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Laboratory analysis of Staphylococcus aureus in Florida from January 1, 2003 to December 31, 2005 with an emphasis on methicillin resistance

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Laboratory Analysis of *Staphylococcus aureus* in Florida From January 1, 2003 to December 31, 2005 with an Emphasis on Methicillin Resistance

by

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ABSTRACT

The Staphylococci are gram-positive bacteria that cause infections in humans and can produce severe morbidity and mortality. Methicillin resistant *S. aureus* (MRSA) isolates are resistant to all β-lactam antibiotics, such as methicillin, and cephalosporins making treatment of these infections more difficult. MRSA has become prevalent throughout the United States, spreading in the health care setting and the community.

The purpose of this study is to examine methicillin resistance among *S. aureus* isolates in an outpatient population in the state of Florida and asses possible associations between methicillin resistance and age group, gender, and geographic area. It is important to define methicillin resistance in a population so that clinical practice can adjust to the prevalence of resistance.

The dataset used for this analysis is a record of all the *S. aureus* isolates tested by a large lab company in the state of Florida from January 1, 2003 to December 31, 2005. This is the first study to asses methicillin resistance with a population based dataset and not patients from hospitals

The percent of isolates that were methicillin resistant increased as year increased. This increase in the number of methicillin resistant isolates was significant for both the

crude and adjusted analysis. When treated as a continuous variable and adjusted for age category, gender, and county of residence the odds ratio for year is 1.446, 95% CI: 1.410-1.484. In 2005, 49.7% of the isolates were methicillin resistant. Methicillin resistance also varied by age category, gender, county, and region. For age group and gender the differences were not large and may not be clinically significant. However, there was substantial variation in methicillin resistance by region and county of residence.

With nearly half of the *S. aureus* isolates being methicillin resistant, the β-lactam antibiotics may no longer be an ideal choice for treating *S. aureus* infections in Florida. The percentage of MRSA isolates that were resistant to trimethoprim-sulfamethoxazole, tetracycline, gentamycin, and rifampin was low. These antibiotics may be feasible alternatives to treat outpatient *S. aureus* infections in Florida.

Introduction

Background

The Staphylococci are non-motile, gram-positive bacteria that can cause infections in humans. They have a characteristic appearance that resembles "a bunch of grapes" (Ruben & Muder, 1998). The organism has the ability to survive in distressed environments such as acidic conditions, high sodium concentrations, and large temperature variations. It can persist on contaminated objects in the environment for more than a week (Daum & Seal, 2001).

The genome of *S. aureus* is a circular chromosome with prophages, plasmids, and transposons (Lowy, 1998). *S. aureus* achieves a genetic flexibility through small and large-scale horizontal transfer of genetic determinates (Buescher, 2005). The genes responsible for antibiotic resistance are found on the chromosome or extra chromosomal elements. These genes can be transferred not only between different *S. aureus* strains, but also between other gram-positive bacterial species via the extra chromosomal elements.

S. aureus also produces many surface proteins, which may play an important part in its ability to colonize host tissue. *S. aureus* produces a variety of toxins, which can cause proinflammatory changes in mammalian cells, toxic shock syndrome, food poisoning, and skin erythema and separation. The organism also produces enzymes that can destroy host tissue (Lowy, 1998).

Staphylococci can produce severe morbidity and mortality. The major concern for public health is the organisms potential to cause epidemics (Ruben & Muder 1998). Once established in a hospital or long term care facility, *S. aureus* can be difficult to control (Gemmell et al., 2006). *S. aureus* is currently the most common cause of skin and soft tissue infections in the United States, with the mortality of serious infections at twenty to twenty five percent (Fridkin et al., 2005).

Antibiotic Resistance

Before the introduction of antibiotics the mortality rate of invasive *S. aureus* infections was about 90% and about 70% of those who were infected developed metastatic infections (Daum & Seal, 2001; Fuda, Fisher, & Mobashery, 2005). With the introduction of penicillin in the 1940s the spread of the organism was initially checked, however the first resistant isolates were found only 1-2 years later. Penicillin resistance is conferred by a serine protease that hydrolyzes the β-lactam ring and inactivates the antibiotic. The prevalence of penicillin resistant *S. aureus* rapidly increased and the organism caused widespread outbreaks in hospitals and nurseries. Within 6 years of penicillin's introduction the prevalence of resistance reached 25% in hospitals and in 15-20 years the prevalence reached 25% in the community. Penicillin resistance continues to be highly prevalent and less than 5% of isolates are currently susceptible (Ruben & Muder, 1998; Chambers, 2001; Lowy, 1998).

Methicillin is a synthetic penicillin that is not susceptible to hydrolysis by staphylococcal β-lactamase. Methicillin was introduced in 1961 to combat penicillin resistant *S. aureus* strains(Kowalski, Berbari, & Osman, 2005). The first methicillin

resistant *Staphylococcus aureus* (MRSA) strain was reported in the United Kingdom of the same year (Chambers, 2001). Since then it has become prevalent in the Unites States and Europe and occurs worldwide. Diekema et al. (2001) used data from the SENTRY Antimicrobial Surveillance program collected from January 1997 to December 1999 to characterize the prevalence of MRSA and methicillin susceptible *S. aureus* (MSSA) from 52 nations across the world. They found MRSA prevalence rates of 46% (657/ 1427) in the Western Pacific region, 34.2% (2455/7169) in the United States, 34.9% (682/1956) in Latin America, 26.3% (916/3477) in Europe, and 5.7% (81/1410) in Canada. The prevalence of resistance for different countries varied within regions. In Europe resistance rates ranged from <2% in Switzerland and the Netherlands to 54.4% in Portugal. In the Western Pacific the prevalence ranged was from 23.6% in Australia to >70% in Japan and Hong Kong.

MRSA isolates are resistant to all β-lactam antibiotics: penicillins, carbapenems, and cephalosporins. The β-lactams act by interfering with the enzymes required for synthesizing the peptidoglycan layer of the bacterial cell wall. Methicillin resistance is mediated by the penicillin binding protein PBP2a, which is encoded by the *mec*A gene (Daum & Seal 2001; Tenover, 2006). PBP2a confers resistance to β-lactams in two ways. It sterically hinders the approach to the active site and it impedes the nucleophilic attack by the active site serine on the β-lactam ring (Fuda et al., 2005).

The difference in morbidity and mortality between MRSA and MSSA infections remains controversial. Studies of the association of MRSA and mortality have had inconsistent results. Abramson and Sexton (1999) examined the difference between patients with MRSA and MSSA primary blood stream infections. They found that

median attributable excess length of stay was longer for MRSA patients compared to MSSA patients (p= 0.23) and the median attributable total cost was greater for MRSA infections (p= 0.43). When examining *S. aureus* bacterimia (SAB) Conterno, Wey, and Castelo (1998) found a rate of mortality for MRSA infections that was four times that of those with a MSSA infection. Romero- Vivas, Rubio, Fernandez, and Picazo (1995) found that methicillin-resistance was an independent predictor of mortality in those with SAB. In a recent study of *S. aureus* bacterimia, Lodise and McKinnon (2005) found that those who had a MRSA infection spent 1.5 times longer in the hospital (p=0.005) and the cost of hospitalization was 2 times that (p=0.001) of patients who had MSSA, controlling for disease severity. This study did not find a significant association between MRSA and mortality. Other studies have also failed to find an association between MRSA infections and increased mortality (Mylotte & Tayara, 2002; Blot, Vandewoude, Hoste, & Colardyn, 2002).

In a study of methicillin resistance on the outcome of patients with surgical site infections (SSIs) Engemann et al. (2003) found that when compared to patients with MSSA, MRSA patients had a significantly greater 90 day mortality rate (OR= 3.4, 95% CI: 1.5- 7.2), a greater duration of hospitalization (2.6 additional hospital days, p= 0.11), and a 1.19 fold increase in hospital charges (mean extra cost due to methicillin resistance $13,901, p= 0.03).

Many MRSA strains are multi-drug resistant. Vancomycin and teicoplanin belong to the glycopeptide class of antibiotics and are currently the antibiotics of choice for treating multi-drug resistant MRSA infections, however strains of *S. aureus* have been found that have intermediate or full resistance to vancomycin (Waldvogel, 2000;

Appelbaum 2006a). Strains of *S. aureus* that are resistant to teicoplanin have been reported in France (Gemmell et al. 2006).

The glycopeptides act by inhibiting the synthesis of the bacterial cell wall. Thickening of the cell wall and the transfer of genetic material are hypothesized to be the cause of the development of vancomycin resistance. Vancomycin binds to the terminal D-alanyl-D-alanine of the bacterial cell wall precursors and inhibits cell wall synthesis. Resistance in vancomycin intermediate *S. aureus* (VISA) strains is thought to arise by the synthesis of extra D-alanyl- D-alanine residues, which bind vancomycin molecules and sequester them in the outer cell wall. Genetic analysis suggest that the first vancomycin resistant *S. aureus* (VRSA) case occurred from the in-vivo transfer of the vancomycin resistant genes, *van*A , from *E. faecalis* to a MRSA stain. This VRSA isolate was resistant through changing the D-alanyl- D-alanine termination residue to D-alanyl-D-lactate, which has a reduced affinity for vancomycin (Applebaum, 2006a).

In 1995 and 1996, two isolates from Japan were found to have reduced susceptibility to vancomycin. The first from the sputum of a 64-year-old male who underwent lung cancer surgery and the other from the surgical wound of a 4-month old infant. Both had been treated unsuccessfully with vancomycin. The first isolate had a pattern of heterogeneous resistance to vancomycin, on culture it produced sub-populations of cells with varying degrees of resistance (Appelbaum, 2006a; Hiramatsu, 1998).

A 1998 study of 195 non-university and 7 university hospitals in Japan, found that of the 970 isolates from the non-university hospitals 1.3% had heterogeneous resistance

to vancomycin and of the 129 isolates from the university hospitals 9.3% had heterogeneous resistance to vancomycin (Hiramatsu, 1998).

In June of 2002 the first VRSA isolate from the United States was identified from a 40-year-old man in Michigan with diabetes, peripheral vascular disease, and chronic renal failure. He had been treated for prior infection with vancomycin. Vancomycin-resistance *Enterococcus faecalis* and *Klebsiella oxytoca* were also isolated from the culture of an ulcer.

In September of 2002 another VRSA isolate was reported from a 70-year-old man who was morbidly obese and hypertensive in Hershey, Pennsylvania. This patient had also had multiple courses of treatment with vancomycin and the culture was also from an ulcer. In 2004, a third VRSA isolate was obtained from the urine sample of an elderly patient in New York. Other vancomycin-resistant enterococi were also isolated from this patient.

In 2005 a forth isolate was cultured from a 78-year-old man with coronary artery disease, diabetes, peripheral vascular disease, neuropathy, chronic renal insufficiency, and obstructive uropathy. This patient had received vancomycin therapy and vancomycin-resistant *E. faecalis* was isolated from a surveillance culture (Appelbaum, 2006b; Appelbaum, 2006a).

The in-vitro and in-vivo conjugative transfer of vancomycin resistance from *E. faecalis* to *S. aureus* has been demonstrated in the laboratory setting (Noble, Virani, & Cree, 1992). Three of the four VRSA isolates from the U.S. have been cultured from patients whose cultures also grew vancomycin-resistant enterococci, only the patient from Hershey did not have vancomycin-resistant enterococci isolates found. This could

indicate the in-vivo transfer of the *van*A gene from the enterococcus to *S. aureus* (Appelbaum, 2006b; Appelbaum, 2006a).

Using data from the SENTRY program Diekema et al. (2001) found that less than 1% of *S. aureus* isolates had reduced susceptibility to Vancomycin and only one isolate was resistant. There were also 5 isolates with reduced susceptibility to teicoplanin.

Currently there are three effective drugs on the market to treat multi-drug resistant MRSA infections other than vancomycin. Linezolid, which blocks the assembly of the initiation complex required for protein synthesis, and daptomycin, which promotes the efflux of potassium out of the cell, are both effective treatments for MRSA and since they have unique mechanisms of actions there is no cross-resistance to other antibiotics (Anstead & Owens, 2004). In a open-label, randomized, multinational study conducted among hospitalized patients with MRSA surgical site infections to compare vancomycin to linezolid, significantly more patients that received linezolid were microbiologically cured (87% versus 48%, p= 0.0022). Unlike vancomycin, linezolid has an oral formulation that is completely bioavailable and can be used on an outpatient basis(Weigelt, Kaafarani, Itani, & Swanson, 2004).

Quinupristin-dalfopristin has been approved for the treatment of *S. aureus*, however due to high cost and adverse effects it is has not been widely used since linezolid and dapomycin have come onto the market. There are also three other promising drugs currently undergoing clinical trials that should enter the market within the next couple of years. Two new glycopeptides, oritavancin and dalbavancin, which inhibit cell-wall formation and are not affected by the *van*A, *van*B, and *van*C encoded alterations that impart resistance to vancomycin. The third drug under development is

tiglecycline, a glycycycline that is an analog of tetracycline. These new drugs are also effective against VISA and VRSA isolates (Anstead & Owens, 2004).

Transmission

The major source of outbreaks is due to carriers. Carriage of *S. aureus* is strongly associated with subsequent infection. The anterior nares are the major site of carriage in children and adults. The rate of nasal carriage is estimated to be from 20% to 40%. Vaginal carriage has also been reported and is estimated to be about 10% among pre-menopausal women. About 20% of the population will be prolonged carriers of *S. aureus*, 60% will be intermittent carriers, and 20% will never be colonized. Some people may become colonized with several different strains at the same time (Waldvogel, 2000; Kuehnert et al., 2006).

As part of the National Health and Nutrition Examination Survey, 2001- 2002, Kuehnert et al. (2006) examined the nasal colonization rates of 9,622 people. The weighted prevalence of *S. aureus* colonization was 32.4% (95% CI: 30.7%- 34.1%). Colonization was highest among those 6-11 years of age (OR= 2.7, 95% CI: 2.0- 3.6, reference group: 1 to 5 year olds). The weighted prevalence of MRSA colonization was 0.8% (95% CI: 0.4%- 1.4%). They found that MRSA colonization was associated with being female (OR= 2.0, 95% CI: 1.2- 3.4) and age greater than 60 (OR= 4.3 95% CI: 1.2- 14.8). No other factors investigated, such as poverty, education, birth outside the US, military service, health-care exposure, or presence of diabetes or dermatologic conditions, were found to be statistically significant.

Certain groups of people are more prone to carriage. Physicians, nurses, and hospital ward attendants may have higher nasopharyngeal carriage rates than the general population. Other groups at higher risk of carriage include; diabetics receiving insulin injections, those undergoing chronic hemodialysis or continuous ambulatory peritoneal dialysis, those with a variety of dermatologic conditions, illicit intravenous drug users, and HIV positive patients (Waldvogel, 2000). Nasal carriers are predisposed to postoperative infection (Ruben & Muder 1998).

From the anterior nares carriage site, the bacteria can be transferred to the skin. The mucous membrane and skin are an effective barrier to tissue infection. Trauma provides *S. aureus* with a portal of entry and leads to a local or generalized infection. In the case of hospitals and long term care facilities, the bacteria are usually introduced into an institution via an infected or colonized patient or by a colonized health care worker. The bacteria are then transferred from one patient to another by the hands of health care workers or the inanimate environment. This has led to major epidemics in hospitals and other chronic care facilities (Waldvogel, 2000). In a study of contact transmission, McBryde, Bradley, Whiteby, and McElwain (2004) found that 17%(9- 25%) of contacts between a health care worker and a patient colonized with MRSA resulted in the transmission of MRSA onto the gloves of the health care worker.

Other routes of transmission have also been reported. Embil et al. (2001) investigated an outbreak of MRSA on a burn unit from September 19 to November 20, 1996. They found that a hand held shower and stretcher for showering in the hydrotherapy room were culture positive for the outbreak strain of MRSA and the most likely route of transmission. In an investigation of a MRSA outbreak on an Intensive

Therapy Unit Cotterill, Evans, and Fraise (1996) found that the source may have been the air-exhaust component of the isolation room's ventilation system. The exhaust grille for the ventilation system of the isolation room was in close proximity to a window above another patient bed. It is hypothesized that it would be possible for particles being exhausted from the ventilation system to be blown into the air and through the window.

Kluytmans et al. (1995) studied an outbreak of MRSA between November 1992 and April 1993 affecting 27 patients and 14 health-care workers at the University Hospital Rotterdam, Dijkzigt, The Netherlands. MRSA was subsequently found in a banana and the outbreak strain was detected in a culture of a dietary workers nares that had prepared food for the hematology unit. This is the first reported incident of a food initiated MRSA outbreak. In 2002, Jones et al. (2002) reported a cluster of gastroenteritis in a community setting. Three adults became ill after consuming shredded pork and coleslaw from a convenience-market delicatessen. The likely source of contamination was determined to be a food handler who was a nasal carrier of the outbreak MRSA strain.

There is also growing evidence of animals as potential sources of infection. Potential human to animal and animal to human transmission have been reported among veterinary personal and pet owners (Weese et al., 2005; Weese et al., 2006; O'Mahoney et al., 2005; Manian, 2003). Weese et al. (2005) investigated an outbreak of MRSA among veterinary personal that had worked with a neonatal foal colonized with MRSA at the Ontario Veterinary College Veterinary Teaching Hospital. MRSA skin infections were found in three of the neonatal intensive care unit personnel and 10 of 103 other veterinary personnel were found to be nasaly colonized. All of the isolates were

indistinguishable by pulsed field gel electrophoresis (PFGE) analysis and were classified as CMRSAA-5. While it is difficult to determine the direction of transmission, it appears that there was transmission between humans and horses at this institution.

Weese et al. (2006) investigated six cases of MRSA infection in 8 animals. In each case a MRSA isolate with an indistinguishable PFGE pattern was isolated from at least one human. All of the isolates were found to be the Canadian epidemic MRSA-2 strain.

Manian (2003) examined a case of MRSA in a diabetic patient and his wife who had repeated MRSA infections and nasal colonization despite antibiotic therapy and decolonization attempts. Nares cultures of the pet dog grew MRSA with an identical PFGE pattern. The recurrent MRSA infections in the patient and his wife were resolved once MRSA had been eradicated from the pet dog. In this case it is likely that the dog served as a reservoir of MRSA, which led to repeated infections in the couple.

O'Mahony et al. (2005) documented the recovery of MRSA from 25 animals and 10 veterinary personnel from different locations throughout Ireland. PFGE analysis showed that most of the non-equine isolates (14 dogs, one cat, one rabbit, and one seal) were indistinguishable from each other and from the personnel caring for the infected animals. This strain was indistinguishable by PFGE analysis from the most prevalent MRSA strain in the Irish population. The eight isolates from horses and the isolates from their personnel were indistinguishable from each other and were unlike those from the other animals.

Concurrent colonization with indistinguishable PFGE patterns suggests that human to animal and animal to human transmission of MRSA is possible. The

possibility of household pets as a reservoir of MRSA should be considered in patients with recurrent Community Acquired-MRSA (CA-MRSA) infections in which no other source can be identified (Weese et al., 2006; Manian, 2003).

Types of Infections

S. aureus can cause a variety of infections. Skin infections of *S. aureus* include folliculitis, furuncles (boils), impetigo, hydradenitis suppurativa, mastitis, wound infection, and spreading pyodermas. Treatment for these localized skin infections is the removal of hair from the area, repeated cleansing with an antiseptic solution alternating with a moist dressings, and covering the infected area with a sterile dressing. Parenteral vancomycin and teicoplanin may also be considered as part of the treatment for skin infections of MRSA if there is a high risk of serious infection such as bacteremia or endocarditis (Gemmell et al 2006; Waldvogel, 2000).

Two localized infections with diffuse skin rash are Staphylococcal Scalded Skin Syndrome (SSSS) and Toxic Shock Syndrome (TSS). SSSS is treated with parental antibiotics and supportive skin care. TSS treatment requires aggressive fluid replacement followed by intravenously administered antibiotics (Waldvogel, 2000).

S. aureus can spread to other sites including the bones, joints, kidneys, and the lungs. In severe cases, infection can lead to septicemia and endocarditis. Factors such as advanced age, immunosuppression, chemotherapy, and invasive procedures have been found to increase the risk of sepsis (Lowy, 1998). In most instances, *S. aureus* bacteremia is the result of a localized infection gaining access to the blood stream. Patients initially experience chills and occasionally frank rigors. Patients are often obtunded and have joint pain and more rarely pleuritic chest pain. Antibiotics should be

promptly administered and in some cases endocarditis warrants surgical intervention (Waldvogel, 2000).

Methicillin Resistance Patterns

Diekema et al. (2004) performed a representative survey of 670 US hospitals, stratified by number of beds, geographic region, and teaching status to examine the antibiotic resistance trends for major pathogens, the frequency of outbreaks, and control measures. In the 494 hospitals that responded, 36% of the *S. aureus* isolates were found to be MRSA. There were significant differences in oxacillin resistance by region ($p = 0.0001$), with higher rates of MRSA in the south. MRSA was the most common resistant pathogen in US hospitals to cause outbreaks and was increasing in more than two thirds of the hospitals surveyed.

Li et al. (2005b) used statewide, population-based antimicrobial susceptibility test data collected from both outpatients and inpatients in Hawaii to examine the epidemiologic trends of MRSA in the state. Data was collected retrospectively from the State of Hawaii Antimicrobial Resistance Project from 2000 to 2002. After removal of duplicate data 31,482 isolates remained in the analysis, of which 8,206 (26%) were found to be MRSA. They found that the proportion of MRSA isolates during the study period was significantly higher among pediatric patients then among the adult population ($p<0.01$) and that a significantly higher proportion ($p< 0.01$) of the adult isolates were resistant to non-β-lactam antibiotics compared to the pediatric isolates. Most of the pediatric isolates were susceptible to the non-β-lactam antibiotics except for erythromycin (24% of isolates were resistant). They also found a significant increase in

the proportion of MRSA isolates, from 24% to 30% ($p<0.01$), among the adult population and an increasing, but not significant trend among pediatric patients.

Moran et al (2006) performed a prospective prevalence study of adult patients who presented to hospitals in the EMERGEncy ID Net, a network of emergency departments in 11 US cities, to examine methicillin resistance in skin and soft tissue infections in August of 2004. Of 422 patients with skin and soft tissue infections, 320 were due to *S. aureus*. Of the *S. aureus* isolates, 78% were methicillin resistant. Among those who received antibiotics, antibiotic therapy given was not active against the infecting bacterium in 57% (100 of 175) of cases. Only presence of abscess at enrollment was significantly associated with MRSA compared to MSSA. Twenty seven percent of MRSA patients had an established risk factor for methicillin resistance.

Community Associated MRSA

Initially infections of MRSA were primarily a problem of hospitals, nursing homes, and long term care facilities. Traditional risk factors for Health care Associated-MRSA (HA-MRSA) infections include frequent contact with the health care environment, prolonged hospitalization, recent hospitalization or surgery, living in a long-term care facility or nursing home, advanced age, immunocompromise, dialysis, use of anti-microbial agents within the previous 60 days, and indwelling medical devices. In the early 1980s, cases of MRSA began to emerge in the community, mostly among those with a history of injection drug use and other patients at high risk. Recently, CA-MRSA infections have been found in adults and children who did not have exposure to hospitals or other established risk factors. These infections acquired in the community are referred to as community-acquired MRSA (Daum & Seal, 2001; Jones, Kellum, Porter, Bell &

Schaffner, 2002; Niami et al., 2003). Certain populations in the US have been found to have an increased risk of CA-MRSA. CA-MRSA outbreaks have been reported among IV drug users, children (particularly those in daycare), soldiers, competitive sport players, the disadvantaged, native Americans and Alaskan natives, prisoners, men who have sex with men, and the urban homeless. Lack of hygiene and basic-infection control may contribute to outbreaks among these populations (Kowalski et al., 2005; Appelbaum, 2006a).

There are several features that distinguish CA-MRSA from HA-MRSA strains. CA-MRSA lacks the presence of hospital-associated risk factors. CA-MRSA is generally susceptible to most antibiotics other than β-lactam antimicrobial drugs, unlike HA-MRSA strains that exhibit multi-drug resistance. CA-MRSA has distinct genotypes that differ from the *S. aureus* strains commonly found in hospitals. CA-MRSA predominantly carries the type IV staphylococcal chromosomal cassette *mec (SCCmec)*, whereas HA-MRSA carries cassettes I, II, or III. The HA-MRSA strains typically carry a *mec*A gene that is positioned next to other genetic elements, which confers resistance to other antibiotics. The type IV *SCCmec* is smaller and lacks the additional genetic elements, which confer multi antibiotic resistance. *CA*-MRSA typically carries genes encoding for toxins such as Pantone-Valentine leukocidin (PVL),a leukocyte-killing toxin, and many other staphylococcal enterotoxins. The PVL cytotoxins can cause tissue necrosis and leukocyte destruction. Pantone-Valetine leukocidin toxin is thought to contribute to the virulence of CA-MRSA strains (Charlebois et al., 2004; Weber, 2005; Rihn, Michaels, & Harner, 2005; Appelbaum, 2006b).

The most common clinical manifestation of CA-MRSA are furunculosis and cutaneous skin abscesses. Approximately 90% of CA-MRSA infections are non-invasive skin and soft tissue infections. These skin and soft tissue infections account for most of the morbidity of CA-MRSA, with mortality very uncommon (Kowalski, 2005; Stankovic & Mahajan, 2006).

The origins of CA-MRSA are unknown and may have arisen by different pathways. Hospital strains of MRSA may be carried into the community, where they can then spread from person to person. CA-MRSA may also arise de novo when the methicillin-resistance gene complex is acquired by a methicillin-susceptible strain of *S. aureus* (Charlebois et al., 2004).

Naimi et al. (2003) compared HA-MRSA and CA-MRSA. Twelve sentinel laboratories in Minnesota were used to identify cases of MRSA from January 1, 2000 to December 31, 2000. A medical record review was conducted to determine whether they met the definition of health care associated or community acquired MRSA. During the study period 4612 patients were identified with an *S. aureus* clinical culture, of these 25% were identified as MRSA infections. Of the MRSA infections 131 (12%) were classified as community acquired, 937 (85%) as health care associated, and 32 (3%) could not be classified due to lack of information.

The CA-MRSA patients were significantly younger ($p<0.001$) than health care associated patients with a median age of 23 years versus 68 years. Race was documented for 72% of the CA-MRSA patients and 64% of the health care related patients. The CA-MRSA patients were significantly more likely to be nonwhite, OR= 3.13, 95% CI: 2.16-4.32.

The distribution of the type of clinical infections was also different between the community acquired and health care associated cases. The CA-MRSA cases were significantly more likely to involve skin and soft tissue, OR= 4.25, 95% CI 2.97- 5.90. The CA-MRSA cases were also significantly less likely to be respiratory infections, OR = 0.22, 95% CI 0.09- 0.49 and urinary tract infections, OR = 0.04, 95% CI 0- 0.24. The CA-MRSA isolates were significantly more likely to be susceptible to ciprofloxacin, clindamycin, gentamicin, and trimethoprim-sulfamethozazole, OR= 5.88, 95% CI 4.86- 6.64.

PFGE testing identified 119 distinct subtypes. From these subtypes, five clonal groups containing 3 or more isolates were identified and accounted for 96% of the isolates. Clonal group A accounted for 62% of the CA-MRSA isolates, but only 9% of the health care related ones (OR= 4.61, 95% CI 3.82- 5.16). Clonal group B accounted for 14% of the CA-MRSA isolates and 2% of the health care related isolates (OR= 2.43, 95% CI 1.61- 2.93). Clonal group H was associated with health care related MRSA, accounting for 80% of the isolates in that group versus 16% of the CA-MRSA isolates (OR= 2.83, 95% CI 2.60- 2.97).

Fridkin et al. (2005) used data from the MRSA Active Bacterial Core Surveillance project which monitored all MRSA isolates from 11 Baltimore hospitals, Health District 3 in greater Atlanta, and 12 sentinel hospitals in Minnesota to examine the incidence of endemic CA-MRSA infection, racial disparities in incidence, patterns of anti-microbial susceptibility, and clinical outcomes. Surveillance was from February 2002 to February 2003 in Baltimore, from July 2001 to January 2003 in Atlanta, and from January 2001 to January 2003 in Minnesota.

During the study period 12,553 MRSA isolates were reported. Of these 2,107 were classified as confirmed or probable CA-MRSA. In Atlanta and Baltimore the incidence was significantly higher among those who were less than two years old, OR=1.51 (95% CI: 1.19- 1.92). In Atlanta the incidence was significantly higher among blacks than whites, age-adjusted relative risk = 2.74 (95% CI 2.44- 3.07).

Groom et al. (2001) used a retrospective cohort study design to examine the occurrence of CA-MRSA and risk factors associated with CA-MRSA compared with MSSA among a rural American Indian community during 1997. They found that of the 112 isolates 55% (62) were MRSA and 45% (50) were MSSA. Of the 62 MRSA infections 74% (46) were classified as community acquired. CA-MRSA isolates were more likely to be susceptible to other antibiotics than non-community acquired isolates, only resistance to ciprofloxacin was significant however. No significant differences in risk factors were found between CA-MRSA and CA-MSSA.

The current approach to treatment of community acquired *S. aureus* infections commonly includes the use of β-lactam antibiotics. In communities where MRSA has become a significant proportion of *S. aureus* isolates this treatment approach may need to be reevaluated. Clinicians need to consider the possibility of MRSA infection in the community setting and obtain material for bacterial culture to determine the susceptibility of the infecting organism (Fridkin et al., 2005).

Molecular typing studies have found that in the U.S. most of the CA-MRSA infections are caused by two clones, USA 300 and USA 400. These clones have also been associated with the Panton-Valentine leukocidin (PVL) virulence factor and the SCCmec type IV. These CA-MRSA clones are typically resistant to β-lactams and

erythromycin, but remain susceptible to clindamycin, trimethoprin-sulfamethoxazole, and fluoroquinolones. King et al. (2006) conducted active laboratory surveillance from August 1, 2003 to November 15, 2003 of *S. aureus* isolates from patients with skin and soft tissue infections at a 1000-bed urban hospital and its outpatient clinics in Atlanta, Georgia. This hospital provides for a medically indigent inner-city population, about 80% of which are African American.

The isolates were defined as USA 300 or USA 400 based on PFGE or antimicrobial susceptibility pattern (anti-biogram) if PFGE was not available. If the isolates with no PFGE were resistant to the β-lactams and erythromycin, but susceptible to clindamycin, trimethoprin-sulfamethoxazole, vancomycin, and fluoroquinolones they were classified as USA300/USA400.

There were 389 episodes of *S. aureus* skin and soft tissue infection among 384 patients, of which 72% (279) were found to be MRSA and 28% (100) were MSSA. Of the 389 infections, 279 were classified as CA-MRSA and of those 87% (244) were classified as the USA 300/USA 400 CA-MRSA group. When only isolates with a PFGE were considered (175 isolates), 91% (159) could be classified as USA 300/ USA 400. Of the CA-MRSA isolates with PFGE patterns that matched the USA 300 or USA 400 pattern, 87% (136) met the antimicrobial susceptibility pattern of resistance only to the β-lactams and erythromycin. Of the isolates with PFGE patterns that had a pattern other than the USA 300/USA 400, 88% (14 of 16 isolates) met the antimicrobial susceptibility pattern of HA-MRSA with resistance to the β-lactams, erythromycin, and at least one additional antibiotic.

It was also found that inadequate empirical and definitive antibiotic therapy was more common among the CA-MRSA group (65% inadequate empirical and 43% inadequate definitive) versus the MSSA group (1% and 1%). When comparing the CA-MRSA USA300/USA400 group to the MSSA group black race (prevalence ratio 1.53, 95% CI: 1.16- 2.02), female sex (prevalence ratio 1.16, 95% CI: 1.02- 1.32) and previous hospitalization within 12 months (prevalence ratio 0.80, 95% CI: 0.66- 0.97) were found to be significantly associated in multivariate analysis. In areas with a high prevalence of CA-MRSA non-β-lactam agents should be used as empirical therapy (King et al., 2006).

In a study of *S. aureus* infections at Texas Children's Hospital from August 1, 2001 to July 31, 2004 Kaplan et al. (2005) found that the percentage of community associated *S. aureus* isolates that were methicillin resistant significantly increased during the study period, from 71.5% in 2001 to 76.4% in 2004 (p=0.008). They also found that in the beginning of 2000 the percentage of CA-MRSA isolates that were the USA300 clone was 50%. In 2003 the percentage of CA-MRSA isolates that were USA300 was >90% and carried the PVL gene. This indicates that the USA300 strain is capable of rapidly spreading in the community.

The risk factors for community acquired MRSA differ from those for health-care related MRSA infections. Factors that have been associated with a higher risk for nosocomial acquisition of MRSA (HA-MRSA) are prolonged hospitalization, care in an intensive care unit, prolonged antimicrobial therapy, surgical procedures, dialysis, presence of an indwelling catheter, use of injectable drugs, residence in a nursing home or long term care facility, and close proximity to a patient in the hospital who is infected or colonized with MRSA (Salgado, Farr, & Calfee, 2003; Li et al., 2005b).

While hospital admission within the past twelve months has been identified as a risk factor for CA-MRSA in some populations, there have been reports of CA-MRSA in patients with no identifiable risk factors (Ellis, Hospenthal, Dooley, Gray, & Murray, 2004).

High Risk Populations

There have been reports of infections and outbreaks of CA-MRSA among children. Hunt et al. (1998) reported four pediatric deaths due to CA-MRSA. All four lacked traditional risk factors and were initially treated with a cephalosporin to which the MRSA strain was not susceptible. Delayed use of an antibiotic to which the MRSA strain was not susceptible may have contributed to the fatal outcomes.

Herold et al. (1998) conducted a retrospective review of medical records to examine CA-MRSA in children with no predisposing risk factors admitted to the University of Chicago Children's Hospital. Cases of MRSA infection were classified as community acquired if they were obtained within 72 hours of admission, if they were obtained after 72 hours they were classified as nosocomially acquired. Children with CA-MRSA were classified as those without identified risk if they lacked a traditional risk factor and those with an identified risk factor if they had a previous hospitalization of antimicrobial therapy within the previous 6 months, history of endotracheal intubation, an underlying chronic disorder, an indwelling venous or urinary catheter, history of surgical procedure, or a notation of a household contact with an identified risk factor in the medical record.

From 1988 to 1990 there were 32 cases of MRSA identified and from 1993 to 1995 there were 56 cases of MRSA identified. For 1988 to 1990, 8 of the MRSA cases

were determined to be community-acquired and for 1993 to 1995, 35 of the MRSA cases were identified as CA-MRSA. Only one of the cases from 1988 to 1990 lacked an identified risk factor while 25 of the cases from 1993 to 1995 lacked a traditional risk factor. In this population the prevalence of CA-MRSA with no identified risk factors increased from 10 per 100,000 admissions in 1988 to 1990 to 259 per 100,000 admissions in 1993 to 1995.

The clinical spectrum of disease associated with MRSA and MSSA isolates from 1993 to 1995 was also examined. Among children with CA-MRSA without identified risk none of the 22 children had bacteremia without a focus of infection and 27% (6 of 22) had a diagnosis of abscess. Among children with an identified risk factor 20% (2 of 10) had bacteremia without a focus and none of the 10 had a diagnosis of an abscess.

The distribution of clinical syndromes for CA-MRSA was also compared to CA-MSSA. In children with CA-MRSA without identified risk the distribution of clinical syndromes was similar to that of CA-MSSA. Cellulitis and abscess were the predominate clinical syndromes for both CA-MRSA and CA-MSSA.

Creech et al. (2005) examined the prevalence of *S. aureus* colonization in healthy children attending health maintenance visits in 2004. Nasal swabs were collected from 500 children. They found that 36.4% (182) children were colonized with *S. aureus* and 9.2% (46) were colonized with MRSA. When compared to the prevalence rate of colonization in 2001, the rate in 2004 was significantly higher ($p< 0.001$). The only significant risk factor found was having a family member that worked in a hospital (OR= 2.0, 95% CI: 1.03- 4.1).

Infections of athletes, such as football players, rugby players, wrestlers, and fencers have been reported. Among this group risk factors for infection that have been identified are skin trauma, contact with lesions of infected players, and sharing equipment or clothing (Barrett & Moran, 2004). Studies of athletes suggest that players involved in frequent, repetitive contact are more likely to develop an infection, supporting the hypothesis that transmission occurs via direct person-to-person contact (Rihn et al., 2005).

Kazakova et al. (2005) conducted a retrospective cohort study of 84 St. Louis Rams football players and staff members. From September 1 through December 1, 2003 8 MRSA infections were identified in 5 of 58 Rams players. All were skin abscesses and developed at turf burn sites. Only the lineman or linebacker position and body-mass index was significantly associated with MRSA infection, RR=10.6 (95% CI 1.3- infinity) and p= 0.03 . This may be due to the frequent contact among linemen during play. The outbreak strain carried the SCCmec type IV and PVL genes. Comparison of PFGE patterns of the outbreak strain with those of other isolates revealed that the outbreak strain had an indistinguishable PFGE pattern from strains of two other professional football teams. Eighty-four nasal cultures were obtained. Of these none grew MRSA and 42% grew MSSA. MSSA was also cultured from environmental samples and was found to have an identical PFGE pattern to isolates obtained from nasal swabs.

Nguyen, Mascola, and Bancroft (2005) examined recurring MRSA infections among a college football team in Los Angeles. Using a case-control design they found that sharing bars of soap and having preexisting cuts or abrasions were associated with an increase risk of becoming infected. In a 'carrier-control' study they found that having a

locker near a teammate with an infection, sharing towels, and living on campus were associated with nasal carriage.

In another study of college football players, Begier et al. (2004) investigated an outbreak of MRSA in Connecticut, which had resulted in 2 hospitalizations. Ten case patients were identified from the 100 players on the team. Of the six isolates available for PFGE, all had the pattern of USA 300 and carried the PVL gene. Analysis revealed an increased risk among those who had cosmetically shaved the groin area (RR 9.3, 95% CI: 2.3- 37.6) or shared the whirlpool greater than or equal to 2 times a week (RR 12.2, 95% CI: 1.4- 109.2). Those who had sustained turf burns had a RR of 7.2, 95% CI: 1.0- 54.4.

In a retrospective cohort study of a MRSA outbreak among a high school wrestling team and the surrounding community Lindenmayer, Schoenfeld, O'Grady, and Carney (1998) found that none of the risk factors investigated (demographics, wrestling history, use of Jacuzzi, sharing towels or clothing, and contact with health care facilities) was associated with a significant increase in risk for developing a MRSA infection. Eleven non-wrestlers also developed MRSA infections, six had a connection with the high school while the other five had no known connection with the wrestling team or the high school. The authors can only speculate that MRSA may be transmitted by less direct contact than that which occurs among wrestlers or that some unknown mode of transmission was responsible for the community cases.

Military recruits are another high risk group. Ellis et al. (2004) examined the prevalence and risk factors for CA-MRSA among 812 U.S. Army recruits. Recruits had a nasal swab at the beginning of their training and 8- 10 weeks later. At the first

sampling 3% (24) of the recruits were colonized with MRSA and 28% (229) were colonized with MSSA. Antibiotic use within the previous 6 months was the only risk factor for CA-MRSA colonization that was significant (p=0.03). Colonization with MRSA at the initial sampling was a significant risk factor for developing a soft-tissue infection. During the study period 38% (9) of MRSA carriers developed soft-tissue infections while only 3%(8) of MSSA carriers developed infections (RR= 10.7, 95% CI 4.6- 25.2, p< 0.001).

Zinderman et al. (2004) investigated an outbreak of MRSA among military recruits at a training facility in the Southern U.S.. From August to December 2002 there were 235 cases of MRSA identified. Most of the infections (73%,) occurred on an extremity. Nasal swabs of 874 workers who had direct contact with recruits found that 2.7% (24) were colonized with MRSA. Once control measures were implemented such as placing antibacterial soaps at all recruit sinks, enforcing daily showers of adequate duration, and prohibiting sharing of personal items the outbreak subsided.

Outbreaks of MRSA have also been reported among inmates in correctional facilities. A case-control study from a minimum-security detention center in Georgia identified prolonged incarceration (>36 days) and outdoor work duty as risk factors for MRSA acquisition. Another case-control study from a maximum-security prison identified previous antimicrobial use, self-draining of boils, skin laceration, washing clothes by hand, sharing soap, and recent arrival at the prison as risk factors for MRSA infection. A case-control study from the Texas Department of Criminal Justice's (TDCJ) largest intake facility identified previous skin infections and recent close contact with a MRSA-infected inmate as risk factors. In 1998 TDCJ began requiring the culturing of all

draining skin lesions. The proportion of *S. aureus* infections that were found to be MRSA increased from 24% in 1998 to 66% in 2002.

The investigators of these studies identified four factors that contribute to the spread of MRSA in jails and prisons. There are barriers to inmate hygiene such as limited access to soap due to security reasons. Access to medical care can be held up due to co-payments required for care and insufficient supplies and staff. Frequent medical staff turnover make providing education on proper infection-control procedures difficult and MRSA may not be recognized as the cause of skin infections (Tobin-D'Angelo, 2003).

There have also been reports of outbreaks of CA-MRSA among Native Americans, Alaskan natives, and Pacific islanders. Baggett et al. (2003) conducted a retrospective cohort study in rural southwestern Alaska to investigate a large outbreak of community-onset MRSA infections and to determine the extent of the infections and whether the isolates were CA-MRSA. This area of Alaska has a population of 25,000, of which about 85% are Alaskan Natives. Many live in isolated villages with no running water. Isolates from the microbiology laboratory records were identified from March 1, 1999 to August 10, 2000. The percentage of isolates that were MRSA was 82.2% (412 of 501) for the study period.

Through chart review there were 240 isolates recovered from 229 patients. Of the 240 isolates 75% (180) were MRSA, and skin infections accounted for 73% (175) of the isolates. Of the 175 skin infection isolates, 86% (151) were MRSA and 14% (24) were MSSA ($p< 0.01$). When comparing CA-MRSA with non-CA-MRSA, sex was found to be significantly different, OR= 2.5, 95% CI: 1.1- 5.8.

All of the 143 cases of MRSA skin infections during the study period were community onset. There were 109 that met the definition of CA-MRSA infections. Seventy-six percent of the MRSA isolates from this population were community acquired, suggesting widespread acquisition and transmission in the community. The high prevalence of methicillin resistance supports the recommendation that the β-lactams should not be used as first line therapy in this population. The authors recommend that trimethoprim-sulfamethoxazole be used as first line therapy, with vancomycin only used in patients with severe infections.

Melish et al. (2004) conducted a retrospective study of CA-MRSA infections in Hawaii from July 2001 to June 2003. During the study period 1,389 patients from the four study facilities were identified as having MRSA, of which 389 (28%) had an illness that was consistent with CA-MRSA. Racial/ethnic data was available for 346 (89%) of these patients. Pacific Islanders accounted for 51% (178) of the CA-MRSA patients, but they made up only 24% of the population of Hawaii in 2001 ($p<0.01$). In the pediatric and women's center that was included in the study Pacific Islanders accounted for 76%(90 of 118) of the CA-MRSA patients, but only 35% of the patients served by these centers are Pacific Islanders ($p< 0.01$).

Objectives

The purpose of this study is to examine whether or not methicillin and the β-lactam class of antibiotics should continue to be used to treat Staphylococcal infections in Florida. The study will examine the proportion of MRSA versus MSSA infections in Florida and who is becoming infected with methicillin resistant *S aureus* strains. The β-lactam antibiotics are ineffective against MRSA isolates. If the percent of *S. aureus* isolates that are methicillin resistant is high then β-lactam antibiotics may not be the ideal choice for initial treatment of *S. aureus* infections in this population. In clinical practice antibiotic use needs to adjust to changes in the prevalence of resistance (Gemmell et al. 2006). Knowing the prevalence of resistance in Florida will allow clinicians to adapt treatment of *S. aureus* infections based on the likelihood that the strain is resistant.

The study will also examine potential associations between gender, age, county, and region with regard to methicillin resistance to examine whether any subgroup of the population has a higher risk of having infections with methicillin resistance. Secondary descriptive analysis will include examining any association between MRSA and site of infection (ear, eye, nasal, other, respiratory, skin and soft tissue, sinus, and sterile site) and how many of the isolates in the dataset have the USA300/USA400 type anti-biogram (the most common CA-MRSA clones in the U.S.).

The hypothesis is that the proportion of *S. aureus* isolates that are methicillin resistant have increased from January 1, 2003 to December, 31 2005 and that there are differences

in those who contract methicillin-resistant versus methicillin susceptible *S. aureus* infections.

Methods

Database

The dataset is a record of all the *S. aureus* isolates tested by a large lab company in the state of Florida from January 1, 2003 to December 31, 2005. This is the first statewide surveillance of both methicillin resistant and methicillin susceptible *S. aureus* and was conducted over a three year time period. The dataset contained a large number of isolates. Before exclusions there were 67,790 isolates. This dataset consists almost entirely of cultures from outpatient facilities. Only 275 of the total 67,790 isolates, 0.4%, were from a provider with hospital in the name. This is in contrast to previous studies of MRSA that have been based on *S. aureus* isolates taken from emergency room visits or hospitalized patients.

Data Included and Excluded

The study design of this investigation is cross-sectional since information on both the outcome (Methicillin resistance) and exposure (age, gender, county) was collected at the same point in time. The original dataset was cleaned at the Florida Department of Health. In the initial dataset the antibiotic susceptibilities that were tested for each person were listed down in approximately fifteen rows. The antibiotics and the resistance variables were transposed in order to make each observation into one row. First and last name and date of birth were used to determine if two isolates were duplicates. If two observations had the same first and last name and date of birth then they were considered

to be the same person. Address was not use to categorize observations as duplicates due to large variation in the manner in which street addresses were recorded and there is also the possibility that a person could have moved during the study period, which would cause them to be counted more than once.

The dataset was examined manually to look for potential errors in first and last name and date of birth. If it was determined that there was an error in the first and last name or date of birth then it was changed manually. For all three years there were 298 observations that were changed.

Some patients had more than one isolate taken for testing on the same day. A majority of these had the same antibiotic susceptibility profile. For patients who had different susceptibilities for an antibiotic the most resistant isolate was used for the analysis.

Some observations that had two isolates taken on the same day had two different sources. For these observations the isolate from the more serious infection was used. In nearly all of those who had two sources, one of them was a nasal swab or unknown. The other source recorded was used because a nasal swab may be a test for colonization with MRSA and not infection. There were only two observations that had two sources in which one of them was not nasal or unknown.

As per National Committee for Clinical Laboratory Standards (NCCLS) guidelines, only the first isolate per person per analysis period of 365 days was used in the analysis. This approach has been validated by several studies evaluating isolate removal methods (Horvat, Klutman, Lacy, Graver, & Wilson, 2003; Lee et al., 2004; Li et al., 2005; Magee, 2004; Shannon & French, 2002). It is believed that patients with a

methicillin resistant isolate may have repeat cultures taken more often than patients who have susceptible *S. aureus* isolates, leading to a biased resistance estimate (Magee, 2004). Horvat et al. (2003) found that there was a significant difference between de-duplicated methicillin resistance estimates and estimates including all isolates and that 91% of patients tested did not switch between MRSA and MSSA during the 6-year analysis period. Lee et al. (2004) and Li et al. (2005) found that those with MRSA isolates had more duplicates then those with MSSA and including all isolates produced lower susceptibility estimates. Lee et al. (2004) concluded that, "These results suggest that the method of calculating results for the first isolate per patient may remove the effect of duplication, allowing the simple and unambiguous analysis of cumulative susceptibility rates" (p. 4776).

Exposure

The exposures used in the analysis were gender, age, and county and region of residence. Age was put into 10-year intervals of <1, 1-10, 11- 20, 21- 30, 31-40, 41- 50, 51- 60, 61- 70, 71- 80, 81- 90, and >90. Those who were less than one year of age were put into a separate category because they may have different risks than older children. In the analysis age was treated as a nominal variable. Of those who were 11 to 40, the 11 to 20, 21 to 30, and 31 to 40 age groups, the group with the lowest resistance was selected. This age range was chosen because these age categories contain young healthy people. The 11 to 20 age group had a large number of isolates and had the lowest resistance of those who were 11 to 40 and was selected as the reference category. Ten dummy variables were created for the eleven age categories. In a separate analysis age was

divided into those who were less than 18 and those greater then or equal to 18 in order to compare adult and pediatric patients.

Counties were numbered 1 to 67 and also treated as a nominal variable. For selection of the reference category, of the five counties that had the highest number of isolates the one with the lowest resistance was chosen. Of the five counties with the most isolates, Miami-Dade County had the lowest percentage of methicillin-resistance and was used as the reference category.

The state was also divided into 7 regions for analysis (Figure 1). Region 6 was made the reference category because it had the lowest resistance percentage.

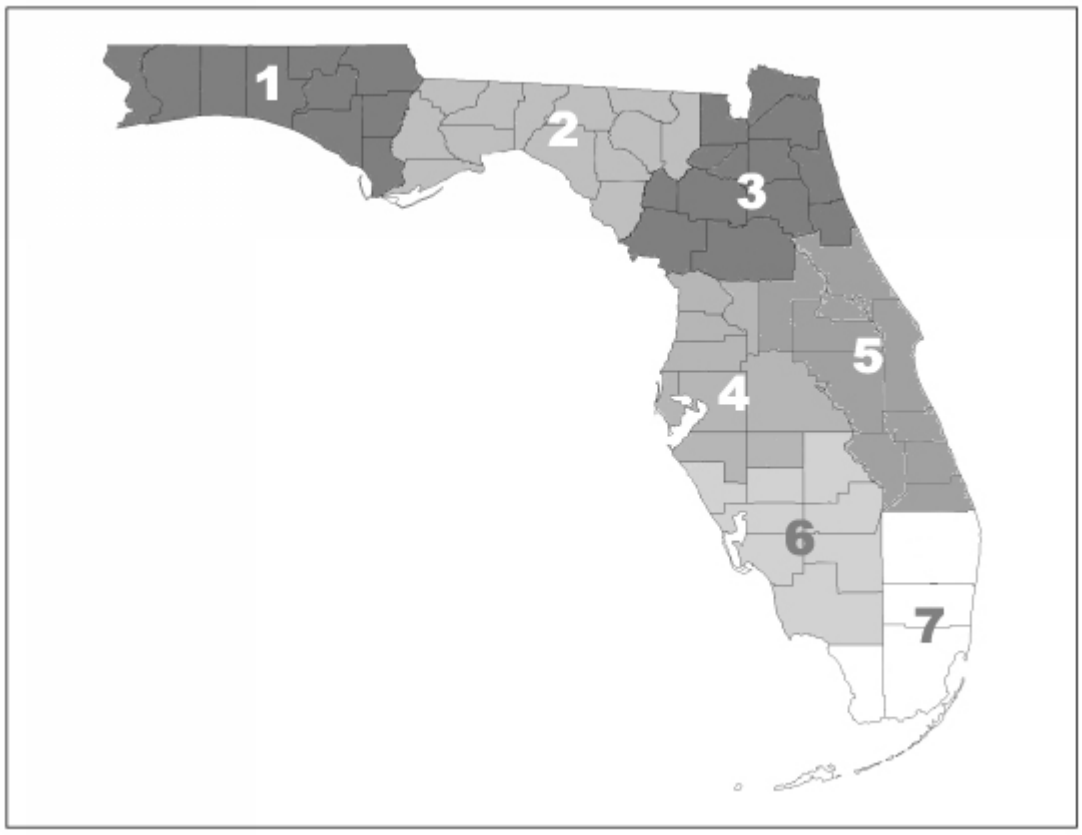

Figure 1. Map of the Seven Regions of Florida.

Outcome

The outcome was whether or not the *S. aureus* isolate tested was methicillin resistant. Resistance for all antibiotics was determined based on NCCLS standards. Oxacillin is used as a surrogate for methicillin resistance and was used for this purpose in this dataset. If the minimum inhibitory concentration (MIC) for oxacillin of an isolate was less than or equal to two then the isolate was coded as susceptible, if the MIC was between two and four then it was coded as intermediate, and if the MIC was greater than or equal to 4 then it was coded as resistant. Those that were methicillin resistant were considered to have the outcome. For the analysis, antibiotic susceptibilities were coded as one if the isolate was resistant or had an intermediate resistance to oxacillin (a surrogate for methicillin resistance) and zero if the isolate was susceptible.

Statistical Analysis

Logistic regression was used to calculate odds ratios and statistical significance of potential risk factors for methicillin resistance to examine the association between the independent variables and the dependent variable. For cross sectional studies, odds ratios may be calculated to evaluate statistical significance of exposure variables. The resulting odds ratio is the odds of having the disease, not developing incident disease

For the logistic regression analysis crude odds ratios were calculated for each variable. A model that included gender, age category, year, and county was used to obtain adjusted odds ratios and 95% confidence intervals for these exposures. In logistic regression any observation that has a missing variable is excluded from the analysis, so a crude odds ratio and 95% confidence interval were calculated for each variable including only those who were not missing any of the exposure variables and were included in the

full model. A second model was used to calculate adjusted odds ratios for region, which also included gender, age category, and year to adjust for these factors.

A second analysis of only patients with a skin and soft tissue infection was also conducted. The data from this subset of patients was analyzed in the same manner as the complete dataset.

To examine whether the proportion of MRSA has changed over time, the data was analyzed in two ways. Year was treated as a nominal variable and two dummy variables were created. The year 2003 was made the reference category because it had the lowest resistance percentage. The second method was to treat year as a ordinal variable and put it directly into the model. The Year 2003 was coded as 0, 2004 was coded as 1, and 2005 was coded as 2. This analysis was performed only including year and in a model with gender, age category, and county.

Secondary Analysis

The site from which the culture was taken, the source of the infection, was divided into nine categories. Isolates which were reported to be taken from the ear were categorized as ear. Isolates which were recorded as eye, cornea, conjunctiva, etc. were categorized as eye. Isolates with a reported source of nasal, nose, and nostrils were categorized as nasal. Isolates were categorized as other is they were from bone, urine, vaginal/cervical, breast fluid, semen, urethra, etc. Isolates that were cultured from throat, oral, nasopharynx, and sputum were categorized as respiratory. Isolates that were recorded as sinus or ethmoid were categorized as sinus. Any isolate that had a reported source of arm, leg, finger, toe, etc. or ulcer, abscess, or wound were categorized as skin and soft tissue. Isolates were categorized as sterile site if they were cultured from blood,

bursa fluid, synovial fluid, etc. If the source was missing or the recorded data was not interpretable then it was categorized as unknown.

This study also examined how many of the isolates in the dataset have the USA300/USA400 type anti-biogram. If the isolate was resistant to methicillin and erythromycin, but susceptible to clindamycin, trimethoprim-sulfamethoxazole, ciprofloxacin and levofloxacin then it was considered to have the USA300/USA400 type anti-biogram (King et al 2006). SAS version 9 statistical software was used for cleaning the raw dataset and to perform statistical analyses.

Results

Year

After removal of duplicates there were 8299 isolates for 2003, 17,309 for 2004 and 35,986 for 2005. There were two isolates that were missing the date of service. The total number of isolates after exclusions for all three years was 61,596.

There were 8,286 isolates for 2003, 16,980 isolates for 2004, and 35,946 isolates for 2005 with a reported susceptibility or resistance to oxacillin and 384 observations that were missing a MIC for oxacillin for all three years. These observations were not included in any of the analysis because they were missing the outcome variable. The percentage of MRSA for all *S. aureus* isolates increased as year increased (Figure 2). The percentage of MRSA increased from 35.1% of all *S. aureus* isolates in 2003 to 41.5% of isolates in 2004 to 49.7% of isolates in 2005.

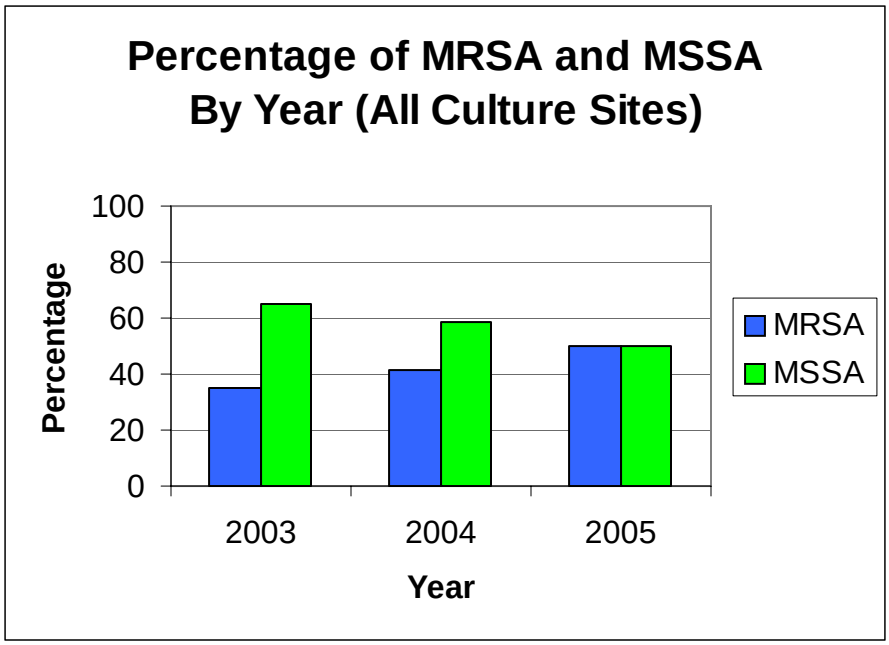

Figure 2. Percentage of MRSA and MSSA for all *S. aureus* isolates from 2003 to 2005 For All Culture Sites.

The increase was significant for both methods of analysis. The crude odds ratio, when treated as a categorical variable, is 1.31, 95% CI: 1.24- 1.38 for 2004 compared to 2003 and the odds ratio for 2005 compared to 2003 is 1.82, 95% CI: 1.73- 1.91. When analyzed as a continuous variable, the crude odds ratio for year is 1.36, 95% CI: 1.33- 1.39. All of these confidence intervals do not include one and are significant at the $\alpha = 0.05$ level.

The crude odds ratios excluding observations that were missing any variable were 1.31, 95% CI: 1.24- 1.39 when comparing 2004 to 2003 and 1.84, 95% CI: 1.74- 1.95 when comparing 2005 to 2003. When treated as a continuous variable the odds ratio was 1.37, 95% CI: 1.34- 1.40. For the full logistic regression model year was included as a continuous variable and put directly into the model. When included with age, gender, and county the odds ratio for year becomes 1.45, 95% CI: 1.41- 1.48.

Age Group

There were 11 age categories, <1, 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, and >90, which were numbered 0 to 10 in the analysis. The 51-60 age group, category 5, had the highest number is isolates, followed by the 11-20 age group, category 3. There were 901 observations that were missing patient age (Figure 3).

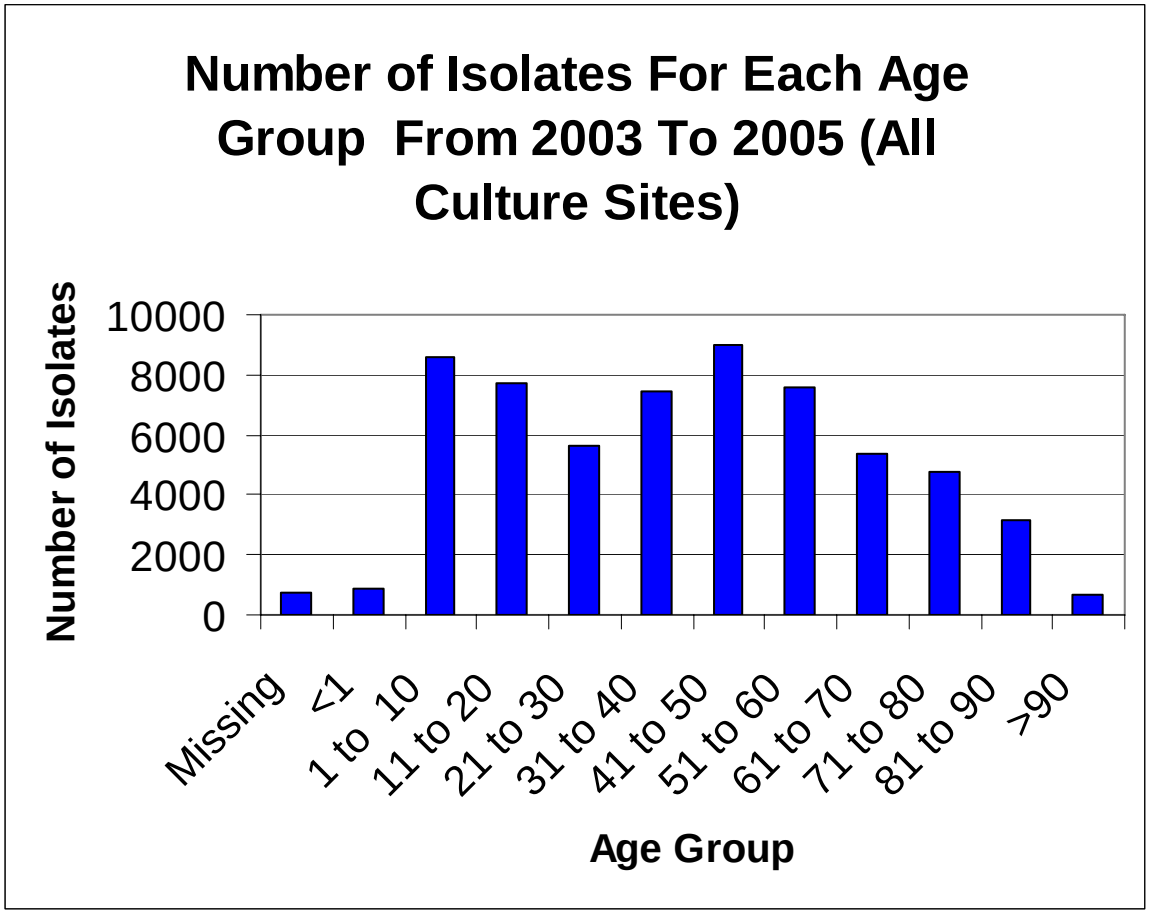

Figure 3. Number of *S. aureus* Isolates for Each Age Group From 2003 To 2005 For All Culture Sites.

When the percentage of MRSA is calculated for each age group, the missing category had the highest percentage of MRSA (61% in 2005). Of all the age groups, the

21- 30 age group has the highest percentage of MRSA (51% in 2005). This group is followed by the >90 age group (50% in 2005) and the 31-40 age group (49% in 2005) (Figure 4). Methicillin resistance for all the age groups and the missing group increased from year to year, except for the >1 age group which had a slight decrease from 2003 to 2004, then an increase from 2004 to 2005 (Figure 5).

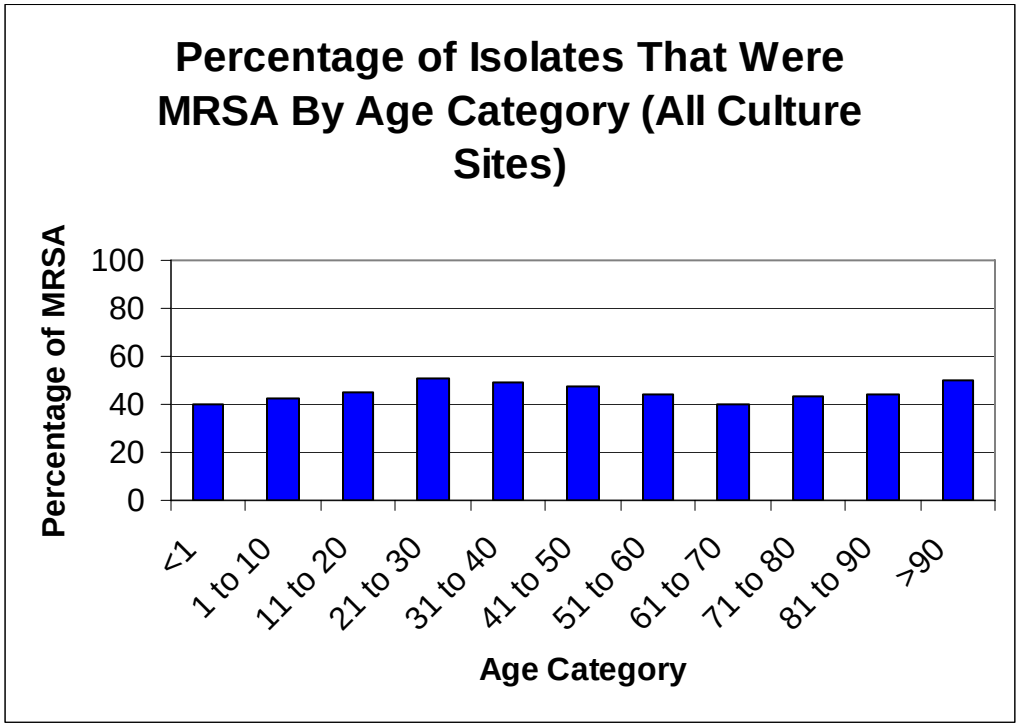

Figure 4. Percentage of *S. aureus* Isolates That Were MRSA By Age Category From 2003 To 2005 For All Culture Sites.

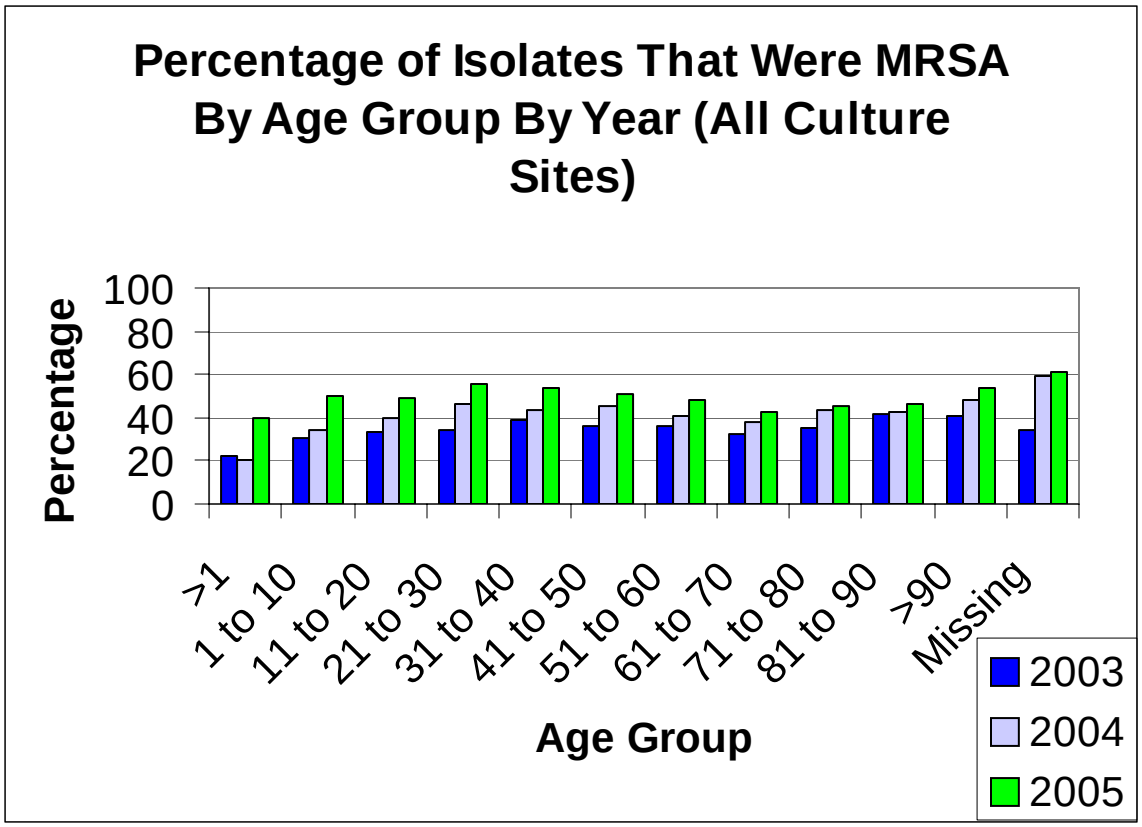

Figure 5. Percentage of *S. aureus* Isolates That Were MRSA By Age Category By Year From 2003 to 2005 For All Culture Sites.

The missing group had the largest change in the percent of isolates that were resistant. Of the age groups, the 21 to 30 year olds had the largest change in the percent of isolates that were resistant. The younger age groups had a greater increase in the percent of isolates that were methicillin resistant (Figure 6).

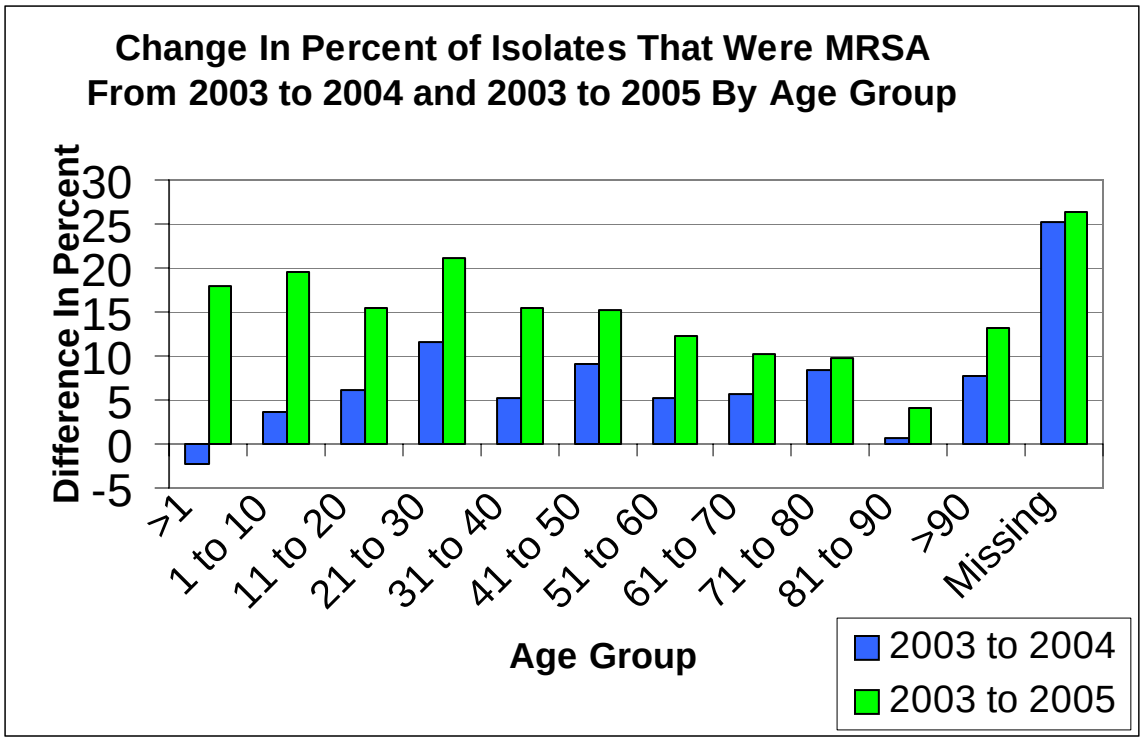

Figure 6. Change in the Percentage of *S. aureus* Isolates That Were MRSA From 2003 to 2004 and from 2003 to 2005 By Age Group For All Culture Sites.

For the crude odds ratios, the less than 1, 1 to 10, and 61 to 70 age groups have a significantly lower risk of being methicillin resistant and the 21 to 30, 31 to 40, 41 to 50, and the greater than 90 age groups have a significantly higher risk of being methicillin resistant when compared to the 11 to 20 year old reference category. For the crude odds ratios excluding those missing any variable the 61 to 70 age group had significantly lower risk of having methicillin resistance and the 21 to 30, 31 to 40, 41 to 50, and greater than 90 age groups have a significantly higher risk of being methicillin resistant when compared to the reference group (Table 3).

Table 3. Crude Odds Ratios and 95% Confidence Interval for Age Categories When Compared to the Eleven to Twenty Age Group.

Age Category	OR	95% CI
<1	0.81	0.70- 0.93
1 to 10	0.92	0.96- 0.98
11 to 20	Reference	
21 to 30	1.27	1.19- 1.37
31 to 40	1.19	1.12- 1.27
41 to 50	1.11	1.05- 1.18
51 to 60	0.97	0.91- 1.04
61 to 70	0.82	0.76- 0.88
71 to 80	0.93	0.86- 1.0
81 to 90	0.98	0.90- 1.06
>90	1.22	1.04- 1.44

When included in the logistic regression model with year, gender, and county, age, those who were 1 to 10, 51 to 60, and 61 to 70 did not have a significantly higher risk of having methicillin resistant *S. aureus* compared to methicillin susceptible *S. aureus* than those in the 11 to 20 age group. The odds ratios and 95% confidence intervals for the 21 to 30, 31 to 40, 41 to 50, 71 to 80, 81 to 90, and >90 age groups all do not include one and have a significantly higher risk of having a methicillin resistant infection than the reference group at the $\alpha = 0.05$ level. The <1 age group has a significantly decreased risk of methicillin resistance. The odds ratio and 95% confidence interval for the <1 category was less than one, this group has a significantly decreased risk of methicillin resistance compared to the reference group (Table 4).

Table 4. Odds Ratios and 95% Confidence Interval for Age Categories When Compared to the Eleven to Twenty Age Group Adjusted for Gender, Year, and County.

Age Category	OR	95% CI
<1	0.76	0.65- 0.88
1 to 10	1	0.94- 1.07
11 to 20	Reference	
21 to 30	1.19	1.10- 1.29
31 to 40	1.21	1.13- 1.30
41 to 50	1.14	1.06- 1.22
51 to 60	1.04	0.94- 1.11
61 to 70	0.93	0.86- 1.00
71 to 80	1.1	1.01- 1.19
81 to 90	1.13	1.04- 1.24
>90	1.46	1.23- 1.74

Adult and Pediatric

Adults had a higher percentage of isolates that were methicillin resistant for all three years. When adults were compared to pediatric patients (those less than 18), the crude odds ratio was 1.17, 95% CI: 1.13- 1.22. The crude odds ratios excluding those missing any variable was 1.12, 95% CI: 1.08- 1.16. The adjusted odds ratio was 1.17, 95% CI: 1.12- 1.22. In this dataset adults had a significantly higher risk of methicillin resistance than those who were under 18 (Figure 7).

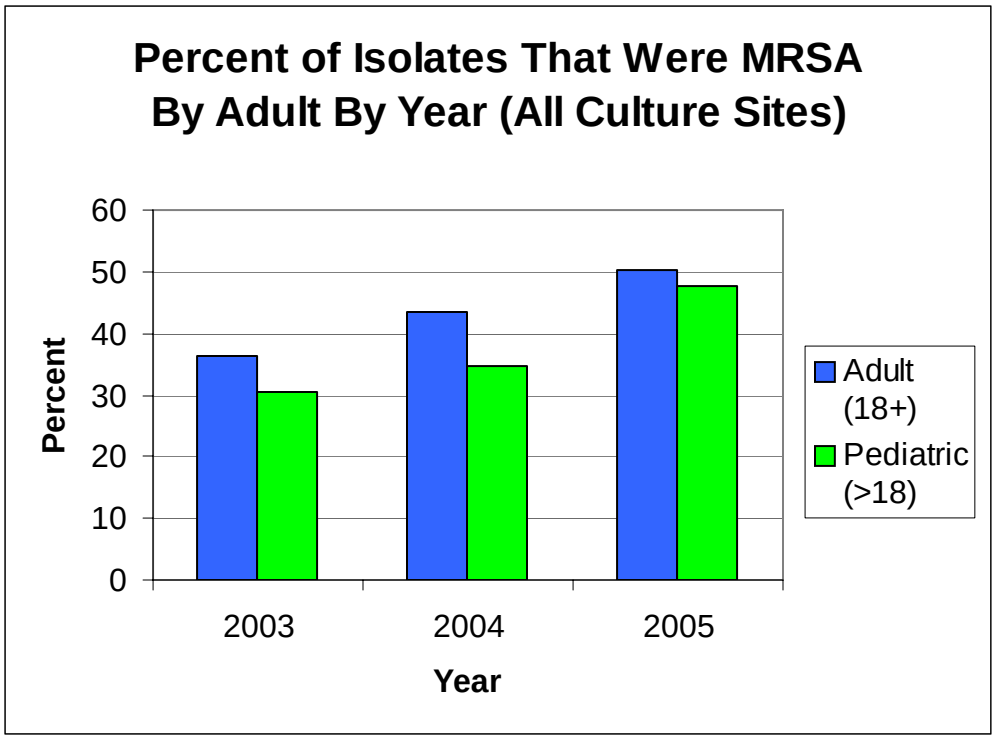

Figure 7. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Adult Versus Pediatric By Year From 2004 To 2005 For All Culture Sites.

Gender

For the gender variable there were 34,536 males and 28,898 females. There were 790 observations that were missing gender. The overall percentage of MRSA for males was 45.2% and the overall percentage for females was 45.7%. When the percentage of MRSA for gender is divided into year, the percentage for both males and females increases from year to year. However, the increase is slightly faster for females than for males. In 2003, the percentage for males is slightly higher than females (35.2% for males and 34.9% for females), then the percentage for males and females are approximately even in 2004 (41.5% for males and 41.4% for females), then the percentage becomes slightly higher for females in 2005 (49.0% for males and 50.2% for females) (Figure 8).

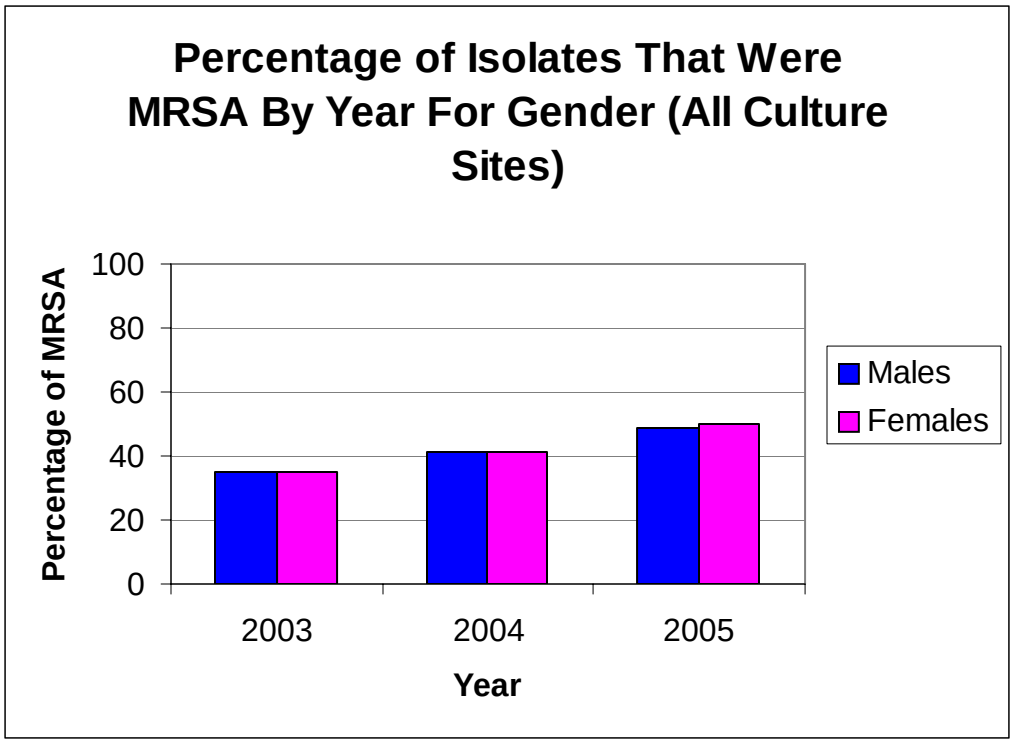

Figure 8. Percentage of *S. aureus* Isolates That Were MRSA By Year For Males and Females From 2003 To 2005 For All Culture Sites.

The crude odds ratio is 1.022 95% CI: 0.99- 1.06. The crude odds ratio excluding observations with any missing variables is 1.05, 95% CI: 1.02- 1.09. When adjusted for age, county, and year, females have a slight, but significantly higher risk of having methicillin resistant *S. aureus* versus methicillin susceptible *S. aureus* compared to males. The odds ratio for females compared to males is 1.05, 95% CI: 1.01- 1.08. When methicillin resistance is plotted by gender for each age group the number and percent of isolates that were resistant are similar for each age group and the missing group (Figure 9 and Figure 10).

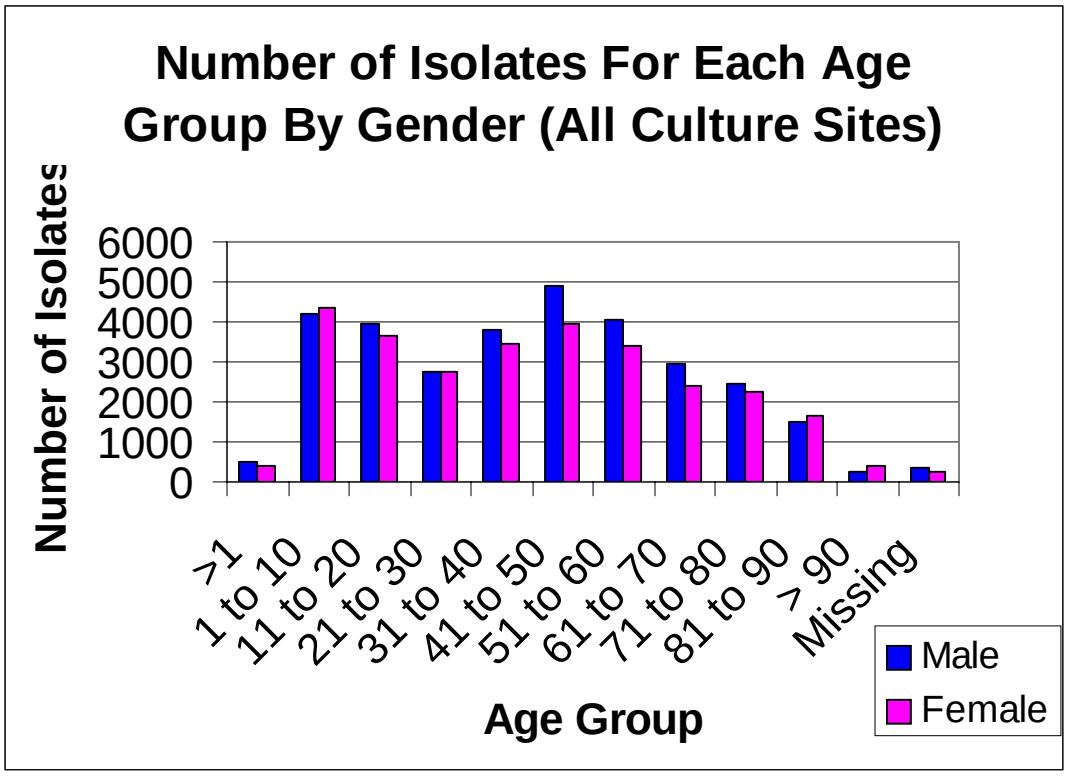

Figure 9. Number of *S. aureus* Isolates For Each Age Group By Gender From 2003 To 2005 For All Culture Sites.

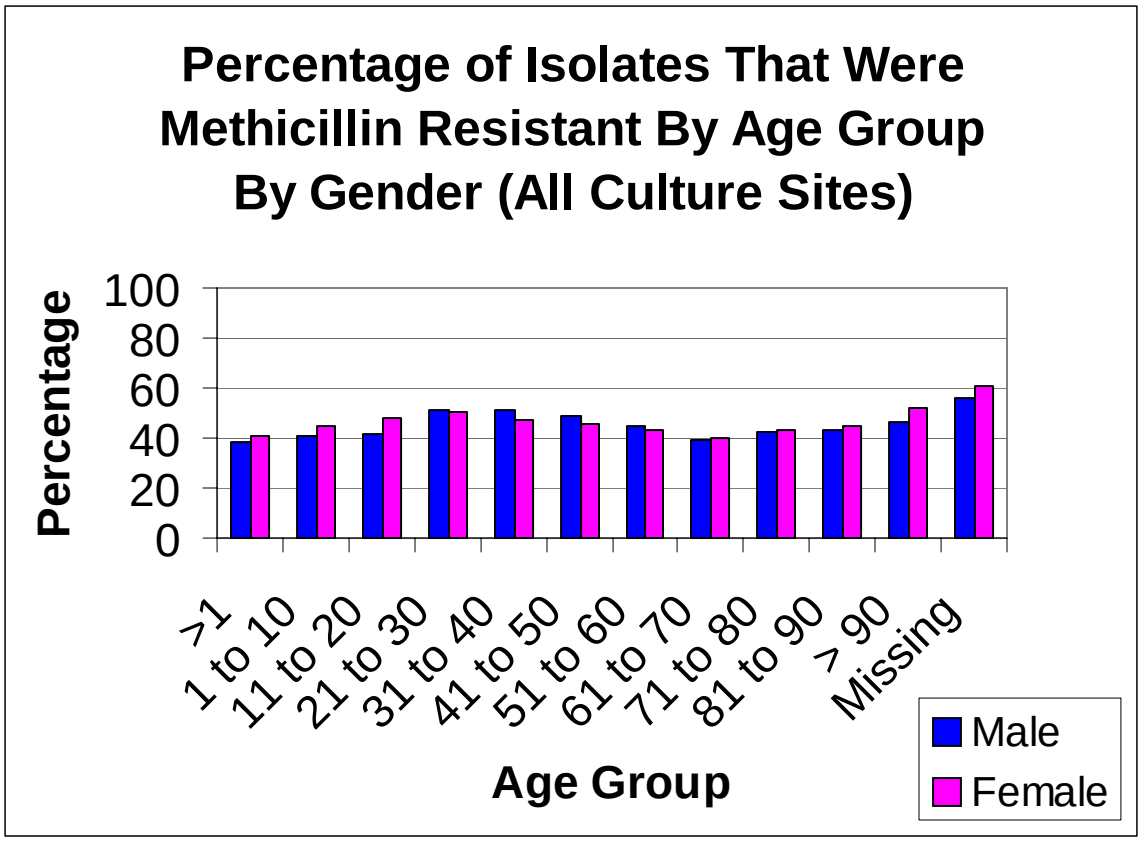

Figure 10. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Age Group By Gender From 2003 To 2005 For All Culture Sites.

Region

The state of Florida was divided into seven regions. There were 1,531 observations for region one (western panhandle), 6,339 for region two (central panhandle), 5,992 for region three (north east), 13,980 for region four (west central, including Tampa), 11,297 for region five (eastern central, including Orlando), 3,358 for region six (southwest, including Ft. Myers), 10,305 for region seven (southeast, including Miami), and there were 8,794 observations which were missing information on region (Figure 11).

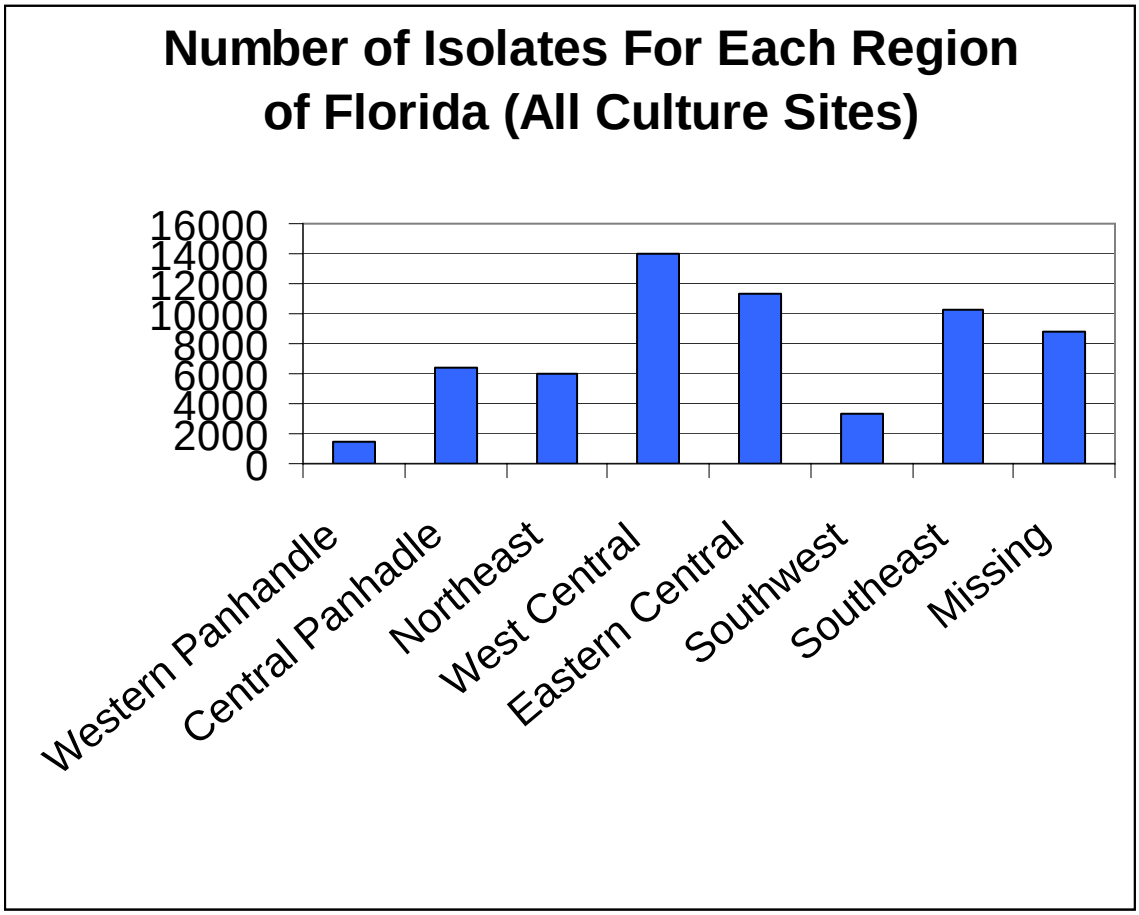

Figure 11. Number of Isolates For Each Region of Florida and Those That Were Missing Region From 2003 To 2005 For All Culture Sites.

Region 1, the western panhandle, had the highest percentage of MRSA (59.9%), followed by the northeast, region 3 (50.5%), west central, region 4 (46.4%), eastern central, region 5 (44.1%), the southeast, region 7 (39.8%), central panhandle, region 2, (38.3%), and the southwest, region 6 (37.4%). For the observations missing region, the percentage of MRSA was 54.2% (Figure 12). The percentage of MRSA increased as year increased for all seven regions and the group that were missing data on region (Figure 13).

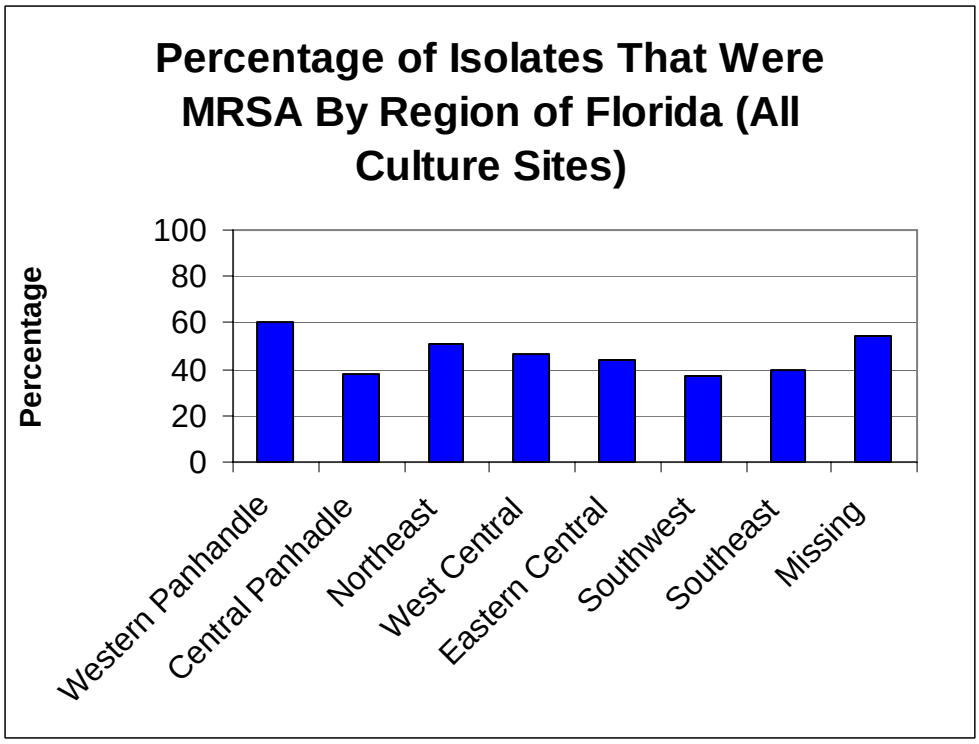

Figure 12. Percentage of *S. aureus* Isolates That Were MRSA By Region of Florida From 2003 To 2005 For All Culture Sites.

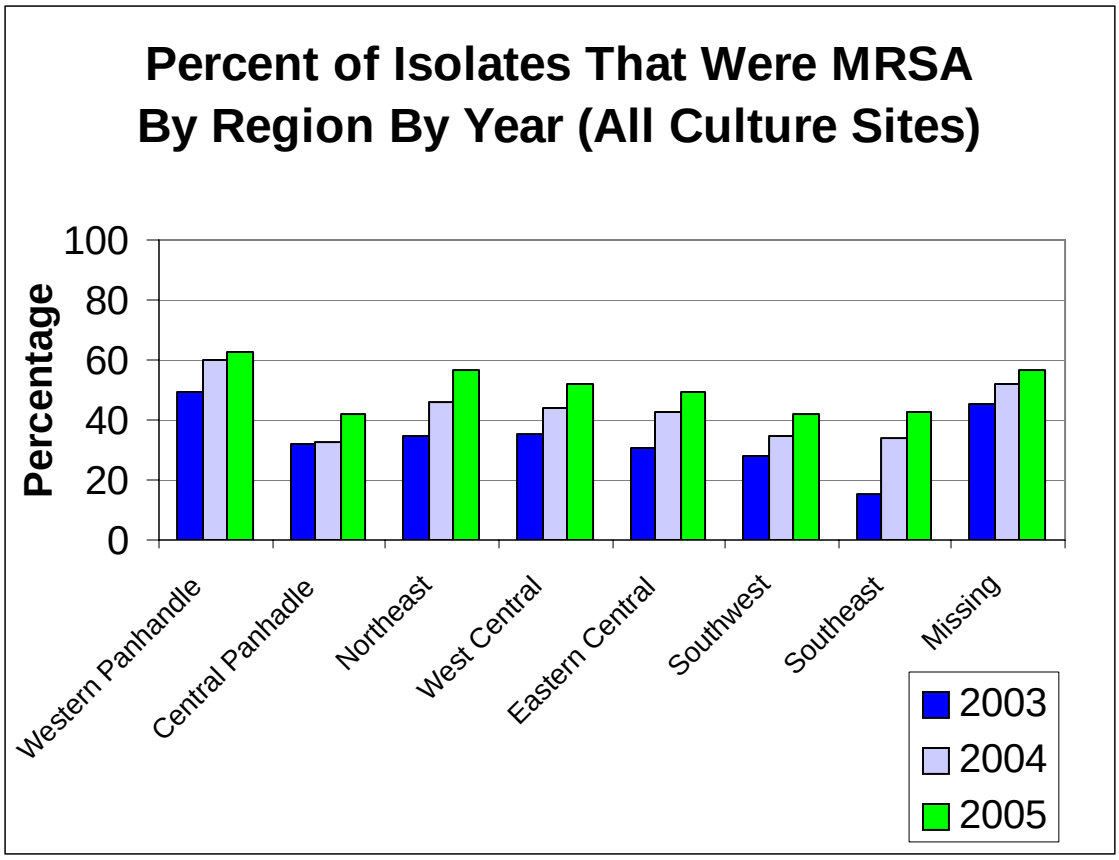

Figure 13. Percentage of *S. aureus* Isolates That Were MRSA By Region Of Florida By Year From 2003 To 2005 For All Culture Sites.

The change in the percent of isolates that were methicillin resistant was the highest for the southeast. The central panhandle had the smallest change in the percent of isolates that were resistant (Figure 14).

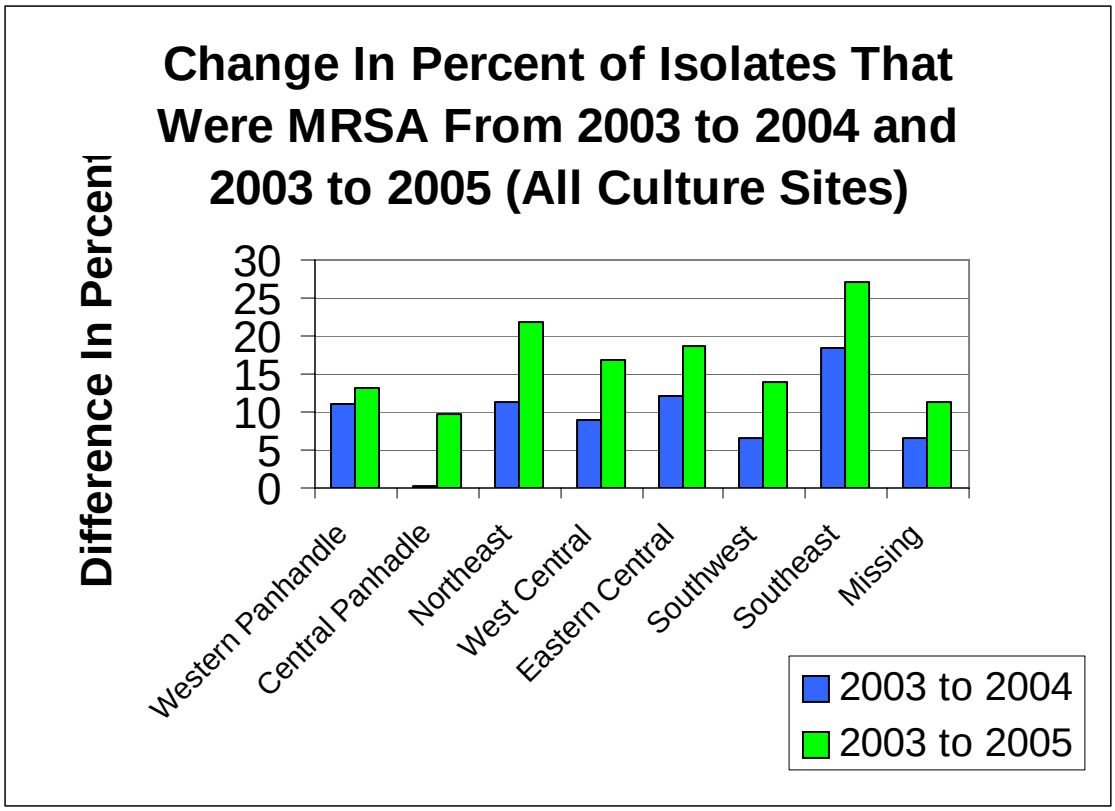

Figure 14. Change in the Percent of *S. aureus* Isolates That Were Methicillin Resistant From 2003 to 2004 and From 2003 to 2005 By Region Of Florida.

For the logistic regression analysis, the crude odds ratios for the western panhandle, the northeast, West central, Eastern central, and the Southeast have a significantly higher risk of having methicillin resistant *S. aureus* strains than isolates from region the Southwest. The crude odds ratios excluding those who were missing any variable were the same as the crude odds ratios (Table 5).

Table 5. Crude Odds Ratios and 95% Confidence Interval For the Seven Regions of Florida Compared to Region Six.

Region	OR	95% CI
Western Panhandle	2.50	2.21- 2.83
Central Panhandle	1.04	0.95- 1.13
Northeast	1.70	1.56- 1.86
West Central	1.45	1.34- 1.56
Eastern Central	1.32	1.22- 1.43
Southwest	Reference	
Southeast	1.11	1.02- 1.20

When adjusted for gender, age, and year, the western panhandle, the Northeast, West central, and the Eastern central have a significantly higher risk of having methicillin resistant *S. aureus* versus methicillin susceptible *S. aureus* when compared to the Southwest (Table 6). The risk of methicillin resistance for the Central panhandle and the Southeast were not significantly different than the reference category.

Table 6. Odds Ratios and 95% Confidence Interval For the Seven Regions of Florida Compared to Region Six, Adjusted for Gender, Age, and Year.

Region	OR	95% CI
Western Panhandle	2.50	2.20- 2.83
Central Panhandle	0.97	0.89- 1.06
Northeast	1.68	1.54- 1.84
West Central	1.51	1.40- 1.64
Eastern Central	1.35	1.25- 1.47
Southwest	Reference	
Southeast	1.00	0.92- 1.09

County

The number of isolates and the percent of isolates that were MRSA varied by county. The number of isolates from each county, for each year, ranged from 0 to 3,654 and the percent of isolates that were MRSA ranged from 0% to 75% (Table 7)

Table 7. Percent of Isolates That Were MRSA and Total Number of Isolates For Each County By Year.

County	Percent of Isolates Which Were MRSA (Total Number of Isolates)		
	2003	2004	2005
Alachua	51.1% (45)	57.5% (80)	62.6% (246)
Baker	0% (7)	50% (20)	68.8% (64)
Bay	39.7% (63)	64.9% (37)	62.4% (117)
Bradford	33.3% (3)	40% (10)	72.2% (36)
Brevard	25.9% (343)	43.1% (445)	49.5% (865)
Broward	20% (5)	33.9% (1520)	42.5% (2981)
Calhoun	40% (15)	46.7% (15)	66.7% (15)
Charlotte	45.9% (61)	34.4% (64)	46.8% (158)
Citrus	30% (36)	50.5% (91)	53.8% (145)
Clay	32.7% (49)	43.2% (81)	60.2% (254)
Collier	14.8% (27)	41.9% (31)	35.3% (85)
Columbia	0% (9)	40% (20)	39.2% (51)
DeSoto	33.3% (3)	25% (8)	33.3% (12)
Dixie	58.3% (12)	64.7% (17)	40% (15)
Duval	33.7% (496)	47.3% (763)	57.4% (2006)
Escambia	65.6% (64)	67.1% (161)	71% (335)
Flagler	42.9% (7)	20% (10)	50% (38)
Franklin	20% (5)	63% (27)	60.5% (38)
Gadsden	38.5% (26)	44.4% (18)	58.3% (36)
Gilchrist	63.6% (11)	45% (20)	73.7% (19)
Glades	0% (0)	0% (2)	20% (5)
Gulf	50% (10)	57.1% (14)	75% (40)
Hamilton	26.5% (34)	52.6% (57)	45.5% (99)
Hardee	30.2% (43)	38.6% (44)	54.1% (98)
Hendry	0% (6)	7.7% (13)	42.9% (56)
Hernando	35% (103)	41.8% (165)	53% (353)
Highlands	43.8% (16)	40.9% (22)	46.9% (32)
Hillsborough	33.8% (1095)	42.5% (1328)	53.3% (2634)
Holmes	33.3% (3)	0% (1)	0% (0)
Indian River	26.4% (87)	43% (93)	43.7% (126)
Jackson	20% (15)	50% (6)	40% (25)
Jefferson	27.3% (11)	50% (2)	56.3% (16)
Lafayette	0% (1)	100% (2)	33.3% (3)
Lake	25.2% (230)	41.1% (292)	43.6% (486)
Lee	26.7% (247)	32.5% (394)	41.6% (927)
Leon	33.3% (60)	40.7% (86)	59.7% (124)
Levy	35.3% (17)	54.3% (46)	45.3% (53)
Liberty	54.5% (11)	50% (8)	66.7% (9)
Madison	33.3% (15)	58.3% (12)	55.6% (18)

(Table 7 continued)

Manatee	34.5% (249)	47.2% (320)	51.5% (618)
Marion	32.3% (201)	42.1% (261)	50.4% (520)
Martin	28.6% (7)	42.6% (108)	46.5% (271)
Miami-Dade	0% (4)	29.1% (1821)	39.9% (3342)
Monroe	0% (1)	36.2% (69)	47.2% (159)
Nassau	35.4% (48)	44.4% (63)	52.1% (163)
Okaloosa	51.5% (33)	63.8% (47)	53.3% (107)
Okeechobe	20% (10)	57.1% (21)	54.8% (62)
Orange	32.7% (740)	43.4% (1028)	50.7% (2039)
Osceola	31.1% (161)	43.1% (255)	49.9% (487)
Palm Beach	15.4% (26)	33.9% (1717)	42.9% (3654)
Pasco	33.2% (328)	37.5% (453)	47% (798)
Pinellas	38.8% (704)	49.4% (881)	53.7% (1677)
Polk	33.8% (385)	46.4% (422)	50.2% (833)
Putnam	31.6% (19)	35% (20)	59.3% (54)
Santa Rosa	65% (20)	56.5% (69)	58.9% (163)
Sarasota	25.5% (216)	35.8% (296)	39.8% (580)
Seminole	30.9% (269)	40% (365)	50.6% (720)
St. Johns	40.4% (47)	36.1% (61)	45.6% (125)
St. Lucie	34.8% (112)	38.6% (166)	47.7% (405)
Sumter	45% (40)	27.7% (65)	44.2% (52)
Suwannee	66.7% (6)	66.7% (6)	60% (30)
Taylor	0% (0)	50% (8)	41.7% (24)
Union	0% (3)	66.7% (6)	46.7% (15)
Volusia	37.4% (187)	48.1% (310)	52.2% (674)
Wakulla	22.2% (27)	63.5% (52)	66.7% (75)
Walton	42.9% (7)	43.5% (46)	48.3% (89)
Washington	0% (3)	50% (2)	66.7% (3)
Missing	45.5% (1142)	52.2% (2017)	56.7% (5587)

When crude odds ratios are calculated there are 45 counties that have a significantly higher risk of methicillin resistance than those in the reference county, Miami-Dade (Table 8). For the crude analysis excluding those who were missing any variable, the same 45 counties had a 95% confidence interval that did not include one (Not shown).

Table 8. Crude Odds Ratio and 95% Confidence Interval For Counties With a Significantly Higher Risk of Methicillin Resistance When Compared to Miami-Dade County.

County	OR	95% CI	County	OR	95% CI
Alachua	2.68	216- 3.32	Levy	1.6	1.11- 2.32
Baker	2.59	1.70- 3.95	Liberty	2.37	1.12- 5.02
Bay	2.28	1.73- 3.00	Manatee	1.56	1.37- 1.77
Bradford	3.06	1.71- 5.48	Marion	1.42	1.24- 1.64
Brevard	1.33	1.19- 1.49	Martin	1.46	1.18- 1.80
Broward	1.16	1.07- 1.26	Monroe	1.38	1.05- 1.80
Calhoun	1.86	1.03- 3.34	Nassau	1.6	1.26- 2.05
Charlotte	1.39	1.09- 1.76	Okaloosa	2.23	1.66- 2.99
Citrus	1.94	1.52- 2.48	Okeechobee	1.89	1.26- 2.86
Clay	2.01	1.63- 2.48	Orange	1.47	1.35- 1.60
Dixie	2.13	1.17- 3.87	Osceola	1.43	1.24- 1.65
Duval	1.88	1.72- 2.06	Palm Beach	1.18	1.09- 1.78
Escambia	4.01	3.32- 4.84	Pasco	1.26	1.12- 1.041
Franklin	2.51	1.56- 4.05	Pinellas	1.73	1.58- 1.89
Gadsden	1.69	1.09- 2.63	Polk	1.48	1.32- 1.65
Gilchrist	2.66	1.51- 4.70	Putnam	1.67	1.10- 2.51
Gulf	3.64	2.15- 6.15	Santa Rosa	2.53	1.95- 3.27
Hamilton	1.41	1.05- 1.88	Seminole	1.38	1.23- 1.57
Hardee	1.45	1.08- 1.94	St. Lucie	1.36	1.16- 1.60
Hernando	1.58	1.33- 1.87	Suwannee	2.89	1.54- 5.39
Hillsborough	1.53	1.41- 1.65	Volusia	1.69	1.49- 1.92
Leon	1.63	1.27- 2.08	Wakulla	2.43	1.76- 3.37
			Walton	1.54	1.10- 2.16

When county is included into a logistic regression model with year, gender, and age category, there are 50 counties whose 95% confidence interval for the odds ratio does not include one. These counties have a significantly higher risk of methicillin resistance than the reference county, Miami-Dade, when adjusted for age, gender, and year (Table 9). When those who were missing data on county are put into a separate category and run in a model with gender, year, and age group, the odds ratio for the missing group is 2.08, 95% CI: 1.93- 2.24. Like 50 of the counties, the missing group has a significantly higher risk of methicillin resistance than the reference county.

Table 9. Odds Ratio and 95% Confidence Interval For Counties With a Significantly Higher Risk of MRSA When Compared to Miami-Dade County.

County	OR	95% CI	County	OR	95% CI
Alachua	2.76	2.22- 3.44	Levy	1.78	1.22- 2.59
Baker	2.74	1.78- 4.21	Liberty	3.11	1.45- 6.64
Bay	2.65	2.00- 3.50	Madison	2.03	1.11- 3.70
Bradford	2.91	1.61- 5.24	Manatee	1.77	1.56- 2.01
Brevard	1.51	1.35- 1.69	Marion	1.61	1.40- 1.86
Broward	1.14	1.05- 1.24	Martin	1.45	1.17- 1.79
Calhoun	2.32	1.28- 4.21	Monroe	1.37	1.05- 1.79
Charlotte	1.53	1.19- 1.95	Nassau	1.75	1.36-2.24
Citrus	2.13	1.66- 2.73	Okaloosa	2.46	1.82- 3.31
Clay	2.07	1.67- 2.55	Okeechobe	1.95	1.29- 2.95
Dixie	2.85	1.55- 5.26	Orange	1.64	1.51- 1.79
Duval	2.01	1.84- 2.20	Osceola	1.6	1.38- 1.85
Escambia	4.29	3.55- 5.19	Palm Beach	1.16	1.07- 1.26
Franklin	2.81	1.73- 4.58	Pasco	1.44	1.28- 1.62
Gadsden	1.96	1.25- 3.07	Pinellas	1.95	1.78- 2.13
Gilchrist	3.3	1.86-, 5.87	Polk	1.71	1.52- 1.92
Gulf	4.23	2.47- 7.24	Putnam	1.9	1.25- 2.88
Hamilton	1.56	1.16- 2.10	Santa Rosa	2.65	2.04- 3.43
Hardee	1.67	1.24- 2.26	Seminole	1.55	1.37- 1.76
Hernando	1.78	1.50- 2.11	St. Johns	1.43	1.09- 1.88
Hillsborough	1.74	1.61- 1.89	St. Lucie	1.48	1.26- 1.75
Indian River	1.32	1.04- 1.68	Suwannee	3.01	1.60- 5.66
Lake	1.3	1.13- 1.49	Volusia	1.83	1.61- 2.09
Lee	1.13	1.01- 1.28	Wakulla	2.76	1.99- 3.83
Leon	1.85	1.44- 2.37	Walton	1.56	1.11- 2.18

As a comparison, an analysis using only the first per patient was performed. Using only the first isolate for each patient, there are 61,050 observations. When the full model is run on this set of patients the results are nearly identical to using the first isolate every 365 days for all variables. No odds ratio was more than 0.05 different than the analysis using isolates from patients every 365 days.

Other Antibiotics

The percentage of isolates that were resistant to other antibiotics varied by year and by whether the isolate was MSSA or MRSA. For MSSA isolates less than 1% of the isolates were resistant to amoxicillin clavuanic and about 85% were resistant to penicillin. These percentages did not vary over the three years. Nearly 100% of the MRSA isolates were resistant to the β-lactams amoxicillin clavuanic and penicillin. For trimethoprim-sulfamethoxazole the percent that were resistant decreased slightly from 1.5% to 0.8% among the MSSA isolates. For the MRSA isolates the percent that were resistant fluctuated from 2% to 4% to 1%. Among the quinolones and floroquinolones group there were differences between the MSSA and MRSA isolates. The MSSA isolates had about 6% of the isolates resistant to ciprofloxacin and about 5% resistant to levofloxacin. Though the resistance rate for ciprofloxacin and levofloxacin decreased from year to year among the MRSA isolates, the percent that were resistance was higher than the MSSA isolates, 39% and 36% in 2005.

For gentamycin the percent of isolates that were resistance among the MSSA isolates was about 1% for the three years. The percent that were resistant among the MRSA isolates was slightly higher, 2.4 % in 2005. MRSA isolates also had a higher resistance rate for tetracycline, erythromycin, and clindamycin. The percent of isolates that were resistant among the MRSA isolates is about double that of the MSSA isolates for tetracycline. For erythromycin the percent that were resistant was about 39% among the MSSA isolates and 93% among the MRSA isolates. The percent that were resistant to clindamycin was only 3% in the MSSA group, however among the MRSA isolates there were 22% that were resistant in 2003 and 2004 and 14% in 2005. The percent that

were resistant to rifampin was low in both groups, <1% among the MSSA isolates and 1% among the MRSA isolates (Table 10).

Table 10. Percent Resistance of MSSA and MRSA Isolates to Other Antibiotics By Class.

Antibiotic	Percent of MSSA Isolates Resistant For 2003, 2004, 2005	Percent of MRSA Isolates Resistant For 2003, 2004, 2005
Penicillins		
Amoxicillin	0.7%, 0.9%, 0.3%	99.9%, 100%, 100%
Penicillin	84.6%, 85.1%, 84.4%	100%, 100%, 99.9%
Folate Pathway Inhibitors		
Trimethoprim Sulfamethoxazole	1.5%, 1.3%, 0.8%	2.3%, 4.1%, 1.2%
Quinolones and Floroquinolones		
Ciprofloxacin	6.1%, 7.8%, 6.6%	48.5%, 46.6%, 39.2%
Levofloxacin	4.5%, 5.6%, 4.8%	46.7%, 43.9%, 36.0%
Aminoglycosides		
Gentamycin	1.0%, 1.3%, 0.8%	3.6%, 5.5%, 2.4%
Tetracyclines		
Tetracycline	4.2%, 6.1%, 5.2%	9.9%, 12.2%, 8.9%
Macrolides		
Erythromycin	38.1%, 38.9%, 39.7%	90.5%, 92.7%, 93.3%
Lincosamides		
Clindamycin	2.5%, 4.4%, 3.2%	22.3%, 22.6%, 14.4%
Rifamycins		
Rifampin	0.07%, 0.7%, 0.5%	1.0%, 1.7%, 1.0%

Source

In this dataset 79.6% (49,060) of the isolates were from skin and soft tissue infections. The other sources each make up no more than 5% of the isolates. There were 1,410 isolates from the ear, 1,166 from the eye, 3,296 from nasal swabs, 568 from other sites, 1,910 from respiratory infections, 657 from the sinus, 289 from sterile sites, and 3,240 that were unknown (Figure 15).

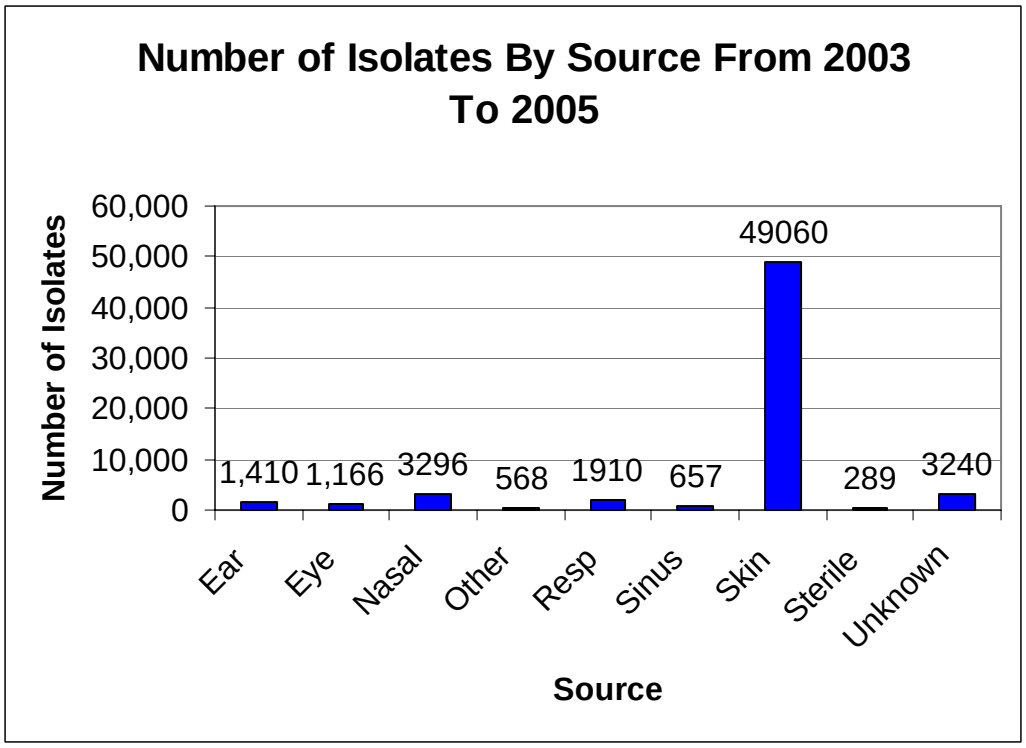

Figure 15. Number of *S. aureus* Isolates For Each Source Site In Florida From 2003 To 2005.

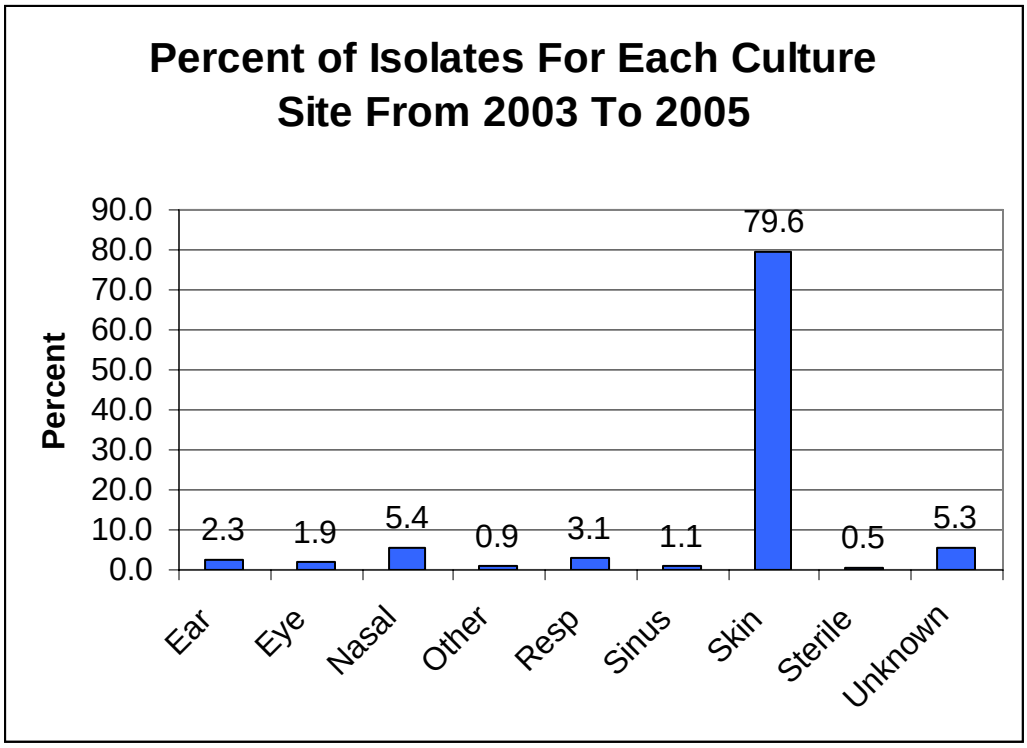

Figure 16. Percent of *S. aureus* Isolates For Each Culture Site In Florida From 2003 To 2005.

The percentage of *S. aureus* isolates that were MRSA was highest for skin and soft tissue infections (48.9%), followed by unknown site of infection (45.5%), sterile site infections (37.1%), other sites of infection (36.2%), eye infections (29.5%), nasal swabs (28.7%), ear infections (25.8%), sinus infections (23.3%), and respiratory infections (21.6%) (Figure 16).

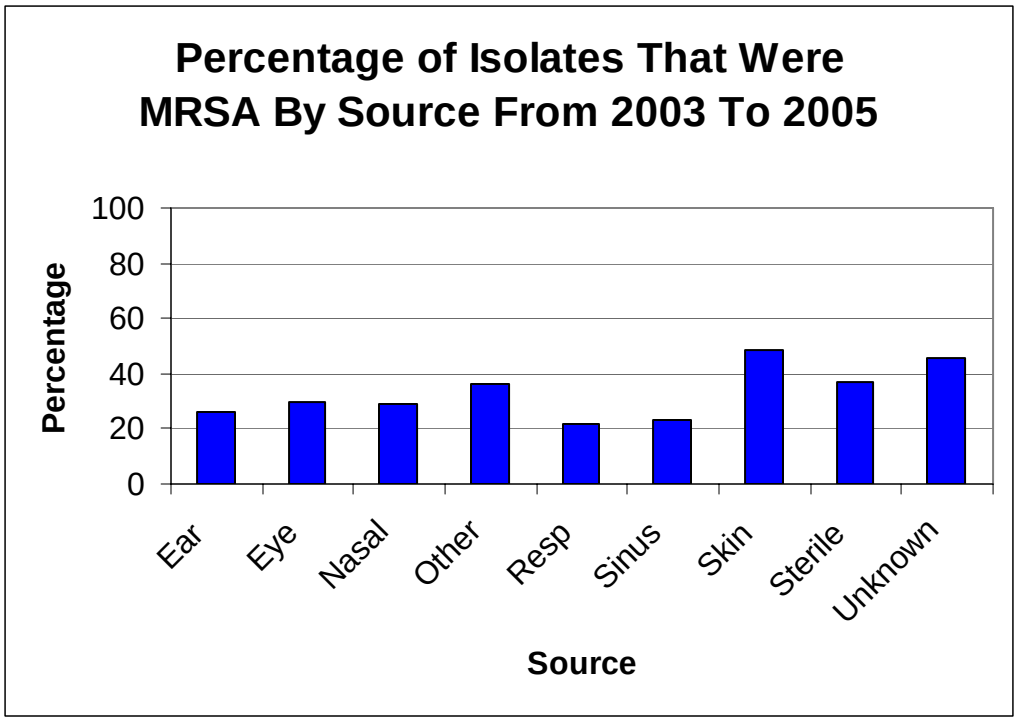

Figure 17. Percentage of *S. aureus* Isolates That Were MRSA By Source of Infection From 2003 To 2005.

Skin and soft tissue, other, and unknown infections increased from year to year. Eye and sterile site infections show an upward trend. Ear, nasal, respiratory, and sinus infections did not show a clear trend over the three years (Figure 18). Among the skin and soft tissue infections, MRSA accounted for 52.7% of the isolates in 2005. This was the highest percent of MRSA of the nine sources. Unknown and other isolates also had a high percentage of MRSA, 49.9% and 43.7% in 2005. The percentage of MRSA in 2005 was 39.7% for sterile site infections, 30.5% for nasal isolates, 30.4% for eye infections, 25.8% for ear isolates, 23.9% for respiratory infections, and 23.8% for sinus infections.

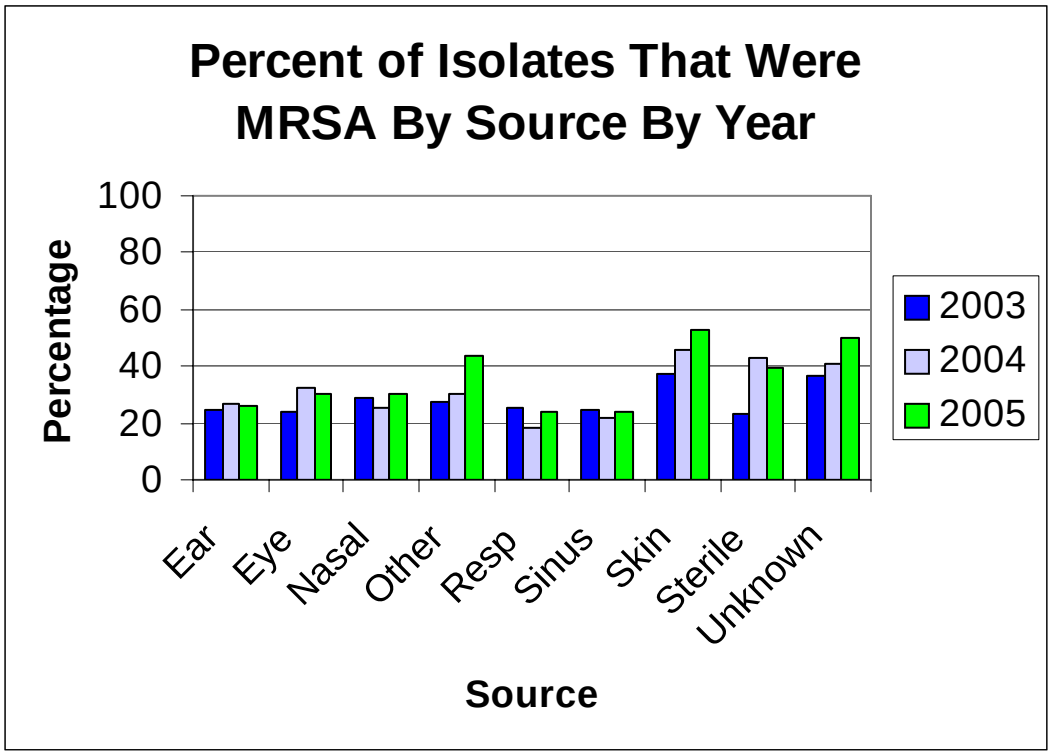

Figure 18. Percent of *S. aureus* Isolates That Were MRSA By Source By Year From 2003 To 2005.

USA300/USA400

In 2003 there were 1,241 isolates with a USA300/USA400 type anti-biogram, in 2004 there were 3,239, and in 2005 there were 9,360 (Figure 19). The percent of isolates that had an USA300/USA400 type anti-biogram increased from 15.0% in 2003, to 19.1% in 2004, to 26.0% in 2005 (Figure 20).

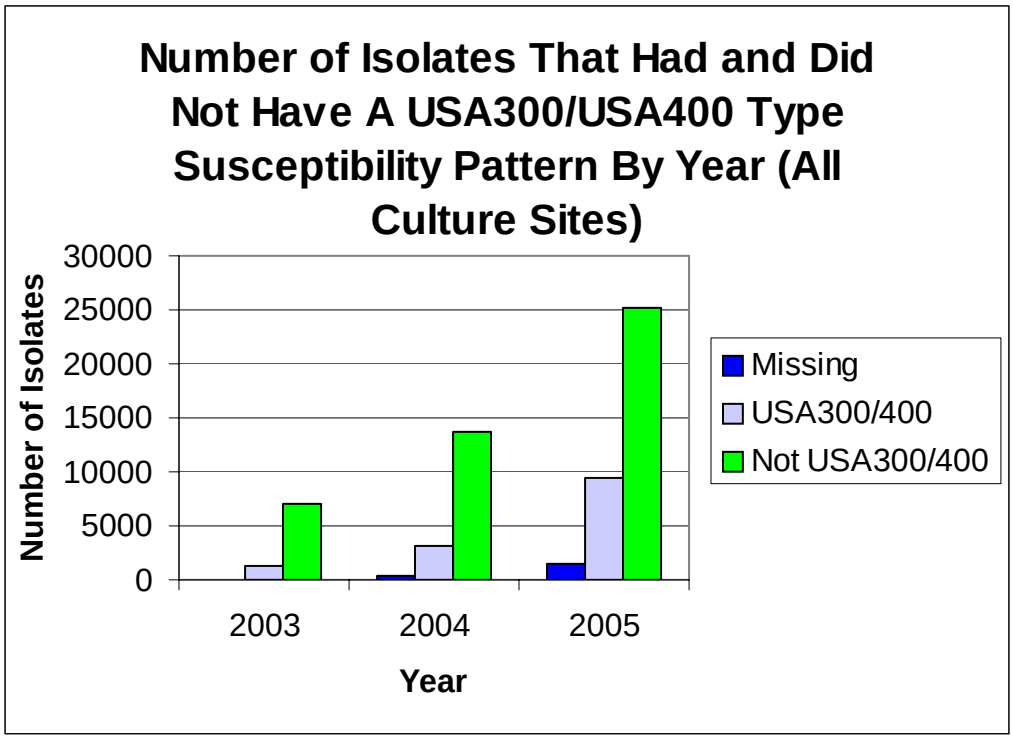

Figure 19. Number of Isolates That Had and Did Not Have A USA300/ USA400 Type Anti-Biogram By Year From 2003 To 2005 For All Culture Sites.

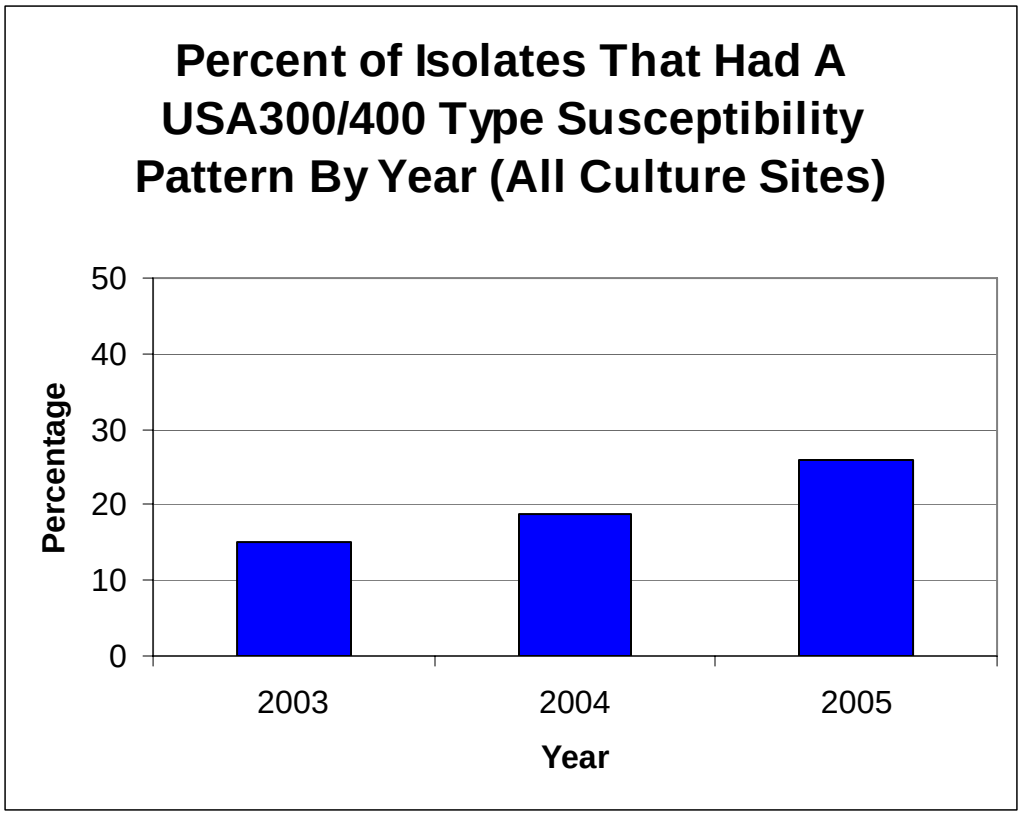

Figure 20. Percent of Isolates That Had a USA300/USA400 Type Anti-Biogram By Year From 2003 To 2005 For All Culture Sites.

There are differences in the percent of isolates that have a USA300/USA400 type anti-biogram by source. The skin and soft tissue category had the highest percent of isolates that were the USA300/USA400 type, 28.7% in 2005. The unknown category had a percent of 25.1% in 2005, among the other categories <11% of the isolates meet the criteria for USA300/USA400 in 2005. Except for ear, nasal, and sterile site the percent of isolates that were USA33/USA400 increased from year to year (Figure 21).

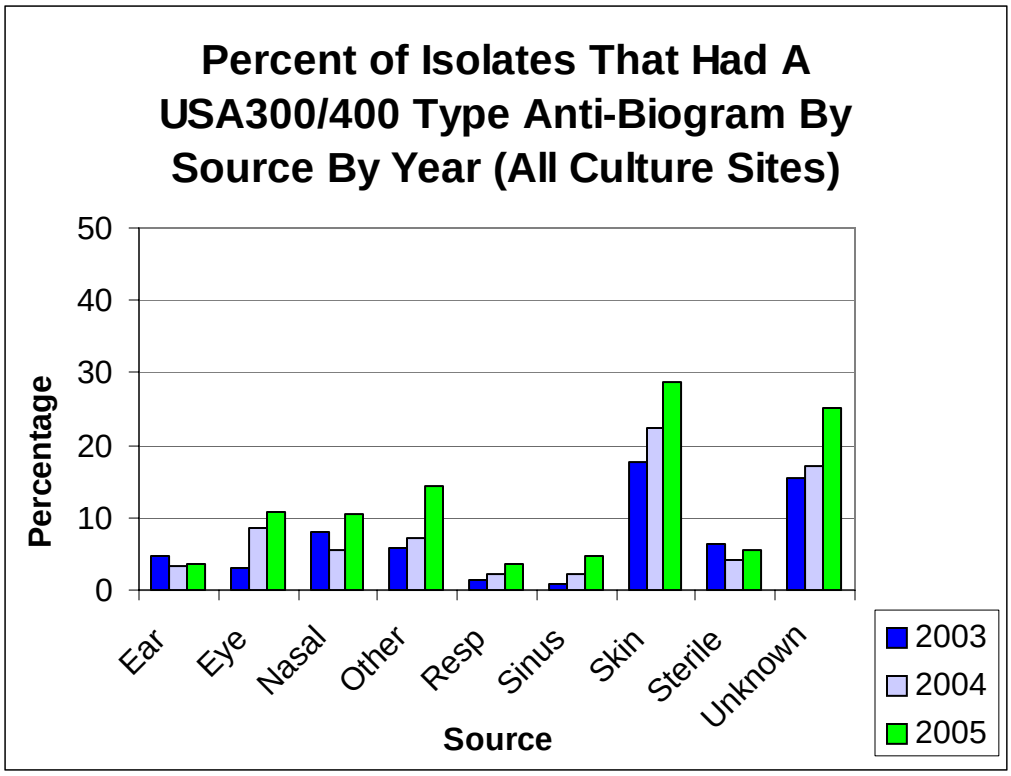

Figure 21. Percentage of Isolates That Had A USA300/USA400 Type Anti-Biogram By Source By Year From 2003 To 2005.

Skin and Soft Tissue Analysis

There were 49,060 skin and soft tissue infections total. There were 48,876 those from patients from a hospital were excluded. When this subset of patients are examined we find that the number and percent of isolates that were methicillin resistant increases from 2003 to 2005 (Figure 22 and Figure 23). In this subset of patients the increase in methicillin resistance is faster than in the complete dataset, 52.7% of isolates were methicillin resistant in 2005.

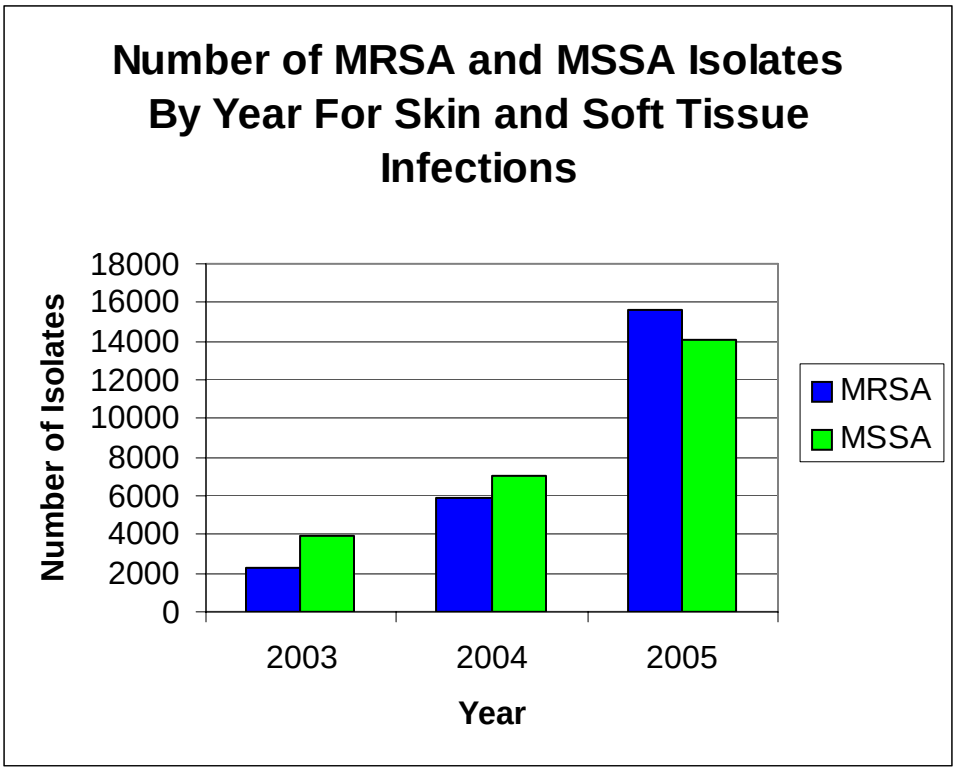

Figure 22. Number of *S. aureus* Isolates That Were MRSA and MSSA By Year Among Skin and Soft Tissue Infections.

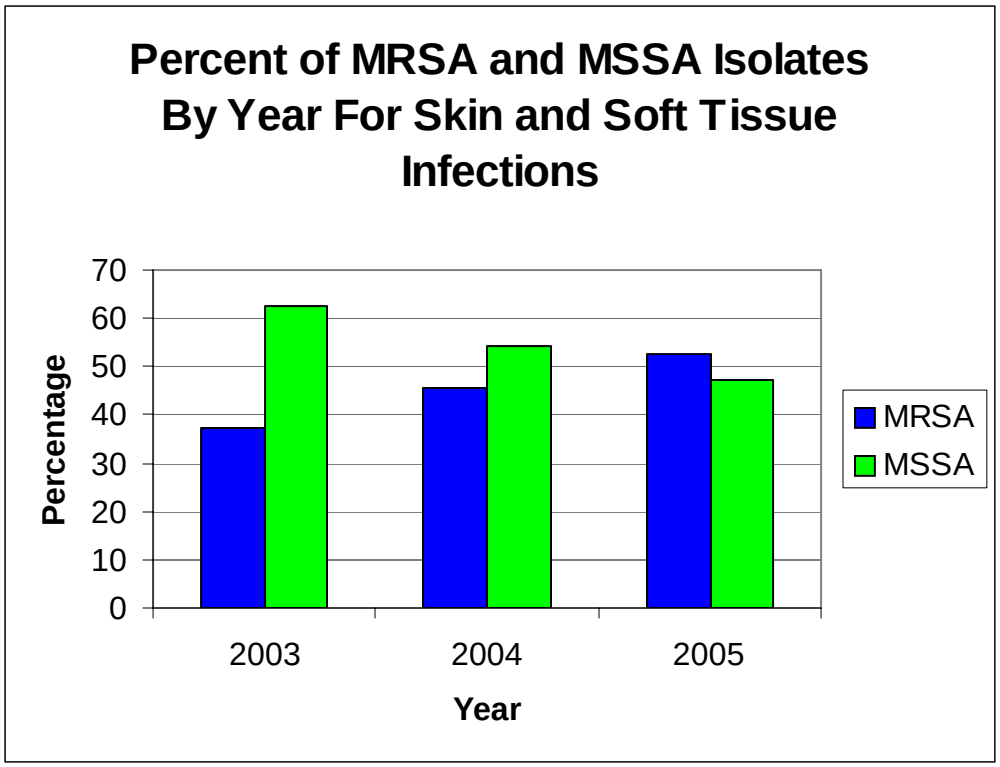

Figure 23. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Year Among Skin and Soft Tissue Infections.

When year is treated as a categorical variable the crude odds ratios is 1.4, 95% CI: 1.32- 1.49 comparing 2004 to 2003 and 1.87, 95% CI: 1.76- 1.97 comparing 2005 to 2003. The crude odds ratio when year is treated as a continuous variable is 1.36, 95% CI: 1.32- 1.39. When year is added to the full model with gender, age category, and county the odds ratio is 1.40, 95% CI: 1.36- 1.44.

In this subset of patients the percent of isolates that were methicillin resistant increases for both males and females, but, as in the full dataset, the increases is slightly steeper for females. In 2003, 36.9% of the isolates from females were methicillin resistant and 37.6% were resistant for males (Figure 24). In 2004, 46.0% were resistant for females and 45.1% were resistant for males. In 2005, 53.8% were resistant for

females and 51.6% were resistant for males.

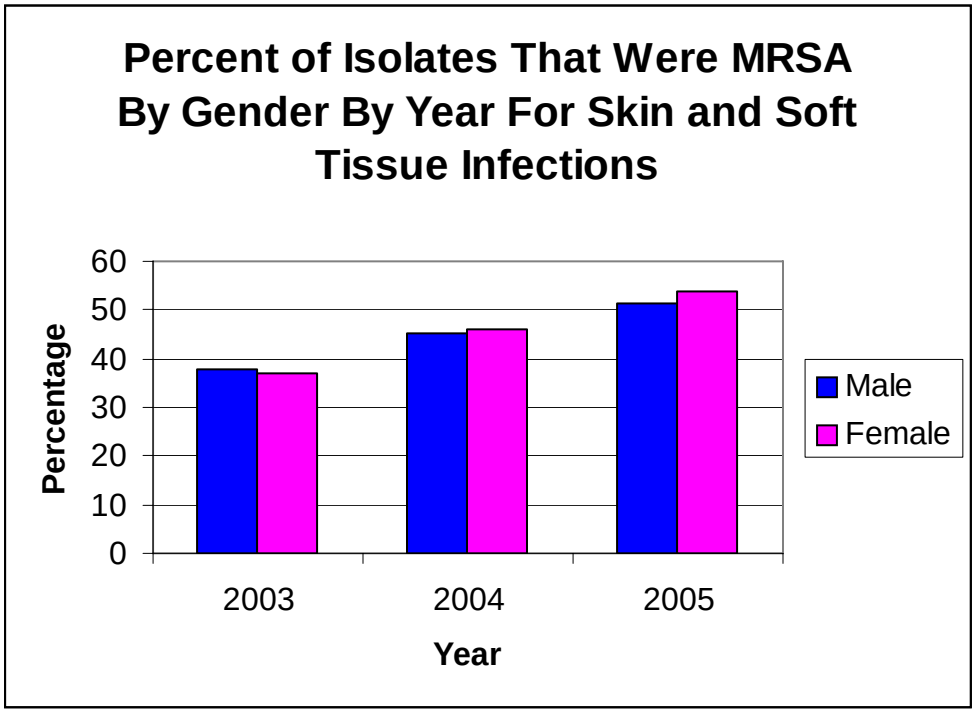

Figure 24. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Gender By Year Among Skin and Soft Tissue Infections.

For gender the crude odds ratio and the crude odds ratio excluding all those that were missing any variable were the same, OR=1.06, 95% CI:1.02- 1.10. When included in the full model the adjusted odds ratio is 1.07, 95% CI: 1.03- 1.12. As in the full dataset we see a slightly higher, but significant, risk for females compared to males.

There was an increase in the percent of isolates that were methicillin resistant for all age groups. The less than one age group had very few isolates in 2003 and 2004, 3 and 10 respectively. The number increased to 560 in 2005. The same trend in methicillin resistance by age group that is seen in the full dataset also occurs in this subset of patients. The missing category had the highest percent of isolates that were methicillin resistant (63% in 2005). The 21 to 30 age group had the highest percent resistant of the

age groups (59% in 2005) followed by the 1 to 10 age group (57.5% in 2005) and the 31 to 40 age group (57% in 2005) (Figure 26).

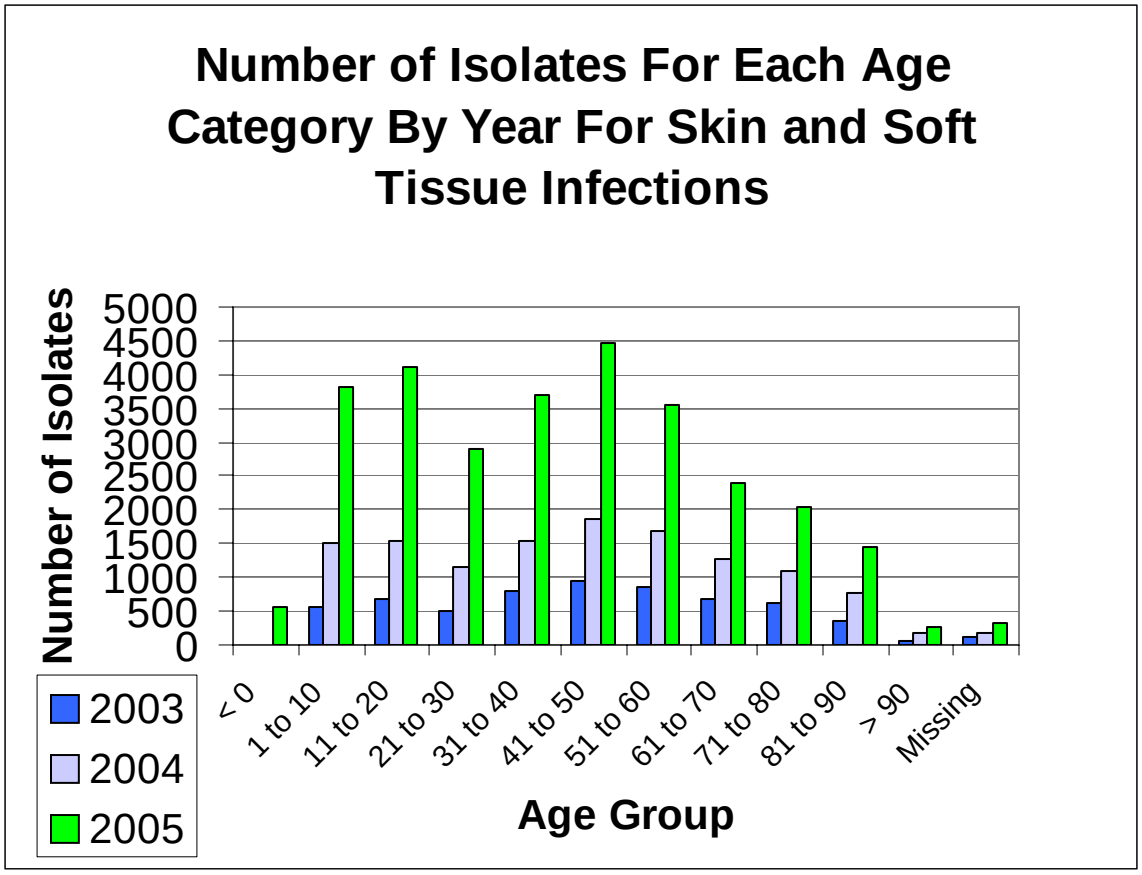

Figure 25. Number of Isolates For Each Age Category By Year For Skin and Soft Tissue Infections

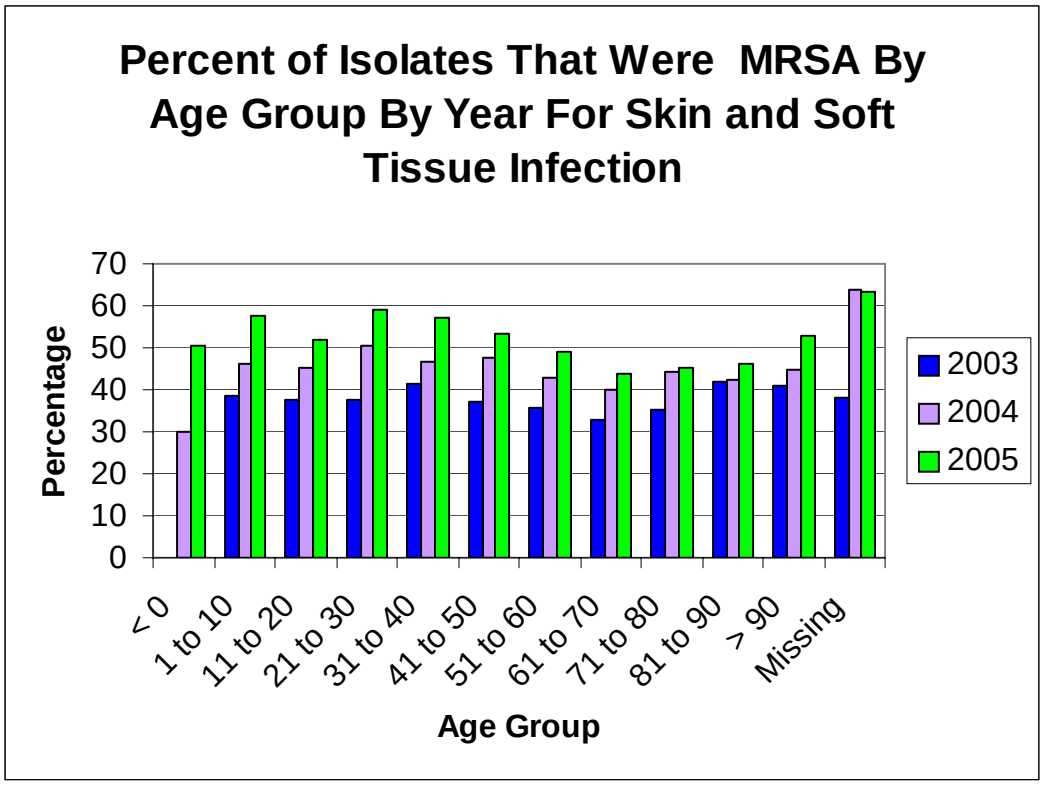

Figure 26. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Age Group By Year Among Skin and Soft Tissue Infections.

The odds ratios that were significant for the crude analysis were also significant for the crude odds ratios that did not include those who were missing any variable. In the crude analysis those who were 1 to 10, 21 to 30, and 31 to 40 had a significantly higher risk and those who were 41 to 50, 51 to 60, 61 to 70, 71 to 80, and 81 to 90 had a significantly lower risk of methicillin resistance than the reference category, the 11 to 20 age group (Table 11).

Table 11. Crude Odds Ratios and 95% Confidence Interval For Each Age Group Compared to The Reference Group For Skin and Soft Tissue Infections.

Age Category	OR	95% CI
<1	1.04	0.877- 1.23
1 to 10	1.17	1.09- 1.25
11 to 20	Reference	
21 to 30	1.25	1.16- 1.35
31 to 40	1.15	1.07- 1.23
41 to 50	1.04	0.97- 1.11
51 to 60	0.87	0.82- 0.94
61 to 70	0.73	0.67- 0.79
71 to 80	0.8	0.74- 0.87
81 to 90	0.84	0.77- 0.92
>90	0.99	0.83- 1.18

When adjusted for year, gender, and county the 1 to 10, 21 to 30, and 31 to 40 age groups have a significantly higher risk of methicillin resistance. Only the 61 to 70 age group has a significantly lower risk of methicillin resistance than the reference category for the adjusted odds ratios (OR= 0.83, 95% CI: 0.76- 0.90).

Table 12. Adjusted Odds Ratios and 95% Confidence Interval For Each Age Group Compared to The Reference Group For Skin and Soft Tissue Infections.

Age Category	OR	95% CI
<1	0.98	0.82- 1.17
1 to 10	1.21	1.12- 1.31
11 to 20	Reference	
21 to 30	1.16	1.06- 1.26
31 to 40	1.15	1.06- 1.24
41 to 50	1.06	0.98- 1.14
51 to 60	0.93	0.86- 1.01
61 to 70	0.83	0.76- 0.90
71 to 80	0.94	0.86- 1.02
81 to 90	0.96	0.87- 1.06
>90	1.19	0.99- 1.44

In this subset the crude odds ratio for adult versus pediatric is 0.93, 95% CI: 0.89-0.97 and the crude odds ratio excluding those who were missing any variable is 0.88, 95% CI: 0.85- 0.93. The adjusted odds ratio is 0.95, 95% CI:0.91- 1.00. Unlike the complete dataset in which adults had a significantly greater risk of methicillin resistance, in this subset adults have a lower, but significant risk of methicillin resistance.

The percent of isolates that were methicillin resistant increased from year to year in all seven regions. The western panhandle (region 1), had the highest resistance for all three years, 64.1% of isolates were resistant in 2005. The southwest, region 6, had the lowest resistance for 2004 and 2005, 42.0% of isolates were resistant in 2005. For the missing category 59.0% were resistant in 2005 (Figure 27).

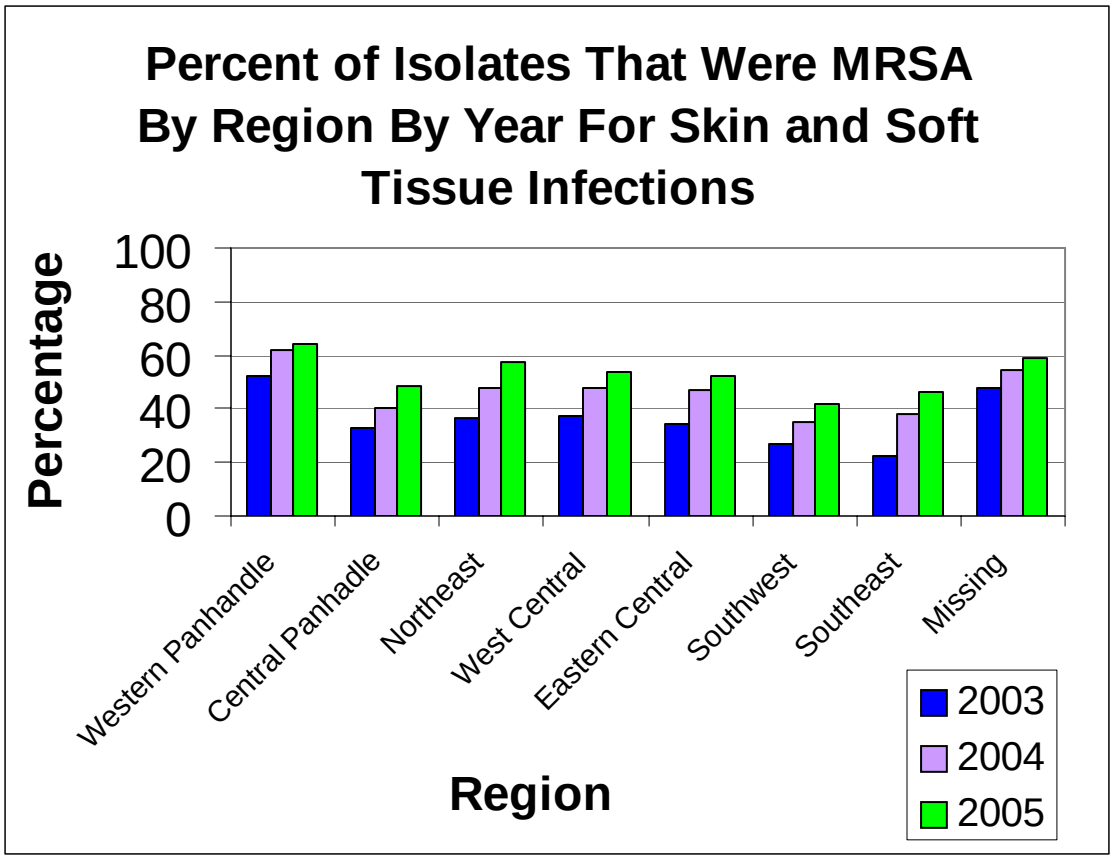

Figure 27. Percent of *S. aureus* Isolates That Were Methicillin Resistant By Region By Year Among Skin and Soft Tissue Infections.

The crude odds ratios for all the other regions were significantly higher than region six, the reference category (Table 13). The crude odds ratios for the analysis excluding those with any missing variables were similar to the crude odds ratios, the same counties were significantly higher than Miami-Dade county. The odds ratios for each region for the full model were also similar to the crude odds ratios, all were significantly higher than region 6 (Table 14).

Table 13. Crude Odds Ratios and 95% Confidence Interval For Each Region For Skin and Soft Tissue Infections.

Region	OR	95% CI
Western Panhandle	2.67	2.33- 3.06
Central Panhandle	1.36	1.23- 1.50
Northeast	1.81	1.65- 2.00
West Central	1.58	1.45- 1.71
Eastern Central	1.48	1.36- 1.62
Southwest	Reference	
Southeast	1.28	1.17- 1.40

Table 14. Adjusted Odds Ratios and 95% Confidence Interval For Each Region For Skin and Soft Tissue Infections.

Region	OR	95% CI
Western Panhandle	2.61	2.27- 3.00
Central Panhandle	1.28	1.16- 1.42
Northeast	1.77	1.61- 1.95
West Central	1.62	1.49- 1.77
Eastern Central	1.50	1.37- 1.64
Southwest	Reference	
Southeast	1.19	1.09- 1.31

For the individual counties the number of isolates for each year ranged from 0 to 2,886 and the percent of isolates that were resistant ranged from 0% to 100%.

Table 15. Percent of Isolates That Were MRSA and Total Number of Isolates For Each County By Year For Skin and Soft Tissue Infections.

County	Percent of Isolates Which Were MRSA (Total Number of Isolates)		
	2003	2004	2005
Alachua	33.3 % (27)	60.0% (65)	62.7% (212)
Baker	0% (5)	62.5% (16)	72.4% (58)
Bay	40.4% (57)	63.6% (33)	63.4% (101)
Bradford	0% (1)	40.0% (10)	76.0% (25)
Brevard	30.0% (243)	45.5% (332)	52.2% (691)
Broward	100% (1)	35.9% (1191)	46.1% (2412)
Calhoun	40.0% (10)	50.0% (12)	64.3% (14)
Charlotte	43.2% (44)	33.3% (45)	47.7% (130)
Citrus	48.3% (29)	56.9% (65)	52.8% (123)
Clay	35.3% (34)	40.0% (70)	61.2% (227)
Collier	21.4% (14)	46.2% (26)	35.2% (71)
Columbia	0% (6)	43.8% (16)	40.0% (45)
DeSoto	50.0% (2)	28.6% (7)	33.3% (12)
Dixie	75.0% (8)	63.6% (11)	54.5% (11)
Duval	37.4% (369)	49.8% (605)	59.4% (1754)
Escambia	71.2% (52)	69.6% (135)	73.6% (288)
Flagler	60.0% (5)	14.3% (7)	48.6% (35)
Franklin	0% (4)	65.2% (23)	61.8% (34)
Gadsden	57.1% (14)	46.2% (13)	55.2% (29)
Gilchrist	75.0% (8)	56.3% (16)	82.4% (17)
Glades	0% (0)	0% (1)	20.0% (5)
Gulf	60% (5)	61.5% (13)	77.1% (35)
Hamilton	34.8% (23)	56.8% (37)	51.3% (76)
Hardee	33.3% (30)	50.0% (32)	56.5% (85)
Hendry	0% (4)	9.1% (11)	41.7% (48)
Hernando	35.4% (79)	41.5% (130)	52.8% (320)
Highlands	55.5% (9)	41.2% (17)	44.0% (25)
Hillsborough	36.8% (785)	46.0% (1016)	56.1% (2158)
Holmes	33.3% (3)	0% (0)	0% (0)
Indian River	22.7% (66)	46.2% (78)	45.1% (113)
Jackson	25.0% (12)	40.0% (5)	31.6% (19)
Jefferson	20.0% (10)	50.0% (2)	53.3% (15)
Lafayette	0% (1)	100% (2)	33.3% (3)
Lake	26.35 (179)	43.0% (244)	45.2% (423)
Lee	24.1% (203)	31.9% (310)	41.0% (788)
Leon	34.8% (46)	44.6% (74)	58.9% (107)
Levy	42.9% (14)	50.0% (28)	46.2% (39)
Liberty	37.5% (8)	42.9% (7)	71.4% (7)
Madison	33.3% (12)	77.8% (9)	61.5% (13)

(Table 15 Continued)

Marion	33.9% (171)	43.3% (224)	50.7% (473)
Martin	40.0% (5)	48.8% (82)	51.2% (205)
Miami-Dade	0% (3)	36.8% (1151)	47.0% (2313)
Monroe	0% (0)	37.9% (58)	49.6% (139)
Nassau	38.2% (34)	50.0% (52)	52.7% (146)
Okaloosa	52.2% (23)	64.3% (42)	56.6% (99)
Okeechobe	25.0% (8)	60.0% (15)	56.4% (55)
Orange	38.4% (524)	48.7% (748)	53.7% (1690)
Osceola	32.5% (120)	48.0% (204)	50.7% (402)
Palm Beach	19.0% (21)	40.5% (1194)	47.0% (2866)
Pasco	36.6% (243)	42.8% (320)	49.9% (655)
Pinellas	39.6% (556)	51.9% (696)	54.8% (1421)
Polk	35.5% (318)	47.9% (365)	51.9% (729)
Putnam	37.5% (16)	38.9% (18)	59.6% (47)
Santa Rosa	85.7% (14)	60.3% (58)	60.3% (146)
Sarasota	25.5% (157)	38.8% (209)	41.8% (469)
Seminole	34.4% (186)	45.2% (263)	53.1% (610)
St. Johns	36.1% (36)	41.7% (48)	44.0% (109)
St. Lucie	34.2% (73)	38.4% (125)	50.9% (344)
Sumter	45.2% (31)	31.1% (45)	46.7% (45)
Suwannee	50.0% (4)	66.7% (6)	63.0% (27)
Taylor	0% (0)	50.0% (6)	50.0% (16)
Union	0% (2)	66.7% (6)	50.0% (14)
Volusia	39.5% (153)	49.8% (261)	53.1% (603)
Wakulla	18.2% (22)	66.7% (45)	68.6% (70)
Walton	33.3% (6)	43.6% (39)	48.8% (80)
Washington	0% (3)	50.0% (2)	66.7% (3)
Missing	47.7% (859)	54.6% (1560)	59.0% (4711)

For the county variable there is a difference between the crude odds ratios and the adjusted odds ratios. The crude odds ratios and the crude odds ratios that did not include any patient with a missing variable were similar, the same counties were significantly higher or lower than the reference county. For the crude odds ratios there were 24 counties that had a significantly higher risk of methicillin resistance than the reference county and there were 2 counties that had a significantly lower risk than the reference county (Table 16). When adjusted for age group, gender, and year there are 32 counties

that had a significantly higher risk of methicillin resistance compared to the reference county and 2 counties that had a significantly lower risk of methicillin resistance (Table 17). There were differences in significance for 10 of the counties between the crude and adjusted odds ratios. Sarasota county has a significantly lower risk for the crude odds ratio, but not in the adjusted. Hendry has a significantly lower risk in the adjusted odds ratio, but not the crude. Brevard, Hardee, Hernando, Leon, Marion, Nassau, Osceola, and Pasco counties have a significantly higher risk of methicillin resistance when adjusted for year, gender, and age group, but not for the crude odds ratios.

Table 16. Crude Odds Ratios For Each County For Skin and Soft Tissue Infections (Counties which had an OR that included one not shown).

County	Odds Ratio	95% CI	County	Odds Ratio	95% CI
Alachua	1.91	1.50- 2.42	Lee	0.74	0.65- 0.84
Baker	2.50	1.56- 4.00	Manatee	1.25	1.08- 1.44
Bay	1.69	1.26- 2.26	Martin	1.31	1.04- 1.67
Bradford	2.29	1.16- 4.54	Okaloosa	1.78	1.30- 2.45
Citrus	1.49	1.13- 1.96	Orange	1.28	1.16- 1.41
Clay	1.53	1.22- 1.91	Pinellas	1.34	1.21- 1.49
Dixie	2.24	1.06- 4.72	Polk	1.16	1.02- 1.31
Duval	1.54	1.39- 1.70	Santa Rosa	2.11	1.59- 2.79
Escambia	3.37	2.73- 4.16	Sarasota	0.79	0.68- 0.93
Franklin	1.87	1.12- 3.12	Seminole	1.19	1.04- 1.37
Gilchrist	3.13	1.59- 6.16	Suwannee	2.13	1.09- 4.15
Gulf	3.28	1.80- 5.99	Volusia	1.31	1.14- 1.50
Hillsborough	1.28	1.17- 1.40	Wakulla	1.93	1.36- 2.74

Table 17. Adjusted Odds Ratios For Each County For Skin and Soft Tissue Infections (Counties which had an OR that included one not shown).

County	Odds Ratio	95% CI	County	Odds Ratio	95% CI
Alachua	1.89	1.48- 2.40	Lee	0.79	0.69- 0.90
Baker	2.53	1.57- 4.08	Leon	1.38	1.05- 1.82
Bay	1.95	1.45- 2.62	Manatee	1.37	1.18- 1.58
Bradford	2.11	1.06- 4.21	Marion	1.25	1.07- 1.46
Brevard	1.25	1.10- 1.42	Martin	1.32	1.04- 1.68
Citrus	1.63	1.24- 2.16	Nassau	1.38	1.05- 1.81
Clay	1.55	1.23- 1.95	Okaloosa	1.997	1.45- 2.76
Dixie	2.83	1.33- 6.00	Orange	1.38	1.25- 1.52
Duval	1.59	1.44- 1.76	Osceola	1.27	1.08- 1.50
Escambia	3.40	2.74- 4.21	Pasco	1.23	1.07- 1.40
Franklin	2.01	1.19- 3.40	Pinellas	1.51	1.37- 1.68
Gilchrist	3.64	1.84- 7.21	Polk	1.29	1.14- 1.47
Gulf	3.46	1.86- 6.42	Santa Rosa	2.09	1.58- 2.78
Hardee	1.41	1.01- 1.97	Seminole	1.28	1.11- 1.48
Hendry	0.58	0.34- 0.997	Suwannee	2.18	1.11- 4.29
Hernando	1.31	1.08- 1.57	Volusia	1.42	1.23- 1.63
Hillsborough	1.39	1.27- 1.53	Wakulla	2.12	1.49- 3.01

Discussion

Methicillin resistance in *S. aureus* has become a problem in the state of Florida. In 2005, 49.7% of all *S. aureus* isolates in this dataset were methicillin resistant. With nearly half of the 2005 *S. aureus* isolates having methicillin resistance, the β-lactam antibiotics may no longer be the ideal choice for treating *S. aureus* infections in Florida.

In this population, the percentage of MRSA isolates that were resistant to trimethoprim-sulfamethoxazole, tetracycline, gentamycin, and rifampin was low. These results are similar to previously reported susceptibilities (Nakamura, Rohling, Shashaty, Lu, Tang, & Edwards, 2002; Sattler, Mason, & Kaplan 2002). These antibiotics may be viable alternatives to treat *S. aureus* infections in Florida.

In 2005, 98% of the MRSA isolates and 99% of the MSSA isolates remained susceptible to trimethoprim-sulfamethoxazole. This antibiotic has oral and intravenous formulations, excellent oral bioavailability, has been used for decades, and is inexpensive (Ellis & Lewis, 2002). For tetracycline, 91% of the MRSA isolates and 95% of the MSSA isolates remained susceptible. Minocycline and doxycycline are two long acting tetracycline derivatives that also have excellent bioavilability, are well absorbed by the gastrointestinal tract and are inexpensive. These two antibiotics may be possible alternatives for treating patients with less serious infections (Ellis & Lewis, 2002; Ruhe, Monson, Bradsher, & Menon, 2005). Rifampin resistance was less than 1% in both the

MRSA and the MSSA groups. If used, rifampin should be used in combination with another antibiotic to treat *S. aureus* infections (Ellis & Lewis, 2002).

The percent of isolates that were resistant to clindamycin was 3% for the MSSA group and 14% for the MRSA in 2005. However, in isolates that are clindamycin susceptible and erythromycin resistant, clindamycin resistance can be induced (Kowalski et al., 2005). In 2005, 40% of the MSSA isolates and 93% of the MRSA isolates were erythromycin resistant. For isolates that are susceptible to clindamycin and erythromycin, clindamycin may be considered for treatment, but in isolates that are erythromycin resistant clindamycin should not be used unless a D-zone disk diffusion is performed to test for inducible clindamycin resistance (Gemmell et al., 2006: Moran et al., 2006). A D-test is not generally performed and in this population 40% of MSSA and 93% of MRSA isolates were erythromycin resistant. Due to a high prevalence of erythromycin resistance, clindamycin may not an ideal choice for initial treatment in this population.

There were 25 isolates with a reported resistance to vancomycin, however the Vitek system that was used to asses antibiotic resistance may give false positives for vancomycin (Raney, Williams, McGowan, and Tenover, 2002). It is believed this is what happened with these isolates and that all isolates were susceptible to vancomycin; however, the non-availability of an oral formulation of any glycopeptide may limit their use in outpatient populations (Gemmell et al., 2006).

There was an increase in the number of isolates over the three years in the dataset. For each increase in year the number of isolates doubled, from 8,286 isolates in 2003 to 16,980 isolates in 2004 to 35,946 isolates in 2005. This may be due to a number of

factors. There may be an actual increase in the number of *S. aureus* infections. The increase in the number of isolates may also be due to other factors such as an increase in cultures being obtained from patients or the lab company's market share may have increased during this time period. This may also be due to a combination of an increase in infections and physician awareness. There was also an increase in the percent of isolates that were methicillin resistant over the three years. This measure may be less susceptible to other extraneous variables causing a change than the number of isolates. It is expected that if more cultures were obtained or the lab was receiving more isolates to test, that the number of MSSA and MRSA isolates would both increase and the percentage of methicillin resistance would not be affected.

The increase in methicillin resistant isolates over the three years was significant. When treated as a categorical variable, the odds ratio for 2004 compared to 2003 was 1.31, 95% CI: 1.24- 1.38 and the odds ratio for 2005 compared to 2003 was 1.82, 95% CI: 1.73- 1.91. In this population the odds that an isolate was methicillin resistant in 2004 was 1.3 times the odds of an isolate being methicillin resistant in 2003 and the odds that an isolate was methicillin resistant in 2005 was 1.8 times the odds of an isolate being methicillin resistant in 2003. When treated as a continuous variable, the odds of an isolate being methicillin resistant increases by 36% for each year(OR= 1.36, 95% CI: 1.33- 1.39). When adjusted for age group, gender, and county the odds of an isolate being methicillin resistant increases by 45% for each year (OR= 1.45, 95% CI: 1.41- 1.48).

For the analysis of only skin and soft tissue infections the percent of isolates that were methicillin resistant was slightly higher than for the entire dataset, 52.7% of isolates

were resistant in 2005. The odds that an isolate was methicillin resistant in 2004 was 1.40 times the odds of an isolate being resistant in 2003 and the odds of resistance in 2005 was 1.87 times the odds of being resistant in 2005. When treated as a continuous variable and adjusted for gender, age, and county the odds of an isolate being methicillin resistant increases by 40% per year. This is a slower increase than for the full dataset, but the skin and soft tissue subset had a higher percent of methicillin resistance in 2003.

For age category, the percent of isolates which were methicillin resistant increases with increasing age group to the 21 to 30 year olds, then decreases with increasing age group to the 61 to 70 year old age group, then increases with increasing age. The difference between the age group with the highest resistance, 21 to 30 year olds, and the age group with the lowest resistance, those less than 1, was 11%. While some of the age groups had a significantly higher risk of methicillin resistance compared to the reference category, there was not a large difference in the percent of isolates that were methicillin resistant between the age groups. Only 1.5% of the total isolates were missing data on age and missing values is not likely to have had a significant impact on the results.

For the skin and soft tissue analysis, the percent of isolates that were resistant in 2005 were different than for the full dataset. In both datasets the missing category had the highest percent of isolates that were methicillin resistant and the 21 to 30 age group had the highest of the age groups. In the full dataset the greater then 90 category had the second highest percent resistant (50% in 2005) followed by the 31 to 40 age group (49% in 2005). In the skin and soft tissue subset the 1 to 10 age group had the second highest percent resistant (58% in 2005) followed by the 31 to 40 age group (57% in 2005). The

same trend of increasing with age to the 21 to 30 age group then decreasing to the 61 to 70 age group then increasing again is seen in both datasets.

For the skin and soft tissue isolates the difference in the percent of isolates that were resistant between the age group with the highest percent resistant and the group with the lowest percent resistant was 15%. In skin and soft tissue infections physicians may need to consider the age of the patient as those who were 61 to 90 had a lower percent of isolates that were resistant than the other age groups.

The odds ratios for age group for the crude and adjusted analysis were different between the two datasets. For the adjusted odds ratios in the full dataset the less than 1 age group had a significantly lower risk of methicillin resistance and the older age groups (21 to 50 and 71 and older) were at significantly higher risk of methicillin resistance than the reference group. In the skin and soft tissue subset the younger age groups (1 to 10 and 21 to 40) had a significantly higher risk of methicillin resistance and the 61 to 70 age group had a significantly lower risk of methicillin resistance. This difference may be due to a difference in risk factors between all *S. aureus* infections and skin and soft tissue infections.

For the full dataset adults had a significantly higher risk of methicillin resistance compared to those under 18. There was only a small difference in the percent of isolates that were methicillin resistant between adults and children. The difference in the percent of isolates that were methicillin resistant was 5.8 in 2003, 8.6 in 2004, and 2.4 in 2005. For the skin and soft tissue infections adults had a lower but not significant risk of methicillin resistance, OR= 0.95, 95% CI: 0.91- 0.995.

Females had a slightly higher risk of methicillin resistance than males in this population, adjusted OR=1.05, 95% CI: 1.01- 1.08. However, the 95% confidence interval was just slightly above one and with such a large dataset even the smallest difference can be significant. With 49.0% of isolates resistant for males and 50.2% for females in 2005 there is not a large difference in methicillin resistance between the sexes. Only 1% difference is not clinically significant. For gender only 1.3% of the isolates were missing data on gender and is not likely to have an impact on the results. The results for the skin and soft tissue analysis were similar to that of the entire dataset. Females had a slight, but significantly higher risk of methicillin resistance, however the difference in the percent between males and females in 2005 was only 2.2%

While there were some differences in methicillin resistance with regards to both age and gender, the differences were not substantial for the entire dataset. Only in the skin and soft tissue analysis was methicillin resistance different depending on age. Age may need to be considered in making initial treatment decisions for skin and soft tissue infections.

The number of isolates varied greatly by region, but the population of the regions varies as well. It is expected that regions with a smaller population will have fewer isolates. There were a large number of isolates that were missing data on region and county, 14.2% of the total number of isolates. This number of missing isolates could have an impact on the results if those who are missing county are not equally distributed among the explanatory variables or methicillin resistance.

The percent of isolates that were methicillin resistant varied by region of the state. The region with the highest percent resistance, the western panhandle (region 1), was

22% higher than the region with the lowest resistance, the southwest (region 6). In the western panhandle there is a much greater chance that an *S. aureus* isolate is methicillin resistant then in the central panhandle (region 2) and the southwest. The odds ratio for region the western panhandle compared to the southwest is 2.50, 95% CI: 2.20- 2.83. The odds of an isolate being methicillin resistant in region the western panhandle was 2.5 times greater than that of region the southwest.

The skin and soft tissue subset was comparable to the entire dataset. The percent of isolates that were methicillin resistant was similar for the seven regions. For the skin and soft tissue analysis, all the regions had a significantly higher risk of methicillin resistance compared to southwest. In the entire dataset however, the central panhandle and the southeast were not significantly different than southwest.

Geographic area was further divided into county. The number of isolates for each county ranged from 4 isolates in Holmes to 5,486 in Palm Beach. There are differences in the populations of the 67 counties and the larger counties are expected to have more isolates (Appendix 1).

In 2005, the percentage of isolates that were methicillin resistant varied from 20% to 75% among the counties. However, some of the counties had a small number of isolates. Glades County had the lowest resistance in 2005 (20%), but there were only 5 isolates from this county. Gulf County had the highest percent of resistant isolates (75%), but there were only 40 isolates. In counties with small numbers of isolates, it is likely that there will be more random variation then in the counties with larger numbers of isolates. The smaller counties may have few providers that use the lab company which provided the data, which could have influenced sampling in these counties. The very

high or low percentage of methicillin resistance could be due to random variation or sampling variations in some counties, particularly those with small numbers of isolates.

There were some counties with a high percentage of MRSA that did have larger numbers of isolates. In Alachua county, 62.6% of the 246 isolates were methicillin resistant. In Escambia county, 71.0% of the 335 isolates were methicillin resistant. In counties such as Alachua, Baker, Bay, Bradford, Clay, and Franklin, where the percent of isolates that were methicillin resistant was greater than 60%, there is a 60% chance that if a β-lactam antibiotic is used as first line therapy it will be ineffective. In Escambia and Gulf counties there is a 70% chance that the infection will be resistant to a β-lactam antibiotic. For most of the counties the percent of isolates that were resistant was in the 40% to 50% range. In 35 of the 67 counties (52%), the percent of isolates that were methicillin resistant was 50% or greater. Methicillin resistance is a problem in all the counties and clinicians in Florida need to be aware of methicillin resistance and consider it when deciding on treatment options.

For the skin and soft tissue dataset there were fewer counties (16) that were significantly different than the reference county. Unlike the entire dataset in which all the counties that were significantly different had a higher risk of methicillin resistance, in the skin and soft tissue analysis there were two counties that had a significantly lower risk of methicillin resistance when adjusted for age, gender, and year (Hendry and Lee Counties). In the full dataset Lee was significantly higher than the reference county and Hendry was not significantly different. In this subset the number of isolates for each county ranged from 0 to 2,886 and the percent that were methicillin ranged from 0% to 100%. The counties with 0% and 100% had very few isolates (1 to 5). Some counties

with a high percentage of MRSA had larger number of isolates. In Alachua, Bay, Clay, and Santa Rosa greater than 60% of the 100 or more isolates were methicillin resistant.

Like region, there were 14.2% of isolates that were missing data on county. This could cause a bias and have an impact on the results if not evenly distributed between the counties and methicillin resistance.

As part of secondary analysis, data on source of infection and whether isolates had a USA300/USA400 type susceptibility profile were also examined. Most of the isolates in the full dataset were from skin and soft tissue infections, nearly 80%. Among the different sources, the percent that were resistant to methicillin varied. The difference in the percent resistant between the highest resistance, skin and soft tissue infections, and the lowest resistance, respiratory infections, was 28%. This indicates that there could be a difference in the percent of methicillin resistance depending on the source of the infection and this should also be taken into account when deciding on antibiotic therapy.

The USA300/USA400 type of isolate is associated with community acquired MRSA. In this dataset there were few isolates that had this type of antibiogram. Of the nine sources, skin and soft tissue isolates had the highest percent of USA300/USA400 type isolates, 38.7% in 2005. For all sources there was an increase in the percent of isolates that had a USA300/USA400 type antibiogram, potentially indicating an increase in CA-MRSA. In this analysis PFGE was not performed, therefore it is uncertain whether these isolates actually were USA300/USA400 strains.

Li et al. (2005b) used statewide, population-based antimicrobial susceptibility test data collected from both outpatients and inpatients in Hawaii from 2000 to 2002. They included 31,482 isolates of *S. aureus* in the analysis, of which 23,550 were from

outpatients. In their dataset, the proportion of MRSA was much lower than in the current study, 22% for outpatients and 39% for inpatients. As in the current study they also found a significant increase in the proportion of MRSA during their 3-year study period. Most of the outpatient MRSA isolates were from skin and soft tissue infections. Among pediatric outpatients 95% MRSA isolates were from wounds and among the adult outpatients 80% were from wounds. In the current study 79.6% of all isolates were classified as skin and soft tissue infections.

Moran et al (2006) performed a prospective prevalence study of adult patients who presented to hospitals in the EMERGEncy ID Net in August of 2004. Of the 320 *S. aureus* skin and soft tissue infections, 78% were methicillin resistant. All patients were seen in an emergency department. Among this population 49% of patients were non-Hispanic black, 25% were non-Hispanic white, 22% were Hispanic, and 4% were other races. This population of ER patients may not be indicative of the entire population of the United States.

This is the first study to use a large outpatient dataset to asses methicillin resistance in Florida. Many previous studies have been conducted on patients in the hospital. These two populations may not be the same. Patients in the hospital may be different than patients attending outpatient clinics. Those in the hospital may have more severe *S. aureus* infections, co-morbid conditions, different risk factors for methicillin resistance, and different demographics.

The prevalence of MRSA also varies with geographic region (Nakamura et al., 2002). There have been no previous studies of *S. aureus* conducted in Florida and one cannot generalize rates from one area to another. For physicians treating outpatients in

Florida, data from this study may be more appropriate for basing treatment decisions on because previous studies used different populations in different geographical areas and this is the most recent data.

There are several limitations to this study. First, data on other factors such as race, economic status, and known risk factors for methicillin resistance was not collected by the laboratory that conducted the resistance testing. Therefore these variables were not in the dataset and could not be included in the analysis. There is the possibility that potential confounding factors and other risk factors not included in the dataset could be causing the associations between year, age, gender, region, and county. Second, there were some patients in the dataset that were missing information on the exposure or outcome variables. In the complete regression model, there were 9,643 observations that were excluded because they were missing one or more variables. This still leaves 51,953 isolates for analysis, but if those who were missing data differed from those who were not then the results may not accurately reflect the true associations in this population. There is also the possibility of data entry error on the part of the lab company. There were some sources recorded which made no sense and must have been typed wrong. There is the possibility that this also occurred with other variables. However, it is unlikely that this would have occurred differentially among the patients and led to a bias. This was also a laboratory only analysis. This population may not represent everyone in the state.

Further studies are needed to assess other explanatory variables such as known risk factors (prolonged hospitalization, care in an intensive care unit, prolonged antimicrobial therapy, surgical procedures, dialysis, presence of an indwelling catheter, use of injectable drugs, residence in a nursing home or long term care facility, and close

proximity to a patient in the hospital who is infected or colonized with MRSA), race, and economic status (Salgado, Farr, & Calfee, 2003; Li et al., 2005b). The changing resistance rates from 2003 to 2005 indicate that studies need to be done periodically to keep up with changes in resistance rates over time.

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Appendix 1

Appendix 1

A-1. Estimated April 1, 2005 Population For the Counties In Florida Based on the 2000 Census (BEBR 4/1/2005 Population for Counties, n.d.).

County	Estimated Population	County	Estimated Population
ALACHUA	240,764	LEE	549,442
BAKER	23,953	LEON	271,111
BAY	161,721	LEVY	37,985
BRADFORD	28,118	LIBERTY	7,581
BREVARD	531,970	MADISON	19,696
BROWARD	1,740,987	MANATEE	304,364
CALHOUN	13,945	MARION	304,926
CHARLOTTE	154,030	MARTIN	141,059
CITRUS	132,635	MIAMI-DADE	2,422,075
CLAY	169,623	MONROE	82,413
COLLIER	317,788	NASSAU	65,759
COLUMBIA	61,466	OKALOOSA	188,939
DE SOTO	32,606	OKEECHOBEE	37,765
DIXIE	15,377	ORANGE	1,043,437
DUVAL	861,150	OSCEOLA	235,156
ESCAMBIA	303,623	PALM BEACH	1,265,900
FLAGLER	78,617	PASCO	406,898
FRANKLIN	10,845	PINELLAS	947,744
GADSDEN	47,713	POLK	541,840
GILCHRIST	16,221	PUTNAM	73,764
GLADES	10,729	ST. JOHNS	157,278
GULF	16,479	ST. LUCIE	240,039
HAMILTON	14,315	SANTA ROSA	136,443
HARDEE	27,333	SARASOTA	367,867
HENDRY	38,376	SEMINOLE	411,744
HERNANDO	150,784	SUMTER	74,052
HIGHLANDS	93,456	SUWANNEE	38,174
HILLSBOROUGH	1,131,546	TAYLOR	21,310
HOLMES	19,157	UNION	15,046
INDIAN RIVER	130,043	VOLUSIA	494,649
JACKSON	49,691	WAKULLA	26,867
JEFFERSON	14,233	WALTON	53,525
LAFAYETTE	7,971	WASHINGTON	23,097
LAKE	263,017	FLORIDA	17,918,227

A-2. Estimated April 1, 2005 Population For the Seven Regions of Florida Based on the 2000 Census. (BEBR 4/1/2005 Population for Counties, n.d.).

Region	Estimated Population
1	966,620
2	556,659
3	2,073,204
4	3,717,196
5	3,491,114
6	1,602,059
7	5,511,375