## Abstract

MOLECULAR EPIDEMIOLOGY OF MRSA AMONG PATIENTS AND EMPLOYEES IN A SURGICAL INTENSIVE CARE UNIT

by

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pervasive drug resistant human pathogen and has become widespread in hospitals around the world. MRSA infections cause approximately 19,000 deaths among hospitalized Americans annually. It is one of the leading causes of healthcare associated, or nosocomial infections, particularly in intensive care units. Hospital acquired MRSA (HA-MRSA) has been a battle for inpatients since the 1960's. However, in the late 1990's, a new strain of MRSA emerged. It appeared outside of the hospital setting and has been termed, community associated MRSA (CA-MRSA). Presently, CA-MRSA has been found to be spreading into the healthcare system presenting a new obstacle for patients and hospitals to overcome.

It has been suggested that employees play a role in transmission of MRSA to hospitalized patients. Since healthcare workers are at the interface between hospitals and the community, they may serve as a potential reservoir for spreading MRSA. However, there are a limited

number of studies that investigate employee MRSA colonization and subsequent transmission to patients.

This study seeks to provide molecular evidence supporting the likelihood that employees play a role in MRSA transmission to patients. Furthermore, with the implementation of a version of "search and destroy", an infection control strategy, we show how reductions of hospital-acquired infections are achieved using this method.

# MOLECULAR EPIDEMIOLOGY OF MRSA AMONG PATIENTS AND EMPLOYEES IN A SURGICAL INTENSIVE CARE UNIT

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Master of Science in Molecular Biology and Biotechnology

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# CHAPTER 1: INTRODUCTION

## *Background*

*Staphylococcus aureus* (*S. aureus*) has been recognized as an important cause of disease around the world. It has become a major pathogen associated with both hospital- and community-acquired infections. One strain in particular, an antibiotic resistant strain termed methicillin-resistant *Staphylococcus aureus* (MRSA), is one of the leading causes of healthcare associated, or nosocomial bacterial infections representing 64% of the *S. aureus* isolates detected in hospital intensive care units (National Nosocomial Infections Surveillance Report, 2004). MRSA has become widespread in hospitals worldwide and is responsible for causing bacteremia, pneumonia, surgical site infections, and other nosocomial infections. MRSA infections cause approximately 19,000 deaths among hospitalized Americans annually, which are higher than the number of deaths due to AIDS, tuberculosis, and viral hepatitis combined (Boucher and Corey, 2008). Klein and colleagues reported an increase of 119% of MRSA related hospitalization between the years of 1999-2005, or a 14% increase per year, with an estimate of 278,000 hospitalizations (Klein et al., 2007). Clearly there is a burden placed on the patient, but one also exists for hospitals and healthcare systems. It is estimated that the mean cost attributable to an MRSA infection is approximately $35,000 each (Stone, 2002), and the annual cost to treat MRSA in hospitalized patients in the U.S. to be between $3.2 billion to $4.2 billion (Rojas, 2005).

However, there are some shifts in the trends. Recent estimates indicate a stabilization trend with only 56% of device-associated infections due to MRSA from 2006-2007 (Hidron et al., 2008). Moreover, the incidence of MRSA central line-associated bloodstream infections reported from hundreds of ICU's has decreased 50-70% between 2001 and 2007 (Burton et al.,

2009). Kallen and colleagues also demonstrated a 34% decrease in blood stream infections from all types of hospitalizations between 2005 and 2008 (Kallen et al., 2010).

### *History*

*S. aureus* was identified as a causative agent in disease as early as the 1880s. Prior to the availability of antibiotics, the mortality rate of an individual with an invasive *S. aureus* infection was about 80% (Skinner and Keefer, 1941). With the introduction of penicillin in the early 1940s, the prognosis for patients with severe infections greatly improved. However, after two years of clinical use, penicillin-resistant *S. aureus* isolates began to appear (Kirby, 1944). By 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant and that percentage grew to 80% by 1960 (Chambers, 2001). In 1959, methicillin, a penicillinase resistant β-lactam, was developed by the United Kingdom (UK) pharmaceutical company, Beecham. Shortly after, it was introduced in the health care settings with the first case of methicillin-resistant *Stapylococcus aureus* emerging in less than two years (Jevons, 1961). Subsequently, it quickly disseminated throughout many countries during the 1960s and early 1970s. By the 1980s, these strains of MRSA became endemic, eventually leading to the worldwide pandemic.

### *Evolution of MRSA*

Penicillin, a β-lactam antibiotic, covalently binds to and inhibits the synthesis, maintenance, and regulation of the peptidoglycan portion of the cell wall (Katayama et al., 2000). *S .aureus* strains have four normal penicillin binding proteins (PBP's) anchored on the cytoplasmic membrane that participate in the crosslinking of the peptidoglycan to the bacterial cell wall. These normal PBPs have a high affinity for β-lactam agents. When bound to

penicillin, the PBPs are not able to function in the assembly of the cell wall, and cause bacterial death. Resistance to penicillin results from the acquisition of a plasmid that encodes the penicillin-hydrolysing enzyme, penicillinase, a form of β-lactamase (Deurenberg et al., 2008).

Methicillin was designed to resist β-lactamase degradation. However, shortly after its introduction in clinical settings MRSA strains emerged that were resistant to all β-lactam antibiotics. The primary mechanism by which *S.aureus* becomes resistant to methicillin is the acquisition of an acquired penicillin-binding protein, PBP2a, which is encoded by the *mecA* gene. PBP2a has a low affinity for β-lactam antibiotics and is capable of substituting the biosynthetic functions of the normal PBPs even in the presence of B-lactams, thereby preventing cell lysis (Tomasz et al., 1989). The *mec*A gene, which was first sequenced in 1987, is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*) (Song et al, 1987). There are different combinations of mec gene complex classes. To date, five types of SCC*mec* elements have been defined and labeled as SCC*mec* type I-V (Zhang, 2005).

*S.aureus* is polyclonal and carries a unique combination of hundreds of genes so that there is variation amongst genomes of different isolates of the organism. 14 fully annotated whole *S.aureus* genomes that have been sequenced reveal a huge diversity between the different strains, particularly in the lineage-determining surface protein genes and the mobile genetic elements (Lindsay and Holden, 2006). These mobile genetic elements move in and out of *S. aureus* horizontally and often encode virulence and resistance genes (Lindsay and Holden, 2006). Remarkably, study of these whole genome sequences also revealed that strains belonging to the same lineage were strikingly similar despite the enormous differences in geography and time (Diep et.al, 2006).

*Healthcare-associated vs Community-Acquired MRSA Strains*

Initially, MRSA strains were hospital-acquired and besides being resistant to all the lactams were also resistant to other classes of antibiotic including macrolides, tetracyclines, sulfonamides, and clindamycin. These hospital-acquired MRSA possess SCC*mec* types I,II, or III. Starting in the mid1990s, MRSA strains have emerged in the community setting. These new strains, termed Community-acquired MRSA (CA-MRSA), are usually resistant to β-lactams but, in contrast to hospital-acquired MRSA, are susceptible to other antimicrobial classes. Additionally, these strains have been found to carry mostly SCC*mec* type IV. In the US, the pulsed-field gel electrophoresis (PFGE) pattern USA300 is the most prevalent strain of the CA-MRSA. CA-MRSA strains are also more likely to possess unique combinations of virulence factors (Tenover et al., 2001; Moran et al., 2006). Genetic analysis and comparison of historical MSSA strains, historical MRSA strains and CA-MRSA strains suggest evolution from a common ancestor (Robinson et al., 2005).

The pathogenicity and virulence of *S. aureus* is associated with the capacity of this organism to produce several virulence factors including enterotoxins and serotypes A through Q (SEA-SEQ), toxic shock syndrome toxin-1 (TSST-1), cytolytic toxins, α and β hemolysins, exfoliative toxins, Panton-Valentine leukocidin (PVL), protein A, and several enzymes (Deurenberg, 2008).

The difference between the two types of MRSA strains also has clinical importance. Healthcare-associated MRSA (HA-MRSA) infections often result from hospitalization of patients who become colonized while in the healthcare setting. The most frequent types of MRSA infections encountered in healthcare settings are those associated with skin and soft tissue, surgery, use of indwelling devices, pneumonia, bacteremia, and sepsis. Klevens et al.

(2007) found that 85% of severe invasive MRSA can be linked to HA-MRSA and the health care system. Bacteremia and pneumonias accounted for 75.2% and 13.3% of these severe invasive infections, respectively.

In contrast, in 2005, there were an estimated 14 million outpatient (i.e., physician offices, emergency and outpatient departments) healthcare visits for suspected *S. aureus* skin and soft tissue infections in the United States (Hersh et al., 2008). The majority of these were due to CA-MRSA. Fridkin and colleagues (2005) found that 77% of CA-MRSA infections were skin and soft tissue infection, mainly skin abscesses and furunculosis, and a mere a 6% caused invasive disease. These infections occur in patients who do not have the risk factors usually associated with HA-MRSA. Yet, severe necrotizing pneumonia and shock resulting in death has also been caused by CA-MRSA strains (Hidron et al., 2009).

### *MRSA Carriage*

Persons may be colonized with *S. aureus* before developing infections. The main reservoir of *S. aureus* resides in the anterior nares. Approximately 20% of individuals are persistently colonized, while 30% are intermittently colonized (Gorwitz et al., 2008). Graham and colleagues performed a large population-based survey of *S.aureus* colonization in the US. They found that 28.6% of the population in their study had *S. aureus* in their nares. 5.2% of those individuals (1.5% of the total study population) had MRSA in their nares (Graham et al., 2006). Colonization provides a reservoir for the introduction when host defenses are breached. Individuals colonized with *S. aureus* are at an increased risk for subsequent infection (Huang et al., 2008). Furthermore, direct contact between non-colonized individuals is common in both the community and healthcare setting. Colonized individuals will most likely be infected by the strains that they already harbor. Moreover, Davis et al. demonstrated that individuals who are

colonized with MRSA are 10 times more likely to become infected with colonized strain than individuals who harbor MSSA (Davis et al., 2004). Depending on the patient population, long-term carriage rates in patients vary between 30% and 60% (Gorwitz et al., 2008).

### *Reducing Risk of MRSA*

To reduce the risk of MRSA transmission is to eliminate the possibility of MRSA as a cause of any disease. Conventional infection control strategies have been shown to help reduce MRSA transmission and infection. These strategies include: hand hygiene programs with institutional education (Pittet et al, 2000); targeted screening of high-risk patients with resulting contact isolation and cohorting of patients cared for by staff that do not care for MRSA negative patients (Jernagan et al., 1996; Mutto et al., 2003); the use of barrier/universal precautions, such as disposable gowns, masks and gloves, with patients identified as MRSA carriers or MRSA-infected (Huang et. al, 2006); and in some institutions decolonization of MRSA carriers (Buehlmann et al., 2008). Often, combinations of these measures are performed collectively and are part of what is called a "MRSA bundle." In order to control the spread of MRSA infections and colonization, multiple simultaneous interventions likely need to be performed due to the multiple routes of transmission.

Previous efforts to lower nosocomial infections by focusing on MRSA surveillance in high-risk units such as intensive care units have shown to be effective. Huang et al. reported a 67% reduction in bacteremias after this implementation (Huang et al., 2008). Other investigators have also experienced a reduction in rate of MRSA infections (Clancy et al., 2006) while others did not achieve the same success (West et al., 2006; Harbarth et al, 2006; Holmes, 2010).

*Molecular Procedures for Epidemiological Studies*

In the USA, PFGE is used by the CDC to assess the USA strain type and each is described by a similar PFGE pattern, antibiogram, mec type, and presence of absence of the PVL gene (Huang and Eells, 2011). The healthcare-associated MRSA strains include USA100, USA200, and USA500. The most common strain type of HA-MRSA is the USA100 strain. CA-MRSA strains are classified as USA 300 and USA 400, with USA 300 being the most common (Tenover and Goering, 2009).

More recently, other methods have been used for strain-typing. These include MLST, protein A gene (*spa*) typing and gene chip-based techniques (Huang and Eells, 2011). Each of the USA strain types has been found to have a corresponding MLST and spa type and rep-PCR pattern (Healy et al., 2005). We have adopted an automated rep-PCR system for our strain comparisons. Study of the genetic relatedness of isolates obtained during the course of an infection in a single patient is becoming a useful practice in many clinical and infection control laboratories. The various techniques used try to determine whether isolates recovered from different patients or sources represent a single strain or multiple different strains. Infection control practitioners use the information provided by molecular procedures to complement their epidemiological investigations.

# CHAPTER II: REDUCING HOSPITAL ACQUIRED MRSA INFECTIONS

## *"Search and Destroy" and Universal Screening*

Many hospitals outside the U.S. take part in the infection control practice of active surveillance of targeted high-risk patients (i.e. ICUs) screening to detect and manage the spread of MRSA by identifying asymptomatic carriers. There are two approaches hospitals will take either "search and isolate" or "search and destroy" (Strasbaugh et al., 2006). The latter strategy, coined by the Australians in the 1980's (Dwyer and Perceval, 1982) but implemented by the Dutch, starts with culturing of all admitted patients for MSRA via a nasal swab of the nares. Those MRSA positive patients are isolated and treated to eradicate nasal carriage (Vandenbroucke-Grauls, 1996). The typical decolonization regimen for MRSA includes topical application of 2% mupirocin twice a day to the colonized nares and chlorhexidine bathing, all for 5-7 days (Hill et al., 1988). The Netherlands, Iceland, and some Scandinavian countries have been able to maintain prevalence levels of nosocomial MRSA infections to <1%, presumably because of nationwide policies of "search and destroy" (van Trijp et al., 2007; Wertheim et al., 2004; Kramer, 2010).

This strategy has been slow to be accepted in the US, due to a lack of confidence that a pathogen-specific strategy would be cost-effective, or induce resistance to mupirocin, and reduce our ability to eradicate carriers with nasal application; however, where versions of "search and destroy" have been implemented, MRSA rates and prevalence have declined (Robicsek et al., 2008; Parada, et al., 2009; Jain et al., 2011).

One 3-hospital health care organization deploying this method found that the MRSA disease rate decreased by >60% in 1 year (Peterson et al., 2007; Robicsek et al., 2008). Additionally, The Veterans Administration (VA) health care system of 153 hospitals performing

universal screening with contact isolation recorded a 77% reduction in the rate of MRSA disease for ICU patients after 21 months. A 24% reduction rate of MRSA disease throughout the system for the patients who were not treated in the ICU was also observed within the first 21-months of implementation (Jain et al., 2011).

### *Screening Employees*

It is traditionally thought that employees could either be victims of or the source of MRSA transmission (Bowler, 1997). However, employees have been implicated as the source in a number of published outbreak reports. Recently, an extensive literature review by Albrich and Harbarth revealed a role of employee carriers of MRSA in the transmission of MRSA. They identified 79 studies that presented the role of employees in transmission of MRSA to patients and estimated the prevalence of MRSA in employees to be 4.6%, with rates ranging from 2%-15% (Albrich and Harbarth, 2008). Irrefutably, 27 studies had clear molecular and epidemiological associations which provided evidence of MRSA transmission from employees to patients, mostly in Europe. Another 52 studies likely had the same presumed direction of transmission. Interestingly, they found 18 studies with proven transmission to patients from employees not clinically infected with MRSA, and another 26 studies with likely transmission (Albrich and Harbarth, 2008). Therefore, there is evidence that colonized employees could act as a reservoir for transmission and potentially cause hospital-acquired infections, (whether displaying symptoms or not).

Most of the evidence for employee screening comes from outbreak reports where the outbreak was brought to an end following the introduction of staff screening as part as a number of infection control measures. As part of the Dutch "search and destroy" program, it is mandatory that employees be screened routinely and may contribute to the low prevalence of

MRSA within the hospital systems. There appears to be a widening pressure of a proposed screening program to include the screening of healthcare workers in other countries as well.

### *"Search and Destroy" Strategy at Pitt County Memorial Hospital*

The numbers of clinical infections due to MRSA began to increase in the late 1990s across North Carolina hospitals, which led to a partnering of Infection Control Departments with that of Dr. Barry Farr of the University of Virginia, known as the Problem Pathogen Partnership (Muto et al., 2003). Each of the participating hospitals, including PCMH, began to empirically culture admitted patients for nasal carriage of MRSA and rectal carriage of Vancomycin Resistant Enterococcus (VRE) and placed into Contact Precautions if they met special criteria that placed them at high risk for these resistant organisms. This approach had been initiated at the University of Virginia hospitals and resulted in decreases of healthcare associated MRSA and VRE transmission (Calfee et al., 2003). The categories of patients to be included were those transfers from Nursing Homes, patients on any form of dialysis, transfers from other healthcare facilities in which they had stayed > 4 days, and any patient with a prior MRSA or VRE positive culture. PCMH added Home Health transfers to the listing. This program was instituted first in the Surgical Intensive Care Unit, then incrementally to the other units in the hospital until all were included by early 2006. In spite of that program, 52% of all *S. aureus* isolates at PCMH were MRSA. While that percentage is not unusual for teaching hospitals, the following data were of concern: (a) MRSA accounted for 1-in-5 Surgical Site Infections among those patients undergoing surgery under the auspices of Medicare surveillance, the Surgical Infection Prevention Project, and up to 25% of the Surgical Site infections following Coronary Artery Bypass Surgery (b) Blood stream infections, or bacteremias due to MRSA exceeded those due to Methicillin-susceptible *Staphylococcus aureus* (MSSA) among patients admitted $\geq$ 48hrs,

suggesting healthcare acquisition of MRSA. Among device-related infections, MRSA accounted for 1-in-5 Ventilator-associated pneumonias (VAPs) in the surgical intensive care unit (SICU), and almost half of the VAPs and 22% of the central-line associated bacteremias in the Medical Intensive Care Unit (MICU). Thus, despite the implementation of the most successful MRSA control methods used in the United States by 2006, plus quality control methods across the hospital, healthcare associated infections with MRSA were a challenge for PCMH (see Fig.1)

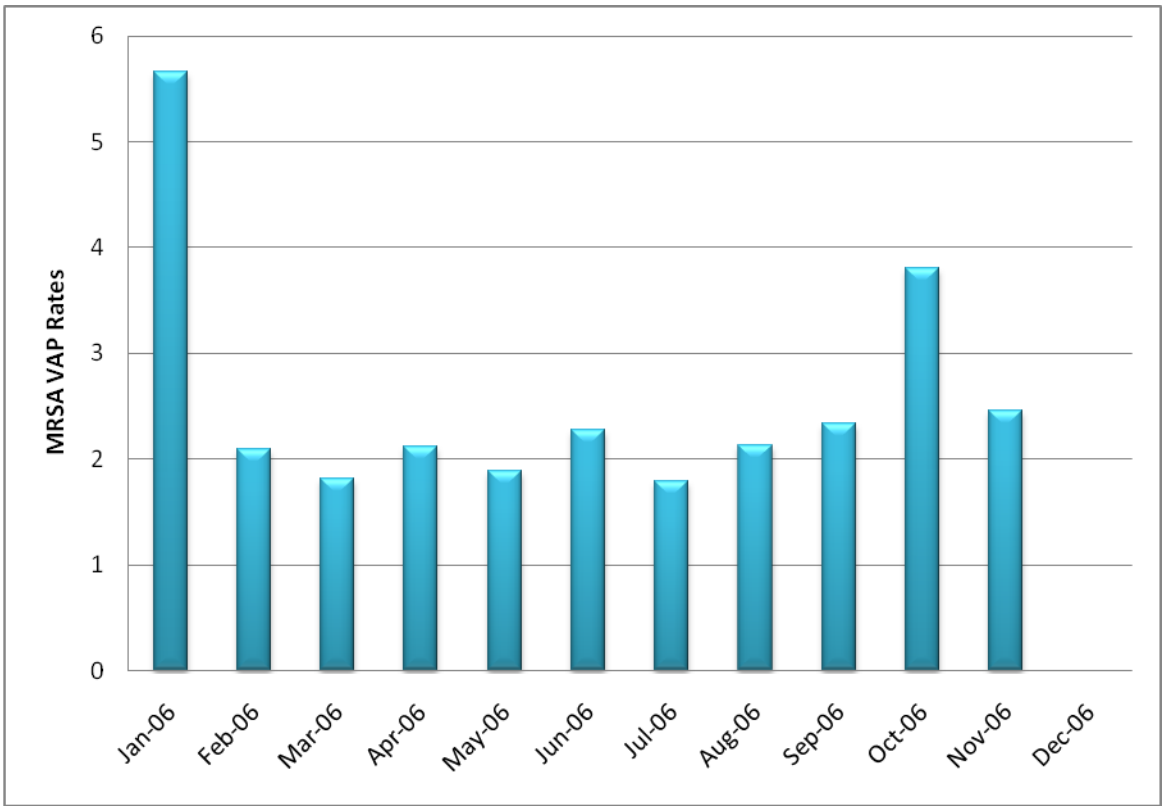



Figure 1. MRSA VAP rates from January to December 2006. MRSA VAP rates for January 2006 through December 2006. Rates are defined as the number of infections per number of devices days per 1000 days.

In response, the Quality Board at PCMH requested that the Medical Director of Infection Control meet and discuss methods to better control MRSA. Various strategies were discussed, including screening of all patients admitted to the Intensive Care Units, screening of all patients scheduled for surgery, and screening of all admissions. After careful consideration, the Quality Board of PCMH directed Dr. Ramsey to proceed with a version of the Dutch "Search and Destroy," with the screening of all admitted patients to PCMH for MRSA (to include those patients undergoing elective surgery). In contrast to the program of the Dutch, in which all employees work for the government, PCMH elected to offer voluntary screening of all employees for MRSA. These programs were to begin in early 2007, after purchasing the necessary rapid testing methods for screening and detecting MRSA from nasal swabs, hiring additional staff, and the devising of educational and clinical processes to implement this large program. Preliminary results detailing the impact of search and destroy with elective surgical procedures at PCMH has been reported by Pofahl and colleagues showing a reduction in surgical site infections, with statistical significant reduction among orthopedic procedures, comparing the pre-intervention infection rates to those of the post- "search and destroy" intervention (Pofahl et al., 2009).

# CHAPTER III: METHODS AND RESEARCH OBJECTIVES

## *Materials and Methods*

### *Study Design and Bacterial Isolates*

Pitt County Memorial Hospital located in Greenville North Carolina, is an academic, tertiary care facility with more than 850 acute care beds. The hospital offers all adult and pediatric medical and surgical services, including intensive care units.

This study was a retrospective comparison of strain typing of clinical and employee isolates. We obtained clinical MRSA from sputum, bronch lavages, tracheal aspirates, or blood after 48 hours of hospitalization beginning January 2006 through February 2011. Employee isolates were obtained via nasal screening beginning January 2007 through Feb 2011.

### *Active MRSA Surveillance Program*

Beginning in February 2007, all patients admitted to PCMH underwent a nasal swab for MRSA testing via PCR within 48 hours of admission. Patients testing positive for MRSA were placed onto contact isolation and their physician notified. The physicians would prescribe nasal mupirocin ointment BID to the nares X 5 days, and the nurses would use chlorhexidine soap for bathing (Pofahl, et al). Infection control retained a portion of the prior screening program by repeating screening on a weekly basis for all MRSA-negative patients receiving antibiotics (Farr et al, 2001). MRSA from either clinical isolates or screening were identified through growth on selective agar, and the results were usually reported within 48 hours. All MRSA isolates were stored at -70° C in the hospital epidemiology laboratory. In parallel, PCMH employees were given the option of voluntary nasal screening, confidentially, via nasal swab and the swabs were also plated onto selective agar for growth. Those healthcare workers positive were not

furloughed from work, and were prescribed 5 days of mupirocin nasal ointment and chlorhexidine supplied for bathing as with the patients. The employees were then re-swabbed for nasal carriage $\geq$ 72hrs post completion of eradication therapy. Data from the patients and employees were tabulated for percent positive.

*Healthcare Associated Infection Surveillance*

Healthcare associated infections were determined at PCMH via definitions from the Centers for Disease Control thru the National Health and Safety Network (NHSN) (Centers for Disease Control, 2005). These include ventilator associated pneumonias (VAPs), central-line associated bloodstream infections (CLABSIs), and catheter-associated Urinary tract infections (CA-UTIs). These infections are tabulated and converted to rates using the formula # infections/ # device days per 1000 device days to achieve rates comparable to other hospitals in the NHSN. These rates are determined each month, and tabulated longitudinally to determine trends in HAIs. The Ventilator-associated pneumonias (VAPs), an extension of infection of MRSA in the respiratory tract from the nares, is the focus of the intervention and correlation of isolates between clinical samples from patients and the healthcare workers who have contact with them.

*Bacterial Culture and DNA extraction*

Frozen isolates were cultured on agar with 5% sheep blood between 18 and 24 hrs at 37°C. This process was repeated two times. A 10µL loop full of bacteria was used in the DNA extraction using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA) according to manufacturer's instructions. The presence and concentration of genomic DNA was estimated with NanoDrop® ND-1000 Spectrophotometer (Isogen, Ijssel stein, The Netherlands) and DNA concentration was adjusted to ~35 ng/µl for each sample.

*Automated rep-PCR DNA Fingerprinting*

All DNA samples were amplified using the DiversiLab™ *Staphylococcus* kit for DNA fingerprinting (bioMérieux, Boxtel, The Netherlands) following the manufacturer's instructions. Briefly, 2 µl of genomic DNA (final concentration 35 ng/µl), 0.5 µl (or 2.5 U) of AmpliTaq® polymerase (Applied Biosystems, Foster City, CA, USA), 2 µl kit-supplied primer mix and 2.5 µl of 10× PCR buffer (Applied Biosystems, Foster City, CA, USA) were added to 18 µl of the kit-supplied rep-PCR master mix (MM1) for a total of 25 µl per PCR mixture. Thermal cycling parameters were as follows: initial denaturation at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 45 °C for 30 s, extension at 70 °C for 90 s; and a final extension at 70 °C for 3 min.

Separation and detection of rep-PCR products were done by micro-fluidic chips of the DiversiLab System (bioMérieux, Boxtel, The Netherlands) and analysis was performed with DiversiLab software version v.r.3.3.40. The resulting DNA fingerprint patterns were viewed on a personal DiversiLab website as electropherograms. The reports included a dendrogram with similarity matrix, scatterplot and a virtual gel image of the fingerprint for each sample.

The DiversiLab software used the Pearson correlation coefficient to analyze and calculate genetic similarity coefficients among all samples. The unweighted pair-group method of averages (UPGMA) was employed to automatically compare the rep-PCR profiles and create corresponding dendrograms (Healy et al., 2005). Reports included computer-generated virtual gel images, scatterplots and selected demographic fields to aid interpretation of the data. Guidelines have been suggested by the manufacturer for determining the strain-level discrimination (subtyping). Cluster analysis, based on peak height and presence or absence of

the peaks, was done by DiversiLab software. Percentage similarity for *S. aureus* was set at 98% similarity.

*Statistical Analysis of Data*

Fisher's exact test was used to see if there is evidence that the distributions of genotypes differ for clinical and employees and a Poisson regression of the data was carried out to see if there was a stastically significant change in the number of ventilator-associated pneumonias post implementation of universal screening.

## *Research Objectives*

1. To determine the prevalence and genotypes of patient isolates upon admission.

2. To determine the prevalence of nasal carriage among employees voluntarily screened.

3. Correlate the incidence of nosocomial MRSA VAPs on the surgical intensive care unit with the nasal colonization of employees working on this unit during the year 2006.

4. Determine the genotypes of MRSA clinical samples (VAP and bacteremias) from the SICU from January 2007 to February 2011.

5. Determine the genotypes of employees colonized with MRSA working on the SICU from January 2007 to February 2011.

6. Correlate potential route of transmission from employees to patients by molecular strain typing.

# CHAPTER IV: RESULTS

## *Prevalence of Patients with MRSA on Admission and Prevalence of Employee MRSA Carriage*

On February 1, 2007, PCMH implemented a universal screening program. The first 8 months of admission screening data showed an approximate 8% (3129 out of 39,600) of MRSA nasal colonization among patients admitted to the hospital (Figure 2). This rate has steadily remained constant for subsequent years and to the present. In contrast, the employee MRSA nasal carriage was found to be 3.4% (Table 1).

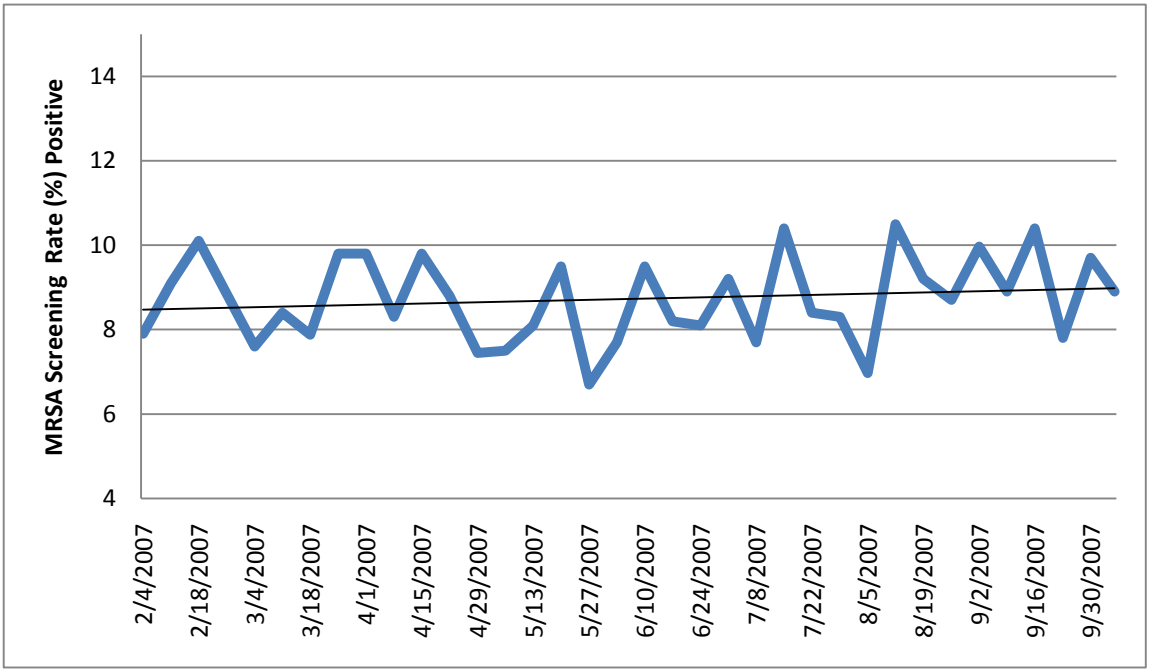



Figure 2. Prevalence of MRSA Colonization or Infection at Admission. Weekly rates of positive MRSA screens within an 8-month period following the implementation of universal screening at PCMH. Trendline is shown in black.

| | Healthcare Workers Tested | | | %Positive |
|---|---|---|---|---|
| | # Negative | #Positive | Total # Tested | |
| **2007** | **2442** | **107** | **2549** | **4.4** |
| **2008** | **3031** | **79** | **3110** | **2.6** |
| **2009** | **2420** | **69** | **2489** | **2.9** |
| **2010** | **2286** | **89** | **2375** | **3.9** |
| **Total** | **10,179*** | **344*** | **10523*** | **3.4** |

***Total number includes duplicate employee samples obtained from different years**

Table 1. Total number of negative and positive employees tested for MRSA carriage from 2007 through 2010 recovered from nasal swabs.

*Distribution of Admission Genotypes*

Genotypic analysis of the admission isolates for February, 2007, 2008, and 2009 showed that a majority of the samples were USA 100, the predominant strain causing HA-MRSA infections (Figure 3). These USA 100 strains represent approximately 50% of all isolates that were tested during the three years. However, between February 2007-2009, there was an increase in the percentage of USA 300 strains from 24% to 31%.

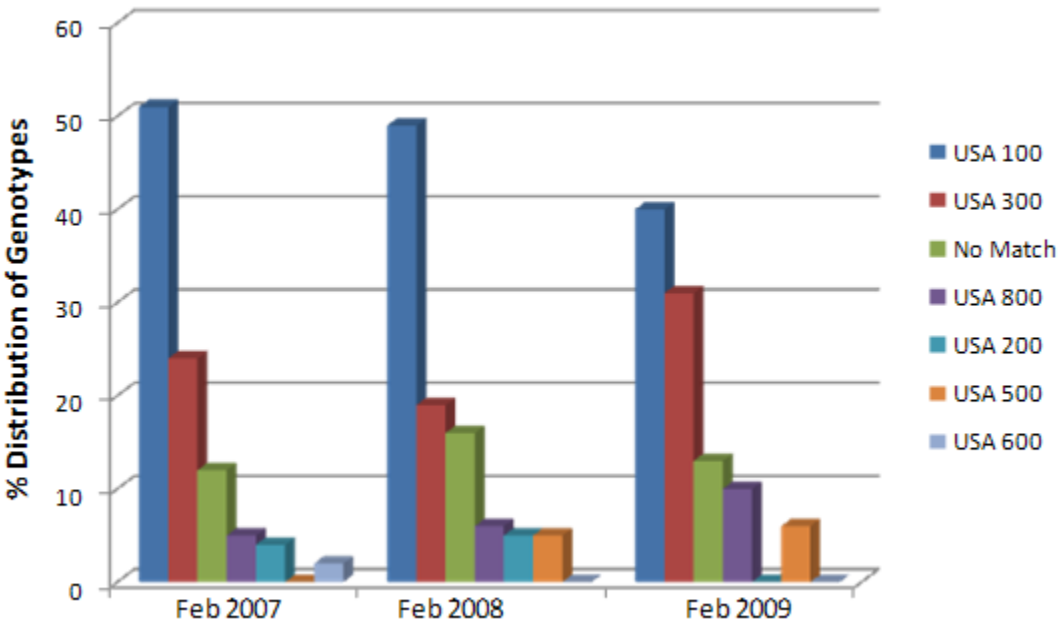



Figure 3. Genotypic analysis of the admission isolates for February 2007, 2008, and 2009. Percent distribution of USA types are given for subsequent years. Genotypes are color-coded.

*Comparison of Genotypes between Employees MRSA Nasal Carriage and MRSA isolates from Ventilator-associated Pneumonias from the SICU*

The availability of MRSA positive employee nasal screen isolates, allowed us to test a hypothesis to see if employee colonization could be responsible for the high rates of VAPs seen in the SICU. SICU employee isolates from 2007 were genotypically compared to the saved MRSA VAP isolates from 2006. A total of 35 samples, 10 VAP clinical (sputum and bronchial lavages) samples and 25 employees, were tested. Results showed 13 different rep-PCR patterns demonstrating strain diversity (Figure 5). 19-of-35 samples (54%) were described as USA 100, with representation from 5 different USA 100 strains. This also describes strain diversity within a particular sub-type. Five isolates were USA 300, five were USA 800, three were USA 200, and three were classified as "No Match", meaning they are unique rep-PCR types by the

Diversilab system. We found a correlation (identical banding of rep-PCR patterns) between clinical samples and employee samples (Cluster III and Cluster VIII). There were also clusters of indistinguishable strain types within both groups of employees and clinical isolates.

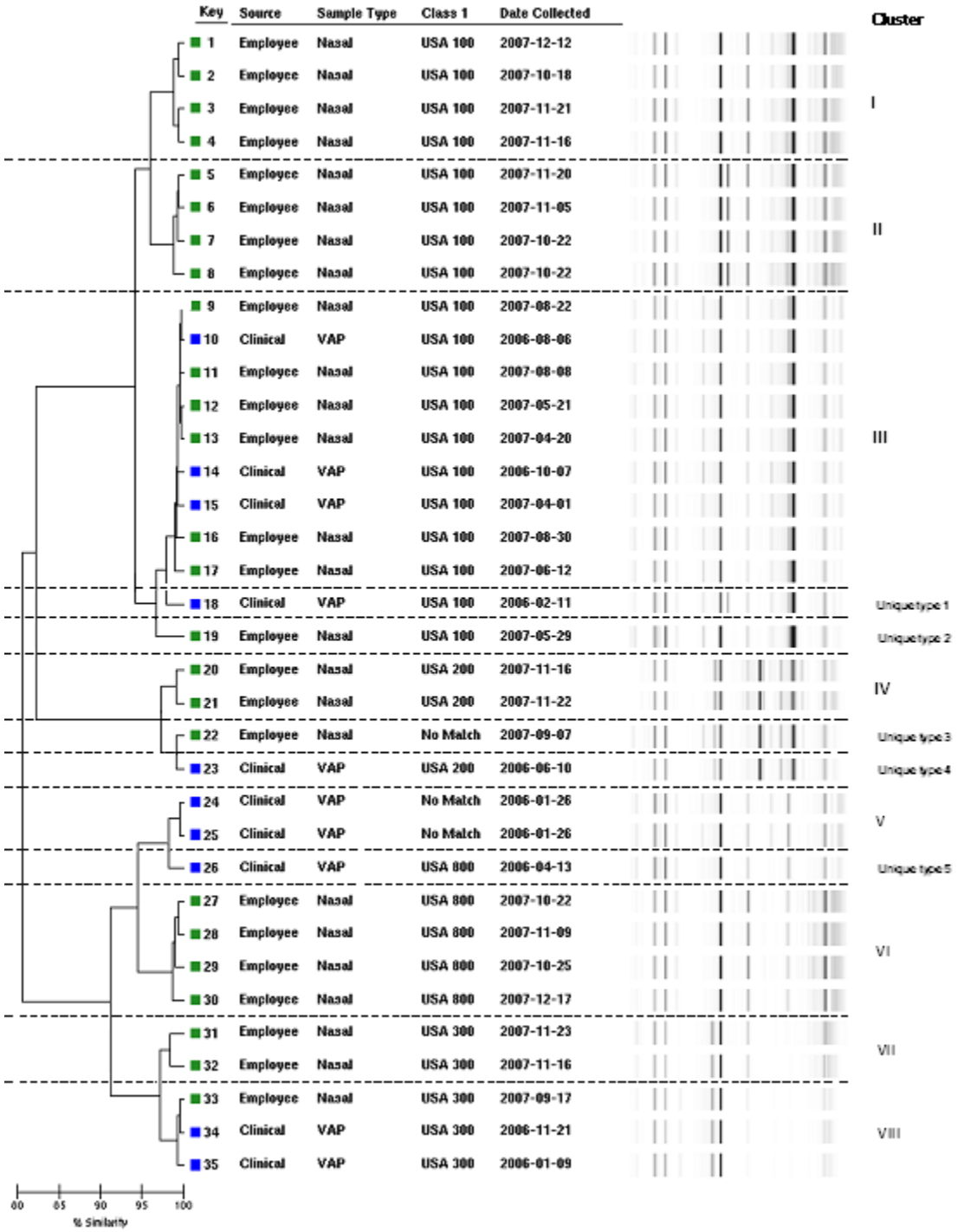


Figure 4. Comparison of the diversity of ventilator-associated pneumonias isolates (blue squares) and employee isolates (green squares). Dendograms and virtual gel images showing the presence of 13 rep-PCR patterns. Clusters I-VIII include multiple isolates in each cluster which are indistinguishable. The bar at the bottom left indicates the present similarity coefficient with the strains.

*Genotypes of Clinical MRSA Infection isolates from the SICU*

Subsequent molecular typing of MRSA bacteremias was performed for comparison of strain types among other clinical specimens in the SICU, along with the original VAP sample set. The results are shown below in Table 2 and Figure 5. Isolates from 36 patients revealed 31 out of 36 clinical isolates were differentiated into four USA types. Five isolates were classified as "No Match. There were 13 patterns observed among isolates. Each pattern has groups of MRSA strains that are indistinguishable from one another, suggesting either common sources or localized spread. USA 100 (50%) was the predominant strain, followed by USA 300 (25%). There were five different strains of USA 100 and three different strains of USA 300 which grouped accordingly in distinct patterns on the virtual gel-image as seen in Fig.1. The remaining strains USA 200, USA 800 and No Matches accounted for 2.8%, 8.3% and 13.9%, respectively.

| | | | Group | | |
|---|---|---|---|---|---|
| | | | Clinical | Employees | Total |
| Type | USA100 | Count | 18 | 26 | 44 |
| | | % within Group | 50.0% | 47.3% | 48.4% |
| | USA200 | Count | 1 | 2 | 3 |
| | | % within Group | 2.8% | 3.6% | 3.3% |
| | USA300 | Count | 9 | 10 | 19 |
| | | % within Group | 25.0% | 18.2% | 20.9% |
| | USA800 | Count | 3 | 8 | 11 |
| | | % within Group | 8.3% | 14.5% | 12.1% |
| | No Match | Count | 5 | 9 | 14 |
| | | % within Group | 13.9% | 16.4% | 15.4% |
| Total | | Count | 36 | 55 | 91 |
| | | % within Group | 100.0% | 100.0% | 100.0% |

Table 2. Distribution and percentages of rep-PCR types found in clinical and employee samples.

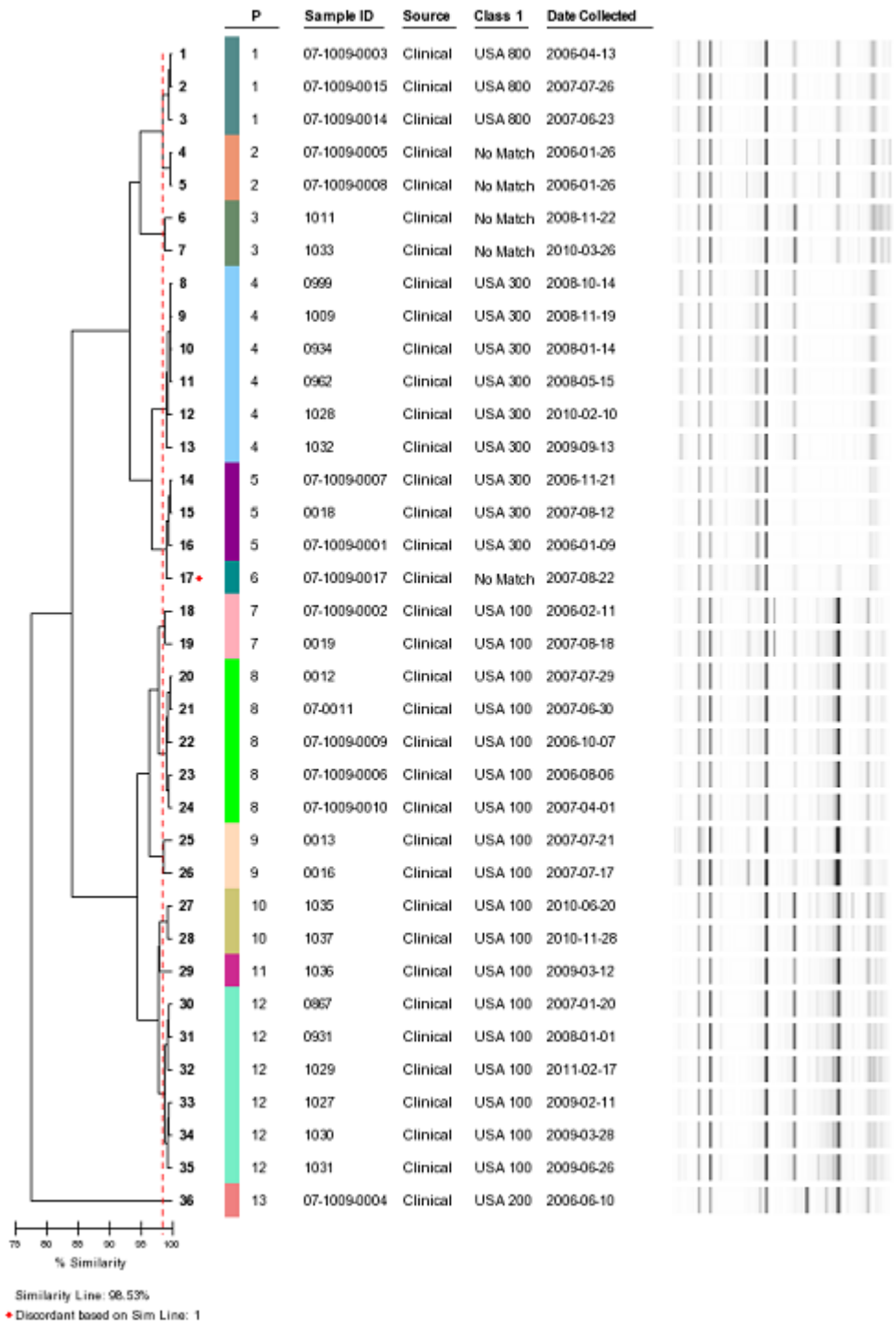
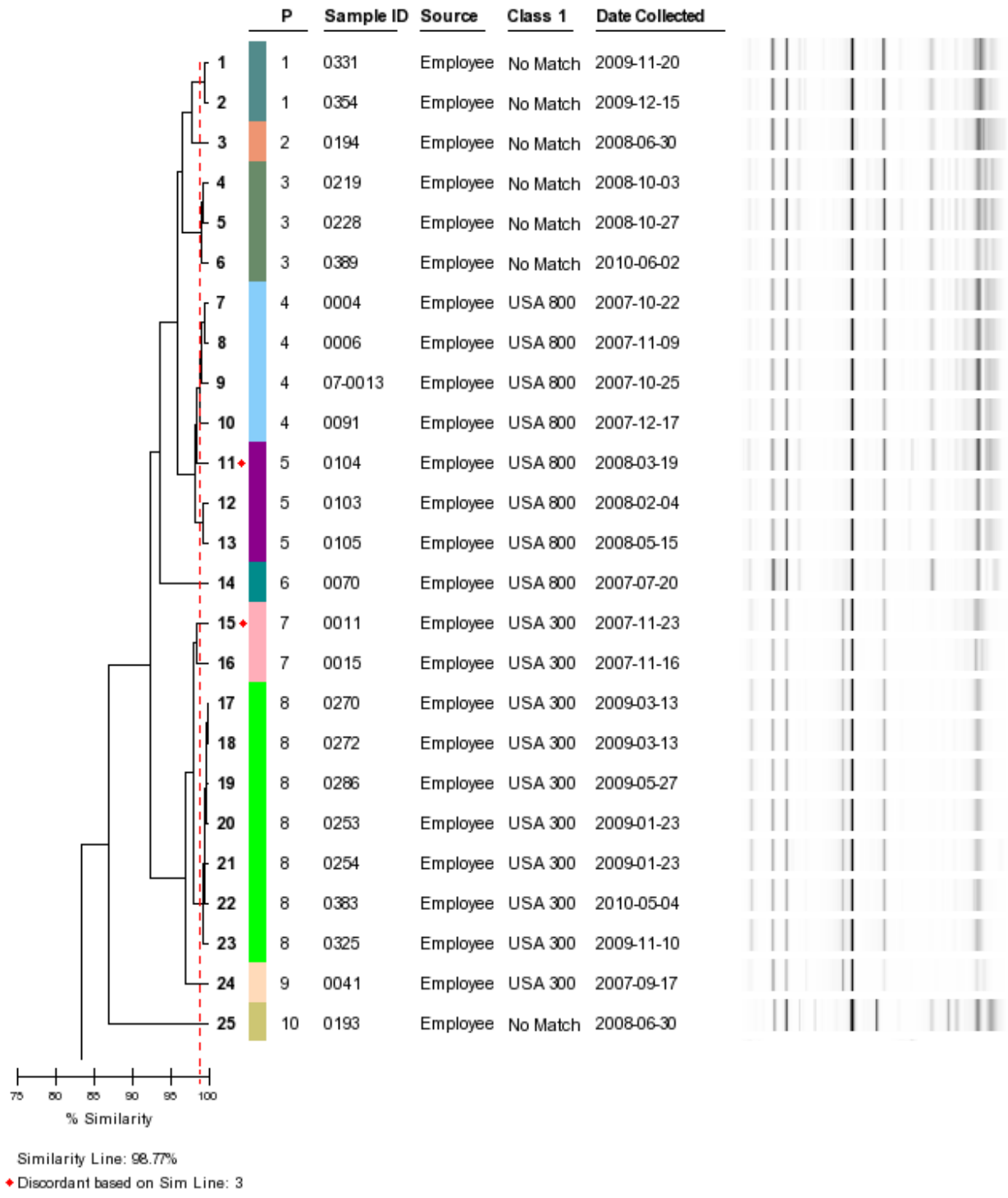



Figure 5. Dendogram and virtual gel images of 36 clinical isolates (ventilator-associated pneumonias and bacteremias) obtained from the surgical intensive care unit from January 2006 until February 2011. Thirteen patterns (P1-P13) are represented by different colors located in Column "P". Patterns with

multiple isolates indicate indistinguishable strains. Sample ID, source, USA type, and collection date are located in between the dendogram and virtual gel images. A similarity line of 98.5% is signified by a red dotted line. The red diamond indicates a discordant sample.

*Genotypes of Employees MRSA Nasal Colonization from the SICU*

The genotyping data for fifty-five employee samples were collected from among 41 of the employees (Table 2 and Figure 6); thus, a minority of nine employees had different sequential isolates. There was a considerable amount of strain diversity indicated by 21 different patterns. The isolates were characterized as being USA 100, USA 200, USA 300, or USA 800 for 46 out of 55 employee samples. Nine isolates were considered "No Match". As in the case with the clinical isolates, approximately 50% of the isolates from among the employees were found to be USA 100, while the second prevalent genotype was USA 300 (approximately 18% ). USA 200, USA 800, and "No Matches" resulted in 3.6%, 14.5%, 16.4%, respectively of the remaining isolate strain types.


P
Sample ID
Source
Class 1
Date Collected
1 1 0331 Employee No Match 2009-11-20
2 1 0354 Employee No Match 2009-12-15
3 2 0194 Employee No Match 2008-06-30
4 3 0219 Employee No Match 2008-10-03
5 3 0228 Employee No Match 2008-10-27
6 3 0389 Employee No Match 2010-06-02
7 4 0004 Employee USA 800 2007-10-22
8 4 0006 Employee USA 800 2007-11-09
9 4 07-0013 Employee USA 800 2007-10-25
10 4 0091 Employee USA 800 2007-12-17
11 5 0104 Employee USA 800 2008-03-19
12 5 0103 Employee USA 800 2008-02-04
13 5 0105 Employee USA 800 2008-05-15
14 6 0070 Employee USA 800 2007-07-20
15 7 0011 Employee USA 300 2007-11-23
16 7 0015 Employee USA 300 2007-11-16
17 8 0270 Employee USA 300 2009-03-13
18 8 0272 Employee USA 300 2009-03-13
19 8 0286 Employee USA 300 2009-05-27
20 8 0253 Employee USA 300 2009-01-23
21 8 0254 Employee USA 300 2009-01-23
22 8 0383 Employee USA 300 2010-05-04
23 8 0325 Employee USA 300 2009-11-10
24 9 0041 Employee USA 300 2007-09-17
25 10 0193 Employee No Match 2008-06-30
75 80 85 90 95 100
% Similarity
Similarity Line: 98.77%
Discordant based on Sim Line: 3

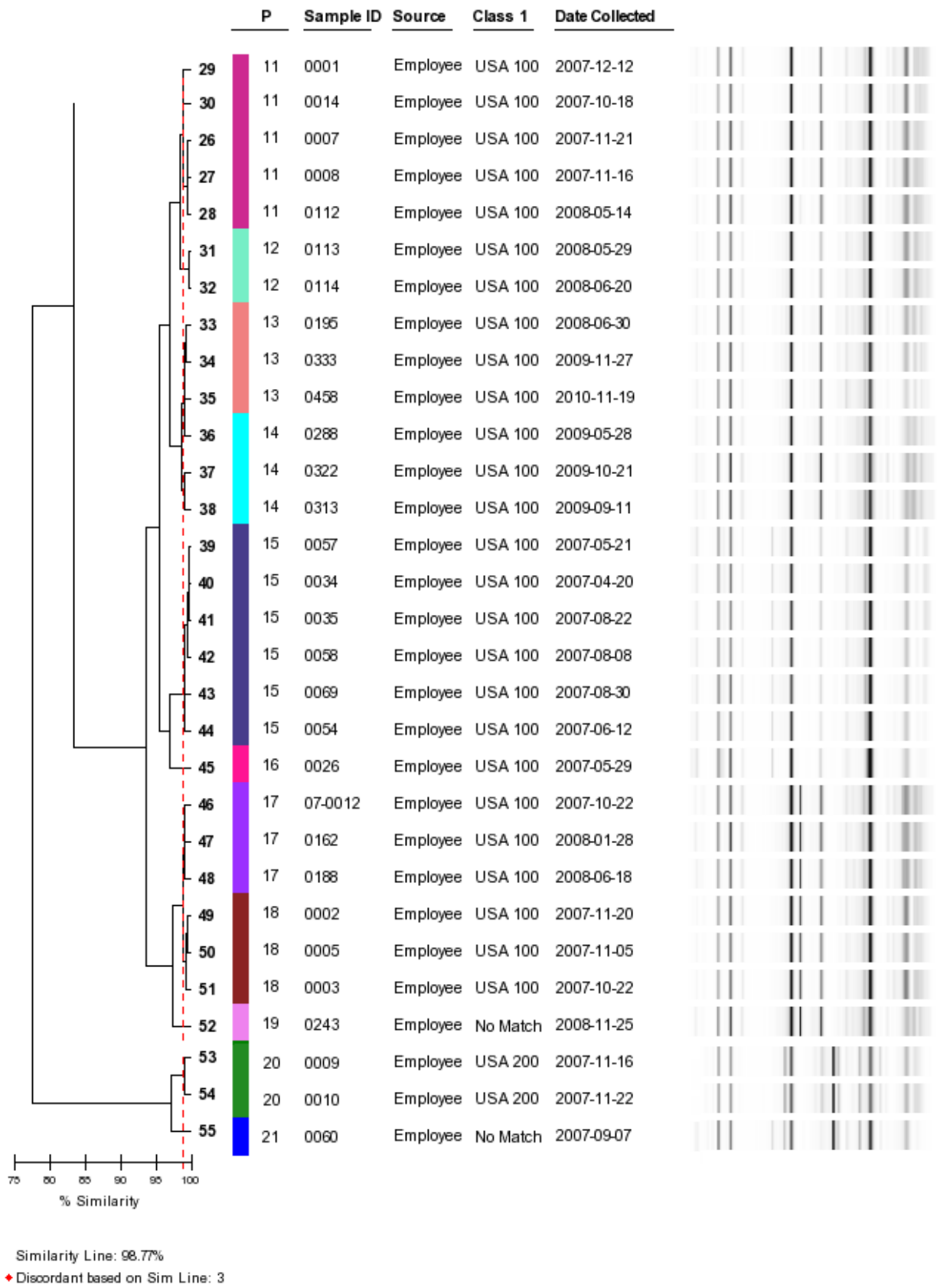
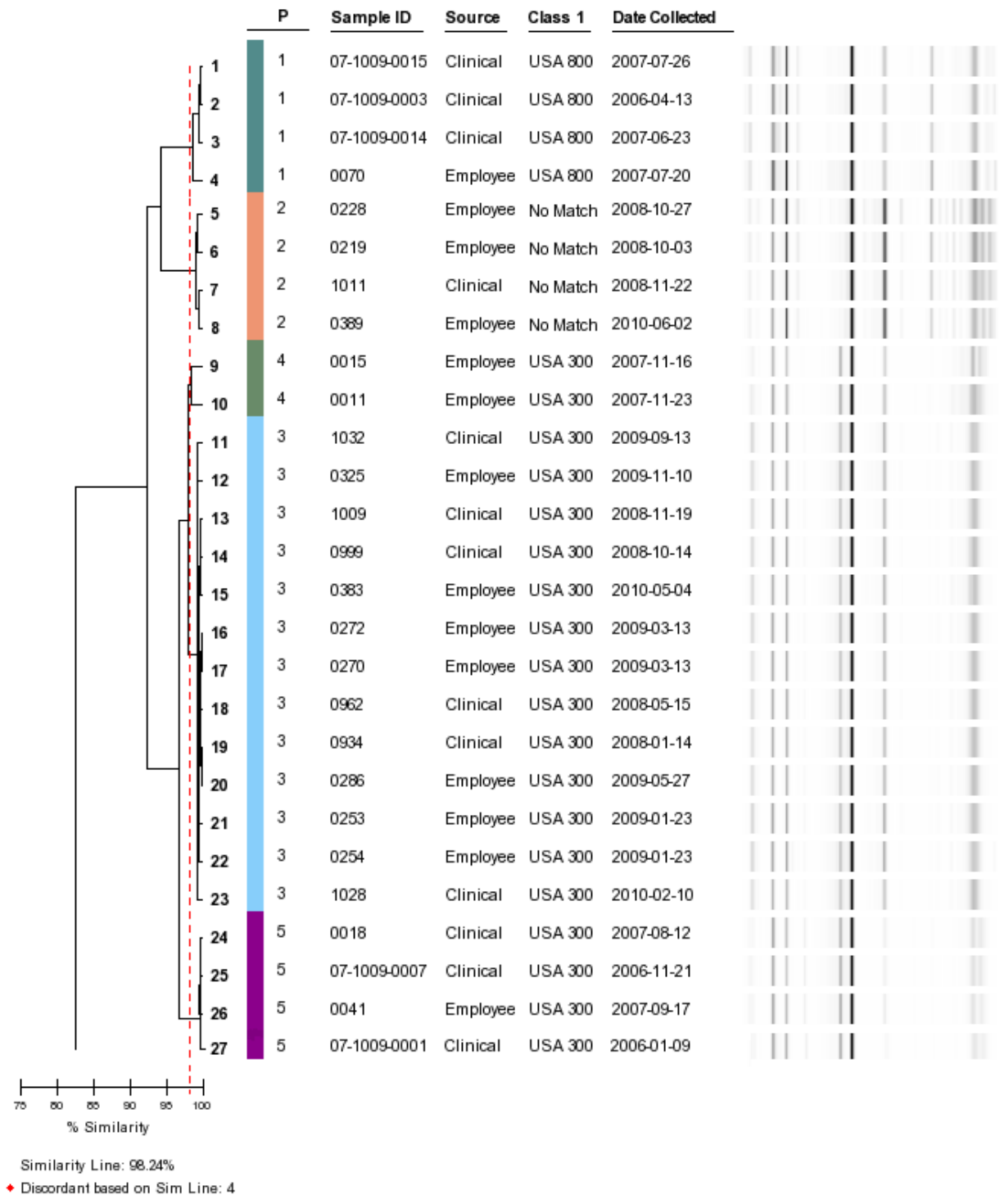

P
Sample ID
Source
Class 1
Date Collected
29 11 0001 Employee USA 100 2007-12-12
30 11 0014 Employee USA 100 2007-10-18
26 11 0007 Employee USA 100 2007-11-21
27 11 0008 Employee USA 100 2007-11-16
28 11 0112 Employee USA 100 2008-05-14
31 12 0113 Employee USA 100 2008-05-29
32 12 0114 Employee USA 100 2008-06-20
33 13 0195 Employee USA 100 2008-06-30
34 13 0333 Employee USA 100 2009-11-27
35 13 0458 Employee USA 100 2010-11-19
36 14 0288 Employee USA 100 2009-05-28
37 14 0322 Employee USA 100 2009-10-21
38 14 0313 Employee USA 100 2009-09-11
39 15 0057 Employee USA 100 2007-05-21
40 15 0034 Employee USA 100 2007-04-20
41 15 0035 Employee USA 100 2007-08-22
42 15 0058 Employee USA 100 2007-08-08
43 15 0069 Employee USA 100 2007-08-30
44 15 0054 Employee USA 100 2007-06-12
45 16 0026 Employee USA 100 2007-05-29
46 17 07-0012 Employee USA 100 2007-10-22
47 17 0162 Employee USA 100 2008-01-28
48 17 0188 Employee USA 100 2008-06-18
49 18 0002 Employee USA 100 2007-11-20
50 18 0005 Employee USA 100 2007-11-05
51 18 0003 Employee USA 100 2007-10-22
52 19 0243 Employee No Match 2008-11-25
53 20 0009 Employee USA 200 2007-11-16
54 20 0010 Employee USA 200 2007-11-22
55 21 0060 Employee No Match 2007-09-07
75 80 85 90 95 100
% Similarity
Similarity Line: 98.77%
Discordant based on Sim Line: 3

Figure 6. Dendogram and virtual gel images demonstrating the diversity of MRSA strains among employees in the surgical intensive care unit from January 2007 until February 2011. Twenty-one patterns (P1-P21), indicated by different colors in "Column P", were observed. Patterns with multiple isolates indicate indistinguishable strains. Sample ID, source, USA type, and collection date are located in between the dendogram and virtual gel images. A similarity line of 98.7% is signified by a red dotted line. The red diamonds indicate discordant samples.

### *Comparison of Genotypes between Clinical MRSA Isolates and Employees with Positive MRSA Nasal Screens*

The comparison of fingerprints between the isolates of employees and clinical samples revealed that 10-of-34 patterns were shared between the two groups (Figure 7). This indicates a correlation between employee carriage and the possibility of transmission to patients and subsequent clinical infection. Since some healthcare workers have had the same strain type for a number of years, these strains likely represent endemic strains that are circulating in the surgical intensive care unit.


P
Sample ID
Source
Class 1
Date Collected
1 1 07-1009-0015 Clinical USA 800 2007-07-26
2 1 07-1009-0003 Clinical USA 800 2006-04-13
3 1 07-1009-0014 Clinical USA 800 2007-06-23
4 1 0070 Employee USA 800 2007-07-20
5 2 0228 Employee No Match 2008-10-27
6 2 0219 Employee No Match 2008-10-03
7 2 1011 Clinical No Match 2008-11-22
8 2 0389 Employee No Match 2010-06-02
9 4 0015 Employee USA 300 2007-11-16
10 4 0011 Employee USA 300 2007-11-23
11 3 1032 Clinical USA 300 2009-09-13
12 3 0325 Employee USA 300 2009-11-10
13 3 1009 Clinical USA 300 2008-11-19
14 3 0999 Clinical USA 300 2008-10-14
15 3 0383 Employee USA 300 2010-05-04
16 3 0272 Employee USA 300 2009-03-13
17 3 0270 Employee USA 300 2009-03-13
18 3 0962 Clinical USA 300 2008-05-15
19 3 0934 Clinical USA 300 2008-01-14
20 3 0286 Employee USA 300 2009-05-27
21 3 0253 Employee USA 300 2009-01-23
22 3 0254 Employee USA 300 2009-01-23
23 3 1028 Clinical USA 300 2010-02-10
24 5 0018 Clinical USA 300 2007-08-12
25 5 07-1009-0007 Clinical USA 300 2006-11-21
26 5 0041 Employee USA 300 2007-09-17
27 5 07-1009-0001 Clinical USA 300 2006-01-09
75
80
85
90
95
100
% Similarity
Similarity Line: 98.24%
Discordant based on Sim Line: 4

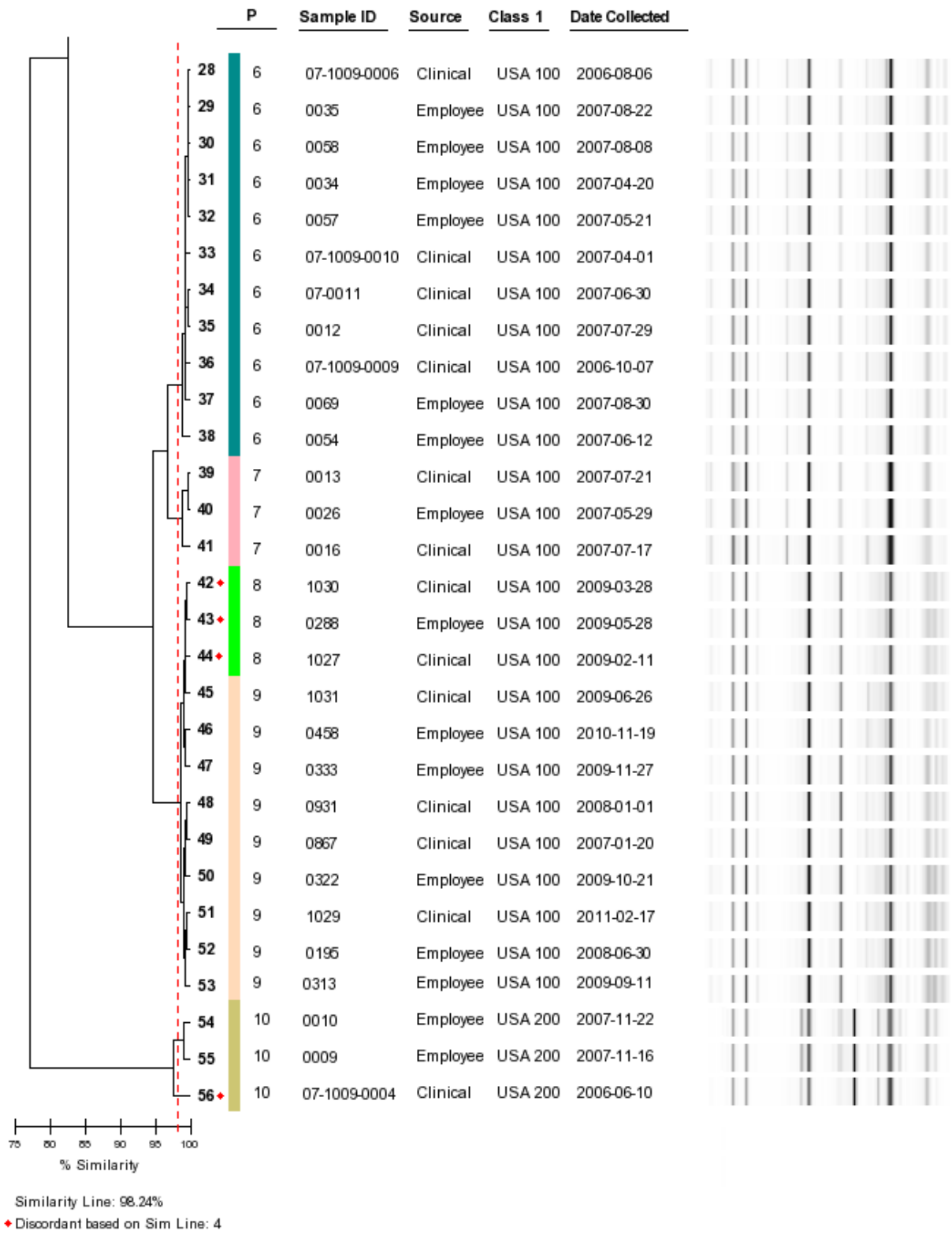

P
Sample ID
Source
Class 1
Date Collected
28 6 07-1009-0006 Clinical USA 100 2006-08-06
29 6 0035 Employee USA 100 2007-08-22
30 6 0058 Employee USA 100 2007-08-08
31 6 0034 Employee USA 100 2007-04-20
32 6 0057 Employee USA 100 2007-05-21
33 6 07-1009-0010 Clinical USA 100 2007-04-01
34 6 07-0011 Clinical USA 100 2007-06-30
35 6 0012 Clinical USA 100 2007-07-29
36 6 07-1009-0009 Clinical USA 100 2006-10-07
37 6 0069 Employee USA 100 2007-08-30
38 6 0054 Employee USA 100 2007-06-12
39 7 0013 Clinical USA 100 2007-07-21
40 7 0026 Employee USA 100 2007-05-29
41 7 0016 Clinical USA 100 2007-07-17
42 8 1030 Clinical USA 100 2009-03-28
43 8 0288 Employee USA 100 2009-05-28
44 8 1027 Clinical USA 100 2009-02-11
45 9 1031 Clinical USA 100 2009-06-26
46 9 0458 Employee USA 100 2010-11-19
47 9 0333 Employee USA 100 2009-11-27
48 9 0931 Clinical USA 100 2008-01-01
49 9 0867 Clinical USA 100 2007-01-20
50 9 0322 Employee USA 100 2009-10-21
51 9 1029 Clinical USA 100 2011-02-17
52 9 0195 Employee USA 100 2008-06-30
53 9 0313 Employee USA 100 2009-09-11
54 10 0010 Employee USA 200 2007-11-22
55 10 0009 Employee USA 200 2007-11-16
56 10 07-1009-0004 Clinical USA 200 2006-06-10
75 80 85 90 95 100
% Similarity
Similarity Line: 98.24%
Discordant based on Sim Line: 4

Figure 7. Dendogram and virtual gel images of 27 clinical isolates and 29 employee isolates. With the exception of Pattern 4 (employee samples only), clinical and employee samples share indistinguishable strains among a variety of patterns. Sample ID, source, USA type, and collection date are located in between the dendogram and virtual gel images. A similarity line of 98.2% is signified by a red dotted line. The red diamonds indicate discordant samples.

*Impact of "Search and Destroy" on Ventilator-associated Pneumonias*

After universal screening of patients and voluntary screening of employees, plus eradication therapy for those positive began in February 2007, there was a statistically significant decline in the amount of VAPs due to MRSA on the SICU (Figure 8). This indicates that screening and eradication of MRSA carriage among patients upon admission, plus screening and eradication of MRSA among HCWs decreases the chance of clinical infection among the MRSA nasal screen negative patients.

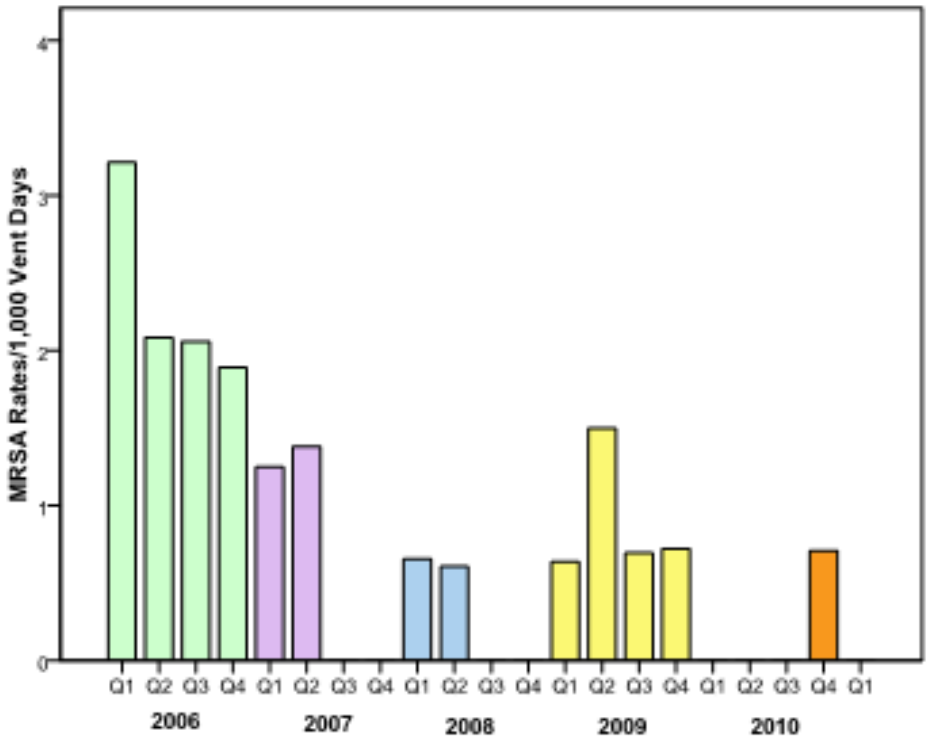



Figure 8. Quarterly rates of MRSA ventilator-associated pneumonia between the years 2006-2010. Downward trend for ventilator-associated pneumonia was significant (P=0.0002, with the use of Poisson regression).

# CHAPTER V: DISCUSSION

## *Study Conclusions*

Despite the implementation of the most successful MRSA control methods used in the United States by 2006, plus quality control methods across the hospital, healthcare associated infections with MRSA were a challenge for PCMH, particularly ventilator associated infections.

On February 1, 2007, PCMH initiated a universal admission screening or surveillance program for MRSA, making it one of the four institutions or hospital systems in the United States to screen all inpatient hospital admissions for MRSA (Robicsek et al., 2008; Parada et al., 2009; Javin et al., 2011). Whereas the other participating organizations have adopted a version of this strategy, the University Health System (UHS) Quality Board adopted a novel version of this "search and destroy" strategy by offering voluntary screening and eradicating of nasal carriage of MRSA among employees. This is the first hospital in the United States to employ or implement this part of the program. The initial six months of admission screening revealed the prevalence of patients admitted to the hospital carrying MRSA was approximately 8%, with approximately 3390-of-42,375 of patients testing positive upon admittance to the hospital the first year. This prevalence rate is higher than the 6.3% admission rate found at Northwestern among 40,000 annual admissions in a 3-hospital organization (Robicsek, et al., 2008); however, the percent MRSA positive upon admission is lower than the percentages seen for the VA-hospital system with an admission colonization rate of 13.6% (Javin et al., 2011). The latter differences are perhaps due to differences in age, and severity of illness, but most likely a result of the male predominance of the patients served by the VA medical system.

In contrast, employees at PCMH had a 3.4% positive nasal carriage rate for MRSA, or about half the rate in the patients whom they serve. Potential explanations for the lower

percentage could include the younger age, better health, and better hygiene practices of employees. Unfortunately, few studies in the US have examined employee carriage other than in response to MRSA outbreaks among hospitalized patients, where there is suspicion of carriage and transmission to hospitalized patients.

In one of the few published studies of nasal colonization of employees, screening of 200 healthcare-workers at Johns Hopkins University Medical Center revealed a 2% carriage rate among medical and nursing staff (Milestone et al., 2010). On the other hand, the percentage of nares colonization of employees at PCMH is substantially lower than the 12% carriage rate among 33 employees from Driscoll Children's Hospital, Corpus Christi, Texas (Stein et al.,2006).

Genotypic analysis of the admission isolates for February, 2007, 2008, and 2009 showed that a majority of the samples were USA 100 and there was an increase in the percentage of USA 300 strains from 24% to 31%. This is consistent with the results of other investigators (Gorwitz et al., 2008; Tenover et al., 2009), reflecting the growing importance of USA 300, or community-CA-MRSA. The genotypic analysis of the clinical samples and those from employees from PCMH's SICU also revealed USA 100 as the predominant strain, suggesting this strain has circulated among patients both in the community and hospital. There appears to be a higher incidence of USA 300 emerging in the clinical specimens and employee screens among the SICU in parallel. A population-based study of invasive MRSA infections in the United States from 2005 to 2006 revealed that USA 100 and USA 300 are the predominant MRSA PFGE types (Limbago et al.2009; Klevens et al., 2007), which parallels to the observations in Table 2, Figure 5, and Figure 6. In contrast, in the Johns Hopkins study which is the only published work in the US exploring molecular typing of MRSA strains colonizing

hospital employees, none of the 8 isolates were characterized as CA-MRSA (Milestone et al., 2010).

Along with the large numbers of patients and employee screening for MRSA, another novel feature of our study is the correlation of the genotypes of employee MRSA strains with those of patients with nosocomial infection using rep-PCR for strain-typing. The absence of any significant differences between the distributions of genotypes among the patients and employees (*p*-value = 0.1924 for the Fisher's Exact Test and and $\underline{p} \leq 0.05$) further suggests that the predominant clones of USA 100 and 300 have been circulating among both our patients and employees during, if not before, this study period.

Implementation of universal screening of the nares with subsequent eradication therapy was successful in reducing healthcare-associated lower-respiratory tract infections with a decrease from 2.1157 of MRSA cases 0.4797 MRSA cases/1000 device days. The rate after the intervention is 22.7% of the rate before the intervention. Poisson regression of the data indicated the ratio of the rates (0.227) is significantly different from 1. The p-value was 0.0002, so we conclude that this ratio is significantly different from 1 (p-value=0.0002, 95% CI: 0.105 to 0.490). These decreases in clinical infections defined as pneumonias were also observed by colleagues in the VA hospital system (Jain et al, 2011), suggesting the validity of our approach at PCMH; however in the VA study, no attempt was made to separate non-ventilator and ventilator-associated pneumonias. The MRSA universal screening program at Northwestern also demonstrated a significant decrease in respiratory tract cultures and infections caused by MRSA (Robicsek et al., 2008), but like the VA Study, did not differentiate between types of respiratory tract infections.

Overall, there was a great diversity of strain types found in our institution among both employees and clinical isolates. The existence of genetically indistinguishable strains in both groups supports the idea that employee carriage of MRSA potentially plays a role in clinical infections such as ventilator-associated pneumonias. These data support the role of a screening program for MRSA carriage among employees with subsequent decolonization for the prevention of nosocomial MRSA infections.

Notably, there was implementation of general infection control practices throughout the sample collection. Therefore, with multiple control procedures being performed simultaneously, the exact contribution of the specific surveillance and eradication program is uncertain. Since our study was conducted at a single organization, our findings cannot be generalized to other institutions. Secondly, as we have not performed systematic testing for mupirocin resistance among our isolates due to the absence of non-standardized susceptibility testing, we are not aware of any changes or loss of mupirocin resistance among our populations of treated carriers. Since our study was conducted at a single organization, our findings may not be generalized for other institutions. However, our experience suggests that a multiplicity of measures including employee screening and universal testing of admission for MRSA colonization needs to be in place to account for the multiple potential sources and routes of transmission and to control the spread of MRSA colonization and infection.

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# APPENDIX: IRB APPROVALS

This research has been approved by the Institutional Review Board under the usage of two separate IRB applications. "Retrospective and Ongoing Analysis of MRSA at PCMH" (IRB # 09-0543) approves the use of data from among patients at PCMH, and "Retrospective and Ongoing Analysis of Health Care Workers with MRSA" (IRB# 09-0020) approves the use of data from among employees, or staff with MRSA at PCMH.

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
1L-09 Brody Medical Sciences Building• 600 Moye Boulevard • Greenville, NC 27834
Office 252-744-2914 • Fax 252-744-2284 • www.ecu.edu/irb

TO: Keith Ramsey, MD, Department of IM, BSOM, ECU

FROM: UMCIRB

DATE: September 21, 2010

RE: Expedited Continuing Review of a Research Study

TITLE: "Retrospective and Ongoing Analysis of Colonization and Infection of Patients with MRSA at PCMH"

**UMCIRB #09-0543**

The above referenced research study was initially reviewed and approved by expedited review on 7/7/09. This research study has undergone a subsequent continuing review using expedited review on 9/17/10. This research study is eligible for expedited review because it is research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.). Also, it is research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.) The Chairperson (or designee) deemed this **unfunded** study **no more than minimal risk** requiring a continuing review in **12 months**. Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The above referenced research study has been given approval for the period of **9/17/10** to **9/16/11**. The approval includes the following items:

- Continuing Review Form (dated 9/9/10)
- Protocol summary
- MRSA questionnaire
- Informed consent (received 9/15/10)

The Chairperson (or designee) does not have a conflict of interest on this study.

**The UMCIRB applies 45 CFR 46, Subparts A-D, to all research reviewed by the UMCIRB regardless of the funding source. 21 CFR 50 and 21 CFR 56 are applied to all research studies under the Food and Drug Administration regulation. The UMCIRB follows applicable International Conference on Harmonisation Good Clinical Practice guidelines.**

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TO: Keith Ramsey, MD, Department of IM- Infectious Diseases, BSOM, ECU, Doctor's Park 6B

FROM: UMCIRB

DATE: January 25, 2011

RE: Expedited Continuing Review of a Research Study

TITLE: "Retrospective and Ongoing Analysis of Health Care Workers Colonized and Infected with MRSA"

**UMCIRB #09-0020**

The above referenced research study was initially reviewed and approved by expedited review on 1/15/09. This research study has undergone a subsequent continuing review using expedited review on 1/21/11. This research study is eligible for expedited review because it is research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.) Also, it is research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.) The Chairperson (or designee) deemed this **unfunded** study **no more than minimal risk** requiring a continuing review in **12 months.** Changes to this approved research may not be initiated without UMCIRB review except when necessary to eliminate an apparent immediate hazard to the participant. All unanticipated problems involving risks to participants and others must be promptly reported to the UMCIRB. The investigator must submit a continuing review/closure application to the UMCIRB prior to the date of study expiration. The investigator must adhere to all reporting requirements for this study.

The above referenced research study has been given approval for the period of **1/21/11** to **1/20/12.** The approval includes the following items:

- Continuing Review Form (dated 1/12/11)
- Informed consent (received 1/19/11)
- MRSA carriage risk factors questionnaire

The Chairperson (or designee) does not have a conflict of interest on this study.

**The UMCIRB applies 45 CFR 46, Subparts A-D, to all research reviewed by the UMCIRB regardless of the funding source. 21 CFR 50 and 21 CFR 56 are applied to all research studies under the Food and Drug Administration regulation. The UMCIRB follows applicable International Conference on Harmonisation Good Clinical Practice guidelines.**