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Molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA)

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MOLECULAR EPIDEMIOLOGY OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

by

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ABSTRACT

Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

by

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Staphylococcus aureus is a frequent cause of a myriad of infections in hospitals, healthcare facilities and community settings. It is often related to skin and soft tissue infection, but it can also cause other diseases varying from food poisoning to endocarditis and toxic shock syndrome. According to a study by Kuehnert et al. (2006) there has been a strong association between *Staphylococcus aureus (S. aureus)* carriage and risk factors for subsequent infections. Subsequent infection from *S. aureus*, including methicillin-resistant *S. aureus* (MRSA) infection, can be projected by looking at the presence of *S. aureus* nasal colonization. The purpose of this study was to determine the prevalence of MRSA among adult patients and assess the antimicrobial susceptibility pattern of isolated samples. Specimens were collected from a Las Vegas county hospital during a four-month period from September 2008 through December 2008. Patient demographics and nasal swabs were collected upon admission from screened patients regarded to be at high risk for MRSA colonization. Antimicrobial-susceptibility testing and strain typing were performed. A total of 100 MRSA samples were processed for this study. Among the

MRSA positive samples studied, the average patient age was 58 years, predominantly in Whites (64%) and mostly in male (69%). All isolates showed susceptibility to quinipristin/dalfopristin and vancomycin, 99% to rifampin, 96% to linezolid, and 83% to tetracycline and trimetoprim-sulfamethoxazole. Predominant strain types were USA100 USA300, Brazilian and Iberian. Increased assessment of the population dynamics of MRSA colonization can assist in determining the extent of MRSA prevalence, aid appropriate treatment, identify interventions, and prevent transmission in the population.

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CHAPTER 1

INTRODUCTION

Prior to the development of antibiotics, infection with *Staphylococcus aureus* was often fatal; now *Staphylococcus aureus* usually responds to numerous antimicrobial agents (Kuehnert *et al*, 2006). However, as the number of multidrug-resistant strains of *S. aureus* continues to increase, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), treatment of this infection has been challenging. Recent 2005 nationwide estimates of MRSA invasive disease involved 94,360 cases and 18,650 deaths (Klevens *et al.*, 2007).

Staphylococcus aureus is a gram positive coccus bacterium that is usually carried on the skin and the nose of healthy people. *S. aureus* infection frequently involves the skin and soft tissue but it can also cause other life-threatening systemic illnesses including endocarditis, meningitis, and toxic shock syndrome. Treatment of early infections usually involves incising and draining the lesion supplemented by beta-lactam antibiotic drugs (McCaig *et al.*, 2006).

Background

S. aureus has numerous properties that are believed to play a part in its ability to cause disease (Koneman *et al.*, 1997). Some strains produce an exopolysacharide capsule or slime layer that prevents it from being consumed by the body's immune system. Its

cell wall contains teichoic acids that help it adhere to mucosal surfaces. Enzymes such as hyaluronidase and lipases that lyse the intracellular matrix in tissues allowing it to spread and digest the host cell membranes. *S. aureus* also secretes leucocidins, such as Panton-Valentine Leukocidin (PVL), an exotoxin that creates holes in white blood cell membranes causing them to swell and lyse. The presence of PVL gene has been used as a marker for community-associated MRSA. Another toxin involved in *S. aurues* virulence is toxic shock syndrome toxin (TSST) which causes the release of numerous cell signaling complexes resulting in organ failure, hypovolemic shock, or tissue hypoxia which can lead to rapid death (Koneman *et al.*, 1997).

The first reported case of MRSA emerged from the United Kingdom in 1961 (Huang *et al.*, 2006), only a year after the introduction of methicillin. It has since spread to Europe, Japan, and Australia. In 1968, the first case of MRSA was first reported in the United States (Huang el al, 2006). In 1972, only two percent of *S. aureus* were drug resistant. By 1995 the percentage rose to 22%, and in 2004 the rate rose to 63% (Klevens *et al.,* 2005).

Antimicrobial resistance takes place when bacteria change or adapt in a way that allows them to survive in the presence of antibiotics designed to kill them. Once strains of bacteria surpass the deadly effects of antibiotics, they will probably pass on the genes for resistance to other bacteria. The mechanism of resistance to methicillin was discovered with the identification of reduced-affinity penicillin-binding protein (PBP) in MRSA (Deresinski, 2005). Normal PBP helps stop the synthesis of bacterial cell walls; however, the altered PBP (PBP2A) in MRSA is blocked. This allows continued bacterial multiplication. PBP2A is encoded by the *mecA* gene which is in the genomic site of the

staphylococcal cassette chromosome mec (SCCmec). SCCmec has been discovered to contain other genetic elements which encode for resistance to methicillin (Deresinski, 2005).

MRSA infections occur more often among those who have had an invasive medical procedure, or who have weakened immune systems, or are being treated in hospitals and health care facilities (MRSA in Healthcare Settings, 2007). Healthcare related MRSA infections typically are serious surgical wound infections, blood stream infections, and pneumonia. Currently, MRSA infection has been increasingly found outside healthcare facilities, infecting otherwise healthy individuals in the community. Community related infections are less severe, usually appearing as pimples and boils (Klevens *et al.*, 2007).

Hospital-Associated MRSA

Today, MRSA has been categorized as either hospital-associated (HA-MRSA) or community-associated (CA-MRSA). Hospital-associated 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 mild intermittent abscesses to life threatening systemic diseases. In addition these isolates are also typically resistant to multiple, non beta-lactam antibiotics (Fey *et al.*, 2003).

The predominant pulse-field electrophoresis strain types seen among HA-MRSA are USA100, USA200, USA600, USA700, USA800 and less often USA500 (Klevens *et al.*, 2007; Buck *et al.*, 2005; 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. Pulse-field gel electrophoresis (PFGE) genotyping aids in determining bacterial isolate identification by acting as DNA "fingerprinting." In epidemiology, genotyping is used to help in tracking the spread of infections, monitor trends in types, and track seasonal outbreaks (Healy *et al*, 2005).

Community-Associated MRSA

Community-associated MRSA has been described in patients without the established healthcare 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 (McCaig *et al.*, 2006; Kazakova *et al.*, 2005; Zinderman, 2004). This organism usually presents as skin and soft tissue infections. CA-MRSA isolates are likely to be resistant to fewer antibiotics, produce different toxins, and have genetically distinct gene complexes 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 *et al.*, 2007). Strains most frequently isolated from MRSA infection of community origin include PFGE USA300, USA400, USA1000, and USA1100 (Klevens *et al.*, 2007).

Epidemic MRSA

MRSA is also a leading cause of nosocomial infection worldwide. Molecular epidemiology studies indicate that the massive geographic spread of MRSA resulted from the dissemination of relatively few highly epidemic clones. Five major lineage have been

defined which were mainly disseminated in southern and eastern Europe, Latin America, and the USA (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 indicate 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).

Significance

According to a nationwide study done by Kuehnert *et al.* (2005) there were 126,000 estimated hospitalizations annually related to MRSA. Serious MRSA infections occur in approximately 94,000 individuals annually; leading to 19,000 deaths (Klevens *et al.,* 2007). Healthcare-associated MRSA accounts for 86% of these cases and 14% are community-associated.

It is estimated that 25% to 30% of the population is colonized in the nose with *S. aureus* (Koneman *et al.*, 1997). Colonization occur when bacteria are present, but not causing an infection. Infection occurs when the bacteria is present, and is causing illness. While *S. aureus* colonize the nose of 25% to 30% of the population, MRSA exists in approximately 1.5% of the population (Tenover *et al.*, 2008). Colonization with *S. aureus*, particularly the nares, has been strongly associated as a risk factor for subsequent *S. aureus* infection (Kuehnert *et al.*, 2006; Tenover *et al.*, 2008). Subsequent infection from *S. aureus*, including MRSA infection, can be projected by looking at the presence of *S. aureus* nasal colonization.

Data was collected on healthy, non-institutionalized (i.e. not in hospitals, long-term care facilities, or prisons) adults from 2001-2004 as part of the NHANES (National Health and Nutrition Examination Survey) a survey research program conducted by the NCHS (National Center for Health Statistics) of the CDC (Center for Disease Control and Prevention). Comparing the data gathered from 2001-2002 and 2003-2004. *S. aureus* colonization decreased from 32.4 % to 28.6% (95% CI = 27.2 to 30.0; $p < 0.01$); while the prevalence of MRSA colonization increased from 0.8 % to 1.5% (95% CI = 1.2 to 1.8; $p < 0.05$) (Tenover *et al.*, 2008).

MRSA was traditionally regarded as a pathogen seen only in the healthcare settings, but in the last decades it has appeared in the community. The spread of multi-drug resistant *S. aureus* in the health care setting have made the infection challenging and costly to treat. According to a report from Healthcare Cost and Utilization Project MRSA hospitalization cost was nearly double that for an MRSA stay compared to stay without MRSA infection (Elixhauser & Steiner, 2007).

As of October 1, 2008, new Medicare regulations will no longer pay hospitals at a higher rate for treatment of some conditions that are acquired during a hospital stay (Schwab, 2008). One of the reports on Medicare reforms finds that these new regulations will have a significant impact on antibiotic prescribing and infection control practices. One third of the physicians who participated in the Physician & Payer Forum, a group that presents physicians' prescribing habits, are expecting an increase in their use of antibiotic prophylaxis (preventive measure) to help avoid the development of hospital-acquired infections (Decision Resources Inc., 2008).

Screenings for MRSA is an important surveillance system for hospitals. It aids in preventing disease transmission and helps save resources. Understanding the dynamics of MRSA carriage may assist physicians in selecting appropriate treatment, identifying interventions, and preventing transmission in the population.

Better tracking of MRSA strains nationwide is needed to observe its spread. Different strain typing methods have been utilized over the years to track the spread and provide insight into its control. Australia, Denmark, The Netherlands, Canada and the United Kingdom have been tracking MRSA over the years. However, this practice has not been true for the US (Mc Dougal *et al.*, 2003). 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). According to the Clark County, Nevada 2005-2006 county-wide Antibiogram, which list the bacterial strains and commonly used antibiotics, there were 28,551 reported infections with *S. aureus* of which 58% were MRSA (SNHD Board Meeting Minutes, 2007).

The isolates used in this study were collected from a large urban county, public hospital in Las Vegas, Nevada. It is a 545 bed, level one trauma center, teaching hospital that offers a wide variety of services unique to the area of Southern Nevada and neighboring states such as level I trauma center, burn care center, cardiac rehabilitation, hyperbaric chamber, and infusion clinic (UMC, 2009).

Purpose of Study

The purpose of this retrospective study was to determine the prevalence of MRSA nasal colonization among adult patients admitted at this hospital. Further characterization of each isolate was also performed. This involved: 1) detection of the *mecA* gene which codes for resistance to beta-lactam antibiotics; 2) assessment of antimicrobial susceptibility patterns; and 3) determination of isolate strain types. This study further described and compared the characteristics of CA-MRSA isolates and HA-MRSA isolates. The data gathered from this study were compared to the NHANES nasal colonization study done by Gorwitz *et al.*, (2008) and Tenover *et al.*, (2008). The comparison was performed to give a description of MRSA nasal colonization between sampled patients compared to the general public.

To compare the results of this study to the NHANES results two hypotheses were tested. The first hypothesis was: strains isolated from the screened patients will be predominantly USA100, USA300, and USA 800 as seen in the nationwide nasal colonization study by NHANES as performed by Tenover *et al.* (2008). The second hypothesis was: isolates gathered from this study will display a greater association with elderly age (≥60 years), Black and White ethnicity, and female gender as seen in the nationwide nasal colonization study by NHANES as performed by Gorwitz *et al.*, (2008). The result of this study will give an insight about MRSA nasal colonization among patients screened at the hospital and describe the populations that are at higher risk for subsequent MRSA infection. Furthermore, the information can aid in disease surveillance and appropriate treatments selection for physicians.

CHAPTER 2

MATERIALS AND METHODS

Specimen Handling and Bacterial Isolates

Clinical specimens were collected from newly admitted patients at the participating hospital from September 2008 through December 2008. Specimens were collected during a routine MRSA nasal screening on adult (18 years and older) patients who demonstrated a high risk for acquiring MRSA infection (e.g. dialysis patients, patients with moist tissue infections, all critical care admits, invasive devices in place on admission, known MRSA infection prior to admission).

A total of 1206 MRSA nasal screen swabs were collected by hospital staff during the study period, using a sterile BD CultureSwab™ (BD Diagnostics, Sparks MD) inserted from both anterior nares, rotated for 5 seconds, and inserted into the swab transport container. Specimens were then transported to the hospital laboratory and innoculated onto MRSA ChromAgar (BD & Company, Sparks, MD). Inoculated plates were then incubated aerobically for 24 hours at at 33-37°C. Plates negative for MRSA were incubated an additional 24 hours. Smooth, mauve, moderately sized, convex colonies recovered from the MRSAChromAgar and coagulase (BD & Company, Sparks, MD) positive, were interpreted as MRSA isolates. Atypical mauve colonies were further evaluated. These isolates were subcultured to Tripticase™ 5% sheep blood (BD & Company, Sparks, MD), incubated for 18-24 hours at 33-37°C, and tested for coagulase

activity using coagulase and/or Protein A using Staphaurex (Fisher HealthCare, Houston, TX). A total of 119 were reported by the hospital as MRSA positive. Of these positive isolates, 100 met the criteria to be included in this study and were innoculated onto 5% Sheep Blood Agar (Teknova, Hollister, CA). The isolates were packaged and marked as a Category B Infectious Disease Substance and transported in a biohazard transport container to the Environmental Health Microbiology Laboratory at UNLV, Biosafety Level II (BSL-2). At the laboratory, the SBA plates were incubated for 18-24 hours at 33-37°C. Isolates were then analyzed for strain typing, susceptibility testing, and presence of the *mecA* gene. All used supplies were disposed of according to the IBC protocol (see Appendix I) which followed proper universal precautions.

Data Collection

Detailed patients' information was collected at the time of admission in accordance with the hospital's admission policies. However, for this study the only patient demographics retrieved from the hospital's electronic database included age, gender, and ethnicity. A numerical accessioning scheme was used to identify samples. No direct identifier was released with this study to ensure patient's privacy.

Identification and Antimicrobial Susceptibility Testing

In vitro antimicrobial testing was performed on all methicillin-resistant *S. aureus* isolates using the Vitek II Compact system (bioMérieux, Hazelwood, MO). Isolates were subcultured for two consecutive days on SBA before testing. Isolate identification testing was performed along with susceptibility testing using the Vitek® 2 Compact ID/AST

system (bioMérieux, Durnham, NC). Susceptibilities to clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, moxifloxacin, oxacillin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin were determined. For isolate identification testing, a bacterial suspension of each isolate was prepared, at an optical density of 0.5-0.63 McFarland standard with isolated colonies. Each suspension was loaded onto a cassette containing a Vitek 2 GP cards (bioMérieux, Durham, NC). For isolate susceptibility testing, 280µl of the 0.5-0.63 McFarland standard was transferred to a sterile saline solution and loaded onto a cassette containing Vitek 2 AST-GP66 cards (bioMérieux, Durham, NC). Once loaded, the cassette was placed into the Vitek II Compact and processed according to manufacturer's protocols. Results of the susceptibility profile were stored electronically in the system and evaluated. The reference strains *S.aureus* ATCC 43300 (oxacillin resistant) and *S. aureus* ATCC 25923 (oxacillin susceptible) were used as controls (Roisin *et al.,* 2008). Isolates were classified as susceptible or resistant; intermediate results were considered resistant. In case of discrepancy, Vitek II identification and susceptibility testing were repeated.

Detection of inducible clindamycin resistance was performed on all isolates that demonstrated erythromycin-resistance and clindamycin-susceptible. Erythromycin and clindamycin disks (Becton Dickinson, Sparks, MD) were placed 15-mm apart after innoculating a the Mueller Hinton agar plate (BBL Mueller Hinton II agar, BD Diagnostics) with a bacterial suspension of 0.5 McFarland standard (Hindler,. 2004). Flattening of the zone in the area between the disks indicates that the organism has inducible clindamycin resitance and is then reported as clindamycin resistant. The

reference strains *S. aureus* ATCC 25923 (no induction) and *S. aureus* ATCC BAA-977 (clindamycin inducible) were used as controls.

DNA Extraction and Purification

DNA extractions were conducted using Mobio UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA). This product is designed to isolate bacterial DNA from microorganisms by lysing the microorganisms through the use of heat, detergent and mechanical force against specialized beads. After cell lysis, the DNA is then released onto a silica spin filter. The filter is then washed, and the DNA is recovered for further testing.

A 10µl loop of plated culture was suspended in a MicroBead Solution containing salts and buffer which helps stabilize and homogeneously disperse the cells. A reagent containing sodium dodecylsulfate (SDS) and other disruption agents were then added to lyse the cells. The solution was vortexed which combines both chemical and mechanical lysis condition to release the nucleic acids from the cells. The culture suspension was then centrifuged to clear the solution of cell debris, leaving the DNA in the supernatant. A reagent was then added to remove contaminating organic and inroganic material. The culture supernatant was then loaded onto a spin filter device with a high salt concentrated reagent to help bind the DNA to the spin filter membrane allowing other contaminants to pass through the filter. An ethanol-based washed solution was then used to further clean the filter bound DNA. The filter was then centrifuged to dry and remove any remaining wash solution. The filter was washed with an elution buffer to aid in DNA release. The

DNA was recovered in a 35µg DNA-free Tris buffer and quantitated using a UV spectophotometer (Thermo Fisher Scientific Inc., Pittsburgh, PA).

Automated rep-PCR DNA Fingerprinting

Isolates identified as *S. aureus* with an oxacillin-resistant profile from the antibiotic susceptibility testing were subjected to strain typing using the DiversiLab (bioMerieux, Athens, GA) according to manufacturer's protocol. Isolates were re-streaked onto SBA and incubated overnight as described above. DNA was extracted using the DiversiLab Staphylococcus Fingerprinting kit (Bacterial BarCodes Inc., Athens, GA). The GeneAmp® PCR System 2400 (Applied Biosystems, Foster City, CA) was used for PCR analysis. The materials for amplification included: rep-PCR MM1, GeneAmp 10X PCR Buffer, Primer Mix, AmpliTaq® DNA Polymerase and DNA tempalate. The thermal cycling parameters were initial denaturation of 2 minutes at 94°C, 35 cycles of 94 °C for 30 seconds for denaturation, annealing at 45 °C for 30 seconds, extension at 70 °C for 90 seconds and a final extension at 70 °C for 3 minutes.

The resulting DNA was loaded onto the DiversiLab Caliper® LabChip device (Bacterial BarCodes, Inc., Athens, GA) and processed according to manufacturer's protocols. The DiversiLab chip is first loaded with a gel-dye mix followed by the DNA Marker and the resulting DNA rep-PCR product. Comparisons of strain profiles were made of isolates from this study and isolates in the Biomerieux database. The Diversilab software was used to analyze results using Pearson correlation coefficient to determine the distance matrices and the weighted-pair group method with arithmetic mean to create the dendograms (Healy *et al.*, 2005). The software reports includes the dendogram,

similarity matrix, electropherograms, virtual gel images, scatter plots and demographic fields. Isolates were grouped according to the closests match from the Biomerieux database's top matches showing $\geq$ 90% similarity . Additional review of the virtual gel images, graph overlays and similarity matrix were performed for samples showing similarities to two different strain types.

mecA Gene Assay

Resistance to oxacillin was confirmed by detecting the *mecA* gene. A real-time PCR assay applying the TaqMan® (Applied Biosystems, Foster City, CA) technology was used to detect the mecA gene (Francois *et al.*, 2003). PCR analysis was performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems) using the following *mecA* primers sequence (5'-3'): forward, CAATTGATCGCAACGTTCAATTT and reverse, AGGTCCTTACGTCTTTCTGGT. Primers were acquired from Operon Technologies (Huntsville, AL). The TaqMan® probe sequence was 6-FAM TGGAAGTTAGATTGGGATCATAGCGTCAT-TAMRA (Applied Biosystems, Foster City, CA).

The materials for the amplification were 1X TaqMan® Universal Primer Mix (Applied Biosystems), 100 nM of each forward & reverse primers, 75 nM probe, and genomic DNA template. A volume of 25 µl nuclease-free water (Promega Corp., Madison, WI) was used to adjust the reaction volume. The parameters for thermal cycling were run on standard mode of 2 minutes at 50°C, 10 minutes at 95 °C, and 40 cycles of 15 seconds at 95°C then 1 min at 60 °C. The reference strain *S. aureus* ATCC 43300 (*mecA* gene positive) and *S. aureus* ATCC 25923 (*mecA* gene negative) were used as controls.

Statistical Analysis

To summarize the patients' characteristics and the prevalence of MRSA descriptive statistics were used. Statistical examination of organism characteristics, as determined by laboratory testing, was performed using SPSS (15.0 software) and Microsoft office excel 2003. Data are shown as a raw number or percentage of each group. Categorical data were compared using chi-square test or Fisher's exact test when the categorical variable cells were <5. A p value of less than 0.05 was considered significant.

IRB Approval

A separate IRB approval was requested and granted from each institution (the hospital and UNLV). UNLV Institutional Review Board approved as exempt (9OPRS # 0807-2811, August 7, 2008). The hospital's Institutional Review Board request was also approved as exempt (UMC-IRB00002394, August 2, 2008) for this project. UNLV's Institutional Biosafety Committee (IBC) approval was also obtained prior to initiation of this research.

CHAPTER 3

RESULTS

MRSA Nasal Colonization Prevalence among Hospital Isolates

Within the four-month study period, 119 out of 1206 samples were reported by the hospital as MRSA-positive. The prevalence of MRSA nasal colonization among all samples collected at the hospital was 9.87% during the study period. Out of all the positive isolates, 100 met the criteria to be included in this study.

Table 1. Demographic characteristics of the 100 samples with methicillin-resistant *Staphylococcus aureus* colonization.

Patient characteristics	Hospital isolates n=100
Gender	
Male	69
Female	31
Ethnicity	
Whites	64
Blacks	17
Spanish	15
Other	4
Age group	
18-34	7
35-59	51
≥ 60	42

Patient Demographics among Hospital Isolates

Of the 100 isolates collected for this study, 69% were from male patients, while 31% were from female patients (Table 1, Figure 1). The mean patient age was 58 years (range, 18 to 92). Sixty-four percent of the samples were from patients with White ethnic background, followed by Black at 17%, Spanish at 15% and other ethnic backgrounds at 4% (Table 1, Figure 2). Eight percent of the samples were collected from patients 18 to 34 years, 50% were from patients 35 to 59 years, 42% were from patients 65 years or older (Table 1, Figure 3).

Strain Type Distributions among Hospital Isolates

The hospital MRSA isolates showed a significant difference in proportion between HA-MRSA and CA-MRSA strain types ($p < 0.001$). The most common strain type seen among hospital MRSA isolates was USA100 which was present in 35% out of the 100 isolates tested. The second most common strain type was USA300, present in 25% of all the isolates. The Brazilian and the Iberian were the third and fourth most common strain type isolated at 16% and 14% respectively.

Patient Demographic and Strain Type Distribution among Hospital Isolates

The most common strain type seen among study isolates was USA100 which was mainly collected from patients who were white, male, and 60 years or older (Table 4-6). The second most common strain type was USA300. These isolates were mostly collected from patients who were white, male, and 35 to 59 years of age (Tables 4-6). The third

most common strain type was Brazilian. Isolates that belong in this group were collected from patients who were male, white, and between the ages of 35 to 59 years (Tables 4-6).

No statistically significant association was found between patient ethnicity and MRSA stain type ($p = 0.544$). Patient gender and MRSA strain type also did not show a statistically significant association ($p = 0.335$). However, a statistically significant association was seen between patients' age group and MRSA strain type ($p = 0.02$). Over 65% of USA100 were from older patients age 60 years or over. However, over 75% of USA300 were from patients aged 35 to 59 years.

HA-MRSA and CA-MRSA Comparison among Hospital Isolates

Strain Type and Antimicrobial Susceptibilities Testing among Hospital Isolates

HA-MRSA strains (USA100, USA200, USA500, USA600, USA700, USA800, Iberian, and Brazilian) accounted for 73% of the isolates among hospital samples (Table 3). CA-MRSA (USA300, USA400, USA1000, and USA1100) accounted for 27% of the isolates (Table 3).

USA100 was the most predominant strain type among HA-MRSA hospital isolates. It was highly susceptible (greater than 75%) to gentamicin, quinipristin/dalfopristin, linezolid, tetracycline, rifampin, vancomycin and trimethoprim/sulfamethoxazole. However, USA100 showed to be less susceptible (less than 50%) to levofloxacin, clindamycin, and erythromycin.

USA300 was the most predominant among CA-MRSA isolates. It was highly susceptible to gentamicin, quinipristin/dalfopristin, linezolid, tetracycline, rifampin,

vancomycin and trimethoprim/sulfamethoxazole, clindamycin. It was less susceptible to levofloxacin and erythromycin.

Antimicrobial Susceptibility Testing among Hospital Isolates

All of the hospital isolates tested positive for the *mecA* gene which codes for methicillin resistance. None of the isolates were susceptible to penicillin and oxacillin. All of the study isolates were susceptible to vancomycin, quinipristin/dalfoprinstin, and linezolid. The CA-MRSA and HA-MRSA isolates showed a significant difference in antibiotic susceptibility proportion ($p < 0.001$). CA-MRSA was susceptible to more antimicrobial agents than the HA-MRSA (Table 8).

HA-MRSA and CA-MRSA antimicrobial susceptibility results indicate a high susceptibility to rifampin (98.6% vs. 100%; $p = 0.308$). Although there was a statically significant difference in proportion for gentamicin (80.8% vs. 96.3%; $p = 0.008$), tertracycline (78.1% vs. 96.3%; $p = 0.002$), and trimethoprim/sulfamethaxazole (78.1% vs. 96.3%; $p = 0.002$); both HA-MRSA and CA-MRSA groups indicate a high susceptibility to the antimicrobial agents mentioned. There was a statistically significant difference in susceptibility proportions between HA-MRSA and CA-MRSA for levofloxacin (4.2% vs. 22.2%; $p < 0.001$) and erythromycin (8.3% vs. 18.5%; $p = 0.21$), but MRSA was still less susceptible to levofloxacin and erythromicin in both groups. Susceptibility to clindamycin (36.9% vs. 81.5%; $p < 0.001$) was less susceptible among HA-MRSA than CA-MRSA and this difference was statistically significant.

Table 2. PFGE designation for methicillin-resistant *S. aureus* hospital-associated (HA-MRSA) and community-associated (CA-MRSA) grouped according to USA and European class.

MRSA Pulse-Field Gel Electrophoresis Designation	
HA-MRSA	CA-MRSA
USA100	USA300
USA200	USA400
USA500	USA1000
USA600	USA1100
USA700	
USA800	
Iberian	
Brazilian	

Note. Table was composed from PFGE designation as reported from studies by Klevens *et al.*, (2007); Buck *et al.*, (2005); McDougal *et al.*, (2003) and Bartels *et al.*, (2007).

Table 3. Hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) and community associated MRSA (CA-MRSA) class distribution among hospital isolates.

MRSA Class	No. of isolates	Percentage
HA-MRSA (n=73)		
USA100	35	47.9
USA200	1	1.3
USA700	6	8.2
USA800	1	1.3
Iberian	14	19.2
Brazilian	16	21.9
CA-MRSA (n=27)		
USA300	25	92.6
USA1000	1	3.7
USA1100	1	3.7

Note. G^2=<0.001; p=<0.001 for HA-MRSA and CA-MRSA comparison.

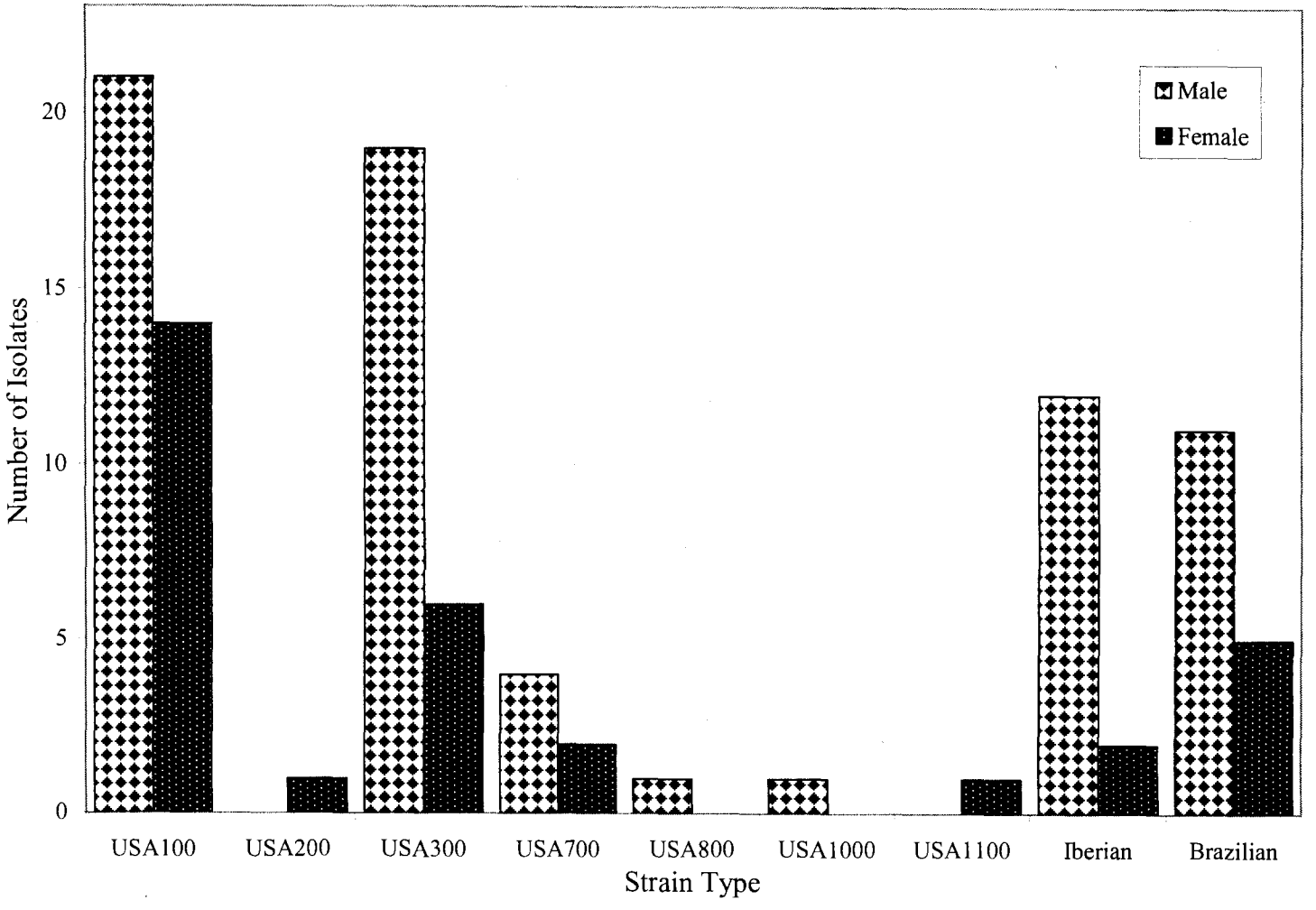

Figure1. Patient gender categorized by strain type among hospital isolates.

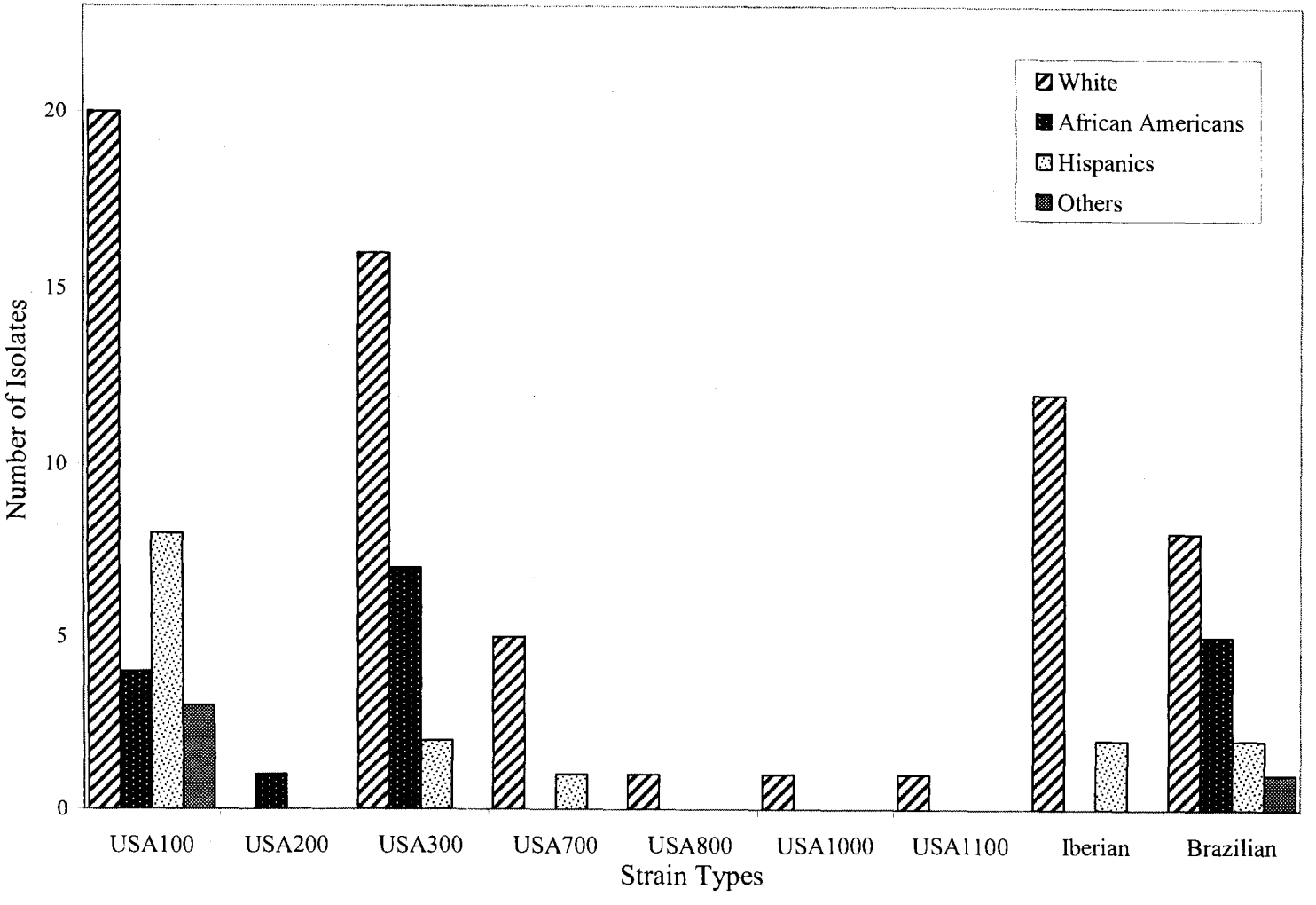

Figure 2. Patient ethnicity categorized by strain type among hospital isolates.

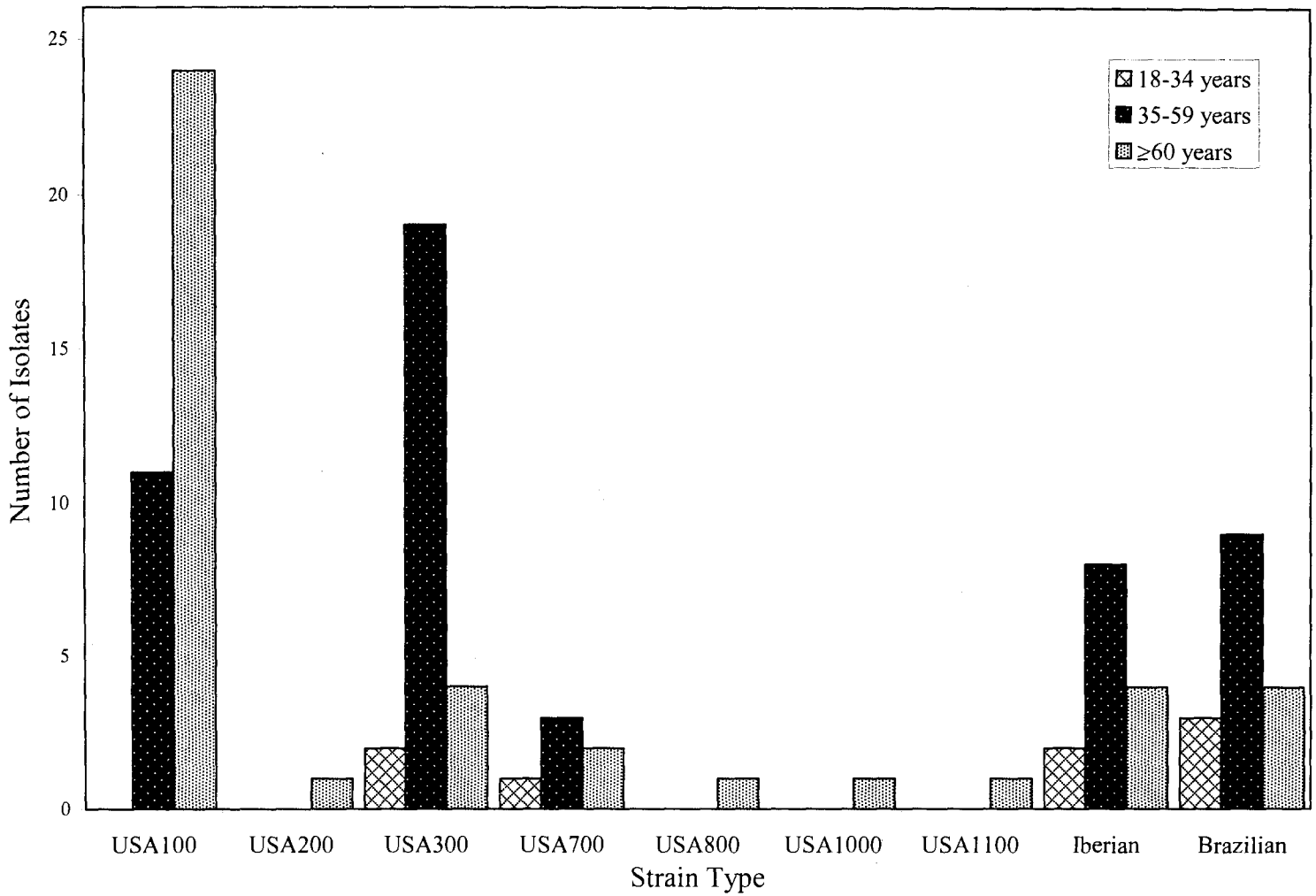

Figure 3. Patient age group categorized by strain type among hospital isolates.

Table 4. Methicillin-resistant *S. aureus* strain type and patient ethnicity distribution among hospital isolates.

Ethnicity	Strain Type									
	USA100	USA200	USA300	USA700	USA800	USA1000	USA1100	Iberian	Brazilian	Total
White	20	0	16	5	1	1	1	12	8	64
Black	4	1	7	0	0	0	0	0	5	17
Spanish	8	0	2	1	0	0	0	2	2	15
Others	3	0	0	0	0	0	0	0	1	4
Total	35	1	25	6	1	1	1	14	16	100

Note. $G^2 = 0.340$; $p = 0.544$ for ethnicity and strain type comparison.

Table 5. Methicillin-resistant *S. aureus* strain type and patient gender distribution among hospital isolates.

Gender	Strain Type									
	USA100	USA200	USA300	USA700	USA800	USA1000	USA1100	Iberian	Brazilian	Total
Male	21	0	19	4	1	1	0	12	11	69
Female	14	1	6	2	0	0	1	2	5	31
Total	35	1	25	6	1	1	1	14	16	100

Note. $G^2 = 0.254$; $p = 0.335$ for gender and strain type comparison.

Table 6. Methicillin-resistant *S. aureus* strain type and age group distribution among hospital isolates.

Age Group	Strain Type									
	USA100	USA200	USA300	USA700	USA800	USA1000	USA1100	Iberian	Brazilian	Total
18-34 years	0	0	2	1	0	0	0	2	3	8
35-59 years	11	0	19	3	0	0	0	8	9	50
>60 years	24	1	4	2	1	1	1	4	4	42
Total	35	1	25	6	1	1	1	14	16	100

Note. $G^2 = 0.006$; $p = 0.020$ for age group and strain type comparison.

Table 7. Susceptibility pattern for each methicillin-resistant *S. aureus* strain types among hospital isolates.

Antimicrobial Agents	HA-MRSA						CA-MRSA		
	USA100 (n=35)	USA200 (n=1)	USA700 (n=6)	USA800 (n=1)	Iberian (n=14)	Brazilian (n=16)	USA300 (n=25)	USA1000 (n=1)	USA1100 (n=1)
GM	35	0	6	1	14	3	24	1	1
LE	0	0	1	0	1	1	5	1	0
E	2	0	1	0	3	0	4	1	0
CC[a]	3	0	5	0	13	3	21	1	0
QDA	35	1	6	1	14	16	25	1	1
LZD	35	1	6	1	14	16	25	1	1
TET	34	1	5	0	14	3	24	1	1
RIF	34	1	6	1	14	16	25	1	1
SXT	34	0	6	0	14	3	24	1	1
VA	35	1	6	1	14	16	25	1	1

Note. GM=Gentamicin, LE=Levofloxacin, E=Erythromycin, CC=Clindamycin, QDA=Quinipristin/Dalfopristin, LZD=Linezolid, VA=Vancomycin, TET=Tetracycline, RIF=Rifampin, SXT= Trimethoprim/Sulfamethoxazole.

[a] Susceptibility due to inducible and constitutive resistance.

The susceptibility patterns for each strain type belonging to CA-MRSA and HA-MRSA groups generally followed a similar pattern with the exception of the Brazilian clone. The Brazilian isolates were less susceptible to more antibiotics than other HA-MRSA isolates, especially to trimethoprim/sulfamethoxazole, gentamicin, and tetracycline (Table 7).

Hospital and NHANES Isolate Comparison

Hospital and NHANES Isolate Strain Type Comparison

USA100 was the most common strain type seen among this study's hospital MRSA isolates. This strain was present in 35% of the 100 isolates tested (Table 8). The second most common strain type was USA300, present in 25% of all the isolates. The most common strain type isolated among NHANES MRSA isolate was USA100, with 44.8% of the 134 isolates tested. This was then followed by USA300 and USA800, each present in 17.1% of all the isolates tested.

Hospital and NHANES Isolate Antimicrobial Susceptibility Comparison

None of the isolates were susceptible to penicillin and oxacillin. All of the sample and NHANES isolates were highly susceptible to vancomycin, rifampin, gentamicin, tetracycline, quinipristin/dalfoprinstin, trimethoprim/sulfamethoxazole, and linezolid (Tenover *et al.,* 2008) (Table 9). Study isolates showed less susceptibility to levofloxacin than NHANES isolates. Both groups were less susceptible to erythromycin.

Table 8. Susceptibility pattern for community-associated (CA-MRSA) and hospital-associated (HA-MRSA) types among hospital isolates.

Antimicrobial Agent	Percentage of isolates for indicated MRSA type		p value
	HA%	CA%	
GM	80.8	96.3	0.008
LE	4.2	22.2	<0.001[a]
E	8.3	18.5	0.21
CC [b]	36.9	81.5	<0.001
QDA	100	100	NT
LZ	100	100	NT
TET	78.1	96.3	0.0026
RIF	98.6	100	0.3086
SXT	78.1	96.3	0.002
VA	100	100	NT

Note. GM=Gentamicin, LE=levofloxacin, ER=erythromycin, CC=Clindamycin, QDA=Quinipristin/Dalfopristin, LZD=Linezolid, VA=Vancomycin, TET=Tetracycline, RIF=Rifampin, SXT= Trimethoprim/Sulfamethoxazole.
G^2=<0.001; p=<0.001 for comparison between HA-MRSA & CA-MRSA.
[a] Fisher's exact test.
[b] Susceptibility due to inducible and constitutive resistance.
[NT] Values are the same, no test of comparison performed.

Table 9. Distribution of strain types of study isolates and NHANES 2001-2004.

Strain type	Study n=100	Study Percentage	NHANES [a] n=134	NHANES Percentage
USA100	35	35	60	44.7
USA200	1	1	6	4.4
USA300	25	25	23	14.1
USA400	0	0	8	5.9
USA600	0	0	3	2.2
USA700	6	6	3	2.2
USA800	1	1	23	17.2
USA1000	1	1	1	0.7
USA1100	1	1	1	0.7
IBERIAN	14	14	0	0
BRAZILIAN	16	16	NR	NR

Note. NR represents values that were not reported.

[a] These value do not include six isolates, each with unique PFGE pattern.

Table 10. Susceptibility profile for MRSA study isolates and NHANES isolates.

Antimicrobial agent	Hospital Isolate [b] n=84	Hospital Isolate Percentage	NHANES [c] n=133	NHANES Percentage
PCG	0	0	0	0
OXS	0	0	0	0
GM	85	85	132	99.3
LE	9	9	60	45.1
E	11	11	24	18.1
CC [a]	46	46	50	37.6
QDA	100	100	133	100
LZD	96	96	133	100
VA	100	100	133	100
TET	83	83	122	91.7
RIF	99	99	131	98.5
SXT	83	83	133	100

Note. PEN=Penicillin, OX=Oxacillin GM=Gentamicin, LE=levofloxacin, E=erythromycin, CC=Clindamycin, QDA=Quinipristin/Dalfopristin, LZD=Linezolid, VA=Vancomycin, TET=Tetracycline, RIF=Rifampin, SXT= Trimethoprim/Sulfamethoxazole.

[a] Susceptibility due to inducible and constitutive resistance.

[b] These values do not include the 16 Brazilian clones. Comparisons were made only on strain types reported by both studies.

[c] These values do not include six isolates, each with unique PFGE pattern.

CHAPTER 4

DISCUSSION

The strong association between *S. aureus* carriage and risk factors for subsequent infections has led numerous researchers to examine the presence of *S. aureus* nasal colonization and project subsequent infections. The rising prevalence of MRSA colonization has several implications for public health and also for clinical diagnosis and treatment of patients (Jung *et al.*, 2006). This retrospective study determined the prevalence of MRSA nasal colonization and described the characteristics of CA-MRSA and HA-MRSA isolates from nasal cultures collected from adult patients screened upon admission at the participating Las Vegas community hospital. Patient isolates were taken from routine nasal screens collected from September 2008 through December 2008. The result of this study will give an insight in the characterization MRSA and the prevalence of MRSA nasal colonization among adult patients at high risk for subsequent infection in the Las Vegas community.

HA-MRSA and CA-MRSA Comparison among Hospital Study Isolates

The characterization between CA-MRSA and HA-MRSA used for this study was performed based on predominant strain type seen in each group as previously reported by Klevens *et al.* (2007), Mc Dougal *et al.* (2003), Bartel *et al.* (2007) Buck *et al.*, (2005), and Bartels et al. (2007). This study demonstrated that there is a higher proportion of HA-MRSA nasal colonization among sampled patients (73%) compared to CA-MRSA

(27%). The CA-MRSA nasal colonization isolates were susceptible to more antimicrobial agents than the HA-MRSA. This result was also consistent with previous MRSA studies as mentioned earlier.

There was also a higher percentage of the EMRSA strain type (e. g. Iberian and Brazilian clones) isolated among MRSA colonized patients from this study compared to the NHANES. This suggests that new clones of MRSA are being introduced or currently exist in the study population. Data from the susceptibility testing suggest that 14 of the Iberian strains isolated follow the same antimicrobial susceptibility pattern as other HA-MRSA. The 16 Brazilian isolates were less susceptible to more antibiotics than the other HA-MRSA isolates, especially to trimethoprim/sulfamethoxazole, gentamicin, and tetracycline.

Hospital and NHANES Isolate Comparison

The most common strain types seen in this study were strain types USA100 (35%) and USA300 (27%). These findings are consistent with the NHANES study, which showed a high prevalence of USA100 (44%) and USA300 (17.1%), in addition to USA800 (17.1%).

Colonization of MRSA was more common among white, males, and aged 50 to 64 years. The NHANES study showed a higher prevalence of MRSA colonization among females and individuals aged 60 years and older. Black and White ethnicity showed an equally high prevalence rate.

The similarity seen between the common strain types observed in both population shows that both USA100 and USA300 are prominent in both populations. The high

proportion of USA800, which is frequently associated with pediatric patients, was probably due to the proportion of pediatric patients included with the NHANES study.

The difference in the demographic distribution of MRSA nasal colonization between the two groups suggests that the sampled population is different from NHANES. The sampled population does not represent the general public but a subset in the Las Vegas community. The NHANES data were used for comparison as it was the largest study performed on the subject of nasal colonization in the US. The data from this nationwide study were used as the best assumption to represent the general public. Nevertheless, the data gathered from this study could be used as a guide to aid in MRSA surveillance, identify interventions and guide physicians in selecting appropriate treatments.

Strain Type Analysis

The most predominant strain type isolated from nasal cultures was USA100, also known as New York/Tokyo clone. It has been the most common stain type found among health-care associated infections in the US (Mc Dougal *et al.,* 2003). In a 2005 population-based surveillance for invasive MRSA, USA100 was the most common strain type seen among invasive health-care onset MRSA infections (Klevens *et al.,* 2007).

The second most common strain type isolated from nasal cultures was USA 300. It is the strain most often isolated in MRSA community outbreaks. It frequently harbors the Panton-Valentine leukocidin. This toxin has been highly linked as an epidemiological marker for CA-MRSA strains. While PVL is a useful marker for strains with the capability to cause severe *S. aureus* infections, its clinical implication has yet to be determined (Voyich *et al.,* 2006).

Mc Dougal et al, (2003) describe the first eight lineages of USA MRSA clones from a previous study, USA100 to USA800. USA800 clones have been found to belong in the same cluster as other MRSA belonging in the pandemic pediatric clone. USA200 is the second most common healthcare-associated US isolate. USA 400 is associated with community onset infections along with USA1000 and USA1100 (Klevens *et al.,* 2007). USA 500, USA 600, and USA800 have been associated with healthcare-associated MRSA. USA700 has been emerging in both community and healthcare settings (McDougal *et al.,* 2003).

International hospital clones of MRSA are known as EMRSA. These epidemic clones are named either by the place where they were first found or by some unique epidemiological property (Oliveira *et al.,* 2002; Bartels *et al.,* 2008). The Iberian clone has been found in several countries in Europe and the Brazilian clone has been reported to be predominant in Portugal (Stefani & Varaldo, 2003).

Antimicrobial Susceptibility

The antibiotics used for this study were efficacious against bacteria through different mechanisms. Penicillins, cephalosporins, and carbapenems belong in the family of antibiotics called beta-lactams. These antibiotics work by disrupting the synthesis of the cell envelope in growing cells, inactivating the penicillin-binding proteins thus inhibiting the synthesis of bacterial cell wall. These antibiotics are considered bactericidal, only growing cells are killed (Singleton, 1992). However, shortly after the introduction of penicillin, bacterial strains developed resistance to beta-lactamases. Therefore, the

development of semi-synthetic penicillin (e.g. methicillin and oxacillin) was used as a replacement for penicillin.

Antibiotics such as gentamicin, tetracycline, erythromycin, clindamycin, linezolid, and quinupristin-dalfopristin work by inhibiting protein synthesis in bacteria (Salyers & Whitt, 2005). Rifampin belongs with the family of antibiotics which prevents prevent bacterial growth by inhibiting the RNA polymerase, halting the bacteria protein synthesis (Gladwin & Trattler, 2004). Levofloxacin works by inhibiting the enzyme DNA gyrase, resulting in the breakage of bacterial DNA structure and inhibition of DNA synthesis (Gladwin & Trattler, 2004). Vancomycin works much like penicillin as it inhibits the synthesis of bacterial cell wall. Another commonly prescribed antibiotic against MRSA is trimethoprim/sulfamethoxazole. This antibiotic inhibits the necessary cofactors for bacterial DNA synthesis (Salyer & Whitt, 2005; Gladwin & Trattler, 2004).

This study demonstrated that study isolates were less susceptible than NHANES isolates, but the over all susceptibility pattern followed a consistent pattern. An apparent difference in susceptibility was seen with levofloxacin. Its susceptibility was substantially less than that of NHANES. This could mean that levofloxacin is losing its effectiveness in both population of high risk patients for MRSA infection and the general public.

Automated Methods

Pulse-field gel electrophoresis (PFGE) is a highly discriminatory tool and is considered as the "gold standard" genotyping method (Healy *et al.,* 2005) However, PFGE is complicated, labor intensive and can produce inconsistent results. For this study, a new automated and commercially adapted repetitive sequence-based PCR (rep-PCR)

genotyping method was used (DiversiLab system). According to a comparison study performed by Reece et al. (2005) and Healey et al. (2005) rep-PCR results were stable, reproducible, and highly concordant with PFGE. The DiversiLab system also offers efficiency, excellent discriminatory power, and reproducibility to allow database building and offers result comparison with other institutions.

To determine the oxacillin resistance of each isolate, numerous methods were performed for verification. First, the isolates were inoculated onto CHROM™ agar, which is selective for MRSA growth. Second, the isolates were susceptibility tested using the Vitek 2. Lastly, the detection of *mecA* gene, which codes for methicillin resistance, was detected using a reference method PCR assay.

Antibiotic-sensitivity testing is used to determine the susceptibility of a pathogen to a range of antibiotics. These results can aid clinicians to select appropriate antibiotic therapy. One common form of this test is the disk diffusion method. This process normally requires a day of incubation. For this study, an automated antimicrobial susceptibility testing system (Vitek 2) was used. According to a study by Roisin et al. (2008) the Vitek 2 was as accurate as the disk diffusion method.

Disease Transmission and Prevention

The increasing prevalence of MRSA infection in the community and healthcare setting continues to be a major public health concern. The association between MRSA carriage and subsequent infection requires further study. Although the prevalence of colonization in the general public remains low, further study is needed to track changes and indicate ongoing trends (Gorwitz *et al.,* 2008). The low prevalence of MRSA

colonization in the general population does not currently warrant surveillance for individuals not at high risk for MRSA infection (Gorwitz *et al.*, 2008)

The high prevalence of nasal colonization and the increased risk for subsequent MRSA infection among hospital patients suggests continued monitoring of MRSA colonization in patients. Screenings for MRSA is an important surveillance system for hospitals. It aids in preventing disease transmission and helps save resources. Patients that are infected or colonized with MRSA are the most important reservoir of MRSA (Siegel *et al.,* 2006). The practice of active surveillance in hospitals can help reduce MRSA infections and related complications. This practice should involve a constituency of departments, including physicians, nurses, infection control, senior hospital personnel, environmental services purchasing and finance (Uettwiller-Geiger, 2008). Hospital personnel can also serve as reservoirs for MRSA, possibly harboring the organism for months (Siegel *et al.,* 2006). Contact and standard precautions should also be used for individuals that have contact with patients. The application of new technology should also be utilized to aid in surveillance and identification of MRSA along with other multiple drug resistant organisms.

New antibiotics are being created to be used against MRSA, although development of new antibiotics has not been promising as an answer to the antibiotic resistance problem. It is estimated that it costs over $800 million and requires 10 to 15 years to take a new antibiotic from conception to approval (Salyer & Whitt, 2005). Alternate drug therapies and combination of drug therapies should also be considered.

The public, especially individuals that are at an increase risk for acquiring MRSA in the community, should be educated about MRSA disease transmission and prevention.

This includes taking medications as directed, keeping lesions covered with clean and dry bandages, practicing good hand hygiene, and avoiding the sharing of contaminated items such as towels and razors (Moran *et al.,* 2006).

Limitations of Study

This study was cross sectional in design and only accounted for point prevalence of nasal colonization among participants. No subsequent cultures were collected and no follow-up study was performed on identified MRSA colonized patients. The study hospital was a public hospital; therefore, a more underserved population at high risk for MRSA may have been included in the study. No patient identifiers were collected so duplication of samples may have occurred. The number of patients that declined testing was also not collected. Characterization between CA-MRSA and HA-MRSA was performed based on predominant strain type seen in each population from previous studies. Additional patient medical history and other genetic marker could help further characterize the isolates into HA-MRSA or CA-MRSA category. Due to limited time and resources, the test for Panton-Valentine leukocidin gene (usually contained among CA-MRSA) was not performed.

The higher prevalence of MRSA colonization among the hospital study population may be due to the difference in risk acquisition for MRSA. Samples used in this study were from adult patients screened upon hospital admission who demonstrate a high risk for acquiring MRSA (i.e. dialysis patients, patients with moist tissue infections, all critical care admissions, invasive devices in place on admission, known MRSA infection prior to admission). Conversely, NHANES samples were collected from non-

institutionalized patients (i.e. not in hospitals, long-term care facilities, or prisons). The sampled population does not represent the national population. Nevertheless, the data gathered from this study could be used as a guide to aid in counseling physicians in selecting appropriate treatments, and prevent transmission and identify interventions.

CHAPTER 5

CONCLUSION

The aim of this study was to determine the prevalence of MRSA nasal colonization among adult patients admitted at a Las Vegas community hospital and further characterize each MRSA isolate. The information gathered from this study was then compared to the NHANES nasal colonization study.

This study showed that the predominant strain types isolated among study isolates were predominantly USA100 and USA300. There also was a high percentage of EMRSA strain sub types (Iberian and Brazilian clones) present among the hospital isolates. This result refutes the first hypothesis that the sample isolates will also show a predominance of USA100, USA300 and USA800 like the NHANES study.

Data from the susceptibility testing of study isolates suggest that 14 of the Iberian strains follow the same antimicrobial susceptibility pattern as other isolates in the HA-MRSA group. However, the 16 Brazilian isolates were less susceptible to more antibiotics than the other HA-MRSA isolates, especially to gentamicin, trimethoprim/sulfamethoxazole, and tetracycline. This suggests that new clones of MRSA are being introduced or currently exist in the study population.

The data from this study showed that the hospital study population had a higher prevalence of nasal colonization than NHANES. Among the hospital study population, patients that were white, male, and aged 35 to 59 years of age were more associated to

being colonized by MRSA. These groups are also at an increased risk for subsequent MRSA infection. The findings from this study and NHANES both described the older population to be more colonized, compared to younger age. However, colonization was more associated among the black and white race and females gender in the NHANES study which was not similar to the hospital study population as initially hypothesized.

The high prevalence of USA100 and USA300 strain types are consistent in both the study isolates and NHANES, suggesting that both strains are prominent in both populations. The high prevalence of USA800 in the NHANES study was probably due to the pediatric patients included in their study. Conversely, the hospital study only included adult patients.

Data from the antimicrobial susceptibility testing demonstrated that the hospital isolates were less susceptible to the antimicrobial agents tested than NHANES isolates. Nonetheless, over all susceptibility patterns follow a consistent pattern. An apparent difference in susceptibility was seen with levofloxacin. Study isolates were substantially less susceptible to levofloxacin than NHANES. This could suggest that levofloxacin is losing its effectiveness more rapidly among colonized patients than colonized individuals in the general public.

The difference in the demographic distribution of MRSA nasal colonization between hospital patients and NHANES patients suggests that the sampled population is different from NHANES. The sampled population does not represent the general public. Nevertheless, the data gathered from this study could be used as a guide to aid to in counseling physicians in selecting appropriate treatment, preventing transmission and identifying possible interventions.

Continued active surveillance, contact and standard precautions practice can help prevent the spread of MRSA colonization and infection in hospitals and other healthcare facilities. The application of new technology should also be utilized to aid in surveillance and identification of MRSA along with other multiple drug-resistant organisms. Healthcare providers should be aware of their local rate of resistance to different antimicrobial agents as the prevalence of MRSA continues to increase and evolve (Ezeanolue *et al.,* 2008). Additionally, the public, especially individuals that are at an increase risk for acquiring MRSA in the community, should be educated about MRSA disease transmission and prevention.

Performing prevalence studies assists in examining the relationship between specific diseases and health-related factors as they exist in individuals in a population at a particular time. Increased assessment of the population dynamics of MRSA carriage may assist in determining the extent of its prevalence. Further study is needed to track changes and observe trends of MRSA colonization and MRSA infections in the Southern Nevada.

REFERENCES

Aramark Healthcare (2005). Aramark Healthcare Website. Retrieved January 10, 2009 from http://www.aramarkhealthcare.com/DetailNoHeader.aspx?PostingID=747&ChannelID=410.

Bartels, M.D., Nanuashvili, A., Boye, A., Rohde, S.M., Jashiahvili, N., and Faria, N. A. (2008). Methicillin-resistant *Staphylococcus aureus* in hospitals in Tsibili, the Republic of Georgia, are variants of the Brazilian clone. European Journal of Clinical Microbiology and Infectious Disease, 27, 757-760.

Buck, J.M., Como-Sabetti, K., Harriman, K.H., Danila, R.N., Boxrud, D.J., and Glennen, A. (2005). Community-associated methicillin-resistant *Staphylococcus aureus,* Minnesota, 2000-2003. *Emerging Infectious Disease Journal*, 11(10), 1532-1538.

Community-Associated MRSA Information for the Public. Retrieved November 16, 2008 from Center for Disease Control and Prevention Web site: http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_public.html.

Decision Resources, Inc. (2008). Retrieved February 21, 2009 from Infection Control Today Web site: http://www.infectioncontroltoday.com/hotnews/increased-patient-screening-antibiotic-use.html.

Deresinski, S. (2005). Methichillin-resistant *Staphylococcus aureus*: An evolutionary, epidemiologic, and therapeutic oddysey. *University of Chicago Press Journal Division: Clinical of Infectious Disease*, 40, 562-569.

Elixhauser, A., and Steiner, C. (2007). Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) in U.S. hospitals, 1993-2005. Healthcare Cost and Utilization Project. Retrieved February 21, 2009 from Agency for Healthcare Research and Quality Web site: http://www.hcup-us.ahrq.gov/db/state/costtocharge.jsp.

Ezeanolue, E.E., Buttner, M.P., Cruz, P., Henry, J.L., Cross, C.L., and Stetzenbach, L.D. (2008). Prevalence and antimicrobial agent susceptibility of Methicillin-resistnant *Staphylococcus aureus* in healthy pediatric outpatients in Las Vegas. *Journal of the Nevada Public Health Association*, 4(1), 1-9.

Fey, P.D., Said-Salim, B., Rupp, M.E., Hinrich, S.H., Boxrud, D.J., and Davis, C.C. (2003). Comperative molecular analysis of community- or hospital-acquired

methicillin-resistance *Staphylococcus aurues*. Antimicrobial agents and chemotherapy, 47(1), 196-203.

Francois, P., Pittet, D., Bento, M., Pepey, B., Vaudaux, P., and Lew, D. (2003). Rapid detection of methichillin-resistant *Staphylococcus aureus* directly from sterile to non-sterile clinical samples by a new molecular assay. *Journal of Clinical Microbiology*, 41(1), 254-260.

Gladwin, M., & Trattler, B. (2004). *Clinical Microbiology.* (3rd. ed). Miami: MedMaster, Inc.

Gorwitz, R. (n.d.). Methicillin Resistant *Staphylococcus aureus* (MRSA) in the Community: Epidemiology and Mangement. Retrieved February 1, 2009 from http://emergency.cdc.gov/coca/ppt/MRSA_Gorwitz_102607.ppt.

Gorwitz, R.J., Kruzson-Moran, D., McAllister, S.K., McQuillan, G., McDougal, L.K., & Fosheim, G.E. (2008). Changes in the prevalence of nasal colonization with Stpahylococcus aureus in the United States, 2001-2004. *The Journal of Infectious Diseases*, 197, 1226-34.

Griffiths, A.J., Miller, J.H., Suzuki, D.T., Lewontin, R.C. & Gelbart, W.M. (2000). An introduction to genetic analysis (7th ed.). New York: W.H. Freeman and Company.

Healy, M., Huong, J., Bittner, T., Lising, M., Frye, S., and Raza, S. (2005). Microbial DNA typing by automated repetitive-sequence-based PCR. *Journal of Clinical Microbiology*, 43(1), 199-207.

Hindler, J. (2004). Dtest. Clindamycin disk induction test for *Staphylococcus spp.* Appendix (to routine disk diffusion test). Retrived on December 20, 2008, from the Center for Disease Control and Prevention Web site: http://wwwn.cdc/gov/nltn/pdf/2004/2_hindler_dtest.pdf.

Huang, H., Flynn, N.M., King, J.H., Monchaud, C., Morita, M., & Cohen, S.H. (2006). Comparison of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and hospital-associated MRSA infection in Sacramento, California. *Journal of Clinical Microbiology*, 44(7), 2423-2427.

Infection Control Today. (2009). Retrieved from http://www.infectioncontroltoday.com/hotnews/increased-patient-screening-antibiotic-use.html.

Jung, S., Shin, D.H., Park, K, H., & Shin, J.H. (2006). Antimicrobial susceptibility and clonal relatedness between community and hospital acquired methicillin-resistant *Staphylococcus aureus* from blood cultures. *The Journal of Microbiology*, 44(3), 336-343.

Klevens, R.M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., & Harriosn, L.H. (2007). Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *Journal of the American Medical Association,* 298(15), 1763-1771.

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., & Winn, W.C. (1997) Color Atlas and Textbook of Diagnostic Microbiology (5th ed.) Philadelphia, PA: Lippincott Williams & Wilkins.

Kuehnert, M., Hill, A., Kupronis, B.A., Tokars, J.I., Solomon, S.L., & Jernigan, D.B. (2005). Methicillin-resistant-*Staphylococcus aureus* Hospitalizations in the United States. *Emerging Infectious Diseases*, 11(6), 868-872.

Kuehnert, M., Kruszon-Moran, D., Hill, H.A., McQuillan, G., McAllister, S.K., & Fosheim, G. (2006). Prevalence of *Staphylococcus aureus* Nasal Colonization in the United States, 2001-2002. *The Journal of Infectious Diseases*, 193, 172-179.

Lodise, T.P., & McKinnon, P.S. (2005). Clinical and economic impact of methicillin resistance in patient with *Staphylococcus aureus* bacteremia. *Diagnostic Microbiology and Infectioius Disease*, 52(2005), 113-122.

Mc Dougal, L. K., Steward, C. D., Killgore, G. E., Chaitram, J. M., McAllister, S. K., & Tenover, F. C. (2003). Pulsed-field gel electrophoreisis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *Journal of Clinical Microbiology,* 41, 5113-5120.

Merrill, R.M., & Timmreck, T.C. (2006). Introduction to Epidemiology (4th ed.). Sudbury: Jones and Bartlett Publishers.

Moran, G.J., Krisnadasan, A., Gorwitz, R.J., Fosheim, G.E., McDougal, L.K., and Carey, R.B. (2006). Methicillin-resistant *S. aureus* infections among patients in the emergency department. *New England Journal of Medicine*, 355(7), 666-674.

MRSA in the Healthcare Settings (2007). Retrieved January 5, 2009, from the Center for Disease Control and Prevention Web site: http://www.cdc.gov/ncidod/dhqp/ar_MRSA_spotlight_2006.html.

Oliveira D., Tomasz, A., deLancaster, H. (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infectious Diseases*, 2, 180-189.

Overview of community associated MRSA. (2008). Division of Healthcare Quality and Promotion. CDC. http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca.html.

Pan, E.S., Diep, B.A., Charlebois, E.D., Auerswald, C., Carleton, H.A., Sensabaugh, G.F. (2005). Population Dynamics of nasal strains of methicillin-resistant *Staphylococcus*

aureus-and their relation to community-associated disease activity. *The Journal of Infectious Diseases*, 192, 811-818.

Roisin, S., Nonhoff, C., Denis, O., and Struelens, M.J. (2008). Evaluation of new Vitek 2 card and disk diffusion method for determining susceptibility of *Staphylococcus aureus* to oxacillin. *Journal of Clinical Microbiology*. doi:10.1128/JCM.00291-08.

Ross, T.L., Merz, W.G., Farkosh, M., & Caroll, K.C. (2005). Comparison of an automated repetitive-based PCR microbial typing system to pulse-field gel electrophoresis for analysis of outbreak of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 43(11), 5642-5647.

Salyer, A.A., & Whitt, D.D. (2005). Revenge of the Microbes. Washington D.C.: ASM Press.

Schwab, R. (2008). Hospital Acquired Conditions Reduce Medicare Reimbursement on October 1, 2008. Retrieved from http://trusted.md/feed/items/system/2008/05/21/hospital_acquired_conditions_reduce_medicare_reimbursement_on_october_1_2008.

Singleton, P. (1992). *Introduction to Bacteria: for students of Biology, Biotechnology and Medicine.* (2nd ed.). West Sussex: John Wiley & Sons.

Siegel J.D., Rhinehart, E., Jackson, M., & Chiarello, L. (2006). Management of multi-drug resistance organisim in healthcare settings, 2006. Retrieved on February 20, 2009 from Center for Disease Control and Prevention Web site: http://www.cdc.gov/ncidod/dhqp/pdf/ar/MDROGuideline2006.pdf.

SNHD Board Meeting Minutes. (2007, November). Southern Nevada Health District, Southern Nevada District Board of Health Meeting. Retrieved November 16, 2008 from http://www.southernnevadahealthdistrict.org/boh/2007/111507m.html.

Stefani, S. and Veraldo, P.E. (2003). Epidemiology of methicillin-resistant taphylococci in Europe. *European Society of Clinical Microbiology and Infectious Diseases*, 9, 1179-1186.

Tenover, F.C., McAllister, S., Fosheim, G., McDougal, L.K., Carey, R.B., & Limbago, B. (2008). Characterization of *Staphylococcus aureus* isolates from nasal cultures collected from individuals in the United States in 2001-2004. *Journal of Clinical Microbiology*, 46(9), 2837-2841.

Trindade, P.A., McCulloch, J.A., Oliveira, G.A., & Mamizuka, E.M. (2003). Molecular techniques for MRSA typing: Current issues and perspectives. *The Brazilian Journal of Infectious Diseases,* 7(1), 32-43.

Tsuji, B.T., Rybak, M.J., Cheung, C.M., Amjad, M., & Kaatz, G. W. (2006). Community- and health care-associated methicillin-resistant *Staphylococcus aureus*: A comparison of molecular epidemiology and antimicrobial activities of various agents. *Diagnostic Microbiology and Infectious Disease*, 58, 41-47.

Tulchinsky, T.T., & Varavikova, E.A. (2000). The New Public Health. San Diego: Academic Press.

UMC (2009). Retrieved January 10, 2009 from University Medical Center of Southern Nevada Web site: http://www.umcsn.com/index.asp.

Uettwiller-Geiger, D. (2008, June 5). *Implementation of a MRSA Surveillance Program in a Community Hospital.* Presented at Clinical Ligand Assay Society's 34th International Meeting.

Vogt, D.U., & Jackson, B.A. (2001). Antimicrobial resistance: An emerging Public Health Issue. Huntington, NY: Novinka Books.

Voyich, J.M., Otto, M., Mathema, B., Braughton, K.R., Whitney, A.R., & Welty, D. (2006). Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicilli70.n-resistant *Staphylococcus aureus* disease? The *Journal of Infectious Diseases*, 194, 1761-17.

Wylie, J.L. & Nowicki, D.L. (2005). Molecular epidemiology of community- and health care associated methicillin resistant *Staphylococcus aureus* in Manitoba, Canada. *Journal of Clinical Microbiology*, 43(6), 2830-2836.

APPENDIX I

UNIVERSITY OF NEVADA LAS VEGAS BSL-2 LABORATORY

STANDARD OPERATING PROCEDURES (SOPS)

This SOP document should include specific information for the laboratories and procedures being performed. It is meant to give details in addition to UNLV's adopted* standard BSL-2 procedures (pages -) and Exposure Control Plan (available through rms.unlv.edu).

All faculty, staff and students should familiarize themselves with these procedures and sign page prior to starting work in this BSL-2 laboratory. Questions should be directed to the Principal Investigator. A copy of the SOP must be forwarded to the UNLV Biosafety Officers and a copy must be retained in the laboratory's Biosafety Manual.

Principal Investigator: Linda Stetzenbach, Ph.D.
BSL-2 Room Number: MPA-231
Biohazards being used: (MSDS attached if available)
Description of Procedures(s):
Speciment Collection:

Clinical specimens will be collected from September 2008 until January 2009 from adult (18 years and older) emergency department patients admitted to the University Medical Center (UMC), Las Vegas, NV. An expected sample size of 200 to 300 is anticipated over the study time period. Methicillin-resistant Staphylococcus aureus (MRSA) positive isolates will be inoculated onto agar slants. Agar slants will be packaged and marked as a Category B Infectious Disease Substance. They will be transported in a biohazard transport container to Dr. Stetzenbach's UNLV Biosafety Level II (BSL-2) laboratory for strain typing and susceptibility testing. All used supplies will be disposed of according to the IBC protocol which will follow proper universal precautions.

Isolation of MRSA:

Isolates will be streaked to 5% Sheep Blood Agar and incubated for 18 – 24 hours at 35°C. A suspension of each isolate, at an optical density of 0.5-0.63 McFarland standard, will be loaded onto a cassette containing a AST cards. Once loaded the cassette will be placed into the in the Vitek II Compact and processed according to manufacturer's protocols. Results of the susceptibility profile will be stored electronically in the system and evaluated.

MRSA Strain Typing:

Isolates identified with an Oxacillin-resistant profile from the antibiotic susceptibility testing will be subjected to strain typing using the DiversiLab (Biomerieux) according to manufacturer's protocol. All manipulations of colonies for strain typing will be manipulated in the BSL-2 biosafety cabinet located in MPE 231. Isolates will be re-streaked onto 5% Sheep Blood Agar and incubated overnight as described above. DNA extraction will be conducted using Mobio UltraClean™ Microbial DNA Isolation Kit (Carlsbad, CA). Once the DNA is extracted the remaining protocol will be conducted in the laboatory as the material no longer is a risk for infection. The GeneAmp ® PCR System 2400 (Applied Biosystems, Foster City, CA) will be used for PCR analysis. The resulting DNA will be loaded onto the DiversiLab chip and processed according to manufacturer's protocols. Comparisons of strain profiles will be made of isolates from this study and isolates in the Biomerieux database.

Hazards: The following materials and/or equipment associated with this procedure may present exposure hazards, health hazards, and/or physical hazard. Identify potential exposures that may occur during sample preparation, and/or experimental manipulation (i.e., use of sharps, aerosol generation during centrifuge, mixing or sonication, etc):

No sharps will be used during this project. Risk of aerosolization during manipulation is minimal due to the closed screw cap. However, following vortexing the vials will only be opened under the BSL-2 biological safety cabinet located in MPE 231. Small biohazard bags will be used for the disposal of contaminated pipette tips and swabs. When finished, the small biohazard bag will be taped shut. Inoculated Petri plates must also be taped shut at the 6 and 12 o'clock position. All agar plates, vials, swabs, pipettes and pipette tips, and identification and AST cards will be autoclaved prior to disposal.

Administration Controls: The following administrative controls are in place to avoid exposures (i.e., training, signage, restricted entry, etc):

All personnel manipulating the swab samples, bacterial isolates, exposed agar plates, and contaminated materials before autoclaving have been trained by the Principle Investigator and will have undergone UNLV Risk Management blood-borne pathogens and BSL-2 training prior to the initiating of sample collection for this project. While personnel in the adjoining School of Public Health Environmental Health Laboratory have access to the shared anteroom of MPE 231, these individuals will be trained not to open the incubators belonging to the Emerging Disease Laboratory that are located in the shared space. These incubators are labeled with the biohazard symbol and verbage of biological hazards, and labeled with verbage of authorized personnel only. The shared refrigerator and ultra freezer in the anteroom will not be used for storage of infectious biological materials. Discarded potentially infectious material (e.g., exposed swabs, pipettes, incubated agar plates) will be disposed of into autoclave bags and autoclaved when the bags are <3/4 filled.

Engineering Controls: The following safety equipment must be used when carrying out these procedures, (i.e., chemical fume hood, biological safety cabinet, sealed centrifuged rotors, etc):

Laboratory coats and eye protection will be worn by all personnel when potentially infectious materials are being manipulated in the MPE 231. The laboratory coats are cloth and will be laundered as necessary by a commercial company recommended by UNLV Risk Management. Additionally, disposable Tyvek arm sleeves will be worn by individuals manipulating MRSA cultures. Protective latex or non-latex gloves will be worn during manipulations of any exposed agar plates, isolates, inoculated suspensions, exposed swabs, and discarded materials prior to autoclaving, and during any manipulations involving DNA extraction, typing, or susceptibility testing.

Additional Special Handling Procedures: Including any transport between labs or buildings (i.e., secondary containment):

MRSA isolates will be transported from UMC hospital to UNLV MPE 231. All primary culture tubes will be individually labeled according to their contents and screwcaps will be Para filmed. The primary tube will be placed inside a sealable bag that has a biohazard symbol on it with an absorbent packing material. The secondary bag will be placed in a rigid sturdy outer container with a lid. This rigid container will then be placed inside an insulated cooler that has a lid that snaps that prevents it from opening. Leak proof ice packs will be placed inside the cooler. A DOT Class 6.2 Infectious Substance and Biohazard label will be placed on the outside of the cooler. A bill of lading, Material Safety Data Sheet and an emergency contact sheet which includes the School of Public Health's information (address, phone number, and contact person) will be placed on the outside of the cooler in a pouch. The graduate student who is also a Medical Technologist at UMC or the PI will accompany the materials at all times. Anyone transporting the isolates will have current DOT 6.2 Infectious Substance Training provided by RMS before transporting the biological culture material. The package will be and marked as a Category B Infectious Disease Substance.

Decontamination/Clean-Up Procedures: Specifics on products and procedures used to clean work areas. Include specifics on when these procedures will be performed and timing involved (i.e. contact time):

MRSA isolates will be transported from UMC hospital to UNLV MPE 231. All primary culture tubes will be individually labeled according to their contents and screw caps will be Para filmed. The primary tube will be placed inside a sealable bag that has a biohazard symbol on it with an absorbent packing material. The secondary bag will be placed in a rigid sturdy outer container with a lid. This rigid container will then be placed inside an insulated cooler that has a lid that snaps that prevents it from opening. Leak proof ice packs will be placed inside the cooler. A DOT Class 6.2 Infectious Substance and Biohazard label will be placed on the outside of the cooler. A bill of lading, Material Safety Data Sheet and an emergency contact sheet which includes the School of Public Health's information (address, phone number, and contact person) will be placed on the outside of the cooler in a pouch. The graduate student who is also a Medical Technologist at UMC or the PI will accompany the materials at all times. Anyone transporting the isolates will have current DOT 6.2 Infectious Substance Training provided by RMS

before transporting the biological culture material. The package will be and marked as a Category B Infectious Disease Substance.

Waste Disposal Procedures: Include specifics on collection, deactivation and transport for disposal:

Upon completion of each experiment, anything to be removed from the biological safety cabinet will be wiped with a 1% sodium hypochlorite-soaked paper towel, and the paper towel will be disposed in a biohazard bag. Gloves and arm sleeves will be removed while hands and arms are still within the biological safety cabinet and will be placed into a biohazard bag. All used biohazard bags will be taped shut.

Biohazard bags containing materials from the biological safety cabinet will be transferred immediately to the dish room for autoclaving. Autoclaving of contaminated materials will be restricted to properly trained personnel.

All personnel in the laboratory will wash their hands with soap and warm water for at least 20 seconds upon removing ones gloves and before leaving the laboratory.

Spill Response Procedures: Procedures to follow if a spill occurs:

Plastic squirt dispense bottles with a 1% sodium hypochlorite solution are strategically located throughout the laboratory for surface disinfection. If a biological spill were to occur, the area would be saturated with the disinfecting solution and soaked for a minimum of 30 minutes contact time. The area would then be wiped dry with paper towels. The paper towels would be discarded into a biohazard bag before autoclaving (see above). All laboratory personnel in the laboratory at the time of the spill will be notified of the event and the decontamination procedures being undertaken. The PI, If not present at the time of the spill, will be notified by telephone.

Injury/Exposure Response Procedures: Steps to be taken in the event of an exposure incident:

Three sinks are located in the laboratory for washing of any exposed skin that would come in contact with hazardous material. An eyewash station is located in the laboratory for flushing of eyes. A laboratory safety shower is located in the adjoining laboratory space for full body rinsing, if needed. Injury/exposure will be reported to the PI. Depending on the severity of the injury/exposure, the affected individual may chose to be transported to a local quick care or emergency room for treatment. UNLV Health and Safety will be notified of any significant injury/exposure warranting attendance of healthcare professionals.

Unattended Operations: Portions of the experiment that may run unattended and steps taken to prevent accidental exposures:

Petri plates are incubated unattended in the 35 degree incubator. The screw-capped vials containing a swab sample and the enrichment broth will also be incubated overnight in the same incubator. The incubator is double doored with an inner glass door and an outer door. The vials will be placed into a size appropriate test tube rack that is placed into a plastic basin to collect any figurative liquid should a spill occur. Should a Petri

plate lid become dislodged, this would be observed through the glass inner door before that door was opened. The individual would already be wearing gloves and a lab coat (see above). The lid would be replaced and all of the contents placed into a biohazard bag for transport to the biological safety cabinet where the exterior of each plate would be wiped with a 1% hypochlorite solution (see above). The interior of the incubator would be disinfected by wiping with a 1% hypochlorite solution before being returned to service. If a liquid spill were to occur from the test tubes, the basin and its contents will be disinfected with a 1% sodium hypochlorite solution as described above in spill response procedures. Liquids stored in the double glass door or other refrigerators will be limited to sterile water, PBT, and the enrichment broth described above. No liquids containing live bacteria will be stored in the refrigerators.

Additional Laboratory Specific Safety Procedures:

1. Long hair will be tied back when working with or near an open flame.
2. No make-up application in the laboratory.
3. Contact lenses can be worn as long as the user wears safety glasses.
4. No open toe shoes in the laboratory.
5. End of the day laboratory lock-up requires a check to ensure that all propane torches, water bath, hot plates, centrifuges and unused laboratory equipment are turned off, incubators doors are sealed shut, and the laboratory doors are locked.

Variations from UNLV Standard Biosafety Level 2 Practices and Procedures and Reasons:

None.

I have read and understood all portions of this SOP. I agree to contact the Principal Investigator should I have any questions or plan on making any modifications to the procedures detailed here.

NAME	SIGNATURE	DATE
Linda Stetzenbach		
Ilene Bautista		
Vanessa Stevens		
Janice Klaassen		

** Original copy of this document has been submitted to the UNLV's Institutional Biosafety Committee (IBC).

VITA

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