques are excellent tools to investigate the genetic relationship between the different HA-MRSA strains and severe infections in health care settings.

CA- MRSA has been described in patients without the established health care risk factors. CA-MRSA was first described occurring in specific populations with distinctive risk factors such as prisoners, intravenous drug users, athletes, military trainees, and men who have sex with men (Kazakova et al*.,* 2005; McCaig et al*.,* 2006). This form of MRSA usually presents as noninvasive infection, such as skin and soft tissue infections. CA-MRSA isolates are likely to be resistant to fewer antibiotics, produce different toxins, and have genetically distinct genes compared to HA-MRSA (McCaig et al*.,* 2006). Genetic markers such as SCCmec IV, *agr* group I and high PVL gene carriage have been used to distinguish CA-MRSA from HA-MRSA (Tsuji, Rybak, Cheung, Amjad, & Kaatz

2007). Strains most frequently isolated from MRSA infection of community origin include PFGE USA300, USA400, USA1000, and USA1100 (Klevens et al., 2007).

Certain genetic differences have been identified in the two types of MRSA, making HA-MRSA strains more resistant to beta lactams and other non-beta lactams groups of antibiotics than CA-MRSA strains. These genetic differences also make CA-MRSA infections more necrotic and easy to spread from person to person (Gillet et al., 2002). Distinct epidemiological, clinical, and pharmacological characteristics are used to classify MRSA infections as either health-care-associated (HA) or community-associated (CA) (CDC, 2005). On the epidemiologic and clinical side, HA-MRSA strains usually infect older, unhealthy people, whereas CA-MRSA strains have the tendency to infect healthy, younger people and to cause distinct clinical syndromes such as soft skin tissue infections (SSTIs) (CDC, 2005). However, an increased number of necrotizing pneumonia and severe sepsis cases have been reported, associated with CA-MRSA (Seybold et al., 2006). From the pharmacological side, HA-MRSA infections usually require intravenous antibiotic therapy administered in a hospital setting, whereas CA-MRSA infections respond more to ambulatory oral antibiotics. The ambulatory treatment option reduces the length of hospitalization, lowers associated costs, and eliminates the potential side effects of intravenous antibiotics (Doebbeling et al., 1993; Fey et al., 2003). These epidemiological, biological, and clinical characteristics of MRSA have pushed hospitals and other health care settings to apply a variety of phenotyping and genotyping methods to classify MRSA infections as either HA-MRSA or CA-MRSA (CDC, 2005).

The distinct characteristics of MRSA guided CDC and the Clinical Laboratory Institute (CLSI) to create the three most popular phenotyping methods used currently to differentiate MRSA as CA-MRSA or HA-MRSA. According to the health care risk factors method, any MRSA infection identified after 48 hours of hospital admission is labeled as HA-MRSA (CDC, 2005). According to the infection type method, any MRSA isolated from an invasive site; blood; cerebrospinal fluid (CSF); pleural fluids from patients who have the following risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year; or the presence of an indwelling catheter or a percutaneous device at the time of culture, is labeled as HA-MRSA. However, any MRSA isolated from patients who lack the above risk factors is labeled as CA-MRSA (CDC, 2005). The last method is the susceptibility pattern method, where MRSA cases resistant only to β-lactams antibiotics are labeled as CA-MRSA, whereas cases resistant to additional antibiotics classes, such as carbapenems, aminoglycoside, and fluoroquinolones, are labeled as HA-MRSA (Clinical and Laboratory Standards Institute, 2005). Such classification is important to monitor trends in antimicrobial resistance among MRSA within health care settings and for the selection of appropriate antibiotics regimens. Although phenotyping methods are widely used, the emergence of invasive and multidrug resistant CA-MRSA strains as a cause of health care associated infection (Gillet et al., 2006; Seybold et al., 2006), and the increased circulation of HA-MRSA in the community (Miller et al., 2007; Seybold et al., 2006) might interfere with the sensitivity of these phenotyping methods.

CA-MRSA has been a growing problem with increasing incidence and prevalence in Saudi Arabia communities, and the migration of CA-MRSA into Saudi health care settings. In the Middle East and in particular in Saudi Arabia, most of the studies conducted between 1990 and 2008 to classify MRSA as CA-MRSA or HA-MRSA were based on phenotyping methods, in particular susceptibility pattern method (Monecke et al., 2012). According to Sievert (2008), only 20 % from 2151 cases in the United States were consistently classified as HA-MRSA by the three phenotyping methods (health care risk factor, infection type, susceptibility pattern) and 25% were consistently classified as CA-MRSA. However, the discrepancy in the classification as CA-MRSA or HA-MRSA exceeded 40% between the three methods. Several studies have shown that phenotyping markers are more prone to change in time than genotyping markers (Devita, Lawrence, & Rosenberg, 2009). Such a change is due to loss of extrachromosomal genetic elements and later their horizontal transmission (Devita et al., 2009) and can explain the discrepancy between the classification methods that depend on the phenotyping markers (Tenover et al., 1994).

The revolution in genotyping methods that are based on DNA analysis has allowed for an accurate and precise MRSA classification due to markers that are less prone to change in time (Tenover et al., 1994). Therefore, genotyping methods have a discriminative power for MRSA typing. A recent genotyping study in Saudi Arabia has found that HA-MRSA resistance markers (e.g., aacA-aphD, aadD) are now common among CA-MRSA strains (Monecke et al., 2012). Clearly, the presence of these resistance markers in both HA and CA-MRSA will negatively affect the accuracy of the

susceptibility pattern method and therefore its role in monitoring the emergence of the new necrotic and invasive CA-MRSA strains. Moreover, these susceptibility methods are essential for the proper administration of the glycopeptide Vancomycin that is routinely used to treat HA-MRSA. However, the inappropriate and excessive usage of this antibiotic can lead to the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) (Hiramatsu, Cui, & Kuroda, 2001).

With the advent of highly sensitive and specific PCR, genotyping methods, in particular real time PCR, and the limitations of phenotyping methods due to the instability of phenotyping markers, new multiplex real time PCR methods have emerged as essential tools for studying the epidemiology of MRSA. These new genotyping methods have the capability to accurately classify MRSA strains as CA-MRSA or HA-MRSA based on their staphylococcal chromosomal cassette mec (SCCmec) and the presence of the PVL virulence genes (Balkhy et al., 2007). CA-MRSA strains harbor the PVL virulence genes and a novel small mobile staphylococcal cassette chromosome mec (SCCmec) Type IV or V genetic element, which in turn harbors the methicillin resistance (mecA) gene. The SCCmec Type IV or V can more easily be transferred to other *Staphylococcus aureus* strains compared to the larger SCCmec Types I, II, and III that usually characterize HA-MRSA strains (Zhang et al, 2005). This easy chromosomal transfer can explain the tremendous increase in the CA-MRSA rate (Zhang et al., 2005). Unlike the conventional PCR methods that usually target one specific gene, multiplex real time PCR methods target several genes specific to either CA-MRSA or HA-MRSA

(Zhang et al., 2005). Therefore, multiplex PCR methods are highly sensitive and specific, minimizing the errors that the absence or deletion of one specific gene may produce.

During the last two decades, several genotyping studies that target different genes were conducted in the United States and Europe to classify accurately MRSA as CA-MRSA or HA-MRSA and to understand the molecular characteristics of the most predominant strains (Monecke et al., 2012). However, the health care settings in the Middle East, including Saudi Arabia, did not introduce the same types of genotyping studies until 2012 (Monecke el al., 2012). Although genotyping methods that target different genes are very sensitive and specific, they are expensive, and the majority of health care centers in Saudi Arabia cannot afford those (Monecke et al., 2012).

Unlike methicillin-sensitive *Staphylococcus aureus* (MSSA) and HA-MRSA, CA-MRSA is characterized by its evolutionary success, a series of events that occurred within its genome, making the new strains more fit, transmissible, and virulent (Rolo et al., 2012). The SCCmec IV allele that characterizes CA-MRSA is probably responsible for such evolutionary success (Rolo et al., 2012). The high level of genetic diversity of CA-MRSA strains in European and American regions and the emergence of new strains every few years can increase the impart of the pathogenicity of CA-MRSA strains in terms of its invasion capability and antimicrobial susceptibility, posing a real challenge to diagnostic and infection control (Hudson et al., 2013). The results of the first genotyping study in the central province of Saudi Arabia were surprising in terms of the high gene diversity of MRSA strains and the high prevalence of the PVL gene that usually characterizes CA-MRSA strains (Monecke et al., 2012). However, this first study was

limited to only the central region and therefore cannot be generalized to all Saudi regions. Similar population strain diversity has been found to be related to the disproportional distribution of CA-MRSA and HA-MRSA in the United States (Klevens et al., 2007; Rolo et al., 2012). Currently in Saudi Arabia, the selection of phenotyping method to guide infection control strategies in Saudi health care setting depends only on the methods validated for hospitals in the United States (Bukharie, 2010). It is essential to enact an evaluation policy implementing phenotyping methods where applicable and to establish new validation rules for health care settings that are planning to apply these methods. Such policy will serve as the basis of a continuous surveillance strategy aimed at detecting and controlling CA-MRSA in Saudi Arabia (Popovich, Hota, Rice, Aroutcheva, & Weinstein, 2007).

Statement of the Problem

Increasing cases of HA-MRSA and CA-MRSA isolated from health care systems and the communities require more effective methods of classification (Klevens et al., 2007). Many investigators have classified MRSA infections into CA-MRSA and HA-MRSA using approaches advocated by the CDC and the CLSI in order to guide decisions about antibiotics empirical treatment, and to contain the spread of MRSA infection (David et al., 2008). However, the circulation of both HA-MRSA and CA-MRSA in the community, the emergence of CA-MRSA as a nosocomial pathogen, and the identification of high-risk groups for MRSA in the community, including athletes, children, and incarcerated people, raise doubt about the utility of such approaches (David et al., 2008). The spread of multidrug resistant *Staphylococcus aureus* strains has made

the infection challenging and costly to treat (CDC, 2013b). The inherent limitations in therapeutic options necessitate implementation of infection prevention and control procedures in an attempt to limit MRSA spread (Monecke et al., 2011). The cost of such procedures and of the use of second-line antimicrobial medications amounts to billions of dollars/euros in the United States and Europe (Monecke et al., 2011). Different countries worldwide have applied different strain typing methods over the last 30 years to track MRSA spread and provide insight into its control (Monecke et al., 2011; Stefani et al., 2012). However, Saudi Arabia still struggles with understanding the distinction between CA-MRSA and HA-MRSA infection types, risk factors and patients' characteristics such as age distribution for each strain type, seasonality, and potential shifts in therapeutic drug resistance. Health care risk factor is currently the method most commonly used by the infectious disease specialists at the Johns Hopkins Aramco Hospital in Saudi Arabia to classify MRSA; however, no unified method has been adopted and classification by phenotype remains subject to individual physicians' perspectives. As the prevalence of MRSA continues to increase along with its geographical diversity in colonization and infection rates, and as the number of available therapies decrease, health care providers should take notice of their local rates of resistance (Ezeanolue et al., 2008).

This study aimed to fill the gap in understanding in Saudi Arabia with respect to distribution of MRSA in the community and the associated need for the development and testing of a rapid, efficient, inexpensive, and reliable method of identification of HA-MRSA and CA-MRSA strains circulating in the region.

Purpose of the Study

The purpose of this quantitative, secondary data analysis study was to characterize the MRSA strains within the Saudi Aramco community and to identify accurately MRSA strains infection as HA-MRSA or CA-MRSA by comparing and contrasting the three existing phenotyping methods against the gold standard Multiplex PCR method.

Research Questions and Hypotheses

The aim of this research study was to answer the following questions and associated hypotheses:

1. What is the distribution of MRSA in Saudi Arabia Eastern Province based on genotyping?

2. What is the concordance between each pair combination of three phenotyping methods (health care risk factor, infection type, susceptibility pattern) used to classify CA- MRSA vs HA -MRSA in Saudi Arabia?

3. What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used in Saudi Arabia as compared to the gold-standard used to classify CA- MRSA vs HA –MRSA?

4. How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?"

H_0: Demographic and phenotyping variables do not significantly predict MRSA genotyping classification.

H_a: Demographic and phenotyping variables significantly predict MRSA genotyping classification.

Theoretical Framework

My research study was grounded in the Ecological theory (Hardin, 1960; Kouyos, Klein, & Grenfell, 2013). In ecology, the competitive exclusion principle, or Gause's law of competitive exclusion, is a proposition that states, "Two species competing for the same resources cannot co-exist if other ecological factors are constant. When one species has even the slightest advantage or edge over another, then the one with the advantage will dominate in the long term" (Hardin, 1960, p. 1292). Based on the Ecological theory, coexistence of HA-MRSA and CA-MRSA would be possible in environments where ecological factors are constant. However, either CA-MRSA or HA-MRSA would out-compete the other and predominate depending on different environmental conditions (Hardin, 1960; Kouyos et al., 2013). HA-MRSA is classically characterized by a broad resistance antibiotic resistance spectrum conferred by its relatively large Staphylococcal cassette chromosome (SCCmec); SCCmec is a complex mobile genetic element found in Staphylococcus *aureus* (Ito et al., 2014; Shore & Coleman, 2013). HA-MRSA usually carries either SSCmec II or III, which have acquired genes for resistance to antibiotic classes beyond the β-lactams (Hiramatsu et al., 2002). CA-MRSA strains tend to carry SSCmec IV and V, which are relatively small. Thus, CA-MRSA strains tend to be susceptible to clindamycin and other non-β-lactam antibiotics (Naimi et al., 2003). Under ecological theory, these factors would favor HA-MRSA in a health care environment where there is common use of antibiotics. The presence of SCCmec II or III correlates

with slower growth than elements IV or V. Thus in the community, in the absence of antibiotics, CA-MRSA carrying SCCmec IV or V may be at a selective advantage (Monecke et al, 2011). However, the contribution of Fluoroquinolones' selective pressure in the emergence of new CA-MRSA strains in the health care settings and their lower biological cost of resistance, makes them excellent candidates to replace HA-MRSA strains in health care settings (Kouyos et al., 2013; LeBlanc et al., 2006). The ecological theory was thus very useful to explain the tremendous increase in CA-MRSA rate within health care settings, and it can justify why there are misclassifications of certain strains of CA-MRSA. Understanding genotypic virulence factors for the two will aid in formulating critical intervention measures to reverse the current incidence trends by geographical location.

In the application of Ecological theory, if the genotyping variables that influence the evolution and transmission of CA-MRSA and HA-MRSA were defined as bases of MRSA gold standard characterization methods (independent variables), and bases of phenotyping methods validation, then accurate MRSA characterization was expected to contribute to the regulation of MRSA acquisition, transmission, and eradication. As shown in Figure 1, two independent variables (mecA gene, and PVL gene) influence two dependent variables (CA-MRSA, and HA-MRSA), mediated by the influence of three intervening variables (Health care risk factor, infection type, and susceptibility pattern). The PVL gene and SCCmec gene influence the three phenotyping methods. For example, the presence of PVL gene makes MRSA more invasive and therefore affects the infection site while the SCCmec gene has direct effects on the antibiotics panel. The infection type,

health care risk factors, and antibiotics panel that were used to phenotypically classify MRSA are not independent of the genetic profile of the organism. The genetic profile influenced the phenotypic characteristics and the behavior of MRSA.

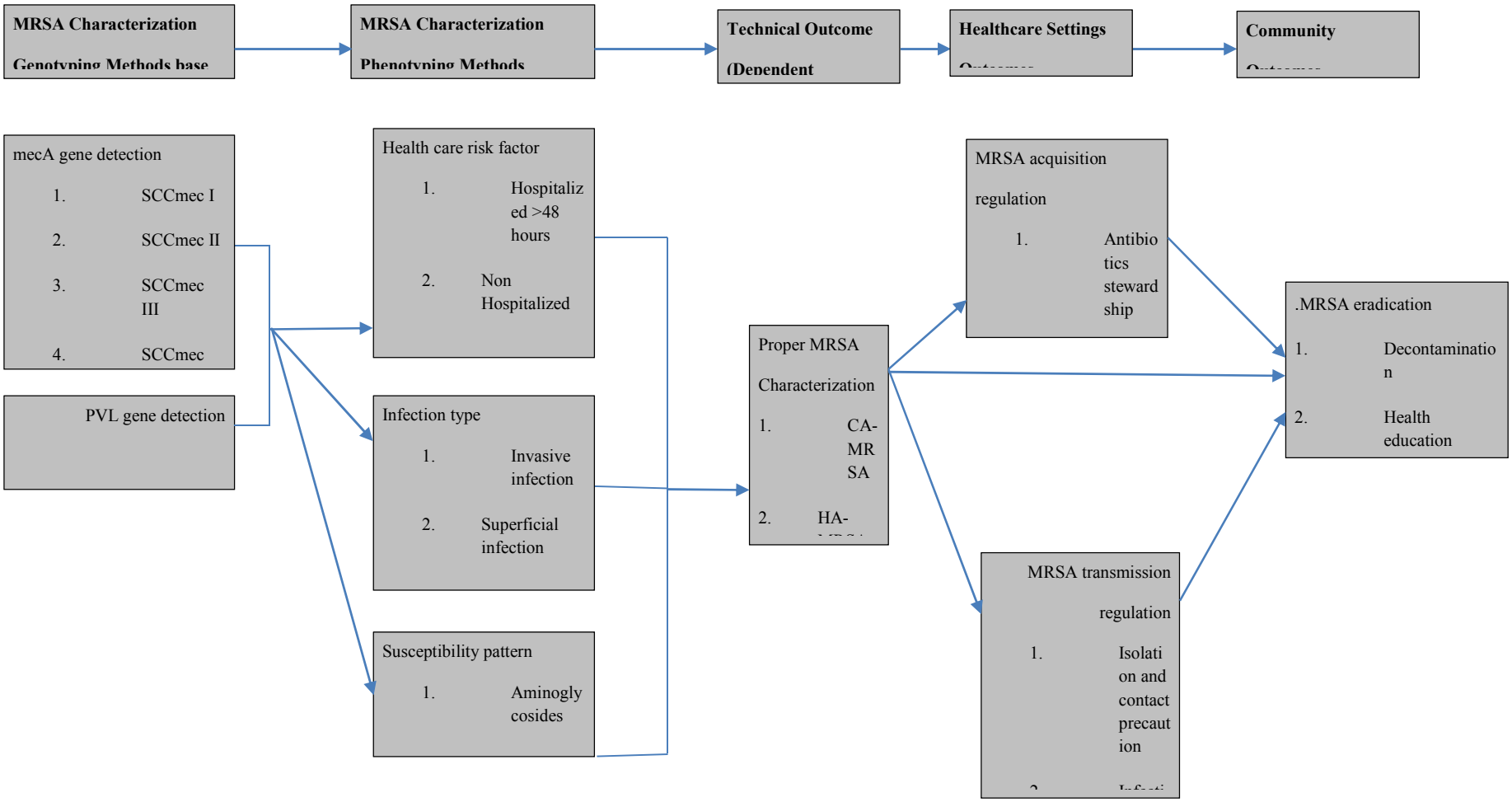

Figure 1. Conceptual framework for the independent, dependent variables, and the expected outcomes of the study.

Nature of the Study

This study was a quantitative, secondary data analysis aimed at describing the current performance of three phenotyping methods used to classify MRSA strains. First, I classifed MRSA cases as either HA-MRSA or CA-MRSA based on the three-phenotyping methods. Then, I measured the concordance of each pair combination of phenotypic methods. Finally, I described each method's accuracy based on their sensitivity and specificity against a reference genotyping method (Multiplex PCR) as the gold standard and then determined the effectiveness of a predictive model using Goodness-of-Fit statistics. Multiplex PCR is characterized by its high sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) (Kimura et al., 2009). In this study, molecular phenotypes of the MRSA strains causing infection within the Saudi Aramco community in the Eastern province of Saudi Arabia were analyzed and compared to the genotypic information generated for the collected samples. It was the second such study in Saudi Arabia, since a study conducted in 2012 in the central province to accurately classify MRSA strains as CA-MRSA or HA-MRSA based on their staphylococcal chromosomal cassette mec (SCCmec) and their PVL genes simultaneously (Monecke et al., 2012). Besides the molecular characteristics of the MRSA strains, I assessed the utility of the current phenotyping methods in infection control and treatment management program by measuring concordance. A poor concordance supported the necessity of a full assessment of each phenotyping method separately through the measurement of its sensitivity and specificity against the genotyping gold standard method (Multiplex PCR) to assess its accuracy, and therefore,

its validity. A phenotyping method with good sensitivity and specificity emerged as a valid method in infection control management that primarily lack epidemiological studies about MRSA diversity in all the regions of Saudi Arabia (El-Mahdy, El-Ahmady, & Goering, 2013; Monecke et al., 2012). In addition, it will provide a simple, less expensive, accurate, and feasible method for full MRSA assessment in Saudi Arabia.

For this study, I obtained the phenotypic characteristics of MRSA samples (admission profile, infection type, and susceptibility pattern) from 133 samples collected between January 2012 and December 2013, and stored within the John Hopkins Aramco Health Center epidemiology and microbiology database. During the period between January 2012 and December 2013, this center started collecting all MRSA samples in order to test the new multiplex PCR system. All isolated MRSA samples were analyzed for PVL gene and SCCmec gene to classify MRSA as CA-MRSA or HA-MRSA using new technology. However, no comparison between phenotyping methods and this new genotyping method had been conducted to date; this was the aim of this study. SAP health care system (SHC) within John Hopkins Aramco Health Center and the Molecular biology and Microbiology sections in John Hopkins Aramco Health Center were the source of demographic information (Age, gender, and survival), medical history of involved patients, and genotyping characteristics, respectively.

Definition of Key Terms

The key terms included are used predominantly by medical and public health sectors and are terms that may require definition in order to be useful to the reader. The key terms used in the study were:

Aminoglycosides: "Antibiotics containing two or more amino sugars in glycoside linkage with a hexose nucleus, which are bactericidal" (Spencer, 1986, p. 216)

Beta-lactam antibiotics: Class of antibiotics with a beta-lactam ring structure that inhibits bacterial growth by altering the synthesis of the cell wall. These antibiotics include penicillins, extended-spectrum penicillins, cephalosporins, imipenem, and aztrenonam (Meriam-Webster, 2013).

Biological cost of resistance: a reduction in bacterial fitness that can be expressed as lower growth, and altered virulence or transmission (Meriam-Webster, 2013).

Fluoroquinolone: Class of antibiotics of fluorinated derivatives (Meriam-Webster, 2013).

Infectivity: Ability to produce infection; tendency to spread rapidly from host to host (Meriam-Webster, 2013).

Polymerase chain reaction (*PCR*): a genotyping technique based on synthesizing large quantities of well-defined DNA segment (Meriam-Webster, 2013).

Pulsed-field gel electrophoresis (*PFGE*): molecular typing method based on the separation of chromosomal DNA molecules after applying an electric field that change their directions (Meriam-Webster, 2013).

Scope and Delimitations

The study scope was to investigate the validity of the three phenotyping methods that are currently used to classify MRSA as CA-MRSA or HA-MRSA within John Hopkins Aramco Health Center in the eastern province of Saudi Arabia. Based on the disproportional distribution of CA-MRSA and HA-MRSA between countries and

populations (Klevens et al., 2007; Rolo et al., 2012) and the emerging of new CA-MRSA strains (Akoglu et al., 2007), I assumed that the Saudi population had distinct MRSA phenotype and genotype that need to be explored in a specific manner.

This research study was delimited to patients at the John Hopkins Aramco hospital in the Eastern Province, Saudi Arabia. The population for this health center consists of approximately 350,000 employees, dependents or annuitants of the Saudi Aramco energy corporation. The study was further delimited to data and samples collected from January 2012 and December 2013.

Assumptions

Assumptions for this research were that the phenotypic and genotyping characteristics data generated for the MRSA samples for the same strains were comprehensive and representative of the distribution across the population under study. I also assumed that the data stored at the John Hopkins Aramco Health Centre of Saudi Arabia had phenotypic and genotypic characteristics distinct from those observed in other countries, consistent with the genetic diversity existing in MRSA strains isolated from different populations (Hudson et al., 2013).

Limitations

Patients' electronic medical records (EMR) were the only source of data. I did not conduct patients' interviews; therefore, information on preexisting health conditions and health care risk factors were not available for some patients due to the recent implementation of the electronic health care information system. However, I attempted to access paper medical records to help minimize missing data after receiving approval from

the infection control department. From the geographical and temporal perspectives, the molecular characteristics of MRSA that are evolving are moving targets. Evolutionary success is an important characteristic of CA-MRSA strains that makes their growth rates 1.33 faster than HA-MRSA strains (D'Agata, Webb, Horn, Moellering, & Ruan, 2009). Therefore, current strains may differ from strains that were analyzed two years before, making the ratio of CA-MRSA to HA-MRSA strains of the year 2012 and 2013 not fully representative for 2014 and 2015. Although, heterogeneity of strains does not have had a significant impact on the genotyping methods, which are based on DNA analysis, it does affect phenotyping methods. The phenotyping methods are based on the organism's physical traits that can be altered by the evolution of new CA-MRSA strains (D'Agata et al., 2009). A comparison between phenotyping methods and genotyping methods minimized the impact of these limitations. The migration of CA-MRSA genes such as PVL gene into HA-MRSA strains makes the physical traits of HA-MRSA similar to CA-MRSA. Thus, the SCCmec gene was included in the genotyping profile to increase accuracy in classification and minimize the impact of this evolution.

Significance of the Research

MRSA is a serious pathogen associated with various severe infections that affect a significant proportion of the world population (CDC, 2013a). However, the greatest cause for concern is the resistant nature of the bacterium (CDC, 2013a). MRSA drug resistance is a serious issue as it may threaten the safety of the world population by increasing morbidity and mortality rates associated with *Staphylococcus aureus* infections (CDC, 2013b). In addition, the resistant nature of the infections has significant cost implications,

related to the treatment and management of the patients, as well as prevention and control measures. (Boyce, Landry, Deetz & DuPont, 1981; Monecke et al., 2011; Stefani et al., 2012).

MRSA infections are more severe when compared with methicillin-susceptible *Staphylococcus aureus*. Severe MRSA infections may require specialized medications and treatments including isolation, ventilation, hyperbaric therapy, and surgical debridement (Klevens et al., 2007). The severity of resistant infections reduces the available antibiotic options, which results in additional risk to the patient due to treatment toxicity and lengthy hospitalization (Lodise & McKinnon, 2005). In some cases, medications and treatments are ineffective and result in morbidity or mortality (Lodise & McKinnon, 2005).

MRSA hospitalization cost is nearly double than that for non-MRSA infections (Elixhauser & Steiner, 2007). Thus, the screening of MRSA to identify strains is important in the prevention of disease transmission and the conservation of hospital resources. Understanding the dynamics of MRSA infection may assist physicians in selecting appropriate treatment, identifying interventions, and preventing transmission in the population. Better tracking of MRSA strains nationwide is necessary to observe and control their spread.

Presently, the management of MRSA infections worldwide has become a priority in health care settings. Susceptibly pattern, patients' age distribution for each strain type, and seasonal pattern of CA-MRSA and HA-MRSA infection are important factors in the management of MRSA infections. CA-MRSA, which is less resistant to non-beta-lactams

antibiotics, has a tendency to infect younger people in the summer months while HA-MRSA, which is also resistant to non-beta-lactams antibiotics has a tendency to infect older people in the winter months (Klein et al., 2007). Most initiatives in MRSA management target reduction of the spread of MRSA in order to relieve the burden of high medical costs and reverse the trend of MRSA morbidity and mortality.

At the molecular level, the role of genetic markers is important in mapping the Saudi Aramco population who may be at high-risk for MRSA infections. Genetic markers increase the accuracy of diagnoses needed to determine appropriate treatments (Healy et al., 2005). Such accuracy is essential for an effective treatment model, feasible and appropriate infection control strategies, and targeted prevention efforts for these emerging strains. My results contributed to the existing body of knowledge about health care settings and environmental factors that contribute to the predominance of CA-MRSA or HA-MRSA infection in Saudi Arabia. The results of this study offered insights about MRSA and the Saudi Aramco populations that are at higher risk for subsequent MRSA infection. Further, the results of this study will ultimately help in the formulation of prevention strategies to reduce MRSA transmission, such as disease surveillance, and raise awareness of the scope of the problem. The potential for positive social change, associated with my study results, lay in its aim to demonstrate the differences between CA-MRSA and HA-MRSA infections types, patients' age distribution for each strain type, the seasonality of MRSA infections, and any potential shifts in therapeutic drug resistance and identification of phenotyping variables that can be applied to distinguish between HA-MRSA and CA-MRSA and hence gauge distribution for each MRSA type,

seasonality, and potential shifts in therapeutic drug resistance. Patients will benefit from an increased understanding of the disease and identification of potential treatment modalities related to each strain. Non-infected individuals will benefit from a better understanding of the source of the strains and the resultant policies aimed at controlling the spread of MRSA. Thus my study contributed to positive social change among the Saudi Aramco population, as better management of existing MRSA will reduce incidence of the infection as well as the morbidity and mortality often associated with MRSA strains. A reliable and affordable MRSA screening program based on concordance of phenotyping classification with a genotyping gold standard provides a rational basis for MRSA surveillance and characterization. Extending the findings of this study to other health care facilities throughout the country would potentially contribute to the development of such a screening program.

Potential strategies towards positive social change deriving from this research are the development of a MRSA screening program, and the education of health workers. The results of the study will help increase understanding of MRSA outbreaks through comparative analysis of data from previous studies worldwide. Findings from comparative analyses will advance knowledge on the diseases' genetic, epidemiological, and clinical characteristics.

Summary

MRSA infections can range from mild to life-threatening in human beings (Monecke et al., 2011; Stefani et al., 2012). The risk factors of HA-MRSA are well known, but the increase in CA-MRSA and the movement of CA-MRSA to health care

settings has changed the epidemiology of the disease. More research is necessary to elucidate the burden and impact of MRSA, in particular invasive MRSA, on communities as well as health care settings. In order to describe and target interventions among populations at risk, several infection control measures are necessary including an antimicrobial stewardship program and an increased understanding the epidemiology of MRSA in specific population. Poor assessment of CA-MRSA and HA-MRSA prevalence within a facility can lead to the administration of unnecessary and/or improper antibiotics, causing a selective pressure on the factors that are responsible for the high rate of multidrug resistance strains (MDR) in Saudi Arabia. Hence, my study was conducted within the Saudi Aramco community in the Eastern province of Saudi Arabia. It was the first study conducted to obtain data to assess the molecular characteristics of the MRSA strains causing infection in this population.

Molecular methods such as genotyping are involved in classifying MRSA infections and identifying MRSA strains to arrive at preventive and control measures. In the absence of molecular methods, three phenotyping methods have been used to differentiate the strains. The purpose of this study was to identify a non-arbitrary, verifiable combination of phenotyping variables to allow reliable classification of MRSA in the Saudi Aramco region of Saudi Arabia into HA-MRSA or CA-MRSA.

The underlying reason for carrying out the study was that this enhanced understanding would help to establish a feasible and effective screening and treatment program. An accurate and less expensive phenotypic method would help the infection control department to monitor and investigate the prevalence of CA-MRSA and HA-

MRSA infections more precisely. The absence of a standardized phenotyping method for assessing MRSA is a real challenge for the infection control program in Saudi Arabia.

Chapter 1 illustrated the gaps in the research this study sought to fill, the purpose and nature of the endeavor relative to the entire body of research, the research questions and hypotheses, the theoretical basis that provided a framework for the study, and an organizing model for the research questions. It also discussed the significance and social change implications this study may have relative to the research topic. This approach allowed more effective establishment of epidemiology of MRSA in the region, in order to help with surveillance and prediction/control of epidemic outbreaks, and allow appropriate antibiotic treatment regimens to be applied in a timely manner.

In Chapter 2, I provide an overview of the current literature on MRSA in Saudi Arabia, Middle East, Europe, and the United States. The chapter briefly outlines the emergence of MRSA bacteria; considers the impact of the infection on populations from a cost, morbidity, and mortality standpoint; and identifies what current research is available regarding the epidemiology of MRSA. The chapter includes a general discussion on the traditional methods for defining CA-MRSA disease and the clinical and molecular epidemiology of CA-MRSA disease.

Chapter 3 details the methodology I used while completing my research. The study design, data sources, abstraction instruments, and details of my analyses are discussed.

In chapter 4, I present the results of my analyses. I utilized John Hopkins Health Care Center MRSA infection data, and MRSA multiplex PCR classification data to conduct the statistical analyses used to answer the research questions in detail.

In chapter 5, I summarize, interpret and discuss key research findings. In addition, I include recommendations for the application of the study findings and future research. Finally, I discuss the impact of my study results and its potential implications for positive social change.

Chapter 2: Literature Review

CA-MRSA and HA-MRSA strains have spread into health care settings in Saudi Arabia, bringing increased morbidity, mortality, and financial burdens (Bukharie, 2010). Unfortunately, current prevention controls in Saudi Arabia rely on MRSA phenotyping methods imported from other countries without testing validity or effectiveness of those methods (Bukharie, 2010). This approach to MRSA typing poses additional health care concerns when attempting to treat the MRSA strains effectively.

International consistency lacks in typing and naming MRSA strains, and uncertainty regarding what constitutes CA-MRSA is prevalent. In Saudi Arabia, there is a lack of studies on locally relevant strains. Currently, there is no MRSA national control program in Saudi Arabia despite increased prevalence of MRSA (Baddour et al., 2007; Bukharie, 2010). This dearth of information is highlighted by the limited number of reports identified by the National Library of Medicine of MRSA infections in Saudi Arabia when compared with other countries such as the United Kingdom or the United States (Baddour et al., 2007). A classification scheme for Saudi Arabia, appropriate for use at local, regional, and national levels, is needed to harmonize surveillance and treatment programs, in keeping with recommended best practice (Stefani et al., 2012). This need was the context for the research problem I addressed in this study.

In this review, I provide an overview of the literature review search strategy, as well as review of the theoretical framework, and the current literature on risk factors,

epidemiology, and genetics for both HA-MRSA and CA-MRSA. I critically analyzed published studies on different genotypic and phenotypic methods used for classification.

Literature Search Strategy

Though I reviewed literature dating back to 1950 to explain the emergence of MRSA, I placed emphasis on including a substantial number of peer-reviewed publications in acceptably high-impact journals from the last 5 years. I also consulted reports and websites of reputable bodies including the CDC.

I used the PROQUEST database and the PubMed search engine to identify the extant literature. Relevant electronic databases were accessed including Medline, Science Direct, and the Institute of Scientific Information (ISI) Web of Knowledge citation indexes. I used various keywords and phrases at different times in compiling sources for the literature review. I remained aware of issues such as the existence of synonyms for the search terms, as well as plurals, alternative spellings (e.g., U.S. versus U.K. English), and alternative and related terms. Having identified keywords, I used them singly or combined as search terms with Boolean operators *AND*, *NOT*, and *OR*. Truncation was often used in searches account for spelling variations, plural versus singular, and root words, commonly by using an asterisk or a question mark to replace a single letter or a string of letters. Keywords searched either singly or in various combinations included *Staphylococcus aureus*; *Methicillin Resistant Staphylococcus aureus (MRSA)*; *community associated* or *community-acquired* or *community onset*; *CA-MRSA*; *HA-MRSA*; *nosocomial, strain**; *clonal complex**; *phenotype*; *phenotyping classification*; *method*; *genotype*; *genotyping classification*; *concordance*; *antibiotic**; *methicillin*; *vancomycin*;

susceptibility; *resistance*; *antibiogram*; *health care*; *risk factor** and appropriate articles with a specific reference to *Saudi Arabia*. Moreover, I also sought and scrutinized distinctive antibacterials through PubMed. Various other articles, which were procured from the reference section of source articles, were acquired through PubMed.

Theoretical Foundation

The ecological theory and antibiotics selection pressure (ASP) theory supported this study. In ecological theory, the competitive exclusion principle or Gause's law of competitive exclusion states that "two species competing for the same resources cannot coexist if other ecological factors are constant. When one species has even the slightest advantage or edge over another, then the one with the advantage will dominate in the long term" (Hardin, 1960, p. 1292). Ecological theory thus dictates that both coexistence and competition can occur between CA-MRSA and HA-MRSA (Kouyos et al., 2013). HA-MRSA is classically characterized by a broad resistance antibiotic resistance spectrum conferred by its relatively large *Staphylococcal* cassette chromosome (SCCmec); SCCmec is a complex mobile genetic element found in *Staphylococcal aureus* (Ito et al., 2014; Shore & Coleman, 2013). In MRSA, it carries the PBP2a-encoding mecA gene responsible for the β-lactam antibiotic resistance, that is absent in resistant MSSA strains (Hiramatsu et al., 2001). HA-MRSA usually carries either SSCmec II or III, which have acquired genes for resistance to antibiotic classes beyond the β-lactams (Hiramatsu et al., 2002). CA-MRSA, in contrast, tends to carry SSCmec IV and V, which are relatively small. Thus CA-MRSA strains tend to be susceptible to

clindamycin and other non-β-lactam antibiotics (Naimi et al., 2003). Under ecological theory, therefore, these factors would favor HA-MRSA in a health care environment where there is common use of antibiotics. Conversely, the presence of SCCmec II or III correlates with slower growth than elements IV or V. Thus in the community, in the absence of antibiotics, CA-MRSA carrying SCCmec IV or V may be at a selective advantage. This was proposed to explain why CA-MRSA out-competes HA-MRSA in the community (Monecke et al, 2011). The classification of HA-MRSA and CA-MRSA has been challenged by studies suggesting that MRSA isolates characterized as containing SCCmec IV and PVL, and. thus genotypically CA-MRSA are emerging in the health care environment (David et al, 2008a). CA-MRSA strains are characterized by a lower biological cost of resistance, making them viable candidates to replace HA-MRSA strains in health care settings (Kouyos et al., 2013). The ecological theory is thus useful in explaining the increase in CA-MRSA rates within healthc are settings, and in particular the emerging multidrug resistant CA-MRSA. This theory can also be used to explain why certain strains of CA-MRSA are misclassified as HA-MRSA.

According to ASP theory, there is a link between increasing use of fluoroquinolones antibiotics and the emergence of new CA-MRSA strains (LeBlanc et al, 2006). This supports the importance of establishing a correlation between commonly prescribed antibiotics and the occurrence of CA-MRSA infection. The theory of ASP is also very useful to explain why some CA-MRSA strains are becoming resistant to different groups of antibiotics, which might interfere with the susceptibility pattern classification of MRSA.

For this study, I focused on the potential CA-MRSA and HA-MRSA strains that could spread into health care settings in Saudi Arabia, specifically in the Saudi Aramco region of Saudi Arabia. Therefore, the study aligned with both the Ecological and ASP theory frameworks, as both of these theories helped explain patterns of CA-MRSA or HA-MRSA incidence and prevalence in the health care setting versus the community. Studies to date in Saudi Arabia, suggest the prevalence of CA-MRSA is increasing and the lines between 'HA-MRSA' and 'CA-MRSA' are blurring in terms of health care risk factors (Al-Tawfiq, 2006; Baddour et al., 2007; Bukharie, 2010; David et al., 2008; Monecke et al; 2102; Moussa & Shibl, 2009; Stefani et al, 2012). The goal of this study was to identify accurate and reliable phenotyping methods to classify MRSA into HA-MRSA and CA-MRSA in concordance with a genotyping gold standard. Previous studies on phenotyping and genotyping concordance suggested that antibiotic susceptibility patterns (Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2005) may be particularly helpful in classification, given the increasingly questioned predictability of health care risk factors (Campanile et al., 2011; David et al, 2008a; Donnio et al, 2004; Hetem et al., 2012; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). This study therefore used the existing Ecological and ASP theories as a suitable framework, but I was mindful of the plasticity of the definitions of what constitutes true HA-MRSA or CA-MRSA.

Literature Review MRSA

Classification

Staphylococcus aureus are Gram-positive cocci that colonize 20% to 30% of the human population (van Belkum et al., 2009) as well as many animal species including livestock and domestic animals (Lindsay 2014; Peton & Le Loir, 2014). It grows either singly, in pairs, or in irregular clusters. *Staphylococcus aureus* is often carried asymptomatically as a commensal organism. However, as a human pathogen it is opportunistic and causes infections ranging from mild skin and soft tissue infections to life- threatening sepsis, pneumonia, and toxic shock syndrome (TSS). The pathophysiology depends on the presence of virulence factors including those present on the cell surface or secreted factors.

MRSA refers to a strain of *Staphylococcus aureus* that is resistant to the antibiotic methicillin. Methicillin and oxacillin resistance are due to the presence of a modified penicillin binding protein (PBP2', or PBP2a), which is encoded by the mecA gene (Lim & Strynadka, 2002). This gene is found on complex mobile genetic elements called staphylococcal cassette chromosomes (SCCmec). In practice, PBP2a confers resistance to all β-lactam antibiotics (Fey et al., 2003), apart from ceftobiprole, including cephalosporins, penicillin derivatives such as ampicillin, amoxicillin, ticarcillin and piperacillin, and carbapenems (National Committee for Clinical Laboratory Standards (NCCLS), 1997). SCC mec also variably contains genes encoding resistance to aminoglycosides or macrolides (Ito et al., 2001; Oliveira, Wu & de Lencastre, 2000).

This high spectrum of antimicrobial resistance complicates treatment options (Wertheim et al., 2005).

Historical Overview of MRSA Emergence

In the 1940s, the major antibiotic available to treat *Staphylococcus aureus* infections was the then newly discovered penicillin (Chambers, 2001). However by 1944 the first penicillin–resistant strain of *Staphylococcus aureus* had already been observed (Chambers, 2001). The prevalence of penicillin-resistant strains began to increase, prompting the development of new semi-synthetic penicillinase-resistant penicillins including methicillin and oxacillin (Lodise & McKinnon, 2005). By 1961, a new *Staphylococcus aureus* strain, which was resistant to methicillin, termed MRSA, had emerged and was observed in the UK (Jevons, 1961). MRSA subsequently spread throughout the world, although it was mainly observed as a hospital-acquired infection (Stewart & Holt, 1963). The first reports of MRSA acquired in the community outside of hospitals and health care facilities were in the 1980s (Levine, Cushing, Jui, & Brown, 1982; Saravolatz, Markowitz, Arking, Pohlod, & Fisher, 1982). However, until the late 1990s such reports remained infrequent. Since then, there has been a steady increase in reports of MRSA isolated from people in the community without recent treatment in a hospital or health care facility (Groom et al., 2001; Herold et al., 1998; Kaplan et al., 2005; Ladhani & Garbash, 2005; Marcinak & Frank, 2003; Naimi et al., 2003; Zaoutis et al., 2006). Whereas at first it was suspected that this marked the spread of HA-MRSA from hospitals into the community, it became clear that in terms of antibiotic susceptibility, epidemiology and genetics, these community associated (CA)-MRSA

strains differed in important respects from HA-MRSA and in fact represented new strains that had arisen in the community (David et al. 2008a).

Epidemiology and Risk Factors of HA-MRSA and CA-MRSA

HA-MRSA and CA-MRSA can be distinguished by their differing epidemiology and risk factors. The CDC defines CA-MRSA based on the hosts' lack of health care risk factors; in the presence of such risk factors the CDC advocates classification as HA-MRSA. MRSA is a common cause of hospitalized infections. Risk factors for HA-MRSA include surgery, hemodialysis, peritoneal dialysis, hospitalization, residency in a long-term care facility within the last year, or presence of indwelling percutaneous devices or catheters at the time of diagnosis or previous isolation of MRSA (CDC 2005). By the CDC definition, CA-MRSA is diagnosed when MRSA is observed in an outpatient or within 48 hours of hospitalization, yet lacks the risk factors outlined for HA-MRSA (Naimi et al., 2003). The rapid and accurate identification of oxacillin-resistance in susceptibility tests (methicillin is no longer in use) is needed to determine appropriate antimicrobial therapy (Louie et al., 2001; Pantosti & Venditti, 2009). Studies on phenotype, genetics, and antibiotic susceptibility of MRSA suggest that the CDC definition of CA-MRSA may be too conservative (David et al., 2008a, 2008b).

Currently, up to 85% of MRSA infections would be classified as HA-MRSA (CDC, 2005). HA-MRSA infection refers to patients isolated after 48-72 hours of admission to a hospital, those present at time of admission or health care workers of long-term care facilities (CDC, 2005). Patients tend to be elderly, although pre-term babies are also susceptible (Cooke & Brown, 2010; Millar et al., 2007). Immunocompromised

patients are susceptible to HA-MRSA (Giamarellos-Bourboulis et al., 2009), as are patients with chronic diseases such as diabetes with skin ulcers, dialysis patients, and people working in health care settings who have poor hygiene (Cooke & Brown, 2010; Millar et al., 2007). HA-MRSA patients may develop pneumonia or serious invasive infections at surgical sites and wounds, and within the urinary tract and bloodstream (Gould et al., 2011). HA-MRSA can also become epidemic MRSA (EMRSA) and spread between hospitals when colonized patients or staff members move from one hospital to another. CDC guidelines suggest that transmission of HA-MRSA could be reduced by practices such as adequate hand hygiene among medical staff, use of protective clothing and equipment, and use of antimicrobial soap and ointment on all intensive-care unit (ICU) patients (CDC, 2005).

The epidemiology of CA-MRSA differs from that of HA-MRSA, as the CDC exclusionary definition implies. Infections often occur among previously healthy, younger individuals either in an outpatient setting or within 48 hours of admission to a health care facility (Cooke & Brown, 2010; Naimi et al., 2003). However, recent studies suggest that these kinds of distinctions may be too limiting as strains of MRSA, which would be genotypically CA-MRSA, also arise within health care settings (David et al., 2008a). CA-MRSA was first observed in the 1980s when it was noted as the cause of a sharp increase in infection among North American intravenous drug users (Minnesota Department of Health, 2004). Later outbreaks were recognized in separate community populations (Table 1) such as children attending day-care centers, soldiers, male homosexuals, Native Americans, athletes in close contact sports, prisoners, and again

intravenous drug users (Cooke et al., 2010; Cooke & Brown, 2010; Davis et al., 2007; Diep et al., 2008a; Hidron et al., 2009; Huang et al., 2008; Thomas et al., 2007).

Table 1

Groups Experiencing Outbreaks of CA-MRSA with Risk Factors

Population or group	Possible risk factors	References- examples
Inmates/prisoners	Crowded living conditions, close contact	MMWR, 2001; Pan et al., 2003
Men who have sex with men	Close contact	Diep et al., 2008a
Athletes	Close contact, skin abrasions, sharing of equipment	Begier et al., 2004; Kazakova et al., 2005
Military recruits	Crowded living conditions, skin abrasions	Ellis et al., 2004
Native Americans	Close contact, crowded living conditions	Groom et al., 2001
Children (especially in day care centres)	Close contact, skin abrasions	Herold et al., 1998; Kaplan et al., 2005
Intravenous drug users	Poor skin hygiene, sharing needles	Cooke et al., 2010
Impoverished adults (inner city areas)	Close contact, crowded living conditions, poor hygiene	Charlebois et al., 2002

Risk factors for development of CA-MRSA include crowded living conditions and poor hygiene, breaks in the skin, and skin-to-skin contact. Infections associated with

CA-MRSA also tend to be less serious, for example skin and soft tissue infections (SSTI), sometimes initially mistaken for an arachnid bite, which can often be treated by excision and drainage rather than antibiotic treatment. However, these conditions can be chronic or recurrent. Moreover, CA-MRSA is also associated with an expression of toxins and if left untreated can lead to serious conditions such as necrotizing pneumonia, sepsis, and osteomyelitis (Lina et al., 1999).

Genetics of HA-MRSA and CA-MRSA

HA-MRSA and CA-MRSA differ at the molecular genetic level. One major difference is in the Staphylococcal cassette chromosome (SCCmec) expressed. SCCmec is a complex mobile genetic element found in *Staphylococcus aureus* (Ito et al., 2014; Shore & Coleman, 2013). In MRSA it carries the PBP2a-encoding mecA gene responsible for the β-lactam antibiotic resistance, which is absent in resistant methicillin-sensitive *Staphylococcus aureus* (MSSA) strains (Hiramatsu et al., 2001). There are currently eleven known SCCmec types for which there are complete nucleotide sequence data available, ranging in size from 20 to 60 kb (Garcia-Alvarez et al., 2011; International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009; Li et al., 2011; Shore et al., 2011; Shore & Coleman, 2013). Each has been assigned a unique Roman numeral, reflecting the order in which it was identified (IWG-SCC, 2009). HA-MRSA is generally associated with expression of SCCmec types I, II or III while CA-MRSA is generally associated with types IV and V. In practice, most MRSA strains are either SCCmec II or IV (IWG-SCC, 2009; Shore & Coleman, 2013).

Another molecular distinction between HA-MRSA and CA-MRSA is the presence or absence of the bi-component toxin PVL (Kaneko & Kamio, 2004) which is harbored by some CA-MRSA strains but not by HA-MRSA (Otto, 2013). Cases of CA-MRSA positive for PVL have been reported worldwide (Monecke et al., 2007). Epidemiologically, PVL expression in MRSA can be approximately divided into three groupings (Monecke et al., 2011). In Europe, the prevalence of PVL-expressing MRSA remains low and its expression in individuals may reflect international travel in and out of European countries. In Australia and Abu Dhabi, prevalence of PVL-expressing MRSA is high and may be the result of mass immigration into these countries by people from all over the world. Finally in the USA, one PVL- expressing MRSA strain, termed USA 300, predominates over all other strains, whether PVL expressing or not.

Although PVL was identified in *Staphylococcus aureus* back in 1932 (Panton & Valentine, 1932), its presence in MRSA is relatively new (CDC, 1999). PVL is a two-component leukocidin toxin that acts by forming pores in the mitochondria (Genestier et al., 2005). PVL subunit expression is considered to contribute to increased virulence in CA-MRSA although there have been conflicting results in animal models in this regard (Otto 2013; Lo & Wang, 2011). However, these PVL-expressing strains have been associated with necrotizing pneumonia in previously healthy young people (Gillet et al., 2007; Labandeira-Rey et al., 2007; Lina et al., 1999).

Genetic Elements of MRSA

SCCmec elements have various well-defined features (Ito et al., 2001, 2014; IWG-SCC, 2009; Shore & Coleman, 2013). They integrate into the *Staphylococcus*

aureus chromosome at the integration site attB within its integration site sequence (ISS), which lies within the 3' end of the orfX gene (Boundy et al., 2013; Ito et al., 2014); this is exploited in some genotyping strategies. SCCmec elements are flanked by repeat sequences and they include a cassette chromosome recombinase (ccr) and mec gene complex. The ccr genes encode the recombinases that facilitate site- and orientation-specific SCCmec excision or integration. It is partially this capacity for mobile genetic element transfer and re-integration that helps the spread of antibiotic resistance among strains.

Notably, in the context of MRSA, the SSCmec also includes the mec region that harbours, where present, the mecA gene and various mec regulatory genes including mecI and mecR1 (Hao et al., 2012; Lim and Strynadka, 2002; Shore & Coleman, 2013). There are currently five known mec classes and eight known ccr classes (Ito et al., 2014). Any regions other than ccr or mec in SCCmec are designated as "joining regions" (J-regions), for which there are three known subgroups, J1-3 (Ito et al., 2014). In terms of the multiple antibiotic resistance profile observed particularly for HA-MRSA, SCCmec also frequently contains integrated insertions, for example plasmids or transposons from other resistant organisms, carrying genes for resistance to other antimicrobial agents such as aminoglycosides or macrolides (Ito et al., 2001; Oliveira, Wu & de Lencastre, 2000).

Although mecA is the gene commonly associated with conferring methicillin resistance, the identification of a novel mecA homologue, now termed mecC, has complicated this association recently (Paterson, Harrison & Holmes, 2013). MecC was originally identified in an epidemiological study of bovine mastitis resulting in the

isolation of a *Staphylococcus aureus* isolate called LGA251, from a bulk tank milk sample in southwest England (Garcia-Alvarez, Webb, & Holmes, 2011). This was phenotypically MRSA but genotyping revealed the absence of mecA.

Subsequent genome sequencing of LGA251 showed that the strain carried a novel mecA homologue initially termed mecALGA251 which was 69% identical to conventional mecA at the DNA level. The strain encoded a modified PBP2a/2′ that was ~63% identical to mecA-encoded PBP2a/2' at the amino acid level (Garcia-Alvarez et al., 2011). This homologue has since been renamed mecC and has been identified in human strains in reports from the UK, Denmark, Belgium and Ireland (Deplano, Vandendriessche, Nonhoff, & Denis, 2014; Garcia-Alvarez et al., 2011; Shore et al., 2011; Tsubakishita, Kuwahara-Arai, Baba, & Hiramatsu, 2010).

SCCmec elements in HA-MRSA versus CA-MRSA. Of the eleven known SSCmec elements, HA-MRSA is generally associated with expression of SCCmec types II or III while CA-MRSA is generally associated with types IV and V. In practice, most MRSA strains are either SCCmec II or IV (IWG-SCC, 2009; Shore, & Coleman, 2013). SSCmec II and III are relatively large and, as mentioned above, contain genes for resistance to antibiotic classes beyond the β-lactams (Hiramatsu et al., 2002). SSCmec IV and V, in contrast, are relatively small and the CA-MRSA strains that carry them tend to be susceptible to clindamycin and other non-β-lactam antibiotics (Naimi et al., 2003). The antibiotic-resistance profiles of CA-MRSA and HA-MRSA are therefore dictated mainly by the SCCmec element.

The presence of SCCmec II or III correlates with slower growth than elements IV or V. It is postulated that in the health care environment, SCCmec II or III confers an advantage due to the multi-antibiotic resistance profile. However in the community, in the absence of antibiotics, SCCmec II or III-containing HA-MRSA may be at a selective disadvantage due to its slower growth. This is proposed to explain why CA-MRSA out-competes HA-MRSA in the community (Monecke et al, 2011). The classification of 'HA-MRSA' and 'CA-MRSA' meanwhile has been challenged by studies showing that MRSA isolates characterized as containing SCCmec IV and PVL, thus genotypically 'CA-MRSA', are arising in the health care environment (David et al, 2008a).

Virulence-associated genetic factors. The key to the success of MRSA lies not only in its antibiotic resistance profile but also in its virulence factors. Important virulence factors expressed by MRSA strain include Panton-Valentine toxin (PVL) (discussed below), phenol-soluble modulins (PSM), and the arginine catabolic mobile element (ACME; Hao et al., 2012; Otto, 2010). PSMs are proinflammatory cytolytic toxins. Seven PSMs are core genome encoded, but there is a novel PSM gene so far detected on SCCmec types II, III and VIII, associated with HA-MRSA (Chatterjee et al, 2011; Monecke et al., 2012; Queck et al., 2009). This is the only SCCmec-encoded toxin gene identified currently.

PSMs are produced by several staphylococcal species. However CA-MRSA produces larger concentrations of PSMs compared to other *S. aureus* types (Wang et al., 2007). CA-MRSA can apparently manipulate neutrophil signaling in the host to mediate PSM pathogenesis (Clarke, 2010). The arginine catabolic mobile element (ACME) is a

pseudo-SCC more commonly observed in *Staphylococcus epidermis* and *Staphylococcus haemolyticus* than in *Staphylococcus aureus* (Shore & Coleman, 2013). However, it has been identified in the genome of SCCmec IV type MRSA strains, in particular in the USA300 strain of CA-MRSA that predominates in the USA (Hao et al,. 2012; Katayama, Ito & Hiramatsu, 2000). ACME consists of arc and opp3 gene clusters that together comprise an arginine deaminase pathway (Diep et al., 2008b). There have been some suggestions that it may contribute to virulence of USA300 but reports from animal models are conflicting (de Lencastre, Oliveira, & Tomasz, 2007; Montgomery, Boyle-Vavra, & Daum, 2009). Carriage of other toxins including staphylococcal enterotoxins such as sec, sel, and sea, endotoxins and toxic shock syndrome toxin (TSST-1) varies considerably between MRSA strains (Monecke et al., 2011).

Panton-Valentine toxin. Panton-Valentine toxin (PVL) is expressed in CA-MRSA strains due to acquisition of the prophage-encoded adjacent lukS and lukF genes. These genes encode the dual leukocidin PVL toxin parts, LukS and LukF (Chambers, 2005; Otto, 2013). PVL belongs to a β-barrel family of pore-forming cytolytic toxins also containing several other leukocidins, gamma-toxin, and alpha-toxin (Szmigielski et al., 1999). In 1932, Panton and Valentine noted that there was an association between PVL production and abscess formation (Panton and Valentine, 1932). Recently, interest in PVL has increased due to the epidemiological association between the lukSF genes and CA-MRSA (Vandenesch et al., 2003). Most CA-MRSA strains contain lukSF genes. They are present in MSSA at a much lower frequency and are absent from HA-MRSA. PVL is now regarded as a relatively stable marker of CA-MRSA.

Agr two-component system. Virulence and resistance are regulated by two-component systems in MRSA. Among the most important of these in controlling both virulence and resistance is the agr system. Four agr groups (I-IV) have been identified in MRSA, whose expressions vary with geography, antibiotic resistance profile, and virulence factors. Agr plays an important role in quorum-sensing and contributes to MRSA pathogenesis, including toxin expression, biofilm formation and heterogeneous resistance (Hao, Dai, Wang, & Yuan, 2012). Generally speaking, agr type I correlates with HA-MRSA and type II correlates with CA-MRSA while types III and IV correlate to other conditions such as TSST-1 and staphylococcal scaled skin syndrome (Hao, Dai, Wang, & Yuan, 2012; Nichol et al., 2011). Both agr types I and II are associated with multidrug resistance (Hao, Dai, Wang, & Yuan, 2012; Nichol et al., 2011). Type I predominates in Europe and South America while type II has been mainly found in Japan and North America (Hao, Dai, Wang, & Yuan, 2012).

Antibiotic Resistance Profiles of MRSA

Antibiotic resistance of MRSA is mainly dictated by elements of the SCCmec. All SCCmec elements carry genes that cause resistant to β-lactam antibiotics, in particular mecA (Lim and Strynadka, 2002; Shore & Coleman, 2013). MecC is a more recently identified homologue of mecA that also encodes β-lactam resistance (Garcia-Alvarez et al., 2011). All SCCmec elements carry genes for resistance to β-lactam antibiotics, in particular mecA, as well as mecRI and mecI genes for the regulation of expression of mecA (Hao et al., 2012). In addition, SCCmec Types II and III carry non-β-lactam antibiotic resistance genes on integrated plasmids and a transposon. These confer

resistance to antimicrobials including aminoglycosides, macrolides, tetracycline, mercury, and cadmium (Hiramatsu et al., 2002). This seriously complicates therapeutic options in HA-MRSA and limits physicians to drugs such as vancomycin, linezolid, clindamycin, sulfamethoxazole-trimethoprim, and rifampin (Monecke et al, 2011; So & Farrington, 2008). All of these drugs have potential issues including ease of administration, penetration, and toxicity (Monecke et al, 2011; So & Farrington, 2008).

Beyond this, new problems are arising with the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA). There are both vancomycin-intermediate *Staphylococcus aureus* (VISA) infections, in which HA-MRSA develops intermediate resistance to vancomycin, and full VRSA (Chang et al., 2003; MMWR, 1997 & 2000; Smith et al., 1999; Weigel et al., 2003). The resistance mechanisms are different and they do not progress from one to the other along a resistance continuum. VISA is suggested to result when an MRSA infection is intensively treated with vancomycin and as a result undergoes mutation in cell wall biosynthesis genes. This leads to a thicker cell wall that can partially exclude vancomycin, resulting in intermediate vancomycin resistance (Cui et al., 2003). VRSA, in contrast, relies on co-infection with both MRSA and vancomycin-resistant *Enterococcus* (VRE), with subsequent transfer of the vanA vancomycin resistance gene on a plasmid or transposon from VRE to MRSA (Weigel et al., 2003). In any case, the rise of these vancomycin-resistant MRSA strains is a worrying development and has contributed to the continuing research efforts to identify new antimicrobials and new delivery strategies to try to combat multi-drug resistant bacteria (Burke & Rose, 2014; Ndieyira et al., 2014).

Examples of new antimicrobials in development include the lipoglycopeptides oritavancin, telavancin, and dalbavancin. The pharmacokinetic and pharmacodynamic properties of these drugs mean that they have shown promise in targeting vancomycin-resistant MRSA (Burke & Rose, 2014). A new strategy for targeting antibiotics to try to overcome resistance and optimize dosing includes use of nanoparticles (Ndieyira et al., 2014). The multiplicity of MRSA strains and continual evolution of these bacteria make development of new antimicrobials and therapeutic strategies imperative and also dictates that there should be some consensus in typing and nomenclature of MRSA clones and strains.

Overview of MRSA Strains

An overwhelming variety of both CA-MRSA and HA-MRSA strains have been identified to date in humans, as well as many more that have been identified in livestock (Monecke et al., 2011). Global epidemiology varies for these strains and there is a need to harmonize methods and nomenclature used to describe strains in order to facilitate surveillance, identification of risk factors and investigation of suspected outbreaks, and identification of possible new strains (Stefani et al., 2011). At present, there is a multiplicity of MRSA typing methods which will be discussed in more detail in the succeeding sections. As summarized by Stefani et al., 2011, these include spa typing, multilocus sequence typing (MLST), SCCmec sequencing, macrorestriction pattern analysis, and multilocus VNTR analysis (MLVA). The genes targeted in these typing methods and their main advantages and disadvantages are summarized in Table 2.

Recently, researchers, involved in a major genotyping effort by DNA microarray analysis in laboratories in nine different countries, proposed assigning of MRSA isolates to 34 distinct lineages based on non-mobile genetic elements (Monecke et al., 2011). Monecke et al. (2011) demonstrated that the epidemiological distribution of MRSA strains could be approximately divided up into three groups based on PVL expression (Monecke et al., 2011). PVL-expressing MRSA prevalence is low in Europe but high in Australia and Abu Dhabi, while in the USA, one PVL expressing MRSA strain, termed USA300, predominates over all other strains.

MRSA strains can be assigned to different clonal complexes based on a 'fingerprint' of core or core variable genes (Monecke et al., 2011). The most commonly observed globally of these clonal complexes (CC) are CC5, CC8, CC22, CC30, and CC45 (Monecke et al., 2011; Stefani et al., 2011). Within these clonal complexes lie strains of both HA-MRSA and CA-MRSA. These strains have different names in different countries, making it difficult to apply uniform approaches to surveillance and treatment worldwide. One suggested nomenclature was to name strains by: sequence type (ST)-MRSA-SCCmec type (Enright et al., 2002). This naming convention would be a relatively simple and transferrable method; however assignment to strains in such a way is also complicated by, for example, very different strains sharing the same ST and SCCmec. Thus additional information, for example on PVL status, should also be considered (Monecke et al., 2011).

Generally, prevalence of HA-MRSA is declining in some European countries or remaining stable in others, but is present at very high rates in parts of East Asia (Stefani

et al., 2011). Europe has remained relatively free of CA-MRSA, although there is now evidence of steady increases in, for example, incidence of USA300, probably as a result of international travel (Tietz, Frei, & Widmer, 2005). Meanwhile in East Asia, incidence of CA-MRSA varies considerably from one country to the next (Song et al., 2011). In terms of CA-MRSA, the number of cases has rapidly expanded in recent years in the USA (Maree et al., 2007; Pan et al, 2003 & 2005; Labandeira-Rey et al., 2007). The majority of cases can be assigned to strain USA300, which expresses the SCCmec type IV and is PVL positive, considered classic hallmarks of CA-MRSA (Fridkin et al., 2005; Pallin et al., 2008; Stefani et al., 2011). The other most commonly observed strain in the USA was from CC5. PVL in these CA-MRSA strains were associated with increases in necrotizing pneumonia (Gillet et al., 2007; Labandeira-Rey et al., 2007).

There is doubt now as to what can be classified 'HA-MRSA' and what can be classified 'CA-MRSA'. s previously mentioned, the CDC advocates an exclusionary definition of CA-MRSA based on the absence of health care-associated risk factors including surgery, hemodialysis, peritoneal dialysis, hospitalization, residency in a long-term care facility within the last year, or presence of indwelling percutaneous devices or catheters at the time of diagnosis or previous isolation of MRSA (CDC, 2005). However, it is increasingly recognized that boundaries are blurring in terms of discriminating between HA-MRSA and CA-MRSA by way of health care-associated risk factors. This is because patients diagnosed with a genotypically CA-MRSA infection, for example expressing SCCmec IV and PVL, have shown to have exposure to a health care environment, indicating spread of CA-MRSA into hospitals both in the USA (David et al, 2008a;

Maree et al., 2007; Nair et al., 2011; Otter & French, 2011) and in Europe (Campanile et al., 2011; Donnio et al., 2004; Hetem et al., 2012). In East Asia, there has been spread in both directions, from the community to the hospital and from the hospital to the community (Song et al., 2011).

It is clear that lack of international consistency in the use of typing methods and classification of MRSA strains brings considerable challenges. These are relevant to the issue addressed in this study, the establishment of a verified MRSA phenotyping method supported by a genotyping 'gold standard' classification method for MRSA strains isolated in the Saudi Aramco community. In terms of Saudi Arabia specifically, there have not been many studies on characterization of locally relevant strains of MRSA. One study, however, was carried out in the King Fahad Medical City in Riyadh on 102 patient isolates (Monecke et al., 2012). These samples were subjected to genotyping by DNA microarray analysis, resulting in identification of five different strains belonging to four clonal complexes of great diversity. Consistent with the findings in other countries, there was evidence of strains in the hospital-acquired samples which would be considered CA-MRSA by the presence of PVL. In Table 2, I present the advantages and disadvantages of MRSA typing methods.

Table 2

MRSA Typing Methods and their Advantages and Disadvantages

Method	Genes targeted	Advantages	Disadvantages
Spa typing	Polymorphisms in the X region of the surface protein P encoding spa gene	Rapid, high throughput, portable, dynamic, standardized nomenclature	Results in misclassification in some lineages
MLST	Core and core variable genes	Defines the core genetic population; portable; standardized nomenclature	Low throughput; expensive
SCCmec sequencing	Mobile genetic elements	Standardized nomenclature	Low throughput; expensive; protocols are not standardized
PFGE	Whole chromosome-restriction polymorphisms	High discriminatory index	Requires good technical skills; portability limited; multiple nomenclatures exist- not standardized; results in misclassification in some lineages
MLVA	Chromosomal VNTR polymorphisms	Rapid, high throughput	No internationally standardized protocols or nomenclature; results in misclassification in some lineages

Note. Adapted from Stefani et al., 2011.

The following sections consider these phenotyping methods in more detail along with currently available genotyping methods.

Phenotyping of MRSA

The most common phenotyping methods for assignment as HA-MRSA or CA-MRSA belong to three categories, health care risk factors, infection type (CDC, 2005), and susceptibility pattern (Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute), 2005). The health care risk factors method advocated by the CDC (CDC, 2005) is a conservative method which dictates that CA-MRSA diagnosis is based on the host's lack of health care risk factors. If such risk factors are present, it advocates classification as HA-MRSA. Risk factors for HA-MRSA include surgery, hemodialysis, peritoneal dialysis, hospitalization, and residency in a long-term care facility within the last year, or presence of indwelling percutaneous devices or catheters at the time of diagnosis or previous isolation of MRSA (CDC, 2005).

CA-MRSA is diagnosed when MRSA is observed in an outpatient or patient within 48 hours of hospitalization that lacks the risk factors outlined for HA-MRSA (Naimi et al., 2003). In terms of the infection method for phenotyping, there is overlap with the health care risk factors method. Any MRSA isolated from deep wounds or sterile body sites such as the blood, CSF, and pleural fluids from patients who have health care risk factors comprising hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, is labelled as HA-MRSA. MRSA isolated from patients lacking those risk factors is labelled as CA-MRSA (CDC, 2005).

Finally, the susceptibility pattern method refers to susceptibility to antibiotics. As mentioned previously, HA-MRSA is associated with the larger SCCmec Types II and III, which feature plasmids and/or transposons encoding antibiotic resistance genes beyond the β -lactam antibiotic class. Thus, according to the susceptibility pattern phenotyping method, MRSA cases that are resistant only to β-lactams antibiotics are assigned to CA-MRSA while those that are resistant to additional antibiotics classes such as carbapenems, aminoglycosides, and fluoroquinolones are designated HA-MRSA (Clinical and Laboratory Standards Institute, 2005).

Accurate phenotyping and the ability to rapidly and efficiently assign MRSA cases to HA-MRSA or CA-MRSA is important in monitoring trends in MRSA within health care settings and in the community in different countries. Accurate phenotyping directly affects choices of appropriate antibiotic treatment, monitoring of outbreaks, and prediction or recognition of epidemics. The phenotyping methods described are widely used within health care settings. Although each has some merit, the suitability of any single method for all situations is now in doubt.

One complicating factor is the emergence of invasive and multidrug resistant CA-MRSA strains in health care settings as a cause of health care associated infection (Gillet et al, 2010; Seybold et al, 2006), both in the USA (David et al, 2008a; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011) and in Europe (Campanile et al., 2011; Donnio et al., 2004; Hetem et al., 2012). There is also evidence of increased circulation of HA-MRSA in the community (Miller et al, 2007; Seybold et al, 2006). In East Asia, meanwhile, there is evidence of the spread of ‘HA-MRSA’ and ‘CA-MRSA’ from the

community to the hospital and from the hospital to the community (Song et al., 2011). All these factors would be expected to interfere with the sensitivity of the types of phenotyping methods described and their concordance with each other. This interference suggests a need to identify elements of the available phenotyping methods that would offer increased sensitivity in local situations and show better concordance with genotyping methods.

In my study, I aimed to examine the suitability of phenotyping methods in the context of the Saudi Aramco community in the Eastern province of Saudi Arabia, test their concordance, and identify phenotyping elements that would contribute to increased sensitivity in diagnosis. I established concordance with information derived from a 'gold standard' genotyping multiplex PCR method.

Genotyping of MRSA

There have been a variety of methods described for genotyping of MRSA, all of which have their advantages and disadvantages (Biendo et al., 2013; Monecke et al., 2011; Stefani et al., 2012; Tenover et al., 1994; Zimmerman et al., 2012). Some of the genotyping methods currently in common use were summarized in Table 2. The success of these methods depends on having an understanding of the basic genome structure of *Staphylococcus aureus*, the variation between *Staphylococcus aureus* strains, and how strains are evolving (Stefani et al., 2012). The basic genetic core of *Staphylococcus aureus* consists of a highly conserved set of core genes interspersed with a set of approximately 700 core variable (CV) genes, which are used to group *Staphylococcus aureus* into lineages. Lineages are currently defined on the basis of multilocus

sequencing type (MLST) clonal complexes (CC) from sequencing of a set of seven housekeeping genes. This is the basis of the 'sequence type' (ST) designation in *Staphylococcus aureus* terminology generally and MRSA terminology specifically. Sequence type refers to single nucleotide polymorphisms in MLST sequences of these seven housekeeping genes. Isolates that are identical will have the same ST number and closely related STs will be grouped together in the same CC (Stefani et al., 2012).

However, as already mentioned, *Staphylococcus aureus* features mobile genetic elements that are essential in the evolutionary success of *Staphylococcus aureus* strains, as they carry genes conferring both increased virulence and resistance to antibiotics. The key to how HA-MRSA and CA-MRSA fit into both the Ecological and ASP framework of the current study is the mobile genetic elements of *Staphylococcus aureus* that have driven the emergence of MRSA. Some genotyping methods for MRSA consider the mobile genetic elements, particularly SCCmec, mecA, mecC, and PVL, as well as additional antibiotic resistance genes conferred by plasmids and transposons. Major methods currently used in MRSA genotyping are described in the following sections.

Pulse Field Gel Electrophoresis. Macrorestriction pattern analysis by PFGE is a widely used genotyping method which scans the whole chromosome for restriction polymorphisms. The method entails lysing the bacterial cell wall, embedding the bacterial cells in agarose and partially digesting the bacterial DNA with a range of restriction endonucleases before separating bands by electrophoresis. This method has been validated epidemiologically and significant correlation has been observed between phenotyping by antibiogram and genotyping by PFGE (Blanc et al., 2001; Chomvarin et

al., 2005). PFGE is considered the gold standard MRSA genotyping method due to its high discriminatory power (Blanc et al., 2001; Chomvarin et al., 2005; Deurenberg et al., 2007).). However, it is a technically complicated and time-consuming, its portability is very limited and there is no major consensus on nomenclature derived from the method (Mehndiratta & Bhalla, 2012; Narukawa et al., 2009; Stefani et al., 2012). As it is an image-based method, standardization and sharing of information between laboratories is challenging.

Spa sequence typing. The spa gene of *Staphylococcus aureus* encodes the cell wall component *Staphylococcus aureus* protein A (Deurenberg et al., 2007). This gene consists of a number of 24 base repeats and exhibits polymorphisms which are exploited in the spa sequencing genotyping method (Deurenberg et al., 2007). This method has major practical advantages in terms of its high throughput, rapid turnaround, and lack of extreme technical difficulty, as it only involves sequencing one locus and it benefits from a standard nomenclature (Stefani et al., 2012). In some cases, it has been shown to have good concordance with other genotyping methods including PFGE and MLST (Melin et al., 2009; Narukawa et al., 2009; Strommenger et al., 2006) and its sensitivity is considered to lie somewhere between these two methods (Deurenberg et al., 2007). Development of the clustering algorithm Based Upon Repeat Patterns (BURP) has facilitated cluster analysis based on spa sequencing data and been used to show concordance of spa sequence typing with other methods (Deurenberg et al., 2007; Stefani et al., 2012; Strommenger et al., 2006;). The spa server database, curated by the SeqNet.org initiative contains information from thousands of spa sequences (Deurenberg

et al., 2007; Stefani et al., 2012). Overall, spa typing is considered to be a good clinical typing option. However, in some cases spa sequencing type leads to misclassification of lineages and it lacks discrimination in local situations where one or a small number of clones are endemic (Stefani et al., 2012).

Multi Locus Sequence Typing (MLST). MLST is a highly discriminatory method used for analysis of clonal evolution of MRSA (Deurenberg et al., 2007; Stefani et al., 2012) As previously mentioned it is used in sequence type (ST) typing in *Staphylococcus aureus* and as such is associated with a standardized nomenclature (Stefani et al., 2012). In MLST typing, seven housekeeping genes are sequenced and the allele combination identified is used to assign an ST number to the isolate under examination (Faria et al., 2008). Closely related STs will be grouped in the same clonal complex (CC) (Faria et al., 2008; Stefani et al., 2012).

SCCmec typing. Methicillin resistance in *Staphylococcus aureus* is achieved via the SCCmec that contains the mec A gene complex and the ccr gene complex. There are currently eleven known SCCmec types ranging in size from 20 to 60 kb (Garcia-Alvarez et al., 2011; IWG-SCC, 2009; Li et al., 2011; Shore & Coleman, 2013; Shore et al., 2011). Each has been assigned a unique Roman numeral reflecting the order in which it was identified (IWG-SCC, 2009). HA-MRSA is generally associated with expression of SCCmec Types I, II or III while CA-MRSA is generally associated with Types IV and V. Each SCCmec type encodes resistance to different antibiotics (Zhang, McClure, Elsayed, & Conly, 2009).

During the last two decades, several genotyping studies that target different genes were conducted in the USA and Europe to accurately classify MRSA as CA-MRSA or HA-MRSA, and to understand the molecular characteristics of the most predominant strains. However, the same type of genotyping studies were not introduced in the Middle East, and in particular in Saudi Arabia, until 2012 when the first genotyping study that targeted different MRSA genes was conducted in the central province area of Saudi Arabia to characterize and classify 107 MRSA strains that were isolated from 107 patients (Monecke et al., 2012).

HARMONY, the International Union of Microbiology Societies' European Staphylococcal typing network, has suggested that a combination of MLST and SCCmec typing should be used as a reference typing system for multicenter MRSA surveillance MRSA (Cookson et al, 2007) and that it should be possible to integrate findings from PFGE and spa typing- economically more affordable methods- with MLST and SCCmec sequencing findings. MLST and SCCmec both have the disadvantage of being low throughput and expensive methods (Stefani et al., 2012). A multi-typing approach is also advocated by the European Centre for Disease Prevention and Control (ECDC) while SeqNet.org urges standardization of laboratory methods to facilitate quality control and comparability (Stefani et al., 2012).

Findings of an expert panel meeting held by the International Society of Chemotherapy in 2011 suggested that within countries, there should be three levels of typing laboratories at local, regional, and national levels, all with different functions and using typing techniques appropriate to their function and available resources (Stefani et

al., 2012). At the local level, where the main function should be new strain detection and identification of species, time, location, and unusual characteristics, PCR-based methods are suggested as they are rapid, relatively inexpensive, and readily communicated to labs at regional and national level. PCR- based methods include multiplex PCR (M-PCR), real-time PCR, hyper variable region (HVR) and the spa typing (Stranden, Frei, Adler, Fluckiger, & Widmer, 2009). The method of choice in my study was based on multiplex PCR.

Multiplex PCR. The technical basis of multiplex PCR is the use of multiple oligonucleotide primers in the same PCR, which allows simultaneous amplification of several target genes. In a study of MRSA, genes targeted included 16S rRNA, mecA and PVL genes, giving rapid and reliable results for example in detection of USA300 (Bonnstetter et al., 2007).

Another study on mecA and coagulase genes showed excellent concordance between disk diffusion tests to measure oxacillin susceptibility and mecA detection by PCR, while results for coag detection by PCR were concordant with phenotypic tests for all isolates (Rallapalli, Verghese, & Verma, 2008). Studies suggest that speedy detection of MRSA using in-house or commercial PCR analysis is substantially compatible for patient management (Malhotra-Kumar et al., 2008). The commercially available Gene Xpert MRSA kit (Cepheid) uses a multiplex, real-time PCR method with primers. This is designed to detect each SCCmec type as well as the chromosomal orfX-SCCmec junction, which simultaneously gives information on both the SCCmec identity and on

whether the SCCmec is correctly integrated with respect to orfX, thus confirming methicillin resistance (Biendo et al, 2013).

A recent update to the Gene Xpert system simultaneously detects spa, thus confirming the presence of *Staphylococcus aureus*, presence of mecA and the junction between SCCmec and orfX, and overcoming previous drawbacks in the system that falsely led to MSSA strains with an 'empty' cassette, lacking mecA, as MRSA (Biendo et al., 2013). In blood cultures this method yielded genotypic results with excellent concordance with phenotypic methods.

Thus, multiplex PCR methods seem to offer the advantages of rapidly obtainable results that are concordant with phenotypic data. Among various other PCR based typing techniques, random amplified polymorphic DNA (AP-PCR/RAPD), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and multilocus variable-number tandem repeat analysis (MLVA) are also suggested to be useful for typing of MRSA strains.

AP-PCR/RAP. This rapid and straightforward technique is potentially applicable to almost all MRSA strains (van Belkum et al., 1995). It involves use of small arbitrary primers of unknown homology to the target sequence to randomly amplify the target DNA segments. The number and size of the fragments produced during PCR are the foundation for MRSA isolate typing of a MRSA isolate. In a study to evaluate AP-PCR for *Staphylococcus aureus* typing, it was suggested that the technique could be useful in studying outbreak strains but not for use as a reference method due to poor inter-laboratory reproducibility (van Belkum et al., 1995).

PCR-RFLP. This technique relies on amplifying a defined fragment of DNA followed by consequent restriction enzyme digestion of the amplified product and analysis of restriction fragment length polymorphisms (Mehndiratta et al., 2009; Stefani et al., 2012). This method has been used on the coagulase (coa) and spa genes to distinguish between MRSA strains (Mehndiratta et al., 2009). PCR-RFLP of spa gene in particular has been reported to be valuable in differentiating between strains which were otherwise difficult to type (Mehndiratta et al., 2009).

Multilocus VNTR analysis (MLVA). MLVA is a PCR-based method that relies on the polymorphism of tandem repeated DNA sequences (Schouls et al., 2009). In terms of MRSA it is seeing increasing use as it is a high-throughput, rapid method (Stefani et al., 2012). However, there is some concern about reproducibility and nomenclature and also there is a lack of any standard methodology, as a number of schemes are in use (Stefani et al., 2012). Against these disadvantages, it has been shown that this method potentially has a discriminatory power in excess of either spa or PFGE-based methods, while at the same time showing good concordance with results from both of these methods (Schouls et al., 2009).

Concordance of Phenotyping and Genotyping of MRSA

Generally accepted characteristics of HA-MRSA and CA-MRSA as inferred from phenotyping and genotyping studies are summarized in Table 3. Phenotyping by criteria including health care risk factors, infection type (CDC, 2005), and susceptibility pattern (Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2005) have been widely used to classify MRSA into HA-MRSA and CA-MRSA.

However, experience throughout the world of the spread of CA-MRSA into health care settings and emergence of HA-MRSA in the community has challenged assignment of MRSA purely in terms of the health care risk factor and infection type phenotyping methods (Campanile et al., 2011; Hetem et al., 2012; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). In Table 3, I present the characteristics of CA and HA-MRSA.

Table 3

Characteristics of CA-MRSA and HA-MRSA

Characteristic	CA-MRSA	HA-MRSA
Common manifestations	SSTI, necrotizing pneumonia	Nosocomial bacteraemia, pneumonia, wound infections
Antibacterial susceptibility	Frequently susceptible to non-β-lactam antibacterials, low prevalence of *iMLS$_\beta$* resistance	Broad resistance to non-β-lactam antibacterials, resistance common *iMLS$_\beta$*
SSCmec type	IV, V	I, II, III
Accessory gene regulator type	agr III	agr I, II
Genotype (PFGE)	USA300, USA400, USA1000, USA1100	USA100, USA200, USA500, USA600, USA800
Sequence type (MLST)	ST1, ST8, ST30, ST59, ST80	ST5, ST36, ST45
Virulence genes/factors	pvl, sea, seb, sec, seh and Type I ACME common; higher expression of PSM; more rapid in vitro growth	pvl uncommon, Type I ACME absent

For example, in a study of 616 patients in University of Chicago hospitals, it was found that many patients with health care risk factors carried MRSA that was clindamycin-resistant, PVL positive, contained SCCmec IV and/or was assigned as ST8 by MLST, all features of CA-MRSA (David et al., 2008a). Therefore, exposure to health care risk factors is becoming increasingly unreliable as a predictor of HA-MRSA versus CA-MRSA. The result is that CA-MRSA becomes underestimated, as the risk factors and infection type methods have a higher tendency to identify HA-MRSA than the susceptibility pattern method or genotyping methods (Sievert 2008).

David et al (2008a) further confirmed that skin and soft tissue infections (SSTI) are the most common types of infections for both CA-MRSA and HA-MRSA, challenging the infection type phenotyping method. Genotyping methods such as multiplex PCR and PFGE are highly reliable in classifying MRSA. It is imperative to identify reliable phenotyping criteria confirmed with genotyping results and thus dependable for use in the field. In some cases, concordance between phenotyping and genotyping methods has been confirmed. For example, in a study where genotype of MRSA was predicted using a fluoroquinolone susceptibility test, the results of antibiogram and PFGE were significantly correlated suggesting that antibiogram can be an effective tool for use as an epidemiological marker (Chomvarin et al., 2005). Concordance between the Gene Xpert multiplex PCR genotyping method and phenotyping by antibiotic susceptibility using the disk diffusion method has also been recently shown (Biendo et al., 2013).

The International Subcommittee on Phage Typing of Staphylococci standardized phage typing as a phenotyping method in 1972. The challenge is to successfully compromise between phenotyping and genotyping methods in a way that achieves the most reliable results but is realistic in terms of available resources in local and regional situations. Genotyping methods have long been recognized as more reliable when compared to phenotypic methods (Weller, 2000). However, phenotyping criteria can be tested against genotyping methods in order to choose the best combination of infection type, risk factors and susceptibility pattern as the phenotypic characters of MRSA keep changing constantly. The challenge in my study was to identify a verifiable MRSA phenotyping method, supported by a genotyping 'gold standard' classification method, for MRSA strains isolated in the Saudi Aramco community in the Eastern province of Saudi Arabia.

Epidemiology of MRSA in Saudi Arabia

Prevalence of CA-MRSA has been rising in Saudi Arabia in recent years (Monecke et al., 2012). As documented in other countries, healthy individuals have been observed to acquire MRSA in the absence of health care associated risk factors (Bukharie, 2010). For example, between 2000 and 2008 in the Eastern Province, CA-MRSA infections increased dramatically in King Fahad Hospital (Bukharie, 2010). In Dhahran Medical Centre, MRSA accounted for 6%. Meanwhile in the western province of Saudi Arabia, the prevalence of CA-MRSA was found to be 15.8% of all MRSA isolates (Bukharie, 2010). A study from seven hospitals in Riyadh indicates that among

all *Staphylococcus aureus* isolates, the prevalence of MRSA ranged from 12% to 49% and the prevalence in tested hospitals ranged from 27%–33% (Baddour et al., 2007).

Comparing all MRSA isolates, community-acquired infections comprise 62 percent (Al-Tawfiq, 2006). The proportion of CA-MRSA dramatically increased from 41.7% in 1999 to 66.6% in 2002 (Bukharie, 2010a, p. 379). By 2008, 73% of MRSA isolates were community acquired strains and the prevalence of CA-MRSA infections rose from 9.9 per 10,000 admissions to 67 per 10,000 admissions between 2001 and 2008 (Bukharie, 2010a, p. 379).

Saudi Arabia lacks a MRSA national control program. A search of the National Library of Medicine identified only 35 reports of MRSA infections in Saudi Arabia from the year 1990 to April 2007 (Baddour et al., 2007). In contrast, the United Kingdom had 480 reports and United States 826 reports over a comparable period (Baddour et al., 2007). Expert advice suggests that within countries there should be three levels of typing laboratories at the local, regional, and national levels, with differing functions and using typing techniques appropriate to their function and available resources (Stefani et al., 2012). A classification scheme for Saudi Arabia which is appropriate for use at local, regional, and national levels is needed to harmonize surveillance and treatment programs. This classification scheme must be both practical and reliable. Current MRSA classification criteria are not guaranteed to be accurate and misclassification is thus a risk. For example, colonization with an infecting organism may last for months or years, causing patients to be misclassified of having HA-MRSA infection, when they actually acquired the endogenous MRSA strains from the community. In addition, patients with a

prior history of MRSA infection may be labelled as having recurrent health care-associated infections, when the infection was acquired in the community (Stryjewski & Chambers, 2008).

International experience suggests that health care risk factors has become a less reliable indicator as CA-MRSA strains merge with health care settings and the boundaries between 'CA-MRSA' and 'HA-MRSA' in terms of these types of risk factors become blurred (David et al., 2008a; Diep & Otto, 2008). Even in terms of antibiotic resistance typing, caution is needed. Despite the fact that the antibacterial susceptibility phenotypes can distinguish between the CA- and HA-MRSA strains, still certain CA-MRSA isolates have been observed to show rising resistivity to the antibiotics (David et al., 2008a; Diep & Otto, 2008). A 2007 study showed that isolates of MRSA that were phenotypically analogous to community associated strains have become predominantly related with HA-MRSA (Maree et al., 2007).

Other pathological traits of CA-MRSA are not traditionally associated with HA-MRSA, such as expression of certain toxins (Diep & Otto, 2008). For example, Panton-Valentine toxin (PVL) is expressed in CA-MRSA strains due to acquisition of the prophage-encoded adjacent lukS and lukF genes which express it (Chambers, 2005; Otto, 2013). It is now regarded as a relatively stable marker of CA-MRSA and is proposed to contribute to increased virulence of CA-MRSA. For example, PVL-expressing strains have been associated with necrotizing pneumonia in previously healthy young people (Gillet et al., 2007; Labandeira-Rey et al., 2007; Lina et al., 1999).

PVL is a two-component leukocidin toxin that acts by forming pores in the mitochondria (Genestier et al., 2005). By studying the MRSA genotypes from Riyadh hospitals in the central province of Saudi Arabia, Moussa et al. (2009) found that, the majority of strains of CA-MRSA carry the PVL genes and SCCmec type IV element (Moussa & Shibl, 2009). More recently, in a study to characterize the population structure of MRSA in Riyadh, microarray analysis was carried out on clinical and environmental MRSA isolates collected in KFMC hospital (Monecke et al., 2012). A great diversity of clonal complexes was identified and this study confirmed the remarkable prevalence of PVL in the population as well as a high rate of antibiotic resistance markers in community-associated strains.

Epidemiological studies, in addition to clinical data, would benefit from molecular genotypic techniques and identification of various marker genes (e.g. SCCmec type, virulence/toxin genes) to achieve a complete and reliable characterization of MRSA isolates in Saudi Arabia. Standard definitions for CA- and HA-MRSA should be used whenever possible, including molecular genotype assignment, SCCmec type, and the presence or absence of various genes such as PVL, type I ACME and agr type.

Since some phenotyping methods for identification of MRSA strains and antibiotic resistance detection can take more than 48 hours, molecular based genotyping techniques such as those discussed above are used for fast and accurate identification and characterization of MRSA isolates (Fluit, Visser, & Schmitz, 2001). To date, among all the genotyping methods, PFGE is recognized as the ‘gold standard’ and is the most extensively used typing technique. (Fluit, Visser, & Schmitz, 2001). PFGE is often

coupled with other molecular typing techniques as a reference method, as it is the most sensitive and specific so far (Molina et al., 2008). Within the last decade, numerous different genotyping methods have been devised and used to distinguish among MRSA types, as described in the section 'Genotyping of MRSA'.

Before the development of PCR, several efficient typing methods were used, which include bacteriophage typing, capsular typing, PFGE, and zymotyping, for discriminating between MRSA strains (Weller, 2000). PCR-based methods are fast and reliable methods, relatively inexpensive, and have high throughput. Therefore, they are considered a method of choice for typing of strains at a local level (Stefani et al., 2012). For my research, I used a multiplex-PCR method as a 'gold standard' genotyping method against which to measure concordance of proposed phenotyping criteria for use in MRSA classification in local situation of the Saudi Aramco community in the Eastern province of Saudi Arabia. It was thus in line with expert international recommendations (Stefani et al., 2012).

Future Considerations

Staphylococcus aureus is a human pathogen that can be found everywhere around us and thus contributes a significant fraction of our public health history (Monecke et al., 2011). . This bacterial organism has developed mechanisms to escape different antimicrobial agents and continues to evolve to ensure its continued existence (Monecke et al., 2011; Stefani et al., 2012). On the basis of current epidemiological data, the incidence and prevalence of MRSA will persist and increase (CDC, 2005). The intermixing of risk groups and environments for HA-MRSA and CA-MRSA has already

begun and boundaries are blurring between the two. Thus an evidence-based characterization and understanding of the existing epidemiology of MRSA is indicated, so that the changes can be tracked and described appropriately. Without this knowledge valuable and successful interceptive and preventive programs cannot be developed or implemented. The accurate assessment of MRSA will be the cornerstone to implement community-based control strategies. These strategies will be designed to minimize the ability of MRSA strains to be transmitted from the community into the health care settings. Also, these strategies will help determine the best approach for containing and preventing the spread of CA-MRSA within the community.

Summary

In this chapter, I covered major themes in the current literature on MRSA with a view to placing my research project in context. The purpose of my study was to establish a set of verified MRSA phenotyping variables, supported by a genotyping 'gold standard' classification method, for MRSA strains isolated in the Saudi Aramco community in the Eastern province of Saudi Arabia. Results of this study allowed reliable classification of MRSA as HA-MRSA or CA-MRSA. The literature review included a historical overview of the worldwide emergence of HA-MRSA and CA-MRSA, of the problem of multiple drug resistance and research aiming to identify new antibiotics and treatment options.

The issue of the multiplicity of MRSA strains and variations in worldwide geographic distribution was covered, including an update on current knowledge of MRSA phenotypic and genotypic data within Saudi Arabia. Considering the worldwide increase in cases of CA-MRSA and the challenges inherent in introducing international

consistency in the use of typing methods and classification, I highlighted the importance of establishing a reliable and inexpensive classification method for use in Saudi Arabia. The new classification method would, in turn, expedite accurate diagnoses and administration of appropriate treatment, and help identify outbreaks and epidemics in a timely fashion. To place genotypic studies in context and show the importance of genotypic identification in accurately classifying MRSA, the genetic elements of MRSA were reviewed with special attention to the staphylococcal cassette chromosome mec (SCCmec) and Panton-Valentine leuokocidin (PVL) genes. Current phenotyping and genotyping methods and concordance or discordance between them were also reviewed.

Limitations in the methods were considered. In particular, the challenges inherent in classification of MRSA as 'HA-MRSA' and 'CA-MRSA', given the limitations of phenotyping methods, such as the health care risk factors method advocated by the CDC, were addressed. These limitations helped me to demonstrate the necessity of identifying phenotypic methods that give results that are concordant with genotyping data in the specific context of the Saudi Aramco community in the Eastern province of Saudi Arabia. Overall, while many studies exist on the risk factors, epidemiology, and genetics of HA-MRSA and CA-MRSA, there are few studies specifically addressing the situation in Saudi Arabia. The literature review presented in this chapter contextualized the need for testing of the current phenotyping methods against a 'gold standard' genotyping method in the Eastern province of Saudi Arabia.

I describe the methodology I used to answer my research questions in chapter 3. This included samples and data to be used, a description of the three most commonly

used phenotyping methods, health care risk factors, infection type, and antibiotic susceptibility; the multiplex PCR genotyping data; analysis of concordance of data from each phenotyping method with each other and with genotyping data; and sensitivity and specificity of the three methods. I also identified the combination of MRSA phenotypical classification methods or individual variables that best predict HA- and CA-MRSA in Saudi Arabia's Eastern Province, compared to the multiplex-PCR data.

Chapter 3: Research Method

The purpose of this quantitative, secondary data analysis study was to characterize the MRSA strains within the Saudi Aramco community and to accurately identify MRSA strains infection as HA-MRSA or CA-MRSA by comparing and contrasting the three existing phenotyping methods against a gold standard Multiplex PCR method. The phenotyping methods tested were the health care risk factor, infection type (CDC, 2005), and antibiotic susceptibility pattern methods (Clinical and Laboratory Standards Institute, 2005).

In this chapter, I explain the overall rationale for the selection of the study design, and I describe the study population, sampling methods, and the data collection procedures. Next, I discuss the methods I used to address each of the four research questions.

Research Design and Rationale

My study was a quantitative, secondary data analysis. The aim of the research was to characterize the two forms of MRSA infections, HA-MRSA and CA-MRSA, in the Saudi Aramco population. Compared with cohort studies that are usually used to study incidence, causes, and prognoses and are preferred when the exposures or the risk factors are rare, case control studies are not suitable to measure multiples outcomes, always need controls, and are more suitable when the outcomes are rare (Newman, 2001). A cohort approach would have been preferable in this study; however, because I used secondary data to determine exposures and outcomes concurrently, my research study was cross-

sectional. Quantitative studies using secondary data are useful for establishing associations rather than causality and for determining prevalence, rather than incidence; further, they are appropriate when the outcomes are frequent, such as with MRSA infection (Newman, 2001). This research design was appropriate for this study because it assessed the prevalence of HA-MRSA and CA-MRSA within the Saudi Aramco population through a rapid characterization of HA- and CA-MRSA infections using pre-existing datasets. The selected design provided a method to assess the relationship between various diagnostic methods across a large population in a system in which it would be unethical to perform controlled experiments.

A combination of methods was used to relate demographic characteristics of the patient population to MRSA infection phenotype and genotype. This combination of methods generated novel findings regarding the specificity and sensitivity of affordable phenotypic tests for differentiating HA- and CA-MRSA by comparing them with the genotypic gold standard (Newman, 2001).

Study Population

The samples for this study were collected from patients attending the John Hopkins Aramco Health Center, a 405-bed, acute-care hospital in Dhahran city, in the Eastern Province of Saudi Arabia. The samples were from patients in the study population, approximately 350,000 people who are employees, or dependents of the Saudi Aramco Energy Corporation, which is headquartered in Dhahran (Saudi Aramco, 2014). Most of the population is of Asian origin, being either Saudis or nationals of other Arab or Asian nations; expatriates from Western countries are a minority.

Power analysis

For this study, all the available data for the period between January 2012 and December 2013, 133 MRSA samples, were used. The power of the study to measure the predictive ability of the model created in response to the final research question was constrained by this fixed sample size. Power is the ability to detect a difference if one exists and specifically relates to the ability to reject the null hypothesis (Newman, 2001). Research Question 4 was the only question in this study with a null and alternative hypothesis, so I conducted a power analysis for logistic regression with the parameters, α = .05, effect size = .15 (medium effect size), and a maximum of eight predictors using G*Power® ((Erdfelder, Faul, & Buchner, 1996). The resulting power from this calculation was .99 and the associated x-y plot is presented in Figure 2.

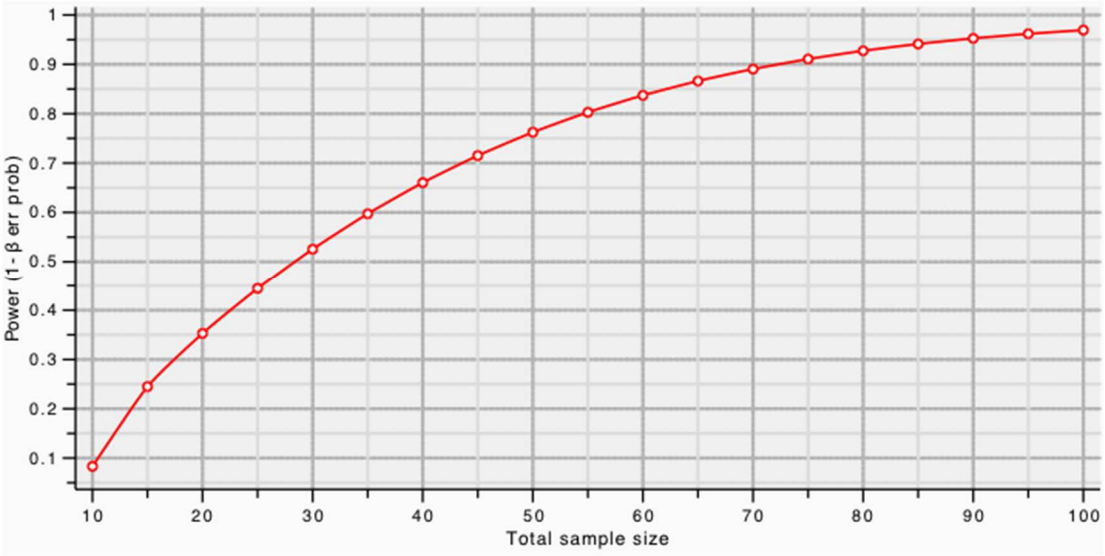

Figure 2. X-Y plot of power based on sample size.

Generally, to reject the null hypothesis, a p Value of .05 is needed. A power of at least 0.8 is commonly considered desirable. Therefore, I could detect a medium difference (effect size = .15) in predictive ability between multiple logistic regression models with a sample of 133.

Data Collection

After attaining formal approval from the Saudi Institutional Review Board, I obtained secondary data from all samples isolated between January 1, 2012, and December 31, 2013, and I stored them in epidemiology and microbiology databases at the Johns Hopkins Aramco Health Center. This time corresponded to the period when the health center began collecting all MRSA samples to validate the multiplex PCR system, for which all isolated MRSA samples were analyzed for the PVL and mecA genes. *Staphylococcus aureus* infections were tested for sensitivity to the following antibiotics: penicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, clindamycin, erythromycin, quinipristin, linezeloid, vancomycin, and tetracyclin. Samples were defined as MRSA if the *Staphylococcus aureus* strain was found to be resistant to cefoxitin; it was assumed that they were also resistant to oxacillin. However, the molecular biologists who analyzed the original samples during data cleaning procedures were further excluded 24 cases , due to borderline resistance to oxacillin. Other data cleaning criteria during the original data collection efforts included samples with no definition of infection site and colonization.

The epidemiological data included the following independent variables: health care risk factors, hospital admission profile, whether they had been hospitalized for at

least 48 hours prior to diagnosis or had been transferred from a different hospital, infection type or bodily location of the infection, and antibiotic susceptibility profile. They also included the covariates age, gender, survival status, and pre-existing illnesses. Table 4 presents a sample data line.

Table 4

Sample Data Line

Variables	Sample values
Date	13 Jun
MR	
Age	Boy
Gender	Male
Health risk factors	HCA
Infection	Pneumonia
Type	Sputum
Hospitalized	No
Visited clinic during the last year	Yes
Survival	Yes
Pre-existing illness	DM
P	≥ 0.5R
OX	≥ 4
GM	≤0.5
CIP	≥ 8
LEV	≥ 8
MOXI	≥ 8 *continued*

Table 4 *continued*

Variables	Sample values
CC	≤ 0.25
E	≥ 8
Quini	≤ 0.25
Vance	15
Tetra	≤ 1
Tigecy	≤ 0.125
Rif	≤ 0.5

Bacterial samples were stored by the molecular biology and microbiology divisions at the Johns Hopkins Aramco Health Centre (formerly Dhahran Health Center), and Multiplex PCR methods were carried out on these samples to allow genotyping information to be extracted. The extraction and analysis of the genotypic information formed part of the methodology of this study (Appendix A).

Study Variables

The major classification variables in this study were the three phenotyping methods: health care risk factors, infection type, and antibiotic susceptibility. These are presented in Table 5.

Table 5

Study Variables

Variable name	Source	Potential responses	Level of measurement
Gender	Medical records	Male Female	Dichotomous
Race	Medical records	Asian White Black	Categorical
Age	Medical records	Age in years	Continuous
Survival	Medical records	Yes No	Dichotomous
Pre-existing condition, Any of the following: Diabetes (DM) Chronic Renal Insufficiency Dialysis Cardio-vascular Disease (CVD) Coronary Heart Failure COPD	Medical records	Yes No	Dichotomous
Health care Risk Factors: Surgery Hemodialysis Peritoneal dialysis, Hospitalization, Residency in a long-term care facility within the last year Presence of indwelling percutaneous devices or catheters at the time of diagnosis or previous isolation of MRSA	Medical records	Yes No	Dichotomous

continued

Table 5 *continued*

Variable name	Source	Potential responses	Level of measurement
Infection type: Bacteremia Pneumonia Skin/Soft Tissue Surgical Site	Medical records	Type	Categorical
Drug resistance: Ciprofloxacin Clindamycin Gentamicin Levofloxacin Trimeth-Sulfamethoxazole	Sensitivity test results	Yes No	Each type is recorded as a Dichotomous variable as there may be more than one per sample
Classification	Genotyping and phenotyping	HA-MRSA CA-MRSA	Dichotomous

The operational definitions of the health care risk factors and infection type methods were as defined in the CDC criteria (CDC, 2005). The following sections explain in detail how each variable was measured and used to designate samples as 'HA-MRSA' (HA=1) or 'CA-MRSA'(CA=0). These designations were then used for concordance/discordance analyses, as described in detail in the relevant sections of the Data Analysis.

Classification by Health Care Risk Factors

Health care risk factors phenotyping is currently the methodology used at the Johns Hopkins Aramco Health Center for designation of MRSA as HA or CA. Cases were labelled as HA-MRSA in the hospital database on the basis of health care risk

factors if the patient had at least one of the following established risk factors: hospitalization >48 hours prior to the current infection (the patient was not MRSA-infected at the time of hospitalization but culture and infection were identified > 48 hours after admission), presence in an intensive care unit (ICU) >48 hours prior to the current infection, hospitalization in the previous year (admitted and discharged from a hospital at any time during the year prior to the current infection), surgery during the previous year, dialysis during the previous year, presence of a percutaneous device or indwelling catheter in the previous year, and status as a resident of a long-term care (LTC), nursing home or rehabilitation facility in the previous year (CDC, 2005).

Cases with none of the established factors for all seven HA-MRSA risk factors were considered CA-MRSA (Naimi et al., 2003). For the purposes of this study, in order to verify that the classification as HA-MRSA or CA-MRSA was correct, I re-assessed the electronic and/or physical records for each sample and verified that the designation was made correctly on the basis of at least one of the established risk factors being present for a designation of HA-MRSA, or none being present for a designation of CA-MRSA.

Classification by Infection Type using Clinical Information

As stated previously, the samples were classified in the database as HA-MRSA or CA-MRSA according to the health care risk factors method, which is the current standard method used in the Johns Hopkins Saudi Aramco Health Centre. In this study, I classified the cases by the infection type method using clinical data available in the hospital database as follows. A sample was designated as CA-MRSA on the basis of infection

type if a skin or soft tissue infection was diagnosed, including abscess, cellulitis, folliculitis, and impetigo, or if a wound infection had "skin" identified as the culture site.

Cases with other, more serious infections, including bacteremia, meningitis, osteomyelitis, pneumonia, septic arthritis, and surgical site infection, were labelled as HA-MRSA (CDC, 2005). CA-MRSA can in some situations cause more serious infections like pneumonia or bacteremia, but these infections are typically caused by HA-MRSA and are usually accompanied by the HA-MRSA risk factors listed previously. Therefore, if a case had both a skin or soft tissue infection and more invasive infection concurrently, it was considered HA-MRSA to give more weight to the more serious infection type (David et al., 2008). This allowed distribution of HA-MRSA and CA-MRSA by the infection type method to be defined by the variable described above and the results compared to the other two methods.

Classification by Susceptibility Pattern Using Clinical Information

The operational definition of the antibiotic susceptibly method was according to the CLSI criteria (Clinical and Laboratory Standards Institute, 2005). Cases were classified as HA-MRSA or CA-MRSA by the antibiotic susceptibility pattern method using clinical data available in the hospital database as follows. *Staphylococcus aureus* samples were primarily tested for antibiotic sensitivity using the VITEK II system. All the antibiotics mentioned in the 'Data collection' section were tested. VITEK II tests for sensitivity were performed by calculating the minimum inhibitory concentration (MIC) of each drug, and the interpretation of each MIC value is assessed based on the Clinical Laboratory Standards Institute (CLSI) guidelines. Cases were classified as CA-MRSA on

the basis of susceptibility patterns if their isolates were resistant only to β-lactams. This is the basic resistance pattern that defines MRSA (Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2005). Cases were labelled as HA-MRSA if resistance to additional antimicrobial classes beyond β-lactams was also reported. This higher resistance included, but was not limited to, aminoglycosides, folate pathway inhibitors, lincosinamide, fluoroquinolones, and tetracyclines (Naimi et al., 2003). This allowed distribution of HA-MRSA and CA-MRSA by the antibiotic susceptibility method to be defined by the variable described above and the results compared to the other two methods.

Data Analysis

Statistical analyses was carried out using SPSS® 21 statistical software (IBM, 2012). The description of the study design was divided into four sections, each pertaining to one of the primary questions I addressed in this research. In Table 6, I present how I used each of the study variables to answer the four research questions.

Table 6

Use of Study Variables per Research Question

Research question	Variable(s)/Type of variable/Level of measurement	Type of analysis	Statistical tests
1. What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?	Age/NA/Continuous	Univariate	Mean and Standard Deviation
	Gender, hospital admission profile, survival, preexisting illnesses, health care risk factors, susceptibility profile(each drug) /NA/Dichotomous		Frequencies
	Infection type/NA/Categorical		Frequencies
2. What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection type, and susceptibility pattern, used to classify CA- vs HA-MRSA in Saudi Arabia?	Genotype Classification as HA-MRSA and CA-MRSA/ Dependent/Dichotomous	Bivariate	Cohen's Kappa
	Three Phenotyping Methods' Classification as HA-MRSA and CA-MRSA/Independent/ Dichotomous		

continued

Table 6 *continued*

Research question	Variable(s)/Type of variable/Level of measurement	Type of analysis	Statistical tests
3. What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?	Genotype classification as HA-MRSA and CA-MRSA/Dependent/ Dichotomous	Bivariate	Sensitivity and specificity
	Three phenotyping methods' classification as HA-MRSA and CA-MRSA/Independent/ Dichotomous		
4. Is it possible to predict HA-MRSA and CA-MRSA in the Eastern Province of Saudi Arabia using a combination of MRSA phenotypical classification factors?	Health Care Risk Factors	Multivariate Block 1	Multiple logistic regression
	Infection Type	Multivariate Block 2	-2 Log Likelihood
	Susceptibility Pattern	Multivariate Block 3	Hosmer and Lemeshow
	Age/Independent/ Continuous; Gender, hospital admission profile, survival, preexisting illnesses/Independent/ Dichotomous	Multivariate Block 4	

Research Question 1

What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?

I previously carried out multiplex PCR on the stored 133 MRSA samples in the molecular biology department in the Johns Hopkins Aramco Health Center study to identify and classify circulating MRSA using the gold standard molecular method (Popovich, 2007). For this study, I classified the samples as CA-MRSA or HA-MRSA according to their mec-A and pvl gene sequences from the results of the multiplex PCR assay. The multiplex PCR methodology was summarized in appendix A. Demographic distribution and clinical information were obtained by matching PCR results to patient information records from the Johns Hopkins Aramco Health Center database. Univariate analysis, including mean and standard deviation for continuous variables and frequencies for dichotomous and categorical variables, was carried out to determine the distribution of HA-MRSA and CA-MRSA as defined by multiplex PCR genotyping results by age, gender, health care risk factors, infection type, hospital admission profile, survival, preexisting illnesses, and susceptibility profile.

The validity of the genotype assignment I used to answer Research Question 1 depended on the accuracy of the PCR genotyping. The method used is standard and well tested, and little error was anticipated (Popovich, 2007; Stefani et al., 2012).

Research Question 2

What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection type, and susceptibility pattern, used to classify CA- vs HA-MRSA in Saudi Arabia?

All cases in the epidemiology dataset were defined as either HA-MRSA or CA-MRSA using one of three phenotyping classification criteria: health care risk factor, infection type, and susceptibility pattern (Sievert, 2008; Sievert et al., 2010). Covariates were age, gender, survival status during MRSA infection period and pre-existing illnesses (specifically diabetes mellitus, chronic renal insufficiency, dialysis, cardiovascular disease, chronic heart failure, and chronic obstructive pulmonary disease). Thus, I used three criteria to classify MRSA type and assessed the similarity between classifications based on these three different characteristics.

I conducted univariate and bivariate analysis to compare the demographic and clinical distributions for the MRSA samples in the Saudi Aramco community, based on HA-MRSA and CA-MRSA.

I defined frequencies and percentages for each of the phenotyping characteristics, gender, health care risk factors, infection type, hospital admission profile, survival, preexisting illnesses, and susceptibility profile. In addition, I evaluated the distribution of age to assess for any patterns. For concordance analysis, I designated samples as HA-MRSA or CA-MRSA and compared results for each of the three phenotyping methods to each other to determine concordance or discordance. I considered $p \leq 0.05$ a statistically significant difference.

Concordance Analysis. I constructed a concordance matrix similar to published literature (Sievert, 2008; Sievert et al., 2010) to examine agreement (i.e., concordance) among all three phenotypic methods and each pair of methods for the Saudi Aramco community specifically. This matrix contained the number and percentage of MRSA cases identified as HA-MRSA or CA-MRSA by all three phenotypic methods, those that were concordant between the three methods. The matrix also denoted the number and percentage of cases identified as HA or CA by each pair of methods or by only one method. The use of all three phenotyping methods and the inclusion of covariates was used to confirm the overall distribution of HA-MRSA and CA-MRSA in the population and added to the validity and reliability of the study instrument. For example in the USA, all three methods yielded demographic, clinical, and microbiological variable distributions that were consistent with patterns in the literature (Sievert et al., 2010).

In addition, Cohen's kappa and its associated confidence interval was calculated for each pair of methods, to provide a metric of concordance that accounted for the fact that sometimes two methods can agree by chance alone (Kwiecien et al., 2011). Thus, the Cohen's kappa measured the normed difference between the rate of agreement that is actually observed between the phenotypic methods and the rate of agreement that one might expect purely by chance (Kwiecien et al., 2011). For a given pair of methods, health care risks, infection type and susceptibility pattern, a two-by-two table was constructed denoting the number of cases identified as HA and CA by each method. Then marginal values, column and row totals, were calculated.

The probability of agreement, p_o, is propA + propD. However, we would expect agreement at random with some probability, p_E (Kwiecien et al., 2011). This can be calculated as the probability with which each method classifies the case as HA independently plus the parallel probability for CA.

$$p_E = propAB*propAC + propBD*propCD$$

The excess agreement, beyond chance, is then pO-pE. This value is normalized to calculate the final Cohen's kappa according to the following equation:

Cohen's kappa = p_0-p_E/(1-p_E).

The 95% confidence interval for Cohen's kappa is calculated according to the following equation:

CI = Cohen's kappa +/- 1.96*sqrt ((p_0(1-p_0)/ (n(1-p_E)^2))

Research Question 3

What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?

The sensitivity and specificity of each phenotyping method was measured using a conventional two-by-two table. Multiplex PCR genotyping served as the gold standard method. For each phenotyping method, I calculated the sensitivity, which is the proportion of actual positives that are detected, as the number of infections identified as positive by both phenotype and genotype, true positives, divided by the number of infections that were identified as positive by genotype alone based on the gold standard

and representing the true number of infections in the population (Newman, 2001). Specificity, which is the proportion of actual negatives that are detected, was calculated as the number of infections identified as negative by both the genotype and phenotype tests, true negatives, divided by the number of infections that were identified as negative by genotyping alone representing the true number of patients infection-free (Newman, 2001).

I also calculated 95% confidence intervals for these proportions, assuming normally distributed error around the estimated sensitivity and specifity, p_hat. This can be written as:

$p_{hat} \pm 1.96(\frac{1}{n} * p_{hat} * (1 - p_{hat})$, where n is the number of observations.

Research Question 4

How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?

H_0: Demographic and phenotyping variables do not significantly predict MRSA genotyping classification.

*H*a: Demographic and phenotyping variables significantly predict MRSA genotyping classification.

I used bivariate statistics, including chi-square and *t* tests to test the sensitivity and specificity of the HA/CA designations generated from each of the major classification variables, health care risk factors, infection type and antibiotic susceptibility, against the outcome variable, HA/CA status based on the genotypic Multiplex PCR test. My main

aim was to validate each phenotyping method against the genotyping method to determine if one of the methods is optimally concordant with the genotypic method in designating samples as HA-MRSA or CA-MRSA.

However, if one or more methods demonstrated superior sensitivity and specificity, I further explored the potentially contributing parameters/variables. For example, the susceptibility method depends on ten or more antibiotics; however, a subset may be identified as crucial.

In the first instance, the statistical significance between the outcome variable and the predictor variables was established by calculating the bivariate correlations of all the possible predictor variables in order to determine those that are significant, age, gender, health care risk factors, infection type, hospital admission profile, survival, preexisting illnesses, and susceptibility profile. The magnitude of the odds ratios were also established using SPSS® to compute a multivariate logistic regression model, with all possible predictor variables initially and a backward conditional method to determine the final model.

Having established the predictive ability of each phenotyping method, I used Multiple Logistic regression. This allowed me to assess the best combination of phenotypic characteristics to predict whether an infection was HA or CA. The response or outcome variable was HA/CA status based on the genotypic Multiplex PCR test, while each of the three classification schemes, health care risk factors, infection type, and antibiotic susceptibility profile, was treated as separate dichotomous classification predictor variables and entered into the model in blocks (Sievert, 2008). Combinations of

these variables were tested to identify the model with the best fit and predictive power. The three methods introduced into the model using separate blocks and a backwards conditional approach allowed me to parse the model and include only those variables from across the three methods in a final model that could be recommended for testing in the Saudi Aramco population. For example, combining individual antibiotic susceptibilities from the susceptibility method with the infection site information could result in a model with increased predictive power over that generated by a model that uses either of these phenotyping methods alone.

Other predictor variables, added to the model generated from testing of the base classification variables were age, gender, survival status during MRSA infection and pre-existing illnesses (yes, if reported any of the following: diabetes mellitus, chronic renal insufficiency, dialysis, cardiovascular disease, chronic heart failure, chronic obstructive pulmonary disease; no, if none of these reported). These constituted an additional block of variables and were therefore controlled for in the final model. Each of the models produced were compared for best fit using -2 log Likelihood, and the final model's relative predictive power using Hosmer and Lemeshow (Newman, 2001). I presented the variables included in each block of the logistic regression in Table 7.

Table 7

Variables in Each Block

Block	Independent variable(s)
1	Health Care Risk Factors: Surgery, hemodialysis, peritoneal dialysis, hospitalization, residency in a long-term care facility within the last year, presence of indwelling percutaneous devices or catheters at the time of diagnosis or previous isolation of MRSA
2	Infection Type: Bacteremia, Pneumonia, skin/soft Tissue, surgical site
3	Antibiotic Susceptibility: Ciprofloxacin, Clindamycin, Gentamicin, Levofloxacin Trimeth-Sulfamethoxazole
4	Covariates: Age, gender, hospital admission profile, survival, preexisting illnesses

The link function was the logit function, defined as:

Logit transformation: ln(mu/(1-mu))

The full model, which included the listed clinical and demographic characteristics as covariates, was as follows:

Genotype = β1*I(ph1) + β2*I(ph2) + β3*I(ph3) + β4*(ph1xph2) + β5*(ph1xph3) + β6*(ph2xph3) + β7*age + β8*gender + β9*survival status + β10*pre-existing illness,

where the βs are the coefficients, I is an indicator function, ph1 refers to phenotype 1 (health care risk factors method), ph2 to phenotype 2 (infection type), ph3 to phenotype 3 (antibiotic susceptibility), etc., and (ph1xph2) indicates an interaction term.

The results of this model yielded the combination of phenotype variables that best predicted genotype, and therefore CA/HA status. The model could then be applied to the study population in the future to quickly and cheaply assess the HA/CA status of a new MRSA case without requiring the time-consuming and expensive procedure of genotyping. The sample size was adequate to conduct multiple comparisons, as needed using Bonferroni to adjust the p value (Newman, 2001). As the main outcome of this research question was the predictive ability of the models, I was not as concerned with the p-values of the individual variables.

Threats to Internal and External Validity

The major threats to internal validity included discrepancies in the medical records, missing information, and information bias. The use of secondary data introduced the potential for misclassifications and missing data, which could contribute bias to outcomes. The potential for information bias could have resulted from non-uniform criteria on the part of health care providers. Health Care professionals have different levels of medical record specificity and accuracy. Individual effort and competency played a role in the completeness of each patient's individual medical records.

While I planned to compare the patient population at the Saudi Aramco facility to the patient population in other facilities in the area, I could not ensure that the samples I

used for this research were representative. This could result in a threat to external validity and reduced my ability to generalize the results.

Ethical Considerations

In order to gain access to the data set, I followed the procedure of applying for Saudi IRB approval through a Saudi Aramco IRB representative. The Saudi IRB is a comittee founded by Saudi Aramco Health Center with the aim of encouraging research. The request was reviewed and I was interviewed to explain the purpose the study. The research was approved and I received an official written approval (see Appendix B). Since the present study had no direct recruitment of any human or animal subject and did not include identifying information regarding the patients, I applied for an expedited IRB review from Walden University. This application was in addition to the official Saudi IRB written approval I had already acquired (see Appendix B).The data continued to be maintained in a secure fashion; the database that contains patient information collected from different resources was stored on a computer without an internet connection in order to avoid misuse by third parties via the internet. The entire data set was then burned to a password protected CD and deleted from the computer. Data was maintained on the secured, password protected CD for the duration of this research and will be further maintained up to 5 years after its completion.

Summary

This quantitative, secondary data analysis study utilized a combination of molecular and phenotypic lab techniques with statistical analysis and modeling to assess the strength of various types of data (i.e., demographic, genotypic, phenotypic) for

differentiating between HA- and CA-MRSA. Specifically, analysis of multiplex-PCR data generated from 133 samples from MRSA patients attending the Johns Hopkins Aramco Health Centre was used to assign HA-MRSA or CA-MRSA genotypic identity to each sample. The genotypic information obtained from this exercise was used as the gold standard to test the results obtained from database information on all of these samples by applying the three phenotyping methods that could be used for MRSA identification. These phenotyping methods were the health care risk factor, infection type (CDC, 2005) and antibiotic susceptibility pattern methods (Clinical and Laboratory Standards Institute, 2005). Other predictive variables including age, gender, and survival status during MRSA infection period (died/survived) and pre-existing illnesses were also tested. In this way, a model was proposed for a combination of phenotypic variables that best predicts genotyping results in terms of identification of MRSA as HA-MRSA and CA-MRSA. After cross validation using new data, this model could then be used as a rapid, reliable, and inexpensive test for MRSA status in the Saudi Aramco population in the Eastern Province of Saudi Arabia.

The analyses I present in chapter 4 utilized John Hopkins Health Care Center MRSA infection data, and MRSA multiplex PCR classification data to compute the results of the statistical analyses used to answer the four research questions in detail.

Chapter 4: Results

Introduction

The purpose of this quantitative, secondary data analysis study was to characterize the MRSA strains within the Saudi Aramco community and to identify them accurately as HA-MRSA or CA-MRSA by comparing and contrasting the three existing phenotyping methods against the gold standard Multiplex PCR method. The three existing phenotypic methods in question were the health care risk factor, infection type (CDC, 2005) and antibiotic susceptibility pattern methods (Clinical and Laboratory Standards Institute, 2005). Currently, the standard phenotyping method used in the Johns Hopkins Saudi Aramco Health Centre, from which the samples for this study were derived, is the health care risk factor method. However, individually each of the existing phenotyping methods has been shown to have the potential for MRSA misclassification (Sievert, 2008; Sievert et al., 2010). In addition, a discordance between methods used in classifying MRSA as HA-MRSA or CA-MRSA has been observed (Sievert, 2008; Sievert et al., 2010). For example, in studies conducted in Michigan, USA, while the distribution of HA-MRSA versus CA-MRSA was similar for the health care risk factor and infection type classifications, it was considerably different for the susceptibility method.(Sievert, 2008; Sievert et al., 2010). There is also a variability in the epidemiology of MRSA between countries (Maree et al., 2007; Pan et al., 2003 & 2005; Labandeira-Rey et al., 2007; Song et al., 2011; Stefani et al., 2012; Tietz, Frei, & Widmer, 2005).

While molecular methods of classification such as Multiplex PCR could be considered the gold standard, these methods are expensive and resource intensive (Stefani et al., 2012). Thus, previous literature suggested a need for a reliable, inexpensive, and readily usable classification method to determine distribution of HA-MRSA and CA-MRSA in the population under study. This classification method should either show the concordance of existing phenotypic methods, or combine elements of the phenotyping methods shown to be concordant, with the results of Multiplex PCR-based genotyping. This need informed the above stated purpose of this study.

To achieve this purpose, I sought answers to the following four research questions:

- Research Question 1: What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?
- Research Question 2: What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection type, and susceptibility pattern, used to classify CA- vs HA-MRSA in Saudi Arabia?
- Research Question 3: What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?
- Research Question 4: How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?

The null and alternative hypotheses associated with Research Question 4 were:

H_0: Demographic and phenotyping variables do not significantly predict MRSA genotyping classification.

H_a: Demographic and phenotyping variables significantly predict MRSA genotyping classification.

In this chapter, I will describe the results of the statistical analyses used to answer the four research questions in detail. In chapter 5, I will interpret and discuss these results. Data collection and baseline descriptive and demographic characteristics of the sample are reported, with consideration of the representativeness of the population sample. The results are then described and organized by research questions/hypotheses. I used tables and figures to illustrate results as appropriate. Finally, I summarized the responses to the four research questions.

Data Collection

I obtained secondary data from all samples isolated between January 1, 2012 and December 31, 2013 and stored in epidemiology and microbiology databases at the Johns Hopkins Aramco Health Center. Data abstraction began after formal approvals from The Saudi and Walden Institutional Review Boards. During the period between January 2012 and December 2013, the Johns Hopkins Aramco Health Center began collecting all MRSA samples for validation of the multiplex PCR system. All isolated MRSA samples were analyzed by this system for the PVL and mecA genes, using the method described in Appendix A. *Staphylococcus aureus* infections were tested for sensitivity to the following antibiotics: penicillin, oxacillin, gentamicin, ciprofloxacin, levofloxacin,

moxifloxacin, clindamycin, erythromycin, quinipristin, linezeloid, vancomycin, and tetracyclin. Samples were defined as MRSA if the *Staphylococcus. aureus* strain was found to be resistant to cefoxitin; it was assumed that they were also resistant to oxacillin. However, 24 cases were excluded by the molecular biologists who analyzed the original samples during data cleaning procedures, due to borderline resistance to oxacillin. Other isolates with no definition of infection site and colonization were also excluded for the original sample. This data cleaning process left 133 MRSA samples, which were used for subsequent data analyses.

Baseline Characteristics of the Sample

The samples for this study were obtained from patients attending the Aramco Dhahran Health Center (now the Johns Hopkins Aramco Health Center), a 405-bed, acute-care hospital in Dharan, in the Eastern Province of Saudi Arabia. These patients are a representative sample of the study population, i.e. approximately 350,000 people who are employees, dependents or annuitants of the Saudi Aramco energy corporation headquartered in Dhahran (Saudi Aramco, 2014). Most of the population are of Asian origin, being either Saudis or nationals of other Arab or Asian nations. While those included in the study represent a sub-population in the Eastern Province, the large size of the population and their wide geographical distribution throughout the Eastern Province makes them representative of the province as a whole.

The epidemiological data included the following independent variables: health care risk factors, hospital admission profile, whether they had been hospitalized for at least 48 hours prior to diagnosis or had been transferred from a different hospital,

infection type, or bodily location of the infection, and antibiotic susceptibility profile. They also included the covariates age, gender, survival status, and pre-existing illnesses. A sample data line is shown in Table 4 of Chapter 3.

The descriptive statistics for the study sample are presented in Table 8.

Table 8

Descriptive Statistics of the Study Sample (N = 133)

Variable	Value	Frequency	Percent
Date	2012	49	36.8
	2013	74	55.6
	2014	14	7.5
Gender	Male	71	51.9
	Female	66	48.1
Admission Profile	≥48 hours	53	39.8
Surgery	Yes	20	15
Catheterized	Yes	19	14.3
Dialysis	Yes	10	7.5
Hx_MRSA	Yes	23	17.3
Bacteremia	Yes	12	9
Pneumonia	Yes	28	21
Skin/Soft Tissue	Yes	40	30.1
Surgical/Deep Wound	Yes	53	39.8
Hospitalized	Yes	47	34.6
Clinic Visit past year	Yes	127	93.2
Survival	Yes	130	97.7
Comorbities	Yes	61	45.9
Drug Resistance	Beta lactams	133	100

continued

Table 8 *continued*

Variable	Value	Frequency	Percent
Drug Resistance	Aminoglycoside	34	25.6
	Cycline	42	31.6
	CIP (Quinolone)	30	22.6
	LEV (Quinolone)	27	20.3
	MOXI (Q)	23	17.3
	Macrolide	36	27.1
	Nitrufuran	2	1.5
	Quinolone	33	24.8
	Sulfa	23	17.3
MRSA Health Care Risk	HA	72	54.1
Factors	CA	61	45.9
MRSA Infection Type Risk	HA	96	69.2
Factors	CA	41	30.8
MRSA Susceptibility Risk	HA	64	48.1
Factors	CA	69	51.9
MRSA Genotyping	HA	63	47.4
	CA	70	52.6

Results

The statistical analyses were carried out using SPSS® 21 statistical software (IBM, 2012). The results will be divided into four sections, arranged according to research question. Table 6 in Chapter 3 presented how each of the study variables was used to answer the four research questions.

Research Question 1

What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?

The results of this analysis are summarized in Table 9.

Table 9

Distribution of HA-MRSA and CA-MRSA as Defined by Genotyping using Multiplex PCR (N = 133)

Variable	Values	HA-MRSA (N=63)	CA-MRSA (N=70)	p Value
Gender	Male	41	28	.004
	Female	22	42	
Age	Mean +/- SD	35.1 +/- 27.3	34.2 +/- 23.9	.839
Admission Profile	≥ 48 hours	33	20	.005
	< 48 hours	30	50	
Survival	No	0	3	.245
	Yes	63	67	
Pre-existing illness	No	34	27	.075
	Yes	29	43	
Health Care risk factors	HA	42	30	.006
	CA	21	40	
Infection type risk factors	HA	48	44	.096
	CA	15	26	
Susceptibility pattern	HA	49	15	<.001
	CA	14	55	

As Table 9 shows, there are observed statistically significant differences in the distribution of the frequencies for all variables except “survival” and “infection type” according to the type of MRSA classification. Of note, are the differences observed for health care risk and susceptibility pattern. For these two phenotyping methods, the frequency of HA and CA are statistically significant. Among the results for susceptibility pattern phenotyping, 49 of 63 cases (77.78%) identified as HA by genotyping were HA-MRSA by phenotyping as well, while 55 of 70 cases (78.57%) identified as CA by genotyping were also CA-MRSA by phenotyping.

Mean age for those designated as HA-MRSA was 35.1 years versus 34.2 years for CA-MRSA (Table 9). In Figure 3, I present the distribution of age for both HA-MRSA and CA-MRSA.

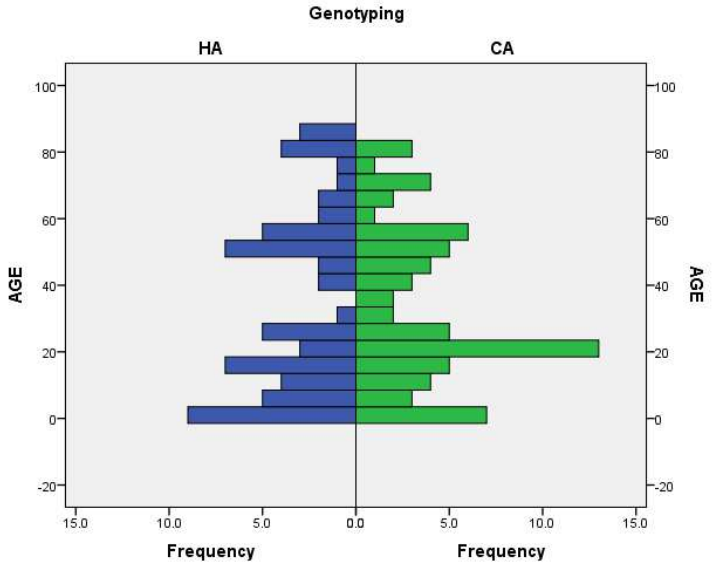

Figure 3. HA-MRSA and CA-MRSA distribution by age.

As suggested by the p Value associated with age in Table 9, there is not a statistically significant difference between mean ages, though there are some minor differences in the distributions. Among cases identified as HA-MRSA, the age distribution is approximately bimodal with the highest frequencies observed under 20 and over 40 years of age. For CA-MRSA, it is a single mode with the majority of cases occurring between 20 and 40, and a defined positive skew.

Research Question 2

What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection type, and susceptibility pattern, used to classify CA- vs HA-MRSA in Saudi Arabia?

Concordance analysis for three phenotyping methods. I present a concordance matrix in Table 10.

Table 10

Concordance Matrix (n = 51)

	Health Care Risk Factors	Infection Type Risk Factors	Susceptibility Risk Factors	% Cases matching
Concordant	HA	HA	HA	29 (22%)
	CA	CA	CA	22 (16%)

In this table, I present the number and percentage of cases that were designated as the same type of MRSA by the three phenotyping methods. Concordance or agreement

on the designation of type of MRSA among all three methods for the designation of HA-MRSA was 22%, while for the designation of CA-MRSA the overall concordance was 16%. Thus, all three methods agree on the designation of MRSA as HA or CA in less than 25% of the cases. As the ideal situation is to have 100% concordance, this is very low, though the statistical interpretation of this requires the use of Kendall's tau, which is the difference between the number of concordant pairs and the number of discordant pairs divided by the total number of pair combinations (Newson, 2002). Like *r*, Kendall's tau varies between -1.0 (all pairs discordant) and +1.0 (all pairs are concordant). I discuss this in more detail following my discussion of discordance.

In Table 11, I indicate in which of the three phenotyping methods the discordance occurs, first for the designation of HA and then for the designation of CA.

Table 11

Discordance Matrix (n =86)

	Health Care Risk Factors	Infection Type Risk Factors	Susceptibility Risk Factors	Number and percent of cases discordant
Discordant HA	HA	HA	CA	31 (23%)
	HA	CA	HA	9 (7%)
	CA	HA	HA	15 (11%)
Discordant CA	CA	CA	HA	17 (13%)
	CA	HA	CA	11 (8%)
	HA	CA	CA	3 (2%)

In each of the three rows identified as “Discordant HA”, one of the three phenotyping methods does not agree with the other two in the designation of HA-MRSA. These are each associated with a percentage that indicates how frequently this type of discordance occurred among the samples. Susceptibility was discordant with the other two methods in 23% of the cases, while Infection Type was discordant in 7% of the cases, and Health Care was discordant in 11% of the cases. Thus, susceptibility was the most frequently discordant with the other two in designating HA-MRSA. I could find no standard for discordance; my observation is a relative one suggesting that susceptibility pattern produces a different distribution pattern than the other two phenotyping methods.

In each of the three rows identified as “Discordant CA”, one of the three phenotyping methods does not agree with the other two in the designation of CA-MRSA. These are each associated with a percentage that indicates how frequently this type of discordance occurred among the samples. The desired discordance is 0%. Susceptibility was discordant with the other two methods in 13% of the cases, while Infection Type was discordant in 8% of the cases, and Health Care was discordant in 2% of the cases. Thus, susceptibility was also the most frequently discordant with the other two in designating CA-MRSA. Again, this is suggestive that susceptibility pattern produces a different distribution pattern than the other two phenotyping methods.

To consider the magnitude of the concordance and discordance for each of the pairs, I computed Kendall’s tau. As discussed previously, Kendall’s tau is the difference between the number of concordant pairs and the number of discordant pairs divided by

the total number of pair combinations (Newson, 2002). Kendall's tau ranged from -.001 to .063, representing HA using susceptibility and CA using health care respectively. All can be interpreted as no agreement between pairs.

Agreement. To further address this research question, I used Cohen's kappa (κ). Cohen's kappa (κ) can range from -1 to +1. Based on the guidelines from Altman (1999), and adapted from Landis & Koch (1977), a kappa (κ) of negative means none, 0-.20 is slight, 0.21-0.40 is fair, 0.41-0.60 is moderate, 0.61-0.80 is substantial, and 0.81-1 is almost perfect agreement. The value for kappa and the associated *p* value for each combination are presented in Table 12. The value for all three phenotyping measures and the genotype is presented in Table 13. Pairwise Cohen's kappa= 0.317, $p < .001$ suggesting fair agreement was obtained for health care risk and infection type methods. Similarly, Cohen's kappa= 0.101, $p = 243$ suggesting slight agreement, was obtained for comparison of the susceptibility pattern and health care risk methods. Finally Cohen's kappa= -0.008, $p = .919$, suggesting no agreement, was obtained for comparison of the susceptibility pattern and infection type methods (Table 12). The results of the comparison of the three phenotyping methods to the genotyping method in terms of either HA-MRSA or CA-MRSA individually gave Cohen's kappa= 0.373, $p <.001$ for both HA-MRSA and CA-MRSA, with an overall agreement with Cohen's kappa= -0.298, $p <.001$. (Table 13). These measures are all suggestive of fair agreement with the genotyping method.

Table 12

Pairwise Agreement Between Phenotyping Methods (n = 133)

Methods compared	Percent pairwise agreement	Cohen's *kappa*	p Value
Health Care Risk and Infection Type	66.9	.317	<.001
Health Care Risk and Susceptibility	54.9	.101	.243
Infection Type and Susceptibility	48.9	-.008	.919

Table 13

Agreement Between 3 Phenotyping Methods and Genotyping Measured by Cohen's kappa (n = 133)

Category	Cohen's kappa	p Value
HA_MRSA	.373	<.001
CA_MRSA	.373	<.001
Overall Agreement	-.298	<.001

Research Question 3

What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?

Sensitivity and specificity of phenotyping methods. The results of the sensitivity and specificity measurements for each phenotyping method are shown in Tables 14, 15, and 16 for health care risk factor, infection type, and susceptibility pattern respectively. I will interpret the results of the evaluation of sensitivity and specificity of

the phenotyping methods in chapter 5 to determine the appropriateness of each of the three phenotyping approaches as a screening tool. In this context, however, sensitivity represents the ability to identify the case as HA if it is indeed HA per the gold standard, while specificity is the ability to identify cases as CA if they are CA. Since true results are desired for cases designated as either HA or CA, in this study, I am looking for methods with both a high sensitivity and a high specificity. FDA approved techniques usually yield 82 to 100 percent sensitivity and 64 to 99 specificity (Marlowe & Bankowski, 2011).

Table 14

Sensitivity and Specificity of Health Care Risk Factors Phenotyping Method

	MRSA (genotyping)	
Phenotyping (Health Care risk method)	HA	CA
HA	True HA = 42	False HA = 30
CA	False CA = 21	True CA= 40
	Sensitivity = 0.6667	Specificity = 0.5714

Table 15

Sensitivity and Specificity of Infection Type Phenotyping Method

Phenotyping (Infection type method)	MRSA (genotyping)	
	HA	CA
HA	True HA = 48	False HA/CA = 44
CA	False CA/HA = 15	True CA = 26
	Sensitivity = 0.7619	Specificity = 0.3715

Table 16

Sensitivity and Specificity of Susceptibility Pattern Phenotyping Method

Phenotyping (Susceptibility Pattern method)	MRSA (genotyping)	
	HA	CA
HA	True HA = 49	False HA/CA = 15
CA	False CA/HA = 14	True CA = 55
	Sensitivity = 0.7778	Specificity = 0.7857

A comparison of the sensitivity and specificity as well as associated 95% confidence intervals is presented in Table 17.

Table 17

Comparison of Phenotyping Methods as Measured Against Genotyping Results

MRSA strain	Phenotyping method	Proportion correctly identified	95% Confidence intervals	
HA-MRSA	HRSA	0.6667	0.5866	0.7468
	IFRF	0.7619	0.6885	0.8343
	Susceptibility	0.7778	0.7071	0.8485
CA-MRSA	HRSA	0.5714	0.4873	0.6555
	IFRF	0.3714	0.2893	0.4534
	Susceptibility	0.7857	0.7160	0.8554

As discussed with Tables 14, 15, and 16, my goal in response to Research Question 3 is to identify the phenotyping method that is most successful in identifying true HA and CA cases. The results presented in Table 17 suggest that among the phenotyping methods, only susceptibility has a sensitivity and specificity similar to the ranges accepted by the FDA ((Marlowe & Bankowski, 2011).

In Figure 4, I present a bar graph, which graphically demonstrates the percentage of HA-MRSA and CA-MRSA correctly identified for each phenotyping method when they are compared to the genotyping method.

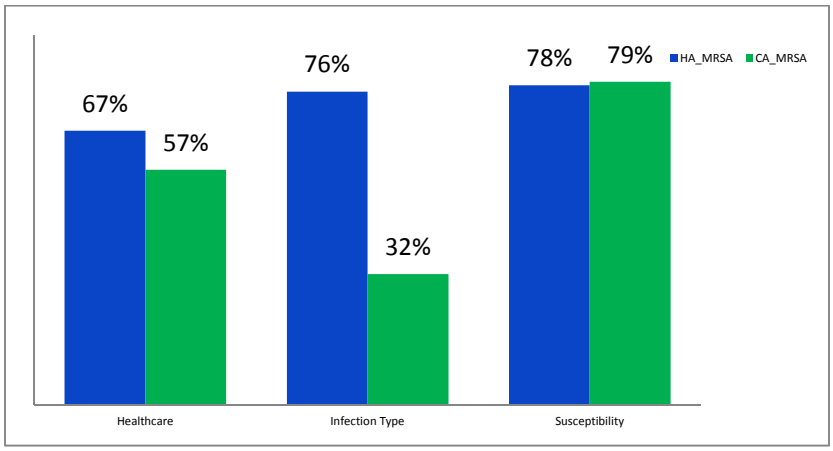

Figure 4. HA-MRSA and CA-MRSA correct identifications by three phenotyping methods.

ROC curve. In Figure 5, I present an ROC curve of the ability of the three phenotyping methods to identify correctly the infection as CA-MRSA. The ROC curve provides a visible image of the sensitivity and specificity of screening tools. The reference is the point where sensitivity and specificity are equal. The lines to the left of the reference line have higher sensitivity than specificity, while those to the right have higher specificity than sensitivity. The lines representing each of the three phenotyping methods can be compared to see which one is most appropriate to use for screening. While all phenotyping methods have higher ability to identify HA-MRSA than CA-MRSA correctly, the Infection type is less likely and the Susceptibility is more likely to identify both HA-MRSA and CA-MRSA correctly.

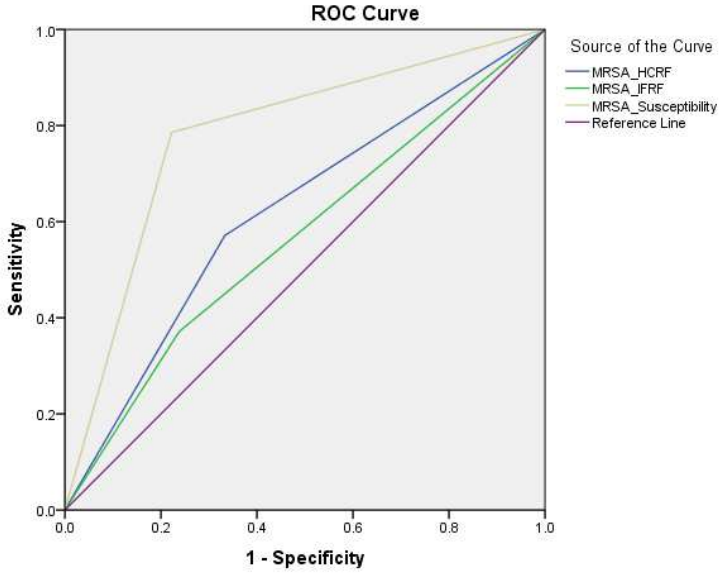

Figure 5. ROC curve comparing phenotyping methods on their ability to identify CA-MRSA.

Research Question 4

How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?

H_0: Demographic and phenotyping variables do not significantly predict MRSA genotyping classification.

H_a: Demographic and phenotyping variables significantly predict MRSA genotyping classification.

To answer this research question, I performed multiple logistic regression using block entry to determine the best model after checking the assumptions required were met. I first determined that the dependent variable was binomial, the independent were continuous or nominal, and that the observations were independent. Using SPSS, I then

determined there was a linear relationship between the dependent and independent variables without multicollinearity or extreme outliers.

In Table 18, I present the blocks of variables that were included for multiple logistic regression.

Table 18

Variables Entered in Each Block for Multiple Logistic Regression

Block	Independent variable(s)
1	Health Care Risk Factors
2	Infection Type Risk Factors
3	Antibiotic Susceptibility: Ciprofloxacin, Clindamycin, Gentamicin, Levofloxacin Trimeth-Sulfamethoxazole
4	Demographics: Age, hospital admission profile, pre-existing illness

Full model using block entry. In Table 19, I present the results of the full model determined using block entry multivariate binary logistic regression. This regression yielded the combination of phenotype variables that best predicted the genotype as defined by multiplex PCR, and therefore CA/HA status.

Table 19

Odds Ratios Computed Using Multivariate Binary Logistic Regression with Block Entry

Step	Variable	B	EXP(B)	95% Confidence Interval	
				Lower	Upper
Block 1	MRSA_HCRF	.100	1.105	.310	3.836
Block 2	MRSA_IFRF	.488	1.630	.542	4.898
Block 3	MRSA_Susceptibility	2.739	15.474	5.995	39.938
Block 4	Gender	.549	1.731	.230	1.450
	Admission Profile	1.056	2.874	.764	10.815
	Pre-existing Illness	.149	1.161	.460	2.932

In Table 19, the lowest OR (EXP (B)) is associated with the first block, which has the health care risk factors phenotyping methods. In contrast, Block 3, which adds the susceptibility phenotyping method, is associated with the highest OR. After adding all the blocks, only susceptibility was found statistically significant, though the OR associated with Admission Profile suggests it may be statistically significant in a reduced model. These results suggest that susceptibility phenotyping is most effective in determining whether MRSA is HA or CA without having to use genotyping methods.

The Cox & Snell and the Negellkerke R Squares comparing the strength of each block included in the multivariate models described above are presented in Table 20.

Table 20

Pseudo R Square Associated with Each Block

Step	Cox & Snell R Square	Nagelkerke R Square
Block 1	.056	.075
Block 2	.061	.081
Block 3	.330	.440
Block 4	.348	.464

Among the Pseudo R Square values presented in Table 20, the largest change occurred after the addition of block three. Using the Cox & Snell R Square, this change was .269, while using Nagelkerke, it was .359. These pseudo R squares can be used to determine which model fits the data best in terms of explaining the variance associated with the dependent variable. While these Pseudo R Square values cannot be interpreted as the percent of the variance explained by the model as a true R square can, they do indicate the relative improvement in the predictive ability associated with the addition of a block. The addition of Block 3 caused the greatest improvement in predictability. There was little improvement associated with Block 4.

In Table 21, I present the results of the Hosmer and Lemeshow Test of Model Fit after the entry of each of blocks 2 through 4.

Table 21

Hosmer and Lemeshow Test of Model Fit

Step	Chi square	Degrees of freedom	Significance
Block 2	5.597	2	.061
Block 3	4.765	5	.445
Block 4	3.688	7	.815

In Table 21, chi square was used to compare the results expected based on the model to those actually observed. The magnitude and significance of the chi square are used to estimate the model fit. Models with lower magnitude and a p value greater than .05 exhibit a better fit than those with greater magnitude and statistically significant differences (Newman, 2001). The model with all four blocks has the best fit, though the model with block 3 is also not statistically significant indicating an acceptable fit.

A scatter plot of the change in deviance by the predicted probability for the full model is presented in figure 6.

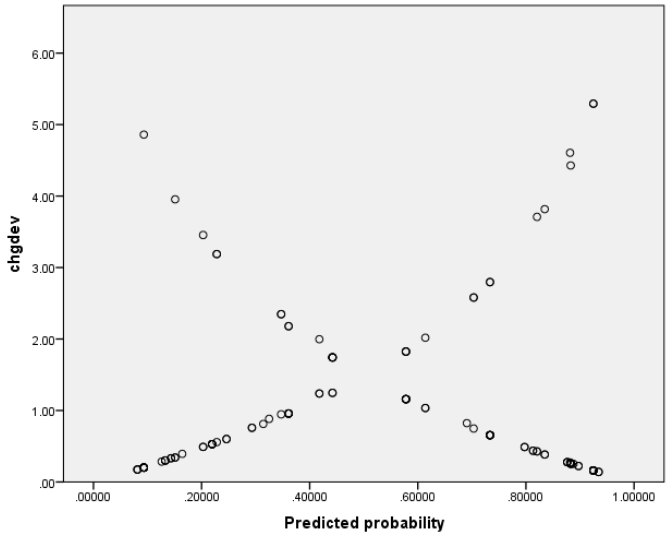

Figure 6. Scatter plot of the change in deviance and predictive probabilities for the full model.

The curve that extends from the lower left to the upper right represents the cases in which the dependent variable was assigned a value of 0. This corresponds to the genotyping designation of HA-MRSA. The curve that extends from the lower right to the upper left represents the cases in which the dependent value was assigned a value of 1. This corresponds to the designation of cases as CA-MRSA. The quadratic like curves of the plots are similar. Each of the scatter dots represents a case, with those that do not fit the logistic regression model, also known as outliers, in the top left or top right corners of the plots. There are nine cases identified as HA-MRSA and eight identified as CA-MRSA with a change in deviance greater than one.

Reduced model. A reduced model was also produced using the backwards conditional method. I also created a multivariate binary logistic regression model using the backward conditional method to yield the combination of variables that best predicts

genotype, and therefore CA/HA status. I present the results in Table 24. This second approach was used to confirm the assignment of odds ratios in the block entry model. In each step 1-5, variables were removed from the original full model to yield the final reduced model. The susceptibility pattern method (MRSA_Susceptibility) had an EXP (B) of 15.549 (95% CI 6.292-38.424) and admission profile had an EXP (B) of 3.942 (95% CI 1.568-9.911).

Table 22

Odds Ratios of Variables included in the Reduced Models at Each Step

Step	Variable	B	EXP(B)	95% Confidence interval	
				Lower	Upper
1	MRSA_HCRF	.100	1.105	.310	3.836
	MRSA_IFRF	.488	1.630	.542	4.898
	MRSA_Susceptibility	2.739	15.474	5.995	39.938
	Gender	.549	1.731	.230	1.450
	Admission Profile	1.056	2.874	.764	10.815
	Pre-existing Illness	.149	1.161	.460	2.932
2	MRSA_IFRF	.486	1.625	.541	4.879
	MRSA_Susceptibility	2.740	15.480	5.997	39.959
	Gender	.570	1.768	.734	4.258
	Admission Profile	1.124	3.076	1.125	8.409
	Pre-existing Illness	.148	1.159	.458	2.931
3	MRSA_IFRF	.436	1.546	.539	4.440
	MRSA_Susceptibility	2.708	15.005	5.995	37.783
	Gender	.576	1.778	.740	4.274
	Admission Profile	1.113	3.004	1.116	9.069
4	MRSA_Susceptibility	2.671	14.460	5.813	35.975
	Gender	.615	1.850	.775	4.415
	Admission Profile	1.268	3.555	1.394	9.069
5	MRSA_Susceptibility	2.744	15.549	6.292	38.424
	Admission Profile	1.372	3.942	1.568	9.911

The ORs (EXP(B)) presented in this table demonstrate to what extent each of the independent variables are associated with the dependent variable. Note that the ORs and

their associated CI change with each iteration. These changes reflect the effect the removal of some of the independent variables from the model have on the observed associations. The magnitude of the OR reflects the direction and strength of the associations, while the lower and upper bounds of the CI suggest the significance. Susceptibility was found to be a statistically significant predictor of MRSA status in all models. This is in agreement with the conclusions drawn from the full model. Unlike the full model, however, Admission profile became significant only after the removal of Pre-existing illness. The final model (5) is the most parsimonious and the only one in which both independent variables have statistically significant ORs. I examined the changes in the predictive ability of the independent variables included in the model using Cox & Snell and Negelkerke R Squares. I also tested if this model can be applied to this population in the future to quickly and cheaply assess the HA/CA status of a new MRSA case without requiring the time-consuming and expensive procedure of genotyping, using the Hosmer and Lemeshow test to evaluate the final model fit after each step in the iterative process.

The results of the comparison of the Cox & Snell and Negelkerke R Squares calculated with each model iteration using the backwards conditional method are presented in Table 23. These values suggest that there is little difference in the models ability to predict HA or CA-MRSA. Though the R square associated with Model 5 is lower it is not low enough to justify including variables in the model which do not have statistically significant ORs, such as those included in models 3 and 4.

Table 23

Pseudo R Square Associated with Each Block

Step	Cox & Snell R Square	Nagelkerke R Square
1	.348	.464
2	.347	.464
3	.347	.463
4	.344	.459
5	.334	.446

Table 24 presents the results of the Hosmer and Lemeshow Test of Model Fit after the entry of each of steps 1 through 5. As with Table 21, these numbers reflect each model's ability to explain the variability of the logit associated with the designation of HA or CA-MRSA.

Table 24

Hosmer and Lemeshow Test of Model Fit

Step	Chi square	Degrees of freedom	Significance
1	3.688	7	.815
2	2.836	8	.944
3	2.967	8	.936
4	2.119	6	.908
5	.807	2	.668

The chi square statistic describes the model fit by comparing the expected outcomes based on the model inputs to the actual assignment of HA and CA-MRSA

using genotyping. All models are not statistically significant suggesting they fit the data. The models with more than two variables have higher chi square, but again the differences observed do not justify including non-significant independent variables in the model.

A scatter plot of the change in deviance by the predicted probability for the reduced model is presented in Figure 7.

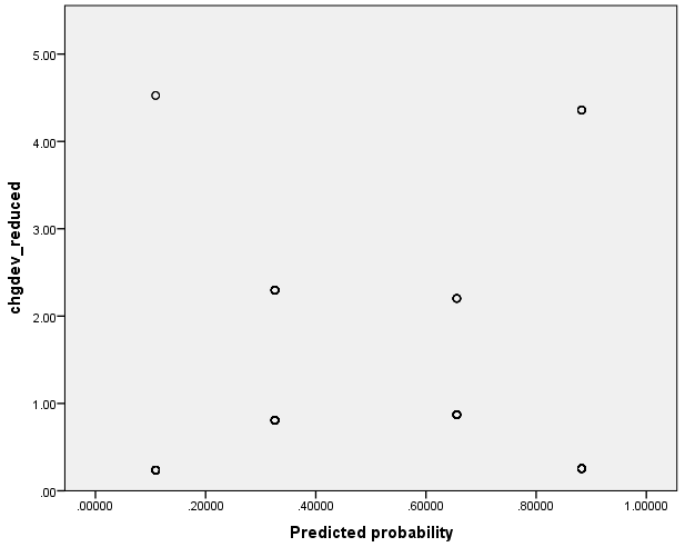

Figure 7. Scatter plot of the change in deviance and predictive probabilities in the reduced model

As with figure 6, the scatter plot of the change in deviance allowed me to identify cases that poorly fit by the model. These are considered outliers. Larger changes in deviance indicate poorer fits. The curve that extends from the lower left to the upper right represents the cases in which the dependent variable was assigned a value of 0. This corresponds to the genotyping designation of HA-MRSA. The curve that extends from

the lower right to the upper left represents the cases in which the dependent value was assigned a value of 1. This corresponds to the designation of cases as CA-MRSA. In this plot there are four outliers, two associated with the designation of HA-MRSA and two with the designation of CA-MRSA. Were there more outliers, further investigation might be warranted, but the existence of four outliers, two in each direction, in a dataset with 133 records is unlikely to have an effect on my conclusions (Newman, 2001).

Finally, the comparison of the results of the final full model to the final reduced model is presented in Table 25.

Table 25

Comparison of Full and Reduced Models

Model	Included variables	Odds ratios (p Value)	Nagelkerke R Square	Hosmer and Lemeshow test results (p Value)
Full	MRSA_HCRF	1.105 (.878)	.464	3.688 (.815)
	MRSA_IFRF	1.630 (.542)		
	MRSA_Susceptibility	15.474 (<.001)		
	Gender	1.731 (.242)		
	Admission Profile	2.874 (.008)		
	Pre-existing Illness	1.161 (.861)		
Reduced	MRSA_Susceptibility	15.549 (<.001)	.446	.807 (.668)
	Admission Profile	3.942 (.004)		

As demonstrated in Table 25, the full and the reduced models both suggest the same model may be most effective in accurately predicting HA and CA designations

based on genotyping using Multiplex PCR. Both the full and the reduced models confirm my previous conclusions that Susceptibility is the phenotyping method of choice. In both models, Susceptibility (OR~15.5, p <.001) is a significant predictor of HA or CA-MRSA, though in the full model it is the only statistically significant predictor, while in the reduced model Admission Profile (OR 3.94 p=.004) was statistically significant as well. In both models, the Nagelkerke R square is similar and suggests an acceptable degree of predictability. Finally, while the chi square is much higher in the full model, after adjustment for the degrees of freedom, those values are also similar. In the absence of significant differences in model fit and predictability, the reduced model is preferable as it requires fewer variables and therefore less data to complete.

Based on the results of the multiple logistic regression, I am able to reject the null hypothesis in favor of the alternative one associated with this research question. Demographic (Admission profile) and phenotyping (Susceptibility pattern) significantly predict MRSA genotyping classification.

Summary

Through the process of this study a verified MRSA phenotyping method supported by a genotyping 'gold standard' classification method for MRSA strains isolated in the Saudi Aramco community in the Eastern province of Saudi Arabia was established. Three commonly used phenotyping methods, health care risk factors, infection type (CDC, 2005), and susceptibility pattern (Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2005) were used to classify the

MRSA strains into CA-MRSA or HA-MRSA. I present a summary of my findings for Research Questions 1, 2, 3, and 4 in Table 26.

Table 26

Summary of Findings Research Questions 1, 2, 3, and 4

Research question	Statistical results	Conclusions
What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?	Health Care Risk Factor HA = 66.67% correct CA = 57.14% correct Infection Type HA = 76.19% correct CA = 37.14% correct Susceptibility Pattern HA = 77.78% correct CA = 78.57% correct	Susceptibility pattern phenotyping had the highest percent of correct designations when compared to genotyping.
What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection type, and susceptibility pattern, used to classify HA or CA-MRSA in Saudi Arabia?	Concordance = 22% Kendall's *tau* -.001 to .063 Cohen's *kappa* = .373 p<.001 HA and CA-MRSA and -.298 p < .001 overall	While the observed concordance between pairs based on percent concordant and Kendall's *tau* suggest little to no agreement between phenotyping methods, Cohen's *kappa* suggests fair agreement between phenotyping and genotyping.

continued

Table 26 *continued*

Research question	Statistical results	Conclusions
What is the sensitivity and specificity of each phenotyping method (health care risk factor, infection type, susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?	Health Care Risk Factor HA = 66.67% (sensitivity) CA = 57.14% (specificity) Infection Type HA = 76.19% (sensitivity) CA = 37.14% (specificity) Susceptibility Pattern HA = 77.78% (sensitivity) CA = 78.57% (specificity)	The calculation of sensitivity and specificity confirms the conclusions drawn in response to RQ 1. That is, Susceptibility has the highest sensitivity (ability to correctly predict HA) and the highest specificity (ability to correctly predict CA)
How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?	Full Model OR Susceptibility = 15.474 (p<.001) Nagelkerke R Square = .464 Hosmer and Lemeshow = 3.688 (p = .815) Reduced Model OR Susceptibility = 15.549 (p<.001) OR Admission Profile = 3.942 (= .004) Nagelkerke R Square = .446 Hosmer and Lemeshow = .807 (p = .668)	While both the full and reduced model were predictive of HA and CA-MRSA classification using genotyping and had a reasonably good fit, only in the reduced model was the OR associated with Admission Profile significant. The reduced model also has the advantage of requiring only two variables and thus increased ease of use. Based on both model results, I am able to reject the null hypothesis associated with this RQ.

As suggested in this table, all of my findings associated with the four research questions suggest that it is possible to predict the designation of HA or CA-MRSA resulting from genotyping using Susceptibility Pattern phenotyping. In the reduced multiple logistic regression model, I also identified Admission Profile as a significant predictor of genotyping results.

In Chapter 5, I summarize, analyze, and interpret key findings from these results and discuss whether they confirm, disconfirm, or extend existing knowledge per the literature review and the Ecological Theory that was the framework for this study. I also acknowledge and discuss the limitations of the study in terms of generalizability and/or trustworthiness, validity, and reliability. Recommendations for further research grounded in the strengths and limitations of the study and the literature reviewed in chapter 2 will be suggested, specifically as related to MRSA testing in Saudi Arabia within the Saudi Aramco community. In this context, implications for positive social change and recommendations for practice will be discussed, along with methodological, theoretical, and/or empirical implications. Finally, conclusions will be drawn to capture the key essence of the study.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The purpose of this quantitative, retrospective cohort study was to characterize the MRSA strains within the Saudi Aramco community and to classify them accurately as HA-MRSA or CA-MRSA by comparing and contrasting the three existing phenotyping methods against the gold standard Multiplex PCR method. My goal was to identify the phenotyping method with the highest sensitivity and specificity for use in this population. Thus, this research will contribute to identify a simple, less expensive, accurate, and feasible method for full MRSA assessment in Saudi Arabia.

In this chapter, I summarize, interpret, and discuss key research findings. My conclusions were drawn in the context of previous research and the theory that framed this research. In addition, I include recommendations for the application of the study findings and future research. Finally, I discuss the impact of my study results and its potential implications for positive social change.

Research Questions

The research questions I formed to address the purpose of my research were the following:

- Research question 1: What is the genotypic distribution of MRSA in a sub-population of Saudi Arabia's Eastern Province?
- Research Question 2: What is the concordance between each pair-wise combination of the three phenotyping methods, health care risk factor, infection

type, and susceptibility pattern, used to classify CA- vs HA-MRSA in Saudi Arabia?

- Research Question 3: What is the sensitivity and specificity of each phenotyping method (health care risk factors, infection type, and susceptibility pattern) used to classify CA-MRSA vs HA-MRSA in Saudi Arabia?
- Research Question 4: How well does a combination of demographic and phenotyping variables of the current three phenotyping methods (health care risk factors, infection type, and susceptibility pattern) predict MRSA genotyping classification as CA-MRSA or HA-MRSA?

  H_0: Demographic and phenotyping variables do not significantly predict MRSA genotyping classification.

  H_a: Demographic and phenotyping variables significantly predict MRSA genotyping classification.

Summary of Key Findings

The key findings from the study can be concisely summarized with respect to individual research questions. For Research Question 1, the key finding was that of the three phenotyping methods, the susceptibility pattern phenotyping method most closely resembled that obtained using the genotyping gold standard. For Research Question 2, the concordance analysis indicated that the susceptibility pattern method was the most discordant of the three phenotyping methods tested. For Research Question 3, the susceptibility pattern method emerged as the most sensitive and specific of the three phenotyping methods. Lastly, for Research Question 4, a reduced model comprising the

combination of susceptibility pattern phenotyping with hospital admission profile emerged as the most effective combination of the variables tested to accurately predict HA-MRSA and CA-MRSA, as defined by the genotyping gold standard. In the remainder of this section, these findings are interpreted and discussed in detail.

Interpretation and Discussion of Key Findings

The statistical analysis in response to Research Question 1 produced the distribution of HA-MRSA and CA-MRSA, as defined by the gold standard multiplex PCR genotyping method, by gender, age, hospital admission profile, survival, pre-existing illness and the three phenotyping methods, health care risk factors, infection type and susceptibility pattern. The distributions are presented in Table 9. Differences, though not tested for statistical significance, were observed in the distributions of all but the survival variable. The findings related to the three phenotyping methods are key to this research, as I am attempting to identify the phenotyping variables which best predict the MRSA profile obtained by genotyping. While the MRSA distribution with regards to each of the three phenotyping method variables was similar for HA-MRSA, the susceptibility pattern data differed from the other two variables for CA-MRSA. Only using the susceptibility pattern did I find a distribution similar to that expected based on the gold standard genotyping, with the majority of cases correctly identified as either HA-MRSA or CA-MRSA according to the comparison to the genotyping data. Neither the health care risk factors method nor the infection type method gave a distribution similar to the genotyping method. This has implications for any use of these methods for classification of MRSA in a Saudi Arabia health facility context, in particular for

designation as CA-MRSA. Genotyping methods based on DNA analysis allow for accurate and precise MRSA classification, due to markers that are less prone to change over time (Tenover et al., 1994). A recent genotyping study in Saudi Arabia has found that HA-MRSA resistance markers (e.g., aacA-aphD, aadD) are now common among CA-MRSA strains (Monecke et al., 2012). It would be expected that presence of these resistance markers in both HA and CA-MRSA would negatively impact on the accuracy of the susceptibility pattern method. Nevertheless, in this case the susceptibility pattern method emerged as the most similar to the genotyping method in the predicted distribution of CA-MRSA and HA-MRSA.

Worldwide experience of the spread of CA-MRSA into health care settings and the emergence of HA-MRSA in the community has challenged assignment of MRSA purely in terms of the health care risk and infection type phenotyping methods (Campanile et al., 2011; David et al, 2008a; Donnio et al., 2004; Hetem et al., 2012; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). The results in response to Research Question 1 in my study extend this pattern in that neither of these phenotyping methods give results consistent with those generated by genotyping. In a study conducted at Chicago hospitals, many MRSA infections in patients with health care risk factors had many features of CA-MRSA, including clindamycin-resistance, PVL positivity, and SCCmec IV (David et al., 2008a). Similarly, in the genotyping study carried out at King Fahad Medical City in Riyadh, there was a high prevalence of PVL-expressing strains, normally considered to be an indicator of CA-MRSA, in the health care setting (Monecke et al., 2012). The effect of the increasing unreliability of health

care risk factors as a predictor of HA-MRSA versus CA-MRSA is that CA-MRSA prevalence is underestimated. The health care risk factors and infection type methods have a higher tendency to identify HA-MRSA than the susceptibility pattern or genotyping methods (Sievert 2008). This is borne out in my results in response to Research Question 1, in which use of the infection type method actually resulted in the majority of CA-MRSA cases being identified as HA. One of the challenges to the infection type method comes from confirmation that skin and soft tissue infections (SSTI) are the most common types of infections for both CA-MRSA and HA-MRSA (David et al., 2008a). Meanwhile for the health care risk factors method, 30 out of 70 of those designated as CA-MRSA by genotyping were identified as HA by phenotyping. Several studies have shown that phenotyping markers are more prone to change over time than genotyping markers (Devita, Lawrence, & Rosenberg, 2009). Such a change is due to loss of extrachromosomal genetic elements and later their horizontal transmission (Devita et al., 2009) and can explain the discrepancy between the classification methods that depend on the phenotyping markers (Tenover et al., 1994). This discrepancy was more closely considered in Research Question 2.

Also notable in my analysis of the data for Research Question 1 was the difference in the distributions of age for HA-MRSA versus CA-MRSA as designated by genotyping, presented in Figure 3. Among cases identified as HA-MRSA, the age distribution is approximately bimodal with the highest frequencies observed under 20 and over 40 years of age (Figure 3). By contrast, for CA-MRSA, it is a single mode with the majority of cases occurring between 20 and 40, and a defined positive skew (Figure 3).

This type of distribution is largely consistent with the results of others, for example in the study by Huang et al. (2006) in California, in which CA-MRSA was most prevalent in those aged between 18 to 49 and HA-MRSA among those aged from 40 years up (Huang et al., 2006). In a study undertaken in Riyadh, Saudi Arabia, the most prevalent HA-MRSA strain identified, CC8/ST239-III, was also found more commonly in older than average patients, with a mean age of 43 years (Monecke et al., 2012).

My results on distribution of HA-MRSA and CA-MRSA with respect to genotyping data are consistent with studies of others on phenotyping and genotyping concordance, which suggested that antibiotic susceptibility pattern (Clinical Laboratory Standards Institute (CLSI), 2005) may be particularly helpful in classification, especially given the increasingly questioned predictability of health care risk. (Campanile et al., 2011; David et al, 2008a; Donnio et al, 2004; Hetem et al., 2012; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). I considered concordance specifically in response to Research Question 2.

In Research Question 2, I further explored the issue of agreement or disagreement between the phenotyping methods, using a concordance and a discordance matrix. These matrices are presented in Tables 14 and 15 respectively. As described in Chapter 4, the results of the concordance analysis indicated that the susceptibility pattern method gives a different classification profile compared to the other two methods, being the most discordant of the three methods. This is compatible with the findings generated in response to Research Question 1, which indicated that the susceptibility pattern method gives a distribution that is closer than the other two phenotyping methods to that obtained

by genotyping. It is also consistent with the results of a study conducted at the Michigan Department of Community Health in the United States, which showed that the health care risk factors and infection type methods were more concordant with each other than either are with the susceptibility pattern method (Sievert et al., 2010). Each of the three classification methods is inconsistent with the other two, for a number of cases. One important reason that contributes to discordance between methods is the evolving and increased rate of invasive CA-MRSA strains (Seybold et al., 2006). Emergence of the health-care-associated community-onset (HACO) group due to the interchange of genetic lineages of MRSA among community and hospital niches, and the evolution of multi drug resistant CA-MRSA strains which can invade hospitals, also affects the distribution of MRSA clones (David et al., 2006; Hudson et al., 2013).

When agreement between methods was formally tested by pairwise Cohen's kappa analysis, presented in Tables 16 and 17, the results confirmed that there was higher agreement between the health care risk and infection type methods than between either of those and the susceptibility pattern method. My Cohen's kappa data confirms that the susceptibility pattern method diverges from the other two methods and that as a result, overall agreement between the three methods is low. The susceptibility pattern method may be divergent from the other two methods either because the rate of multidrug resistant CA-MRSA is significantly increased within the Johns Hopkins Saudi Aramco Health Centre compared to the rate of invasive CA-MRSA, or due to the emergence of invasive CA-MRSA as a nosocomial infection (Hudson et al., 2013). This would be isolated after 48 hours from deep sites, giving it the characteristics of HA-MRSA and

explaining the ‘false’ higher concordance between the health care risk factors method and infection type method. This discordance of the susceptibility pattern method was consistent with what I expected given my results from Research Question 1, in which of the three phenotyping methods only the susceptibility pattern yielded a MRSA distribution pattern similar to that observed for the genotyping method, and the results of others also suggesting that the health care risk factors and infection type methods are more concordant with each other than either are with the susceptibility pattern method (Sievert et al., 2010). My results are also consistent with other studies showing concordance between antibiotic susceptibility phenotyping and genotyping and/or poor predictability of the health care risk factors method (Campanile et al., 2011; David et al, 2008a.; Donnio et al, 2004; Hetem et al., 2012; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). In a study where genotype of MRSA was predicted using a fluoroquinolone susceptibility test, the results of antibiogram and PFGE were significantly correlated (Chomvarin et al., 2005). Concordance between the Gene Xpert multiplex PCR genotyping method and phenotyping by antibiotic susceptibility using the disk diffusion method has also been shown (Biendo et al., 2013). My results also indicate concordance between the susceptibility pattern phenotyping methods and genotyping as carried out by multiplex PCR.

In response to Research Question 3, I considered the sensitivity and specificity of each of the three phenotyping methods. Generally speaking, less invasive screening tools need to have higher sensitivity, i.e. the ability to identify all potential cases. In the context of my study, however, sensitivity represents the ability to correctly identify a MRSA case

as HA-MRSA using a given phenotyping method, if it is indeed HA-MRSA as defined per the genotyping gold standard. Specificity, meanwhile, is the ability to correctly identify a MRSA case as CA-MRSA using a given phenotyping method, if they are indeed CA-MRSA as defined by the genotyping gold standard. Since the goal of my study was to identify a phenotyping method that could be used to accurately classify MRSA cases as HA-MRSA or CA-MRSA, I am looking for methods that have both a high sensitivity and a high specificity.

My tests of the sensitivity and specificity of the three phenotyping methods in response to Research Question 3 indicated that all three phenotyping methods had similar ability to identify HA-MRSA Using the infection type method, however, made it significantly less likely than for the other two that CA-MRSA would be identified correctly. This is compatible with the results of Research Question 1, in which only 26 out of 70 (37.14%) cases identified as CA-MRSA by genotyping were designated CA-MRSA by infection type phenotyping. I found that the susceptibility pattern method is, by contrast, significantly more likely to correctly identify CA-MRSA, compared to the health care risk method sensitivity. This is what I would have expected given the data from Research Question 1, showing that 55 out of 70 (78.57%) cases designated as CA-MRSA by genotyping were also designated as CA by susceptibility pattern phenotyping. It is also consistent with the concordance data from Research Question 2, suggesting that the susceptibility pattern method differs significantly from the other two methods in its ability to classify either HA or CA correctly per the gold standard. Thus, the results from Research Question 3 added another element to the emerging consensus that the

susceptibility pattern method is the most effective of the three phenotyping methods in the context of this study, as it has higher specificity and is therefore less likely to misclassify CA-MRSA cases. This finding suggests that the rate of multidrug resistant CA-MRSA is low, however the rate of invasive CA-MRSA is increased within the Johns Hopkins Saudi Aramco Health Centre population, impacting negatively on the health care risk factors method and the infection type method sensitivity and specificity. Currently, the most commonly used phenotyping method used in Saudi Arabia for MRSA classification is the health care risk factors method. However, the selection of phenotyping method to classify MRSA and guide infection control depends on methods validated for the USA hospitals (Bukharie, 2010). This is despite the fact that prevalence and incidence of HA-MRSA versus CA-MRSA varies widely between countries (Maree et al., 2007; Pan et al, 2003 & 2005; Labandeira-Rey et al., 2007; Song et al., 2011; Stefani et al., 2011; Tietz, Frei, & Widmer, 2005). Consistent with international experience, previous studies in Saudi Arabia have shown that prevalence of CA-MRSA is increasing, while the lines between 'HA-MRSA' and 'CA-MRSA' are blurring in terms of health care risk factors (Al-Tawfiq, 2006; Baddour et al., 2007; Bukharie, 2010; David et al., 2008; Monecke et al; 2102; Moussa & Shibl, 2009; Stefani et al;, 2012). All these factors suggest that the health care risk method is not the most 'fit for purpose' in the Saudi Arabian context; my results add to the evidence that this is the case. The results generated in my study in response to Research Question 3 confirm that the health care risk factors method does not have high specificity in terms of identifying CA-MRSA. Also the MRSA profile generated from the health care risk factors method from research

question 1 showed that only 40 out of 70 (57.14%) of those designated as CA-MRSA by genotyping were also identified as CA by phenotyping. This was highlighted in Figure 4, which is a graphical representation of the correctly identified HA-MRSA versus CA-MRSA distribution generated for each phenotyping method, compared to the genotyping results. To further consider the ability of each of the three phenotyping methods to correctly identify a MRSA case as CA-MRSA, I produced a ROC curve, presented in Figure 5, which clearly shows that the susceptibility method is more sensitive than either of the other two phenotyping methods and therefore has more utility in correctly identifying a MRSA case as CA-MRSA. This is again compatible with the findings from Research Question 1 and 2.

The multivariate binary logistic regression analyses in response to Research Question 4 were also consistent with my other findings for the previous three research questions. In this case, both the block method and the backward conditional method showed that the susceptibility pattern phenotyping variable was significantly more predictive than either of the other two phenotyping methods. By contrast, for both the health care risk factor and the infection type phenotyping methods, the 95% CI include an OR of 1, suggesting that when results of either of these methods are coded '1' for HA-MRSA, it is no more likely that the genotyping method will also give a HA rather than a CA result, or when it is coded '0' for CA-MRSA, it is again no more likely that the genotyping method will also give a CA rather than a HA result.

In using the block entry multivariate binary logistic regression method, I tested the strength of each block using both the Cox & Snell and the Negellkerke R Squares

(Table 20). It emerged that the largest change for each Pseudo R Square test occurred after the addition of block three, which is the antibiotic susceptibility method. This again confirmed the utility of using this phenotyping method to accurately classify MRSA according to a genotyping gold standard as opposed to either of the other two phenotyping methods. Further confirmation was obtained when I used chi square in the Homer and Lemeshow Test of Model Fit to compare the results expected based on the model to those actually observed (Table 21) indicating that both the block containing antibiotic susceptibility pattern and the block containing the variables gender, admission profile and pre-existing illness (Table 18) could contribute to a useful model for prediction of MRSA type.

This was clarified when I used a reduced model by the backwards conditional method. Of the variables included in the reduced model, only admission profile had an OR whose 95% CI did not include 1. The results of the comparison of the Cox & Snell and Negelkerke R Squares calculated with each model iteration using the backwards conditional method show that neither of these Pseudo R Square tests change significantly through each iteration, as might be expected as the most predictive variables, i.e. susceptibility pattern phenotyping and admission profile, remain in each iteration.

The data I generated in response to Research Question 4 identified a reduced model, using the variables susceptibility method phenotyping and admission profile, as effective in prediction of HA-MRSA and CA-MRSA as defined by a genotyping gold standard. The theoretical basis of this study relied on both ecological theory and ASP theory. The ecological theory would favor HA-MRSA in a health care environment,

where the use of antibiotics is common. HA-MRSA usually carries either SSCmec II or III, which have acquired genes for resistance to antibiotic classes beyond the β-lactams (Hiramatsu et al., 2002). CA-MRSA, on the other hand, tends to carry SSCmec IV and V, which are relatively small, leaving it potentially susceptible to clindamycin and other non-β-lactam antibiotics (Naimi et al., 2003). The emergence from Research Question 4 of the antibiotic susceptibility method and hospital admission profile as strong predictors for the classification of HA-MRSA versus CA-MRSA is therefore consistent with ecological theory. However, caution is always needed, as the classification of 'HA-MRSA' and 'CA-MRSA' has been challenged by studies showing that MRSA isolates characterized as containing SCCmec IV and PVL, thus genotypically 'CA-MRSA', are arising in the health care environment (David et al, 2008a). For example in Saudi Arabia, a case has been reported in which a CA-MRSA strain was transmitted directly from a father to his child in a neonatal intensive care unit (Al-Tawfiq, 2006). A genotypic analysis of 107 MRSA isolates from King Fahad Medical City in Riyadh, Saudia Arabia also revealed a high prevalence of PVL-expressing strains, normally considered to be an indicator of CA-MRSA (Monecke et al., 2012). Thus, the relatively limited information available on MRSA profiles in Saudi Arabia health facilities is consistent with international experience of crossover of CA-MRSA strains into hospitals and other health care settings (David et al, 2008a; Monecke et al., 2012). The combination of the antibiotic susceptibility with the admission profile, as proposed from my results in response to Research Question 4, should be effective to help avoid erroneous classification of CA-MRSA in health care settings as HA-MRSA. The high specificity

identified for the antibiotic susceptibility method in Research Question 3 is reassuring in this context.

By contrast, in the multivariate logistic regression analysis in Research Question 4, the health care risk factors phenotyping did not emerge as predictive of genotyping classification results. Genotyping methods such as multiplex PCR are highly reliable in classifying MRSA (Stefani et al., 2012). It is therefore important to identify reliable phenotyping criteria that have been independently confirmed with genotyping results. The results for Research Question 3 and 4 of my study, demonstrating the concordance and predictive value of the antibiotic susceptibility pattern method for genotyping classification, are consistent with other studies showing concordance between antibiotic susceptibility phenotyping and genotyping and/or poor predictability of the health care risk factors method (Campanile et al., 2011; David et al, 2008a.; Donnio et al, 2004; Hetem et al., 2012; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011; Song et al., 2011). In East Asia, there is evidence of spread of 'HA-MRSA' and 'CA-MRSA' from the community to the hospital and vice versa (Song et al., 2011). In the current study, the suitability of the three phenotyping methods in the Saudi Aramco community from the Eastern province of Saudi Arabia, was considered in comparison to a 'gold standard' genotyping multiplex PCR method. Genotyping by this method took into account some of the mobile genetic elements of *Staphylococcus aureus* that have driven the emergence of MRSA, i.e. PVL and mecA, which are key to how HA-MRSA and CA-MRSA fit into both the ecological and antibiotic selection pressure framework. Thus, the

concordance of the susceptibility method with the multiplex PCR genotyping method further confirmed its consistency within the theoretical framework of the study.

While based on the conservative definition of HA-MRSA, CDC estimates suggest that up to 85% of MRSA infections would be classified as HA-MRSA (CDC, 2005), the genotyping results of my study suggest a much more even distribution. Results of previous studies in MRSA assignment in Saudi Arabia have shown that community-acquired infections comprised 62% (Al-Tawfiq, 2006). The proportion of CA-MRSA dramatically increased from 41.7% in 1999 to 66.6% in 2002 (Bukharie, 2010a, p. 379). In my study, genotyping analysis in the Saudi Aramco sub-population indicated that CA-MRSA lies somewhere in between these figures at 70 out of a total of 113 (52.6%) of MRSA infections (Table 9). For men, the percentage is lower, as 28 out of the total 69 men (40.6%) were identified as having CA-MRSA, while for women it is higher as 42 out of the total of 64 women (65.6%) were identified as having CA-MRSA (Table 9). This is not entirely consistent with previous studies in which the proportion of males with CA-MRSA tends to be significantly higher, particularly in urban areas, due for example to factors such as their greater likelihood of engaging in contact sports (Cooke & Brown, 2010; Davis et al., 2007; Hidron et al., 2009; Thomas et al., 2007). While gender did not emerge as a predictive variable in the logistic regression analysis, this data suggests that gender may yet be relevant in CA-MRSA and HA-MRSA distribution; consideration of larger groups of patients over time should clarify the point.

A combination of antibiotic susceptibility pattern and hospital admission profile emerged as the suggested model for phenotyping classification of MRSA in the Saudi

Aramco population. This finding is consistent with both the theoretical framework presented and the literature reviewed in chapters 1 and 2.

Limitations of findings

The study was limited due to the ever-changing molecular characteristics of MRSA from geographical and temporal perspectives, particularly the evolutionary success of CA-MRSA strains that makes their growth rates 1.33 faster than HA-MRSA strains (D'Agata, Webb, Horn, Moellering & Ruan, 2009). Therefore, the profile generated for samples collected in 2012 and 2013 may not be the same as the profile that exists currently. However, the concordance of the proposed phenotyping characterization based on prediction of genotyping results should minimize the impact of this limitation.

Phenotyping by any of the recognized common methods is complicated by factors including emergence of invasive and multidrug resistant CA-MRSA strains in health care settings as a cause of health care associated infection (Gillet et al, 2010; Seybold et al, 2006), as observed both in the USA (David et al, 2008a; Maree et al., 2007; Nair et al., 2011; Otter & French, 2011) and in Europe (Campanile et al., 2011; Donnio et al., 2004; Hetem et al., 2012) and in Saudi Arabia itself (Monecke et al., 2012), and also increased circulation of HA-MRSA in the community (Miller et al, 2007; Seybold et al, 2006).

This study was limited to patients attending the Aramco Dhahran Health Center, an acute-care hospital in Dharan, in the Eastern Province of Saudi Arabia. While these patients were a representative sample of the study population, i.e. approximately 350,000 people who are employees, dependents or annuitants of the Saudi Aramco energy

corporation, which is headquartered in Dhahran (Saudi Aramco, 2014), they may not be representative of Saudi Arabia as a whole.

Recommendations

As this study was confined to a sub-population and results may not necessarily be representative of the country as a whole, future studies should focus on a nationally representative sample of hospitals and other health care facilities in Saudi Arabia. In addition, similar studies comparing phenotypic methods of classifying MRSA as HA-MRSA or CA-MRSA to a gold standard genotyping technique should be performed in hospitals and other facilities, such as long-term care facilities, in all regions of the country. In this way, locally, regionally, and nationally applicable sets of phenotyping variables could be adopted appropriate to urban versus rural areas or different types of health care settings (Stefani et al., 2012)..

Saudi Arabia currently lacks a MRSA national control program. If appropriate phenotyping variables verified against gold standard genotype classification were identified in institutions across the country, it would inform potential implementation of the expert advice. This suggests that within countries there should be three levels of typing laboratories at local, regional, and national levels (Stefani et al., 2012). These laboratories should play different roles and be set up to perform typing techniques within their scope with regards to function and available resources (Stefani et al., 2012). This type of practical and reliable classification scheme, adapted to be applicable to Saudi Arabia rather than imported from other countries, will assist in beginning to harmonize surveillance and treatment programs.

In this study, hospital admission profile emerged as a predictive variable in determining MRSA status. This finding should be further examined in the context of other health care facilities to determine if the result was unique to the Aramco Dhahran Health Center or if it is a general phenomenon in other hospitals in Saudi Arabia. Likewise, the general applicability of the antibiotic susceptibility method of phenotyping in predicting MRSA status needs to be determined.

Long term studies of the susceptibility pattern and hospital admission profile should be performed in the Aramco Dhahran Health Center and periodically checked against the Multiplex PCR screening method, to confirm the long-term robustness of the these two variables in accurately predicting MRSA status. This is of particular importance in view of the constant evolution of CA-MRSA antibiotic resistance profiles, which might also complicate findings from the susceptibility pattern classification of MRSA. These concerns are even more relevant given the rise of CA-MRSA in Saudi Arabia (Bukharie, 2010a, p. 379).

In this study, the genotyping analysis in the Saudi Aramco sub-population for men suggested that the majority of MRSA infections were HA-MRSA; 28 out of the total 69 men (40.6%) were identified as having CA-MRSA (Table 9). For women the opposite was the case; 42 out of the total of 64 women (65.6%) were identified as having CA-MRSA (Table 9). While gender did not emerge as a predictive phenotyping variable in the logistic regression analysis, the difference in gender distribution of HA-MRSA and CA-MRSA in my data suggests that gender may be relevant in CA-MRSA and HA-MRSA distribution in Saudi Arabia. Expansion of studies to cover larger areas of the

country, with larger numbers of people and more institutions included, should clarify whether this type of distribution is consistently observed and has any statistical significance. This possibility should be pursued in any future studies covering other centers and/or data collected over a longer timeframe.

In terms of implications for practice, the underlying reason for this research study was the development and testing of a rapid, efficient, inexpensive, and reliable method of identification of HA-MRSA and CA-MRSA strains circulating in the region. From the results of the study, I recommend that a phenotyping method based on antibiotic susceptibility pattern and hospital admission profile be tested with a view to replacing the existing health care risk factors phenotyping that is currently most common, if this new method proves to be robust in the longer term. Results of the concordance analysis suggest, specificity analysis and multivariate logistic regression suggest that the health care risks factor and infection type methods should be discarded as phenotyping methods in this population. It is also important to consider that the theory of antibiotics pressure is useful in explaining why some CA-MRSA strains are becoming resistant to different groups of antibiotics, which might also interfere with the susceptibility pattern classification of MRSA. The minor discordance between the suggested combination of antibiotic susceptibility profiling and hospital admission profile for MRSA phenotyping, compared to the genotyping analysis, suggests that it would be of benefit to carry out genotyping analysis every two or three years to compare to and verify the new proposed phenotyping method.

Implications for Social Change

The goal of MRSA management programs at any health care facility is the reduction of spread of the disease and hence reduction of the burden of high medical costs and reversal of the increasing trend of MRSA morbidity and mortality. The results of this study have identified phenotyping variables that can be applied to distinguish between HA-MRSA and CA-MRSA and hence gauge distribution for each MRSA type, seasonality, and potential shifts in therapeutic drug resistance. This should help in the formulation of prevention strategies to reduce MRSA transmission and help in understanding and preventing MRSA outbreaks through comparative analyses of data from previous studies worldwide. Such comparative analyses will also advance knowledge on genetic, epidemiological, and clinical characteristics.

The results of my study have implications for positive social change in that antibiotic susceptibility pattern and hospital admission profile have been identified as variables that could form the basis of an MRSA screening program for the Saudi Aramco population. This has the potential to be extended to the Eastern Province of Saudi Arabia, where the Aramco Dhahran Health Center is situated. A reliable and affordable MRSA screening program based on concordance of phenotyping classification with a genotyping gold standard would provide a rational basis for MRSA surveillance and characterization and effective targeting of therapies, as well as education of health workers, in Saudi Arabia. Extending the findings of this study to other health care facilities throughout the country would potentially contribute to the development of such a screening program.

Conclusions

The purpose of this study was to identify which of the three phenotyping methods, health care risk factors, infection type and antibiotic susceptibility pattern, or combination thereof, was best able to characterize the MRSA strains with reference to the profile generated using a Multiplex PCR genotyping method, within the Saudi Aramco community. An additional goal was to help identify the phenotyping variables which were most predictive of genotype and provide an accurate method for development of an effective screening, prevention, control, and treatment program in this population. Accurate phenotyping and the ability to rapidly and efficiently assign MRSA cases to HA-MRSA or CA-MRSA is vital for monitoring trends in MRSA within health care settings and in the community and making choices of appropriate antibiotic treatment, outbreak monitoring, and prediction or recognition of epidemics.

MRSA is on the rise. I carried out the study against a background of rising levels of MRSA in Saudi Arabia, bringing a high financial costs in morbidity and mortality burdens (Bukharie, 2010). The results of my study suggest a potential phenotyping-based method, built on antibiotic susceptibility pattern and hospital admission, with which MRSA could be accurately identified as HA-MRSA and CA-MRSA. As these have different genetic properties which confer different antibiotic sensitivities and virulence properties, the availability of such a method would assist in effective diagnosis and tailoring of therapy. For an effective screening and surveillance program and effective therapy it is vital that a reliable and affordable screening method suited to local

conditions is available. My study lays a foundation on which such a screening method could be built.

Epidemiology of MRSA varies widely between countries (Maree et al., 2007; Pan et al, 2003 & 2005; Labandeira-Rey et al., 2007; Song et al., 2011; Stefani et al., 2011; Tietz, Frei, & Widmer, 2005). The current screening practices in Saudi Arabia depends on the use of phenotyping methods validated for hospitals in the United States (Bukharie, 2010). Thus, the health care risk factor phenotyping method is the most commonly used in the Johns Hopkins Aramco Hospital. However, no unified method has been adopted and the recognized discrepancies in MRSA classification between phenotyping methods have not been taken into consideration (Devita, Lawrence, & Rosenberg, 2009; Sievert, 2008).

The results of my study show that the health care risk factors method is unsuited to the correct classification of MRSA within this community, particularly in regard to CA-MRSA identification. According to the results generated in response to my Research Question 1, only two thirds of the cases designated as CA-MRSA by genotyping were also identified as CA by health care risk factors phenotyping. The total was even lower for infection type phenotyping, for which only 37.14% of cases designated as CA by genotyping were also identified as CA by infection type phenotyping. By contrast, most of the cases designated CA by genotyping were also recognized as CA by susceptibility pattern phenotyping.

The results generated in response to my Research Question 2 confirmed that the susceptibility pattern method was the most discordant of the three methods. Consistent

with these results for Research Question 1 and 2, the data I generated in response to Research Question 3 confirmed that the susceptibility pattern method was significantly more specific for identification of CA-MRSA than either of the other two phenotyping methods. Of several variables tested in this study, I confirmed in response to my Research Question 4 that the antibiotic susceptibility pattern phenotyping method, in combination with hospital admission profile, is the most predictive of MRSA classification obtained by Multiplex PCR genotyping in a sample of the Saudi Aramco population.

My study has fulfilled the original purpose of characterizing the MRSA strains within the Saudi Aramco community and accurately identifying MRSA strains as HA-MRSA or CA-MRSA by comparing and contrasting the three existing phenotyping methods against a gold standard Multiplex PCR method. It has also fulfilled the goal of helping to identify the phenotyping variables which were most predictive of genotype and provide an accurate method for development of an effective screening, prevention, control, and treatment program in this population. It has led to the identification of the antibiotic susceptibility pattern phenotyping method in combination with hospital admission profile as the potential basis of a reliable, inexpensive, and readily usable phenotyping classification method, validated against a genotyping gold standard, for determination of distribution of HA-MRSA and CA-MRSA in the Saudi Aramco community. Based on my study results, I therefore recommend that a phenotyping method based on antibiotic susceptibility pattern and hospital admission profile should be tested in this community with a view to replacing the existing health care risk factors

phenotyping that is currently most common, if this new method proves to be robust in the longer term. Furthermore, I recommend that similar studies should be carried out in other communities and settings across Saudi Arabia with a view to establishing a national phenotyping program underpinned by comparison of phenotyping data to a genotyping gold standard.

The study facilitated a more comprehensive understanding of epidemiological characteristics of MRSA in Saudi Arabia. The standardized phenotyping technique identified in this study to classify MRSA infections as either HA-MRSA or CA-MRSA will provide new evidence to facilitate targeting of control efforts and preventive methods, which will better contend with this adept and evolving bacterial organism.

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Appendix A: Multiplex PCR Methodology

The primers for the amplification of the mec-A gene and pvl gene were MECAP4 (5'-TCCAGATTACAACTTCACCAGG-3') and MECAP7 (5'- CCACTTCATATC TTGTAACG-3'), as described by Oliveria et al. (2002), and luk-PV-1 (5'- ATCATTAGGTAAAA TGTCTGGACATGATCCA-3') and luk-PV-2 (5'- GCATCAA GTGTATTGGATAGCAAAAGC-3'), as described by McLure et al. (2006), respectively.

PCR was performed using a Qiagen Multiplex PCR kit (Hilden, Germany) with the following slight modifications. A 25-µl final reaction volume consisted of 12.5 µl mastermix, 2.5 µl primer mix (0.2 µM of each primer), 3 µl DNA template and 7 µl RNase free water. DNA samples were subjected to thermocycling conditions with an initial inactivation step (95o C, 15 min), 35 repetitions of a three-step cycle of denaturation (94o C, 30 sec), annealing (60o C, 90 sec) and extension (72o C, 90 sec) with a different final extension (72o C, 10 min) and a step that involved soaking at 4oC. After these steps, 5 µl of amplified products were mixed with 2 µl of ethidium bromide (Fermentas, St. Leon-Rot, Germany) and loaded on a 2% agarose gel (Amresco, Solon, USA) along with GeneRuler TM 100 bp Plus DNA Ladder (Fermentas, St. Leon-Rot, Germany); electrophoresis was performed at 100 volts for 50-60 min and visualized under a UV transilluminator (Bio-Doc analyzer, Biometra, Goettingen, Germany).