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Signature:

_____ _____

Russell Ryan Kempker Date

Approval Sheet

An Association of MRSA USA 300 with Mortality in MRSA Bacteremia

By

Russell Ryan Kempker
Masters of Science

Clinical Research

_____
Monica M. Farley, M.D.
Advisor

_____
Susan Ray, M.D.
Advisor

_____
John Boring, III, Ph.D.
Committee Member

_____
Thomas Ziegler, M.D.
Committee Member

_____
Henry M. Blumberg, M.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

Abstract Cover Page

An Association of MRSA USA 300 with Mortality in MRSA Bacteremia

By

Russell Ryan Kempker
B.S., University of Florida Honors College, 1999
M.D., University of Miami School of Medicine, 2004

Advisor: Monica M. Farley, M.D.
Advisor: Susan Ray, M.D.

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Abstract

An Association of MRSA USA 300 with Mortality in MRSA Bacteremia

By Russell Ryan Kempker

Introduction: Evidence suggests MRSA USA 300 strains may have increased virulence; however, there are no clinical studies evaluating outcomes of MRSA bacteremia based on MRSA strain.

Methods: Population based-surveillance for MRSA Bacteremia in 8-county Atlanta from 2005-2008. Cases, defined as MRSA isolated from blood, were classified as healthcare-associated with hospital-onset (HAHO), healthcare-associated with community-onset (HACO), or community-associated (CA) disease. An epidemiological analysis was performed on the entire cohort while a survival analysis was performed on a nested cohort consisting of all MRSA cases for which isolates were evaluated with pulse field gel electrophoresis (PFGE). A cox proportional hazards model was used to determine the association of USA 300 genotype and other risk factors with mortality.

Results: A total of 4344 cases of MRSA bacteremia were identified; 2579 (59.4%) were HACO, 1144 (26.3%) HAHO; 601 (13.8%) CA, and 20 (0.5%) unclassified. Incidence rates of MRSA bacteremia showed a significant decrease over time from 33.9/100,000 in 2005 to 24.8/100,000 in 2008. Rates per 100,000 were highest in persons ≥ 65 years (133.0), blacks (45.2), males (33.7), and persons with AIDS (650.5). PFGE testing was performed on 1104 MRSA isolates. In multivariate analysis, USA 300 genotype was associated with increased in-hospital mortality (HR 1.63, 95% CI 1.19, 2.23). Increasing age, chronic liver disease, AIDS, pneumonia, bacteremia without an associated clinical syndrome, and septic shock were also risk factors for death. In a sub-analysis comparing MRSA bacteremia due to USA 300 vs. USA 100 strains, USA 300 was also associated with increased mortality (HR 1.79, 95% CI 1.24, 2.58).

Conclusions: MRSA bacteremia incidence declined over 4 years but remained high; the proportion with CA disease was significant and persons with HIV, ≥ 65 years old, and blacks were disproportionately affected. Bacteremia due to USA 300 MRSA isolates was associated with increased case-fatality, suggesting that USA 300 strains may be more virulent.

Cover Page

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By
Russell Kempker, MD.
Master of Science in Clinical Research

Advisor: Monica M. Farley, MD
Advisor: Susan Ray, MD

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INTRODUCTION

Staphylococcus aureus (SA) bacteremia is associated with a high mortality rate and places a substantial cost and burden on healthcare systems. The evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) has further complicated the treatment of SAB and led to increased rates of associated mortality and costs as compared to methicillin-sensitive SA (MSSA) [1]. Over the last three decades the incidence of MRSA bacteremia in the United States and worldwide has increased dramatically[2]. Traditionally, the majority of MRSA bacteremia has been acquired in the hospital or in persons with well-defined health care risk factors. More recently MRSA infection has begun to emerge in the community setting, with some cases occurring in persons who have no recent history of hospitalization or other established risk factors for MRSA infection. While the majority of community-associated MRSA (CA-MRSA) infections are localized skin and soft tissue infections, CA-MRSA may also cause severe invasive disease including bacteremia [3-5].

Initially, MRSA strains causing epidemiologically defined CA- and healthcare associated (HA)-MRSA disease were shown to be genetically distinct. Molecular typing studies using pulse field gel electrophoresis (PFGE) have defined one PFGE type that accounts for most CA-MRSA disease in the United States, designated PFGE type USA 300[6]. While animal studies suggest the USA 300 MRSA strain may be associated with increased virulence as compared to other strains [7, 8], there are currently no published clinical studies evaluating outcomes in persons with MRSA infection based on strain type.

Thus, the main purpose of this study was to look for an association of USA 300 with mortality in persons with MRSA bacteremia. In addition, we sought to define the incidence and epidemiology of MRSA bacteremia over a four year period. The study included 4334 persons with MRSA bacteremia who were identified through the Active

Bacterial Core Surveillance (ABCs) Program of the Georgia Emerging Infections Program (GA EIP). The entire cohort was used for epidemiological analysis while the survival analysis was conducted using the 1104 cases for which PFGE results were available. Results from this study may shed light on whether the recently emergent CA-MRSA strain, USA 300, is more virulent than other circulating strains and provide valuable information on the epidemiologic features of MRSA bacteremia over time. A better understanding of invasive MRSA infections, particularly CA-MRSA may contribute to improved prevention and treatment strategies for MRSA disease.

BACKGROUND

Evolution of MRSA

Infection with MRSA was first reported in the early 1960s shortly after the introduction of methicillin and evolved into an important cause of nosocomial infection, including bacteremia. By the late 1970s, outbreaks of MRSA infection were being reported in hospitals in the United States and over the next 10 years these strains became endemic in the hospital environment. Since then the increasing trend of HA-MRSA infection has continued and currently MRSA causes a significant portion of nosocomial infections in the US and worldwide [2, 9].

In a study by the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) Project, SA was found to be the second most common causative organism of nosocomial bloodstream infections in the US between 1995-2002, responsible for 20% of 24,179 cases [10]. The overall rate of SA bacteremia (SAB) was 10.0 cases per 10,000 admissions and the overall crude mortality rate was 25%. Methicillin-resistance was detected in 41% of SA isolates tested overall and there was a significant increase in the proportion of SA isolates with methicillin-resistance from 1995 (22%) to 2001 (57%)[10].

A more recent report by a surveillance network composed of over 300 clinical microbiology laboratories in the U.S. provides further alarming data about the rising trend of MRSA disease [11]. Using an electronic surveillance data collection system, SA was found to be the most prevalent species isolated from inpatient specimens (18.7% of all bacterial isolates) with the annual rates of MRSA increasing steadily from 1998-2005. In 1998 MRSA constituted less than 40% of SA isolates, whereas in 2005, MRSA accounted for 59.2% of SA isolates for non-ICU in-patients and 55% for ICU patients [11].

A recent study by Klevens et al, estimated the overall burden of invasive MRSA disease in the U.S. Based on active surveillance for invasive MRSA in nine sites it was estimated that 94,360 cases of invasive MRSA infection with 18,650 associated deaths occurred in the United States in 2005. Over 85% were found to be healthcare associated and the majority of these invasive infections were bacteremia [12].

The spread of MRSA disease globally is illustrated by a number of studies [13-15]. A population-based surveillance study of all patients with SA bacteremia in Calgary from 2000 to 2006 documented dramatically increasing rates of healthcare-associated MRSA bacteremia while at the same time showing a decrease in cases caused by MSSA [13]. In a report by the European Antimicrobial Resistance Surveillance System for 2007, MRSA isolates were found to constitute a significant proportion of all cases of SA bacteremia throughout Europe with the highest prevalence occurring in Greece (48%), Cyprus (48.2%), Portugal (48.4%), and Malta (52.4%) [14]. While most countries showed an upward trend in MRSA infection rates, decreasing rates were observed in a small number of European countries [14]. More concerning are the results of the International Nosocomial Infection Control Consortium (INICC) surveillance study, which evaluated the cause of nosocomial infections in 98 intensive care units throughout Latin America, Asia, Africa, and Europe from 2002-2008 [15]. In addition to finding SA to be one of the most common causes of nosocomial infections, over 80% of the 1473 SA isolates tested were MRSA [15].

These data and many other reports provide evidence of the establishment of MRSA as an important nosocomial pathogen, with increasing prevalence worldwide.

CA-MRSA Epidemiology

A defining feature of early CA-MRSA cases was the association with otherwise healthy, community dwelling persons without traditional risk factors for MRSA. The first infections were reported in indigenous populations in Western Australia in the early

1990s [16]. In 1999, an early report in the United States described 4 fatal cases of CA-MRSA in otherwise healthy children [4]. These initial reports heralded the rising epidemic of CA-MRSA infections and lead to subsequent reports of outbreaks among American Indian and Alaska Natives [17], sports teams [18] , prison inmates [19], child care attendees [20], military trainees [21], and men who have sex with men [22]. In a population-based study of three communities in 2001-2, the CDC found between 8-20% of all MRSA infections were community-associated and the annual incidence of CA-MRSA disease was as high as 25.7 per 100,000 persons in Atlanta [23].

MRSA strains causing epidemiologically defined CA-MRSA had features quite distinct from isolates traditionally associated with HA-MRSA. In a study comparing CA- and HA-MRSA infection, isolates from the community were found to be more susceptible to non-β-lactam antimicrobial agents including clindamycin, trimethoprim-sulfamethoxazole, and tetracyclines and were more likely to possess the staphylococcal chromosomal cassette (SCC) *mec* type IV [3]. SCC*mec* type IV is smaller than SCCs found in hospital strains of MRSA mainly due to the omission of non-β-lactam resistance genes, explaining the increased susceptibility to antimicrobial agents among CA-MRSA isolates. Additionally, CA-MRSA strains were found to more frequently contain genes encoding for Panton-Valentine leukocidin (PVL) toxin than HA-MRSA strains [3]. PVL is a cytotoxin associated with tissue necrosis and leukocyte destruction and has been suggested to be one factor that may contribute to enhanced virulence of CA-MRSA.

Molecular typing studies with PFGE have defined one PFGE type that accounts for most CA-MRSA disease in the U.S., designated type USA 300. Other PFGE types of community origin include USA 400, USA 1000, and USA 1100. In contrast, strains causing HA-MRSA disease have more frequently been found to be of PFGE types USA 100, USA 200, and USA 500, with USA 100 being the most common [6, 24]. The HA-MRSA strains generally have more multi-drug resistance and carry SCC*mec* type II [3].

The most common presentation of CA-MRSA disease is in the form of skin and soft tissue infections. In a 2004 study, CA-MRSA was found to be the most frequent cause of skin and soft tissue infections in persons presenting to emergency departments in eleven U.S. cities [25]. In another analysis of all community-onset skin and soft tissue infections presenting to a county hospital emergency room in Atlanta, King et al. found that CA-MRSA, specifically USA 300, caused approximately two thirds of the cases [26].

A recent U.S. population-based study provided data on the rising importance of CA-MRSA as a cause of invasive MRSA disease finding that CA-MRSA was responsible for 13.7% of all invasive MRSA cases, of which most were bloodstream infections [12]. Furthermore there have been reports of CA-MRSA causing fatal necrotizing pneumonia [27], necrotizing fasciitis [28], and other invasive clinical syndromes including the Waterhouse-Friderichsen syndrome and empyema.[29] . These reports demonstrate that CA-MRSA is an important cause of invasive MRSA disease and is responsible for an increasing proportion of all cases of MRSA bacteremia.

While CA-MRSA disease and strains were first identified because of characteristics that were not typical of MRSA as previously understood, recent reports have shown the line separating CA-MRSA and HA-MRSA is becoming increasingly blurred. CA-MRSA strains have been introduced into hospitals by the 15-23% of patients who require hospitalization for management of their infection, in addition to people who are colonized and admitted for another reason [23, 25]. In the last few years there have been reports of outbreaks involving the transmission of CA-MRSA strains in healthcare facilities [30]. There are also reports of CA-MRSA strains replacing traditional HA-MRSA strains as a major cause of healthcare associated infections. In a prospective study of MRSA blood stream infections (BSIs) done at Grady Memorial Hospital in Atlanta, MRSA USA 300 accounted for 28% of healthcare associated and 20% of nosocomial MRSA BSIs [31]. In another report by Klevens et al, 18-28% of patients with healthcare

risk factors were infected with CA-MRSA strains, primarily USA 300[32]. A recent study using a deterministic mathematical model, predicted that based upon current trends, MRSA USA 300 will likely become the dominant MRSA strain in hospitals and healthcare facilities replacing MRSA USA 100 [33].

A developing concern is the emergence of multidrug resistant CA-MRSA. Early USA 300 isolates were only resistant to semi-synthetic penicillins, mediated by *mecA*, and macrolides. However, over the last few years USA 300 isolates resistant to clindamycin and tetracyclines have arisen [34]. An outbreak of multidrug resistant CA-MRSA was recently described in a population of men who have sex with men. All of the isolates were resistant to clindamycin and mupirocin, and 63% were resistant to tetracycline [22]. Several reports have described USA 300 isolates with reduced susceptibility to vancomycin and in some cases daptomycin [35, 36]. These reports fuel the fear that USA 300 strains will soon come to resemble the more multidrug resistant HA-MRSA strains.

MRSA USA 300 and Virulence

The first cases of CA-MRSA in the U.S. were caused by a USA 400 strain [4]. However, as described above, USA 300 rapidly emerged to become by far the most common cause of CA MRSA disease in the U.S. [4, 6]. While other strains of CA-MRSA are responsible for infections in other parts of the world, USA 300 has been reported internationally in many countries including Australia, Austria, Switzerland, Denmark, Germany, Italy, the UK, and Japan [9, 34]. As a sign of its biological fitness, the introduction of USA 300 into a new geographic area is often followed by the displacement of existing endemic CA-MRSA strains [37].

In addition to the rapid and disseminated spread of CA-MRSA there have also been reports of worse clinical outcomes with CA-MRSA infections as compared to HA-

MRSA and CA-MSSA infections [38]. These reports along with those of unusually severe disease cause by CA-MRSA (mentioned above [27-29]) suggest an increased virulence of CA-MRSA strains and have prompted many investigations to search for virulence factors that may be associated with USA 300.

Genome sequencing analysis has revealed that USA 300 contains a high amount of unique genetic material in the form of mobile genetic elements, including prophages, plasmids, and pathogenicity islands, which have been acquired by horizontal gene transfer and encode many specialized virulence and resistance factors. The unique genetic elements and molecules that have received the most attention as possibly contributing to increased USA 300 virulence are PVL, the arginine catabolic element (ACME), and phenol-soluble modulins (PSM) [8, 9] .

PVL is a leukocidal toxin encoded by the prophage ΦSA2 that acts by forming pores in membranes of host leukocytes. PVL has been widely postulated to be a virulence factor in CA-MRSA given its striking epidemiologic association with genetically and geographically diverse CA-MRSA strains [25]. However, other observational and experimental evidence has called its role in the pathogenesis of CA MRSA into question. PVL deficient CA-MRSA strains have been reported to cause endemic disease and only 10-40% of community MSSA isolates have genes encoding PVL [25, 39]. While most of the animal models of abscess, pneumonia, and sepsis show no difference in lethality between USA 300 wild type and PVL-deficient strains, a few studies have shown increased virulence of the USA 300 wild type strain in mouse pneumonia models [40, 41]. The role of PVL in MRSA pathogenesis needs to be further defined.

The ACME is unique to USA 300 and is thought to have been acquired from *Staphylococcus epidermidis*. It is located adjacent to SCC*mec* type IV and contains two genetic clusters, a cluster of arginine catabolism genes (*arc*) and *opp*3, which encodes an oligopeptide permease; both clusters have the potential of contributing to virulence. The

arginine deaminase system may act by depleting host L-arginine which is important in the formation of nitric oxide, an important host antibacterial compound . The products of *opp*3 are thought to increase the survival and transmission of USA 300 strains. In a rabbit bacteremia model, it was found that the deletion of ACME but not type IV SCC*mec* resulted in attenuated pathogenicity of USA 300 [42].

The α-type PSMs are a recently discovered class of peptides that are related in part to the PSMs of *Staphylococcus epidermidis.* In a study by Wang et al, much higher levels 0f PSM production were detected in CA-MRSA strains, including USA 300, than in traditional HA-MRSA strains. In their mouse abscess and bacteremia models, isogenic PSM-deleted strains of USA 300 and USA 400 were found to be less pathogenic than wild type strains. Further experiments elucidated the ability of PSMs to recruit, activate, and then lyse human neutrophils, a main defense against staphylococcal infection [7]. Based on these experiments, PSMs seem to play a role in the enhanced virulence of CA-MRSA.

While the epidemiological and experimental data both confirm the pathogenic capacity of USA 300, there have been no published clinical studies evaluating an association of USA 300 with increased mortality. Clinical data is needed to determine whether USA 300 is indeed more lethal than non-USA 300 strains in persons with MRSA bacteremia.

Mortality in SA Bacteremia

SA bacteremia (SAB) is associated with significant morbidity and mortality and is a significant burden on the healthcare system. In an analysis of a large U.S. database, SAB was associated with longer hospital stays, increased mortality, and higher costs as compared to bacteremia from any other organism [43]. The overall mortality rate in SAB ranges widely, with rates > 80% being reported, likely due to differences in the

underlying patient population [44] and infecting organism. A meta-analyses evaluating 31 cohort studies compromising > 3900 cases of SAB, found a significantly higher risk of associated mortality in persons with MRSA bacteremia compared to MSSA bacteremia, with an OR of 1.93 (95% CI, 1.54-2.42; P<.001) [44]. Another study demonstrated that as compared to MSSA bacteremia, MRSA bacteremia was associated with a significant increase in the length of hospitalization (1.29 fold; P=.016) and hospital charges (1.36 fold; P=.017) [1].

Patient characteristics including age and co-morbidities can strongly influence the outcome of SAB. Increasing age has repeatedly been demonstrated to be associated with higher mortality in persons with SAB [31, 45-47]. Co-morbidities that have been found to be associated with increased mortality in persons with SAB include: cirrhosis [48], cancer [48], alcoholism [47], immunosuppression [47], and HIV [31]. Lesens et al, demonstrated the contribution of multiple co-morbidities in the outcome from SAB, by finding that a Charlson weighted index of co-morbidity score of 3 or more was significantly associated with increased mortality (OR 3, 95% CI 1.3 to 5.5; P=.006) [49]. Various clinical characteristics have been identified that increase the risk of mortality in SAB including the presence of shock [47, 50], persistent bacteremia [51], and a non-eradicable source of bacteremia [48].

A lack of highly effective antibiotics and just as importantly a delay in appropriate treatment may also play a role in the poor outcomes of MRSA bacteremia. The mainstays of treatment for MRSA bacteremia are glycopeptides. Frequently, persons with MRSA are treated with inappropriate antibiotics before culture and drug susceptibility results are available. Studies evaluating the influence of initial inappropriate treatment of MRSA bacteremia on outcomes have yielded conflicting results, with a majority finding a non-significant effect on mortality [52-56] . Also, it remains controversial whether MRSA bacteremia treated with vancomycin in the setting of increased (but susceptible)

vancomycin minimal inhibitory concentrations is associated with worse outcomes [56-58].

No studies have been published comparing the mortality of MRSA bacteremia based on MRSA PFGE type. One study abstract reported the results of a retrospective review of 256 patients with MRSA bacteremia, finding no increased 90 day-mortality in disease due to USA 300 strains compared to non-USA 300 strains [59]. As MRSA USA 300 continues to emerge and evolve into a major MRSA strain in the USA, well-conducted studies are needed to evaluate the outcomes MRSA bacteremia due to USA 300 strains. This study's primary aim was to determine whether USA 300 strains as compared to non-USA 300 strains were associated with increased risk of death in persons with MRSA bacteremia and secondarily to document the incidence and epidemiological characteristics of MRSA bacteremia in a large metropolitan area over a 4 year period.

METHODS

The main purpose of this study was to perform a survival analysis to assess whether MRSA USA 300 strains as compared to other MRSA strains, while controlling for other risk factors, are associated with increased in-hospital mortality in persons with MRSA bacteremia. An additional goal was to assess the epidemiology and incidence of MRSA bacteremia over time.

Null hypothesis

MRSA USA 300 is not associated with increased mortality as compared to non MRSA USA 300 strains in persons with MRSA bacteremia.

Alternative hypothesis

MRSA USA 300 is associated with increased mortality as compared to non-USA 300 strains in persons with MRSA.

Study Design

A prospective observational cohort study design was used for the epidemiological analysis while a nested cohort from the prospective population cohort was used for the survival analysis.

Patient selection

Patients included in the study were identified through the Active Bacterial Core Surveillance (ABCs) Program of the Georgia Emerging Infections Program (GA EIP). From 2004 onward the GA EIP has performed prospective, population-based, laboratory-based surveillance for all invasive MRSA isolates in Georgia Health District (HD3), the 8-county area of Atlanta. The study period was defined as surveillance

January 1, 2005 through December 31, 2008. The total population under surveillance was approximately 3.5 million in 2005 3.8 million in 2008.

Cases were defined as MRSA isolated from a blood culture in a resident of HD3, including those institutionalized in long term care facilities. ABC case finding was both active and laboratory based. Primarily cases were collected using clinical laboratory printouts provided by all 31 hospitals and reference laboratories within HD3. Laboratory audits were performed monthly to evaluate reporting accuracy and identify cases not originally reported.

Medical records were reviewed for all case-patients. The *minimum* sources of information that were used to complete the data forms were 1) the admission history and physical or Admission Summary, 2) the discharge summary, 3) the face sheet, and 4) laboratory report. Cases were classified into 3 categories, as healthcare-associated with hospital onset HAHO, healthcare-associated with community onset (HACO) or community-associated (CA) based on abstracted information and predefined criteria. The following information on healthcare risk factors for MRSA was collected: presence of a central venous catheter (CVC) at the time of evaluation or admission; culture obtained > 48 hours after admission; and a history of MRSA infection or colonization, surgery, dialysis, or residence in a long-term care facility within a year before index culture date. Healthcare associated cases are defined by having one of the above mentioned health care risk factors and are further classified as community-onset (cases with a health care risk factor but with a culture obtained < 48 hours after hospital admission) and hospital onset (cases with culture obtained >48 hours after admission, regardless if they had other health care risk factors). For those cases categorized as HAHO, a new variable was created, hospital time, which represented the number of days in the hospital before a positive MRSA blood culture. For CA and HACO cases this variable was given a value of

one. The variable was created to help control for the increasing morbidity that comes with prolonged hospital stays.

Trained personnel used a standard case report form to abstract data on demographics (including race), clinical characteristics (including co-morbidities), and outcomes. Race was collected from information available in the medical record. The site of isolation was defined as the first sterile site from which MRSA was isolated. Bacteremia was defined as a case with a positive blood culture for MRSA. All cases in this analysis had a diagnosis of bacteremia. Associated clinical syndromes for each case were recorded based on their documentation in the medical record. The clinical syndrome of endocarditis included all cases of prosthetic and native valve endocarditis and one case of an aortic valve abscess. The clinical syndrome of pneumonia included all cases of pneumonia or empyema. The clinical syndrome of septic arthritis included all cases of septic arthritis and bursitis and one case of tenosynovitis. The clinical syndrome of deep tissue abscess included all cases with an internal body site abscess. The clinical syndrome of osteomyelitis included all cases of osteomyelitis plus all cases of discitis, lumbar infection, and spinal infections. The clinical syndrome of surgical site infection included cases reported as either internal surgical site infection or surgical incision site infection plus all cases of mediastinitis. The clinical syndrome of skin and soft tissue infection (SSTI) included all cases of cellulitis, traumatic wound infection, and pressure ulcer related infections plus all obvious cases of SSTI as recorded in free text under a specific syndrome variable. A category called miscellaneous clinical syndromes was created which included all clinical syndromes listed as free text and not fitting into any of the above clinical syndromes. All cases without any clinical syndrome were grouped together into a category named bacteremia without any associated clinical syndrome. For the purposes of this analysis cases could only have one clinical syndrome. For cases with overlapping clinical syndromes the following hierarchy based on severity of

syndrome was used to decide which clinical syndrome to use: Endocarditis > Pneumonia >Septic Arthritis > Deep Tissue Abscess > Osteomyelitis > Surgical Site Infection > SSTI >Miscellaneous. Septic shock was utilized as a measure of severity of illness and was defined by the recording of septic shock or symptoms associated with septic shock in the medical chart. The hospital where each patient was administered initial treatment was recorded and variables were created for each hospital that treated > 10% of the study population. Persistent invasive MRSA infection was defined as a same site positive culture between 7 and 30 days from the initial culture for cases that were hospitalized ≥ 7 days. Recurrent invasive MRSA was defined as a positive culture result obtained for the same case > 30 days after the initial culture. Patient outcomes were recorded as either survival to discharge or death prior to discharge. Mortality represented crude, in hospital deaths and was divided into overall death and death within 7 days of a positive culture. Dates of survival or death were recorded.

Information on underlying illnesses was obtained from the medical chart. A case was recorded to have AIDS if reported in the medical chart or if HIV was recorded and the CD4 count was ever <= 200 cells/µl. Peripheral vascular disease was defined as disease of blood vessels outside the brain or heart. Congestive heart failure included cardiomyopathy. Stroke was defined as a history of a cerebrovascular event and did not include a history of a transient ischemic attack. Chronic obstructive pulmonary disease also included chronic bronchitis. Chronic renal insufficiency was defined as a creatinine level of 1.6 mg/dl or higher and included end stage renal disease but did not include acute renal insufficiency or failure. Chronic liver disease was defined as cirrhosis or chronic liver failure and did not include Hepatitis A, Hepatitis B, or Hepatitis C without liver failure of acute liver failure. A case was considered to be on immunosuppressive therapy if chemotherapy was ongoing, if the case was between cycles, or if within 2 weeks of completion. Use of steroids was considered an underlying disease or condition only if

they were on long-term systemic steroids. The presence of the following co-morbidities was based on their inclusion in the medical chart: alcohol use, intravenous drug use, solid organ malignancy, hematologic malignancy, systemic lupus erythematosus, sickle cell anemia, rheumatoid arthritis, asthma, and atherosclerotic heart disease.

Isolate Collection and Laboratory Testing

All laboratories in the Atlanta EIP surveillance area were asked to submit isolates from invasive MRSA infections for further testing and particular emphasis was placed on those laboratories whose staff was willing to save, store, and process large numbers of isolates linked to cases. Preference was given to isolates from blood. Of the 3o laboratories in the surveillance area 11 (37%) contributed isolates. These 11 laboratories contributed 55% of the overall cases (N=4344) during the surveillance period. Isolates were sent to the CDC and Georgia EIP reference laboratory for storage and further testing. Antimicrobial susceptibility testing and toxin profiles were performed centrally at CDC. The GA EIP reference laboratory performed PFGE, staphylococcal chromosome cassette (SCC*mec*) typing, and PVL gene detection. PFGE was done using the restriction endonuclease SmaI, and patterns were analyzed using Bionumerics version 4.01 and then grouped into pulse field types using Dice coefficients and 80% relatedness.

Database Management

Data was entered into a Microsoft Access 2007 database (Microsoft Corp., Redmond, WA) and statistical analyses were performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Data Analysis

U.S. census bureau bridged-race vintage post census population estimates, provided by the National Center for Health Statistics for HD3, were used to calculate

annual MRSA bacteremia incidence rates and incidence rates after stratification by MRSA epidemiological type, age, and race. The Georgia Division of Public Health HIV/AIDS surveillance summary estimates of persons living with HIV and AIDS were used to calculate MRSA bacteremia incidence in persons with HIV and AIDS. Logistic regression, with year as the predictor variable and number cases/total population as the dependent variable, was used to perform a trend analysis on incidence rates over the four year surveillance period. A p-value <.05 was considered significant.

Descriptive statistics were used to compare the cases for which isolates were sent for PFGE testing to those cases that did not have isolates sent for PFGE testing. The purpose of this comparison was to determine whether bacteremia cases with PGFE testing were representative of the overall cohort of bacteremia cases. Differences in proportions of categorical variables (including demographic and clinical characteristics and co-morbidities) were tested using χ^2. If expected cell counts were less than 5, Fisher's exact test was utilized. For continuous variables (age), a two sample *t* test was used to evaluate for any difference in mean age of the two groups. A p-value <.05 was considered significant.

For the nested group of bacteremia cases with PFGE testing, a survival analysis was used to assess risk factors of in hospital mortality. The primary outcome variable was in-hospital mortality and the main predictor variable was MRSA PFGE type (USA 300 vs. non-USA 300). Other risk factors (Appendix A) that were assessed were those with possible biological significance in influencing outcomes in MRSA bacteremia and those risk factors that have been shown in the literature to be associated with mortality in individuals with MRSA bacteremia. A cox proportional hazards model was used to perform the survival analysis. For all subsequent analyses a p value < .05 was considered significant.

Prior to undertaking the survival analyses a scatter plot of time versus age was generated to evaluate the appropriateness of using age as a continuous variable. The graph showed no obvious cutpoint for age and mortality and thus age was included as a continuous variable.

Descriptive statistics were used to compare the study population stratified by PFGE type (USA 300 vs. non-USA 300). Differences in proportions of categorical variables (including demographic and clinical characteristics and co-morbidities) were tested using χ^2. If expected cell counts were less than 5, Fisher's exact test was utilized. For continuous variables (age and days in hospital before positive culture), a two sample *t* test was used to evaluate for differences in mean age and days in hospital before positive culture between the two groups.

Model building and selection was based on the purposeful selection of covariates strategy (Appendix B) proposed by Hosmer, Lemeshow, and May [60]. The first step was to perform a univariable analysis for each variable under consideration. This was done utilizing a cox PH model using the PROC PHREG statement in SAS. A hazard ratio (HR), 95 % confidence interval (CI), and p-value for the wald chi-square value were recorded for each variable. Any variable that had a significant univariate test with a p value <.20 plus PFGE type was selected for multivariate analysis. The cox PH model was then used to perform a multivariate model with all variables significant in univariate analysis and now a p value <.10 was considered significant. Again HRs, 95% CIs, and p values were generated. As each non-significant variable was removed from the multivariate model, a change of 20% in any of the remaining parameter estimates was considered confounding. Any variable found to be a confounder was kept in the model. The next step was to add back the variables that were not included in the initial multivariate model one at a time to evaluate for significant variables (p value <.10) or confounders (change in parameter estimates > 20%). This step can be helpful in identifying variables that, by

themselves, are not significantly related to the outcome but make an important contribution in the presence of other variables. At the end of this step the preliminary main effects model was generated.

Interaction was evaluated between the PFGE type and each of the other variables in the preliminary main effects model. An interaction term consisting of PFGE type and each of the variables was added to the model one by one and a p value < .05 was considered significant.

Once the final model was obtained, a cox PH model using the PROG PHREG statement in SAS was used to generate HRs, 95%, and p values for each variable in the multivariate analysis. PROC GPLOT was used to obtain survival plots stratified by PFGE type.

Model adequacy was evaluated by using a graphical approach to assess the PH assumption. Specifically, plots of estimated log-log survival curves were compared stratified on PFGE type to assess the PH assumption. If the log-log curves were parallel then the PH assumption was assumed to be valid.

A subsequent survival analysis was also performed comparing USA 300 vs. USA 100 isolates following the same method used for the primary survival analyses. The purpose of this sub-analysis was to compare the effect of the two most common PFGE types without any potential bias from the remaining PFGE types.

IRB Approval

The population-based MRSA surveillance study was approved by the Emory University and the Georgian Department of Human resources Institutional Review Boards, the Grady Memorial Hospital Research Oversight Committee and the VA Research and Development Committee.

RESULTS

Incidence of MRSA Bacteremia

There were 4344 identified cases of MRSA bacteremia reported from January 1, 2005 through December 31, 2008. Most cases were healthcare-associated, with 2579 (59.4%) healthcare-associated community-onset infections (HACO), 1144 (26.3%) healthcare-associated hospital-onset (HAHO) infections, 601 (13.8%) community-associated (CA) infections, and 20 (0.5%) unclassified infections. Incidence rates of MRSA bacteremia showed a significant decrease over time from 33.9 per 100,000 in 2005, 30.5 per 100,000 in 2006, 28.5 per 100,000 in 2007, to 24.8 per 100,000 in 2008 (TABLE 1). While there was also a significant downward trend in rates of HACO and HAHO cases over time, the rates of CA MRSA bacteremia remained relatively constant (TABLE 1). Incidence rates per 100,000 were highest in persons 65 year and older (133.0), blacks (45.2) compared to all other races, male (33.7) compared to females and in persons with AIDS (650.5) (TABLE 2). The incidence rates remained higher in blacks than whites across all age groups (TABLE 3).

Comparison of Study Population to Overall Cohort

From the overall cohort, 1104 cases (25.4%) had isolates sent for further laboratory testing including PFGE. A similar percentage of cases were sent for further testing in 2007 (24.8%) and 2008 (24.4 %), with a higher amount in 2006 (31.7%) and a lower amount in 2005 (20.8%). A comparison of the cases with and without isolates sent for laboratory testing is made in Table 4. The bacteremia cases with PFGE typing as compared to the remainder of cases had a younger average age (53.8 years versus 56.5 years, $P < .01$), were more likely to be black (68.6% versus 55.6%, P value <.01), have HIV or AIDS (13.3% versus 9.0%, $P < .01$), and less likely to have congestive heart failure (14.3% versus 17.5%, $P < .05$), coronary heart disease (13.6% versus 16.1 %, $P < .05$), chronic pulmonary disease (8.8% versus 12.3%, $P < .01$), to be immunosuppressed (6.4%

versus 8.5%, P <.05), have been hospitalized in the last year(51.6% versus 56.0%, P<.01), to have no associated clinical source (64.2% versus 67.5%, P <.05), and less likely to have had surgery in the last year (20.6% versus 27.4%, P<.01). There were no significant differences between two groups with regard to gender, MRSA epidemiological types, prior residence in a long term care facility, and the remainder of co-morbidities (Table 4). There were also similar rates of all associated clinical syndromes and the following clinical characteristics (hospitalization, presence of septic shock, relapsed and persistent disease) among the two groups (Table 4). Overall, there were 767 deaths (17.7% case-fatality) with 204 deaths (18.5% case-fatality) in cases with PFGE typing and 563 deaths (17.4% case-fatality) in cases without PFGE typing (P =0.40).

PFGE Types

The nested study group consisted of 1104 bacteremia cases with PFGE testing. The most common pulse field types identified were USA 300 (37.5%), USA 100 (33.9%), USA 500 (15.2%), and Iberian (7.2%). A total of 10 other pulse field types accounted for the remaining 6.2% of cases (Table 5).

Comparison of USA300 versus Non-USA 300

Of the 1104 cases, 414 were USA 300 and 690 were non-USA 300. A descriptive comparison of the two populations (Table 6) revealed many significant differences between the two groups. USA 300 cases were considerably younger (47.1 versus 57.7 years old, P <.01) and more likely to be male (64.5% versus 54.5, P<.01) and black (73.0% versus 65.9%) as compared to non-USA 300 cases. Additionally, USA 30o isolates were more frequent among community-associated cases (30.7% versus 7.4%, P<.01) and less prevalent among healthcare associated cases than non-USA 300 isolates.

In regards to co-morbidities, USA 300 isolates were more associated with HIV (4.8% versus 2.5%, P<.05) and intravenous drug users (3.4% versus 0.1%, P<.01) while

non-USA 300 isolates were more likely to be found in cases with chronic medical diseases, recent hospitalization (57.4% versus 41.8%, P<.01) or surgery (23.5% versus 15.2%, P<.01), or residence in a long term care facility (24.4% versus 10.6%, P<.01). There was no significant difference in the rates of alcoholism, AIDS, hematological malignancy, or receipt of immunosuppressive drugs between the two groups (Table 6).

A similarly high rate of USA 300 and non-USA 300 cases were hospitalized and no differences among rates of recurrent or persistent disease were found. For HAHO cases, those with non-USA 300 isolates were in the hospital much longer before developing MRSA bacteremia than those with USA 300 isolates (33.2 versus 16.1 days, P<.01).

Rates of pneumonia (10.1% versus 10.7%) and endocarditis (4.1% versus 3.5%) were similar between the two groups while the following clinical syndromes were more associated with bacteremia from USA 300: deep tissue abscess, osteomyelitis, and skin and soft tissue infection. The majority of non-USA 300 cases had no identified clinical source (71.9%).

During the follow-up period from admission to discharge, there was a total of 204 in-hospital deaths with no significant difference found between USA 300 and non-USA 300 cases (16.4 versus 19.7%, P=0.17). However, more USA 300 cases died within the first 7 days after positive culture than non USA 300 cases (73.5% versus 49.3%, P <.01) and this also held true when looking at only HAHO cases (58.8% versus 35.3%, P <.01).

USA 300 versus Non-USA 300 Survival Analysis

Utilizing a cox proportional hazards model, a univariate analysis was first performed to calculate unadjusted hazard ratios for all potential risk factors of mortality in persons with MRSA bacteremia. The results are summarized in Table 7. In the univariate analysis, our main predictor USA 300 was not found to be associated with mortality (HR 1.02, 95% CI 0.76, 1.37). The following factors were found to be

significantly associated with mortality: increasing age (HR 1.03, 95% CI 1.02, 1.04), white race versus all other races (HR 1.60, 95% CI 1.20, 2.14), solid malignancy (HR 2.08, 95% CI 1.38, 3.12), peripheral vascular disease (HR 1.91, 95% CI 1.26, 2.88), congestive heart failure (HR 1.62, 95%CI 1.16, 2.26), coronary heart disease (HR 1.57, 95% CI 1.10, 2.25), cerebrovascular accident (HR 1.57, 95% CI 1.07, 2.30), chronic obstructive pulmonary disease (HR 1.72, 95%CI 1.15, 2.59), chronic liver disease (HR 2.23, 95% CI 1.18, 4.22), residence in a long term care facility (HR 1.79, 95%CI 1.31, 2.44), cases with no associated clinical syndrome (HR 1.79, 95% CI 1.31, 2.46), and septic shock (HR 3.99, 95% 2.57, 6.20). Additionally, the following clinical characteristics and syndromes were associated with decreased mortality, recurrent MRSA disease (HR 0.59, 95% CI 0.39, 0.91), osteomyelitis (HR 0.21, 95% CI 0.05, 0.85), and skin and soft tissue infection (HR 0.37, 95% CI 0.17, 0.79).

A purposeful selection strategy including assessment of confounding and interaction was used to select the covariates for multivariate analysis. The final model is listed in appendix C. The hazard ratios, 95 % confidence intervals, and chi-square p values for all variables included in the multivariable analysis are listed in table 8. After adjustment of confounding variables, our main predictor variable USA 300 was associated with increased mortality (HR 1.63, 95% CI 1.19, 2.23). The main confounder of USA 300 was to found to be age (50% change in USA 300 HR with addition of age to model). A cox adjusted survival graph for USA 300 versus non-USA 300 isolates is shown in figure 1. Other factors in the final model significantly associated with mortality in persons with MRSA bacteremia included increasing age (HR 1.04, 95% CI 1.03, 1.05), chronic liver disease (HR 2.48, 95% CI 1.28, 4.81), AIDS (HR 2.04, 95% CI 1.28, 3.27), bacteremia without an associated clinical syndrome (HR 3.26, 95% CI 2.04, 5.20), pneumonia (HR 2.54, 95% CI 1.44, 4.46), and septic shock (HR 5.07, 3.21, 7.99). There were no variables added to the final model solely as confounders and no interaction was

detected (Table 9). Additionally, using a graphical approach the covariates in our final model met the proportional hazards assumption (Figure 2).

USA 300 vs. USA 100 Survival Analyses

Given that USA 100 was by far the next most common pulse field type (33.5%) after USA 300 and the fact that it is the most frequent healthcare-associated MRSA PFGE type in the U.S., a secondary analysis was performed comparing only USA 300 and USA 100 isolates. These two pulse field types made up 71.4% of all cases. A comparison of the two groups as shown in Table 10 was almost identical to the comparison of USA 300 versus all non-USA 300 isolates. USA 100 was more commonly healthcare associated, and found in older persons with chronic medical conditions. In contrast, USA 300 was more likely to be associated with younger persons, AIDS, intravenous drug use, and with the clinical syndromes of skin and soft tissue infections and osteomyelitis. While there was a higher overall case-fatality rate in USA 100 versus USA 300 cases the difference was not statistically significant (20.3% versus 16.4%). In contrast, USA 300 had a much higher 7 day morality rate than USA 100 for all cases ((73.5% versus 47.4%, P<.01) and when analyzing only HAHO cases (58.5% versus 33.3%, P<.01).

The purposeful covariate selection strategy was again used to build a multivariate model for factors associated with mortality. The adjusted hazard ratios, 95% confidence interval, and chi square p values of all variables included in the final multivariable model are shown in Table 11. USA 300 was even more associated with mortality when compared to USA 100 alone (HR 1.79, 95% CI 1.24, 2.58). The survival curves of USA 300 and USA 100, adjusted for all other variables in the final model are shown in Figure 2. Each additional year of age imparted an increased risk of mortality (HR 1.04, 95%CI 1.03, 1.05), as well as did the co-morbidities of alcoholism (HR, 2.16, 95% CI 1.10, 4.27) and chronic liver disease (HR 2.13, 95% CI1.02, 4.45), and the clinical syndrome of pneumonia (HR 2.58, 95% CI 1.32, 5.02), bacteremia without an associated clinical

syndrome (HR 3.18, 95% CI 1.83, 5.53), and septic shock (HR 6.09, 95% CI 3.67, 10.11). While time in the hospital before a positive culture was included in the final model its p value did not meet criteria (<.05) for significance. There was no evidence of interaction between our main predictor variable, pulse field type, and all other variables and the proportional hazards assumption was satisfied for all categorical variables by the graphical approach.

DISCUSSION

The results of this study suggest that USA 300 MRSA strains may be more virulent than non-USA MRSA strains in persons with MRSA bacteremia. There was a significantly increased risk of death in persons with MRSA bacteremia due to USA 300 MRSA strains compared to non-USA 300 strains and an even greater increase when USA 300 strains were compared specifically to USA 100 strains. The fact that there was no significant association between USA 300 and increased mortality identified in univariate analysis was due to negative confounding by age, which is a well described risk factor for mortality in persons with MRSA bacteremia [31, 45-47]. Persons with USA 300 MRSA bacteremia were significantly younger than persons with non-USA 300 MRSA bacteremia and increasing age was a significant risk factor for mortality. This study is one of the largest evaluating risk factors for mortality in persons with MRSA bacteremia and to our knowledge is the first clinical study comparing outcomes in MRSA bacteremia due to USA 300 versus non-USA 300 strains.

The decision to analyze the impact of specific MRSA strain type on mortality in bacteremia was based upon the growing opinion and evidence that CA-MRSA strains, in particular USA 300, may have increased virulence as compared to more traditional HA-MRSA strains [7, 8, 61]. Since 2001, USA 300 has evolved into the major cause of CA-MRSA related disease in the U.S. and has been associated with fulminant and lethal invasive infections and an important cause of MRSA bacteremia [6, 9, 12]. This association with fatal infections and also reports of worse clinical outcomes in CA-MRSA infections as compared to CA-MSSA and HA-MRSA infections suggest that CA-MRSA strains, especially USA 300, may have increased virulence [38, 47] . This has led to numerous studies investigating the pathogenicity and potential virulence determinants of CA-MRSA strains, most of which have been in in-vitro models and have focused on USA 300 and USA 400 strains. Studies in animal models of MRSA bacteremia have

demonstrated an increased virulence of USA 300 strains as compared to HA-MRSA PFGE types and evidence suggests that the underlying mechanism may be the differential expression of key pathogenic determinants [61, 62]. Wang et al, identified a new class of staphylococcal cytolytic peptides, α-type phenol soluble modulins that were more highly expressed in CA-MRSA strains including USA 300, than in HA-MRSA strains, and subsequently demonstrated the dramatic influence of these peptides in the pathogenicity of USA 300 and USA 400 strains in mouse abscess and bacteremia models [7]. A more recent study Li and colleagues, provided additional evidence that the increased virulence of USA 300 in a mouse bacteremia model was due in part to the increased expression of virulence factors, including α-type phenol soluble modulins, more so than the acquisition of new pathogenic factors [61]. The role of the much studied CA-MRSA associated toxin, PVL, and also the less studied USA 300 unique ACME in MRSA virulence is still unclear [8] . In contrast to the active research in animal models, there has been a lack of clinical data comparing the outcomes of serious infections with MRSA USA 300 and other MRSA strains. Our results are the first suggesting that MRSA bacteremia from a USA 300 strain may be more virulent than that due to a non-USA 300 strain. The sub-analysis comparing outcomes of bacteremia from USA 300 and USA 100 strains was done to directly compare USA 300 with the most common HA-MRSA strain in the U.S. without any confounding from either other CA-MRSA strains (<2% of total) or the USA 500 strain (15% of all strains), which is the progenitor strain of USA 300 and may have more similar virulence to USA 3oo than other HA-MRSA strains [61]. Without these potential confounders the effect of USA 300 on associated mortality was more even pronounced (HR 1.79 versus 1.63). Further animal studies of CA-MRSA virulence and clinical studies comparing outcomes of MRSA bacteremia by strain will be important to help elucidate the mechanisms of CA-MRSA

virulence and to confirm the impact of CA MRSA strains, especially USA 300, on mortality.

Several other risk factors in multivariate analysis were found to be associated with a higher risk of mortality in persons with MRSA bacteremia including septic shock, pneumonia, bacteremia without an identified clinical source, AIDS, and chronic liver disease. Septic shock was the most strongly correlated with mortality and has been shown to be a risk factor for death in many other outcome studies of MRSA bacteremia [46, 47, 50] . AIDS, chronic liver disease, and MRSA bacteremia with pneumonia have all also been previously associated with mortality in persons with MRSA bacteremia, likely due to immunosuppression-related increased disease severity [31, 48, 50, 63] . The reason bacteremia without an associated clinical syndrome was associated with increased mortality is less clear. Potential explanations are that it was a marker for increased underlying morbidity given that it was more common in people with chronic medical diseases. Also, the lack of an identifiable associated clinical syndrome may have hindered timely diagnosis and thus delayed proper antibiotic therapy. The only additional variable found to be a significant risk factor for death when comparing USA 300 with USA 100 was the presence of alcoholism. Co-linearity was tested for but not found between alcoholism and chronic liver disease. Alcoholism is known to suppress the immune system and has been identified as risk factor for mortality in MRSA bacteremia in a prior study [47, 64]. We found no significant association between some factors that have been linked to mortality in persons with MRSA bacteremia including community versus hospital onset, endocarditis, cancer, time in hospital before infection, and immunosuppressive therapy.

A comparison of demographic and clinical characteristics between MRSA bacteremia from USA 300 and non-USA 300 strains revealed many significant differences. In agreement with other reports, we found USA 300 strains to be more

common as compared to non-USA 300 strains in younger persons from the community without chronic medical diseases and in persons with AIDS, chronic liver disease, abusing intravenous drugs, and with skin and soft tissue infections [3, 31]. In agreement with other studies demonstrating the emergence of USA 300 strain in the hospital environment, we found that a significant percentage of USA 300 MRSA bacteremias (17.6%) were HAHO. This is consistent with the rates of HAHO MRSA bacteremia caused by USA 300 strains found at other hospitals by Jenkins et al (3-33%), and Seybold et al (20%) [31]. These collective results give credence to the theory that USA 300 strains may have an improved biological fitness as compared to HA-MRSA strains and support a recent modeling study that suggests given current trends, USA 300 strains may eventually become the predominant HA-MRSA strain [33]. While rates of pneumonia and endocarditis were similar, osteomyelitis and deep tissue abscess were significantly more associated with MRSA bacteremia due to USA 300 strains. The increased frequency of these metastatic complications of MRSA bacteremia may be a marker of increased virulence of USA 300.

Surveillance for MRSA bacteremia has either generally been limited to hospital onset disease or has not followed the trends of disease over time [10, 12] . While we found a significant decline in MRSA bacteremia rates over the 4 year period, the overall rates remain high. In 2001-2002, the rate of invasive MRSA disease, of which the majority was bacteremia, in Atlanta was 19.3 per 100,000, thus far below current rates [23]. In a population-based study of invasive MRSA disease in 9 U.S. cities including Atlanta in 2005, rates of MRSA disease were between 19.2 to 116.7 per 100,000 [12]. Over 75% of these MRSA infections were bacteremia and thus these findings can serve as a reference for our results and also corroborate the high rates of MRSA disease found in our study. In contrast, a recent population based surveillance of SAB, including both MRSA and MSSA, from 2000-2006 in Calgary showed a much lower overall rate of

MRSA bacteremia of 2.2 per 100,000. In addition, they also found a dramatically rising rate of MRSA bacteremia over the 7 year surveillance period [13]. Other population based studies of SAB, consisting of mainly MSSA, have shown increasing rates over time [65]. Our study did not obtain data on MSSA bacteremia and thus we were unable to determine if the observed decline in MRSA bacteremia was accompanied by an increase or decrease in MSSA bacteremia.

Decreases in healthcare associated bacteremia cases were responsible for the decline in overall MRSA bacteremia rates found in our study. There were significant decreases in rates of both HACO and HAHO related MRSA bacteremia while rates of CA MRSA bacteremia were fairly constant. This may be attributable to an increase in the implementation of strategies to prevent and control MRSA among hospital inpatients, including efforts to reduce central line related blood stream infections and measures focused on interrupting the transmission between hospitalized patients. A recent study evaluating CDC data on central line-associated blood stream infections in U.S. intensive care units from 1997-2007, showed that in the 6 most common types of ICUs the rates of MRSA central line-associated bacteremia have experienced declines of 50% or more since 2001 [66]. The results of this study along with our findings suggest that currently implemented MRSA prevention strategies are working to decrease rates of MRSA bacteremia. However, it is of concern that rates of CA-MRSA did not show any significant decline over the surveillance period. Given current trends, CA-MRSA will likely represent an even more significant portion of all MRSA bacteremias in the near future. More studies will be needed to evaluate the effectiveness of community-based prevention and control interventions.

We found a striking difference among the rates of MRSA bacteremia by race among all age groups, with blacks having much higher rates than whites and people of all other races. This relationship also held over all MRSA epidemiological types. This

disparity was also seen in the 2005 population study of invasive MRSA by Klevens et al [12]. It is unclear if there is race related genetic or immunological predisposition for increased susceptibility to or protection from infection to explain the different rates of disease or if race is a marker for confounders such as co-morbidities or socioeconomic status. One study did find an increased rate of MRSA postoperative infection in persons from deprived areas [67] and a more recent study demonstrated that higher socioeconomic status was significantly associated with lower rates of SAB [68]. Future analyses should focus on reasons for the disparate rates of MRSA bacteremia in people of different races.

We also describe an extremely high rate, over 12-fold higher than the general population, of MRSA bacteremia in persons with HIV and/or AIDS. Another study evaluating MRSA bacteremia among patients enrolled in an HIV outpatient clinic in Baltimore found similarly high rates of MRSA bacteremia, which significantly increased from 2000-1 (5.3 per 1000 person years) to 2003-4 (11.9 per 1000 person years)[69]. HIV has been shown to be a risk factor for nasal colonization with MRSA, and colonization has been demonstrated to be a risk factor for subsequent MRSA infection [70, 71]. While recent data is lacking, a few studies have demonstrated that the neutrophils of HIV infected persons exhibit reduced phagocytosis of *S. aureus* with the effect more pronounced at lower CD4 counts [72, 73]. Our findings highlight the importance of MRSA as an opportunistic pathogen in HIV infected persons and stress the need for vigilance in prevention, early recognition and treatment of MRSA infections in this population.

There are some limitations to our survival analysis that merit discussion. The survival analysis was performed on a nested cohort that was selected using a convenience sampling method. This nonrandom sampling method could introduce significant bias into a risk factor analysis, as the nested group may not be representative

of the overall cohort. We compared the nested study group with the remaining cases to evaluate for similarity. While we did find significant differences in age, and in the presence of some co-morbidities, no significant differences were found in key categories of MRSA epidemiological type, gender, the majority of co-morbidities, rates of recurrent and persistent disease, rates of associated clinical syndromes, severity of illness as measured by septic shock, and mortality rates. Based on the comparison we feel the nested study group is generally representative of the overall cohort and results of the survival analysis are meaningful.

Another limitation is the absence of information on the timing and receipt of antibiotics. Given that delayed or inappropriate empiric treatment of MRSA bacteremia may increase mortality along with high rates of initial improper treatment of MRSA bacteremia our results may have been biased if there was a difference in proper treatment between USA 300 and non-USA 300 strains [52, 56, 74, 75]. Limiting this potential bias is the fact that vancomycin is currently the only standard empiric antibiotic treatment for MRSA bacteremia in the U.S. [76, 77]. Thus, most physicians suspecting MRSA bacteremia will use the same antibiotic. Additionally, while the timely initiation of effective empiric therapy has been shown to improve the outcome of MRSA bacteremia in some studies, many others have found the delay of proper MRSA treatment did not have a significant effect on outcome [52-55].

Lastly, our outcome of mortality represented crude in-hospital mortality. Information collected on whether MRSA was the cause of death was considered unreliable and incomplete and thus overall mortality was felt to be a more valid outcome measure. To help control for competing causes of mortality we included an extensive list of co-morbidities (the presence of which may increase the risk of dying from another cause) as variables in our analysis and used time (in days) to death rather than overall death as an outcome. The rationale for using time to death was that earlier mortality

after the onset of MRSA bacteremia was more likely to be attributable to MRSA than later mortality. In addition, approximately 75% of the cases in the survival analysis were community-onset (CA and HACO) in origin, thus these were persons whose main reason for presentation and hence outcome was most likely due to MRSA bacteremia. The above discussion along with the magnitude and persistence over time of the association of USA 300 with increased mortality strengthens the suggestion of a true association of USA 300 strains with attributable mortality in persons with MRSA bacteremia.

In summary, our results suggest USA 300 strains may be more virulent that non-USA 300 strains, particularly USA 100, in persons with MRSA bacteremia**.** In addition, we demonstrated USA 300 is responsible for a significant portion of HA-MRSA disease. Future studies confirming our findings and further investigation of the role of microbial virulence mechanisms will be needed to better understand the pathogenesis of USA 300 MRSA infections and to develop improved strategies for prevention and treatment.

REFERENCES

1. Cosgrove, S.E., et al., *The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges.* Infect Control Hosp Epidemiol, 2005. **26**(2): p. 166-74.
2. Deresinski, S., *Methicillin-resistant Staphylococcus aureus: an evolutionary, epidemiologic, and therapeutic odyssey.* Clin Infect Dis, 2005. **40**(4): p. 562-73.
3. Naimi, T.S., et al., *Comparison of community- and health care-associated methicillin-resistant Staphylococcus aureus infection.* JAMA, 2003. **290**(22): p. 2976-84.
4. *From the Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus--Minnesota and North Dakota, 1997-1999.* JAMA, 1999. **282**(12): p. 1123-5.
5. Adem, P.V., et al., *Staphylococcus aureus sepsis and the Waterhouse-Friderichsen syndrome in children.* N Engl J Med, 2005. **353**(12): p. 1245-51.
6. Tenover, F.C., et al., *Characterization of a strain of community-associated methicillin-resistant Staphylococcus aureus widely disseminated in the United States.* J Clin Microbiol, 2006. **44**(1): p. 108-18.
7. Wang, R., et al., *Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA.* Nat Med, 2007. **13**(12): p. 1510-4.
8. Diep, B.A. and M. Otto, *The role of virulence determinants in community-associated MRSA pathogenesis.* Trends Microbiol, 2008. **16**(8): p. 361-9.
9. Chambers, H.F. and F.R. Deleo, *Waves of resistance: Staphylococcus aureus in the antibiotic era.* Nat Rev Microbiol, 2009. **7**(9): p. 629-41.
10. Wisplinghoff, H., et al., *Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study.* Clin Infect Dis, 2004. **39**(3): p. 309-17.
11. Styers, D., et al., *Laboratory-based surveillance of current antimicrobial resistance patterns and trends among Staphylococcus aureus: 2005 status in the United States.* Ann Clin Microbiol Antimicrob, 2006. **5**: p. 2.
12. Klevens, R.M., et al., *Invasive methicillin-resistant Staphylococcus aureus infections in the United States.* JAMA, 2007. **298**(15): p. 1763-71.
13. Laupland, K.B., T. Ross, and D.B. Gregson, *Staphylococcus aureus bloodstream infections: risk factors, outcomes, and the influence of methicillin resistance in Calgary, Canada, 2000-2006.* J Infect Dis, 2008. **198**(3): p. 336-43.
14. European Antimicrobial Resistance Surveillance System management team, *EARSS Annual Report 2007*. 2007: Bilthoven, the Netherlands.
15. Rosenthal, V.D., et al., *International Nosocomial Infection Control Consortium report, data summary for 2002-2007, issued January 2008.* Am J Infect Control, 2008. **36**(9): p. 627-37.
16. Udo, E.E., J.W. Pearman, and W.B. Grubb, *Genetic analysis of community isolates of methicillin-resistant Staphylococcus aureus in Western Australia.* J Hosp Infect, 1993. **25**(2): p. 97-108.
17. Baggett, H.C., et al., *Community-onset methicillin-resistant Staphylococcus aureus associated with antibiotic use and the cytotoxin Panton-Valentine leukocidin during a furunculosis outbreak in rural Alaska.* J Infect Dis, 2004. **189**(9): p. 1565-73.
18. Kazakova, S.V., et al., *A clone of methicillin-resistant Staphylococcus aureus among professional football players.* N Engl J Med, 2005. **352**(5): p. 468-75.

19. *Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections--Los Angeles County, California, 2002-2003.* MMWR Morb Mortal Wkly Rep, 2003. **52**(5): p. 88.
20. Adcock, P.M., et al., *Methicillin-resistant Staphylococcus aureus in two child care centers.* J Infect Dis, 1998. **178**(2): p. 577-80.
21. Zinderman, C.E., et al., *Community-acquired methicillin-resistant Staphylococcus aureus among military recruits.* Emerg Infect Dis, 2004. **10**(5): p. 941-4.
22. Diep, B.A., et al., *Emergence of multidrug-resistant, community-associated, methicillin-resistant Staphylococcus aureus clone USA300 in men who have sex with men.* Ann Intern Med, 2008. **148**(4): p. 249-57.
23. Fridkin, S.K., et al., *Methicillin-resistant Staphylococcus aureus disease in three communities.* N Engl J Med, 2005. **352**(14): p. 1436-44.
24. McDougal, L.K., et al., *Pulsed-field gel electrophoresis typing of oxacillin-resistant Staphylococcus aureus isolates from the United States: establishing a national database.* J Clin Microbiol, 2003. **41**(11): p. 5113-20.
25. Moran, G.J., et al., *Methicillin-resistant S. aureus infections among patients in the emergency department.* N Engl J Med, 2006. **355**(7): p. 666-74.
26. King, M.D., et al., *Emergence of community-acquired methicillin-resistant Staphylococcus aureus USA 300 clone as the predominant cause of skin and soft-tissue infections.* Ann Intern Med, 2006. **144**(5): p. 309-17.
27. Francis, J.S., et al., *Severe community-onset pneumonia in healthy adults caused by methicillin-resistant Staphylococcus aureus carrying the Panton-Valentine leukocidin genes.* Clin Infect Dis, 2005. **40**(1): p. 100-7.
28. Miller, L.G., et al., *Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles.* N Engl J Med, 2005. **352**(14): p. 1445-53.
29. Boucher, H.W. and G.R. Corey, *Epidemiology of methicillin-resistant Staphylococcus aureus.* Clin Infect Dis, 2008. **46 Suppl 5**: p. S344-9.
30. Saiman, L., et al., *Hospital transmission of community-acquired methicillin-resistant Staphylococcus aureus among postpartum women.* Clin Infect Dis, 2003. **37**(10): p. 1313-9.
31. Seybold, U., et al., *Emergence of community-associated methicillin-resistant Staphylococcus aureus USA300 genotype as a major cause of health care-associated blood stream infections.* Clin Infect Dis, 2006. **42**(5): p. 647-56.
32. Klevens, R.M., et al., *Community-associated methicillin-resistant Staphylococcus aureus and healthcare risk factors.* Emerg Infect Dis, 2006. **12**(12): p. 1991-3.
33. D'Agata, E.M., et al., *Modeling the invasion of community-acquired methicillin-resistant Staphylococcus aureus into hospitals.* Clin Infect Dis, 2009. **48**(3): p. 274-84.
34. Tenover, F.C. and R.V. Goering, *Methicillin-resistant Staphylococcus aureus strain USA300: origin and epidemiology.* J Antimicrob Chemother, 2009. **64**(3): p. 441-6.
35. Hageman, J.C., et al., *Occurrence of a USA300 vancomycin-intermediate Staphylococcus aureus.* Diagn Microbiol Infect Dis, 2008. **62**(4): p. 440-2.
36. Graber, C.J., et al., *Intermediate vancomycin susceptibility in a community-associated MRSA clone.* Emerg Infect Dis, 2007. **13**(3): p. 491-3.
37. Diep, B.A., et al., *Widespread skin and soft-tissue infections due to two methicillin-resistant Staphylococcus aureus strains harboring the genes for Panton-Valentine leucocidin.* J Clin Microbiol, 2004. **42**(5): p. 2080-4.
38. Davis, S.L., et al., *Epidemiology and outcomes of community-associated methicillin-resistant Staphylococcus aureus infection.* J Clin Microbiol, 2007. **45**(6): p. 1705-11.

39. Goering, R.V., et al., *Molecular epidemiology of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates from global clinical trials.* J Clin Microbiol, 2008. **46**(9): p. 2842-7.
40. Voyich, J.M., et al., *Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant Staphylococcus aureus disease?* J Infect Dis, 2006. **194**(12): p. 1761-70.
41. Labandeira-Rey, M., et al., *Staphylococcus aureus Panton-Valentine leukocidin causes necrotizing pneumonia.* Science, 2007. **315**(5815): p. 1130-3.
42. Diep, B.A., et al., *The arginine catabolic mobile element and staphylococcal chromosomal cassette mec linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant Staphylococcus aureus.* J Infect Dis, 2008. **197**(11): p. 1523-30.
43. Shorr, A.F., et al., *Healthcare-associated bloodstream infection: A distinct entity? Insights from a large U.S. database.* Crit Care Med, 2006. **34**(10): p. 2588-95.
44. Cosgrove, S.E., et al., *Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis.* Clin Infect Dis, 2003. **36**(1): p. 53-9.
45. McClelland, R.S., et al., *Staphylococcus aureus bacteremia among elderly vs younger adult patients: comparison of clinical features and mortality.* Arch Intern Med, 1999. **159**(11): p. 1244-7.
46. Soriano, A., et al., *Pathogenic significance of methicillin resistance for patients with Staphylococcus aureus bacteremia.* Clin Infect Dis, 2000. **30**(2): p. 368-73.
47. Kaech, C., et al., *Course and outcome of Staphylococcus aureus bacteraemia: a retrospective analysis of 308 episodes in a Swiss tertiary-care centre.* Clin Microbiol Infect, 2006. **12**(4): p. 345-52.
48. Kim, S.H., et al., *Outcome of Staphylococcus aureus bacteremia in patients with eradicable foci versus noneradicable foci.* Clin Infect Dis, 2003. **37**(6): p. 794-9.
49. Lesens, O., et al., *Role of comorbidity in mortality related to Staphylococcus aureus bacteremia: a prospective study using the Charlson weighted index of comorbidity.* Infect Control Hosp Epidemiol, 2003. **24**(12): p. 890-6.
50. Conterno, L.O., S.B. Wey, and A. Castelo, *Risk factors for mortality in Staphylococcus aureus bacteremia.* Infect Control Hosp Epidemiol, 1998. **19**(1): p. 32-7.
51. Chang, F.Y., et al., *A prospective multicenter study of Staphylococcus aureus bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance.* Medicine (Baltimore), 2003. **82**(5): p. 322-32.
52. Ammerlaan, H., et al., *Adequacy of Antimicrobial Treatment and Outcome of Staphylococcus aureus Bacteremia in 9 Western European Countries.* Clin Infect Dis, 2009.
53. Kim, S.H., et al., *Outcome of inappropriate initial antimicrobial treatment in patients with methicillin-resistant Staphylococcus aureus bacteraemia.* J Antimicrob Chemother, 2004. **54**(2): p. 489-97.
54. Kim, S.H., et al., *Outcome of inappropriate empirical antibiotic therapy in patients with Staphylococcus aureus bacteraemia: analytical strategy using propensity scores.* Clin Microbiol Infect, 2006. **12**(1): p. 13-21.
55. Fang, C.T., et al., *Early empirical glycopeptide therapy for patients with methicillin-resistant Staphylococcus aureus bacteraemia: impact on the outcome.* J Antimicrob Chemother, 2006. **57**(3): p. 511-9.

56. Soriano, A., et al., *Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant Staphylococcus aureus bacteremia.* Clin Infect Dis, 2008. **46**(2): p. 193-200.
57. Hidayat, L.K., et al., *High-dose vancomycin therapy for methicillin-resistant Staphylococcus aureus infections: efficacy and toxicity.* Arch Intern Med, 2006. **166**(19): p. 2138-44.
58. Musta, A.C., et al., *Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant Staphylococcus aureus bacteremia: trends over 11 years.* J Clin Microbiol, 2009. **47**(6): p. 1640-4.
59. Kreisel, K., et al. *Is USA300 MRSA BSI Associated with increased Mortality?* . in *Society for Healthcare Epidemiology of America*. 2009. San Diego, CA.
60. Hosmer, D.W., S. Lemeshow, and S. May, *Applied Survival Anlaysis:Regression Modeling of Time to Event Data*. 2nd ed. Wiley Series in Probability and Statistics. 2008: Wiley-Interscience. 416.
61. Li, M., et al., *Evolution of virulence in epidemic community-associated methicillin-resistant Staphylococcus aureus.* Proc Natl Acad Sci U S A, 2009. **106**(14): p. 5883-8.
62. Voyich, J.M., et al., *Insights into mechanisms used by Staphylococcus aureus to avoid destruction by human neutrophils.* J Immunol, 2005. **175**(6): p. 3907-19.
63. Shurland, S., et al., *Comparison of mortality risk associated with bacteremia due to methicillin-resistant and methicillin-susceptible Staphylococcus aureus.* Infect Control Hosp Epidemiol, 2007. **28**(3): p. 273-9.
64. Friedman, H., C. Newton, and T.W. Klein, *Microbial infections, immunomodulation, and drugs of abuse.* Clin Microbiol Rev, 2003. **16**(2): p. 209-19.
65. Lyytikainen, O., et al., *Trends and outcome of nosocomial and community-acquired bloodstream infections due to Staphylococcus aureus in Finland, 1995-2001.* Eur J Clin Microbiol Infect Dis, 2005. **24**(6): p. 399-404.
66. Burton, D.C., et al., *Methicillin-resistant Staphylococcus aureus central line-associated bloodstream infections in US intensive care units, 1997-2007.* JAMA, 2009. **301**(7): p. 727-36.
67. Bagger, J.P., D. Zindrou, and K.M. Taylor, *Postoperative infection with meticillin-resistant Staphylococcus aureus and socioeconomic background.* Lancet, 2004. **363**(9410): p. 706-8.
68. Huggan, P.J., et al., *Population-based epidemiology of Staphylococcus aureus bloodstream infection in Canterbury, New Zealand.* Intern Med J, 2009.
69. Burkey, M.D., et al., *The incidence of and risk factors for MRSA bacteraemia in an HIV-infected cohort in the HAART era.* HIV Med, 2008. **9**(10): p. 858-62.
70. Hidron, A.I., et al., *Risk factors for colonization with methicillin-resistant Staphylococcus aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage.* Clin Infect Dis, 2005. **41**(2): p. 159-66.
71. Shet, A., et al., *Colonization and subsequent skin and soft tissue infection due to methicillin-resistant Staphylococcus aureus in a cohort of otherwise healthy adults infected with HIV type 1.* J Infect Dis, 2009. **200**(1): p. 88-93.
72. Pos, O., et al., *Impaired phagocytosis of Staphylococcus aureus by granulocytes and monocytes of AIDS patients.* Clin Exp Immunol, 1992. **88**(1): p. 23-8.
73. Schaumann, R., J. Krosing, and P.M. Shah, *Phagocytosis of Escherichia coli and Staphylococcus aureus by neutrophils of human immunodeficiency virus-infected patients.* Eur J Med Res, 1998. **3**(12): p. 546-8.

74. Marchaim, D., et al., *Case-control study to identify factors associated with mortality among patients with methicillin-resistant Staphylococcus aureus bacteraemia.* Clin Microbiol Infect, 2009.
75. Rodriguez-Bano, J., et al., *Impact of inappropriate empirical therapy for sepsis due to health care-associated methicillin-resistant Staphylococcus aureus.* J Infect, 2009. **58**(2): p. 131-7.
76. Gould, F.K., et al., *Guidelines (2008) for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the United Kingdom.* J Antimicrob Chemother, 2009. **63**(5): p. 849-61.
77. Corey, G.R., *Staphylococcus aureus bloodstream infections: definitions and treatment.* Clin Infect Dis, 2009. **48 Suppl 4**: p. S254-9.

Table 1. Observed MRSA Bacteremia Incidence by Year and MRSA Epidemiological Classification, Atlanta, GA, 2005-2008[a]

Surveillance Year	Total No. of Cases/Year[b]	Community-Associated	Healthcare Associated Community-Onset[c]	Healthcare Associated Hospital-Onset[c]	Total [b,c]
		Incidence per 100,000 (% of total cases per year)			
2005	1198	4.2 (12.4)	19.4 (57.2)	10.1 (29.6)	33.9
2006	1122	4.2 (13.8)	18.5 (60.9)	7.6 (25.0)	30.5
2007	1076	3.7 (12.8)	17.2(60.1)	7.6 (26.6)	28.5
2008	948	4.2(16.8)	14.7(59.5)	5.8(23.4)	24.8

[a]Epidemiological classification of disease consisted of healthcare associated (either hospital-onset with a culture collected > 48 hours after hospital admission or community-onset cases with healthcare risk factors and culture collected ≤48 hours after hospital admission) or community associated cases (no healthcare risk factors)

[b] Unknown cases=20 (2005:9, 2006:3, 2007:5, 2008:3)

[c] P-value <0.01; testing for a trend in MRSA bacteremia over the 4 years

Table 2. Estimated MRSA Bacteremia Incidence Rates by Sex, Age, Race, and HIV/AIDS Status, Atlanta, GA, 2005-2008

Demographic	Actual No.	Incidence of MRSA Bacteremia per 100,000				
		Surveillance Year				Total
		2005	2006	2007	2008	
Sex						
Male	2471	37.9	35.2	33.3	28.9	33.7
Female	1872	30.0	25.9	23.8	20.7	25.0
Age, y						
≤1	79	16.4	13.0	22.1	15.0	16.7
2-17	75	2.6	1.7	2.5	2.4	2.3
18-34	460	15.5	11.7	11.7	11.2	12.5
35-49	970	28.7	30.8	22.8	21.7	26.0
50-64	1256	51.6	51.9	52.4	42.3	49.4
≥ 65	1504	172.6	135.3	125.7	103.4	133.0
Race						
Black	2558	53.3	48.6	42.4	37.2	45.2
Other[a]	207	33.0	21.7	23.3	19.6	24.1
White	1579	21.1	18.9	19.5	16.8	19.0
HIV[b]	118	1002.9	610.4	311.9	226.4	456.2
AIDS	321	656.6	782.8	612.6	565.6	650.5

[a]Other includes American Indian, Asian, and Pacific Island.

[b] Not Including AIDS

Table 3. Estimated MRSA Bacteremia Incidence Rates by Age and Race, Atlanta, GA, 2005-2008

	Incidence per 100,000		
Age (Years)	**White**	**Black**	**Other[a]**
≤ 1	6.8	27.7	29.0
2-17	1.2	3.4	3.7
18-34	4.2	22.7	14.6
35-49	11.0	49.9	15.1
50-64	27.8	95.6	36.0
>65	97.8	226.4	168.7

[a]Other includes American Indian, Asian, and Pacific Island.

Table 4. Comparison of Selected Characteristics among MRSA Bacteremia Cases with and without Pulse Field Gel Electrophoresis Atlanta, GA, 2005-2008

Characteristic	**No PFGE (n=3240) (%)**	**PFGE (n=1104) (%)**	**P-Value[b] (if <.05)**
Age,in years (Mean)	56.5	53.8	<.01
Male Sex	56.4	58.2	-
Race			
White	39.1	28.2	<.01
Black	55.6	68.6	<.01
Other	5.3	3.3	<.01
Epidemiological Type[a]			
HA HO	26.8	25.1	-
HA CO	59.8	58.2	-
CA	13.1	16.1	-
Comorbidities			
Alcoholism	3.8	3.7	-
Intravenous Drug Use	1.0	1.4	-
HIV or AIDS	9.0	13.3	<.01
Solid Malignancy	9.8	8.2	-
Hematologic Malignancy	2.5	1.5	-
Peripheral Vascular Disease	7.9	6.3	-
Congestive Heart Failure	17.5	14.3	<.05
Coronary Heart Disease	16.1	13.6	<.05
Cerebrovascular Accident	11.8	10.9	-
Chronic Pulmonary Obstructive Disease	12.9	8.8	<.05
Diabetes Mellitus	39.0	36.9	-
Chronic Renal Insufficiency	35.7	33.7	-
Hemodialysis in the Last Year	26.3	23.9	-
Hospitalized in the Last Year	57.1	51.5	<.01
Surgery in the Last year	26.6	20.4	<.01

Long Term Care Facility	20.6	19.2	-
Chronic Liver Disease	2.7	2.3	-
Immunosuppression	9.2	6.4	<.01
Clinical Characteristics			
Hospitalized	95.5	94.3	-
Persistent Disease	6.5	7.7	-
Relapsed Disease	16.9	16.9	-
Clinical Syndromes			
Endocarditis	3.5	3.7	-
Pneumonia	9.3	10.5	-
Septic Arthritis/Bursitis	2.2	2.3	-
Deep Tissue Abscess	1.2	1.5	-
Osteomyelitis	3.2	3.6	-
Surgical Site Infection	2.8	2.5	-
Skin and Soft Tissue Infection	9.3	10.7	-
No associated syndrome	67.5	64.2	<.05
Severity of Illness			
Septic Shock	2.8	3.3	-
Outcomes			
Death	17.4	18.5	-

[a]Epidemiological classification of disease consisted of healthcare associated (either hospital-onset with a culture collected > 48 hours after hospital admission or community-onset cases with healthcare risk factors and culture collected ≤48 hours after hospital admission) or community associated cases (no healthcare risk factors)

[b] P Value for Chi Square Test

Table 5. Distribution of Pulsed Field Gel Electrophoresis (PFGE) Types among MRSA Blood Isolates, Atlanta, GA, 2005-2008 (n=1104)

Pulse Field Type	No. (% of total)
USA 300	414 (37.5)
USA 100	374 (33.9)
USA 500	168 (15.2)
IBERIAN	80 (7.2)
USA 800	31 (2.8)
USA 700	12 (1.1)
CAMRSA9	7 (0.6)
USA 1000	6 (0.5)
GROUP D	4 (0.4)
USA 1100	2 (0.2)
USA 400	2 (0.2)
USA200	2 (0.2)
BRAZILIAN	1 (0.1)
USA 600	1 (0.1)

Table 6. Comparison of Selected Characteristics between USA 300 and Non-USA 300 MRSA Bacteremia Cases, Atlanta, GA, 2005-2008

Characteristic	Non USA 300 (n=690)	USA 300 (n=414)	P-Value[b] (If <0.05)
Age,Y (mean)	57.7	47.1	<.01
Mean Hospital Days before + Culture	10.5	3.7	<.01
Mean Hospital Days before + Culture (HAHO)	33.2	16.1	<.01
Male Sex	54.5	64.5	<.01
Race			
White	30.4	24.4	<.05
Black	65.9	73.0	<.05
Other	3.6	2.7	-
Epidemiological Type[a]			
HAHO	29.6	17.6	<.01
Community Onset	70.0	81.4	<.01
HACO	62.6	50.7	<.01
CA	7.4	30.7	<.01
Treating Hospital			
Hospital 1	19.3	16.4	-
Hospital 2	25.5	35.5	<.01
Hospital 3	12.3	11.1	-
Hospital 4	12.8	10.1	-
Comorbidities			
Alcoholism	2.9	5.1	-
Intravenous Drug Use	0.1	3.4	<.01
HIV non AIDS	2.5	4.8	<.05
AIDS	9.0	11.4	-
Solid Malignancy	9.4	6.0	<.05
Hematological Malignancy	2.0	0.7	-
Peripheral Vascular Disease	7.5	4.4	<.05
Congestive Heart Failure	16.8	10.1	<.01
Coronary Heart Disease	15.9	9.7	<.01
Cerebrovascular Accident	14.2	5.3	<.01
Chronic Pulmonary Obstructive Disease	10.7	5.6	<.01
Diabetes Mellitus	40.9	30.2	<.01
Chronic Renal Insufficiency	37.8	26.8	<.01
Hemodialysis in the Last Year	27.0	18.8	<.01
Hospitalized in last year	57.4	41.8	<.01
Surgery in Last year	23.5	15.2	<.01
Long Term Care Facility	24.4	10.6	<.01
Chronic Liver Disease	1.5	3.6	<.05
Immunosuppression	7.5	4.6	-
Clinical Characteristics			
Hospitalized	94.9	93.2	-*
Persistent Disease	8.6	6.0	-

Relapse	17.7	15.5	-
Clinical Syndromes			
Endocarditis	3.5	4.1	-
Pneumonia	10.7	10.1	-
Septic Arthritis	2.5	1.9	-
Deep Tissue Abscess	0.9	2.4	<.05
Osteomyelitis	2.6	5.3	<.05
Surgical Site Infection	2.8	1.9	-
Skin and Soft Tissue Infection	4.6	20.8	<.01
No associated syndrome	71.9	51.5`	<.01
Severity of Illness			
Septic Shock	2.9	3.9	-
Outcomes			
Death	19.7	16.4	-
Death within 7 days	49.3	73.5	<.01
Death within 7 days (HAHO cases)	35.3	58.8	<.01

[a]Epidemiological classification of disease consisted of healthcare associated (either hospital-onset with a culture collected > 48 hours after hospital admission or community-onset cases with healthcare risk factors and culture collected ≤48 hours after hospital admission) or community associated cases (no healthcare risk factors)
[b] P Value for Chi Square Test

Table 7. Univariate Analysis of Potential Risk Factors for Mortality in persons with MRSA bacteremia, Atlanta, GA 2005-8 (N=1104)

Characteristic	Hazard Ratio[a]	95% CI	P-Value[b] (If ≤0.20)
USA 300 vs. Non USA 300	1.02	(0.76, 1.37)	-
Age, (per increasing year)	1.03	(1.02, 1.04)	<.01
Hospital Days before + Culture (per day)	1.00	(1.00,1.00)	-
Female	0.98	(0.74,1.30)	-
Race			
White vs. Black & Other	1.60	(1.20, 2.14)	<.01
Epidemiological Type			
Community Onset	0.98	(0.72, 1.32)	-
Treating Hospital			
Hospital One	1.09	(0.76, 1.57)	-
Hospital Two	0.91	(0.67 ,1.23)	-
Hospital Three	0.73	(0.43, 1.24)	-
Hospital Four	0.93	(0.60, 1.45)	-
Comorbidities			
Alcoholism	1.35	(0.73, 2.45)	-
Intravenous Drug Use	0.71	(0.18, 2.85)	-
HIV non AIDS	0.46	(0.15, 1.43)	-
AIDS	1.11	(0.71, 1.72)	-
Solid Malignancy	2.08	(1.38, 3.12)	<.01

Hematological Malignancy	1.96	(0.81, 4.77)	<.15
Peripheral Vascular Disease	1.91	(1.26, 2.88)	<.01
Congestive Heart Failure	1.62	(1.16, 2.26)	<.01
Coronary Heart Disease	1.57	(1.10, 2.25)	<.05
Cerebrovascular Accident	1.57	(1.07, 2.30)	<.05
COPD	1.72	(1.15, 2.59)	<.01
Diabetes Mellitus	1.08	(0.82, 1.43)	-
Chronic Renal Insufficiency	1.08	(0.81, 1.44)	-
Chronic Liver Disease	2.23	(1.18, 4.22)	<.05
Hemodialysis in the Last Year	0.91	(0.65, 1.28)	-
Hospitalized in last year	0.98	(0.74, 1.29)	-
Surgery in Last year	1.03	(0.75, 1.41)	-
Long Term Care Facility	1.79	(1.31, 2.44)	<.01
Immunosuppression	1.14	(0.65, 2.00)	-
Clinical Characteristics			
Persistent Disease	0.76	(0.36, 1.57)	-
Relapse	0.59	(0.39, 0.91)	<.05
Clinical Syndromes			
Endocarditis	0.66	(0.29, 1.48)	-
Pneumonia	1.30	(0.89, 1.92)	<.20
Septic Arthritis/Bursitis	0.16	(0.02, 1.17)	<.10
Deep Tissue Abscess	0.26	(0.04, 1.84)	<.20
Osteomyelitis	0.21	(0.05, 0.85)	<.05
Surgical Site Infection	0.59	(0.19, 1.86)	-
Skin and Soft Tissue Infection	0.37	(0.17, 0.79)	<.01
Bacteremia without clinical syndrome	1.79	(1.31, 2.46)	<.01
Severity of Illness			
Septic Shock	3.99	(2.57, 6.20)	<.01

[a]Unadjusted hazard ratios

[b] P Value for Chi Square Test

Table 8. Multivariate Analysis to Determine Predictors of Mortality in Persons with MRSA Bacteremia, Atlanta, GA 2005-8 (N=1104)

Parameters	Hazard Ratio[a]	95% CI	P-Value[b]
USA 300 vs. Non USA 300	**1.63**	(1.19, 2.23)	<.01
Age (per increasing year)	1.04	(1.03, 1.05)	<.01
White vs. Black & Other	1.36	(1.00, 1.85)	0.05
Chronic Liver Disease	2.48	(1.28, 4.81)	<.01
Alcoholism	1.79	(0.95, 3.38)	0.07
AIDS	2.04	(1.28, 3.27)	<.01
Bacteremia without clinical syndrome	3.26	(2.04, 5.20)	<.01
Pneumonia	2.54	(1.44, 4.46)	<.01
Septic Shock	5.07	(3.21, 7.99)	<.01

[a]Adjusted hazard ratios

[b] P Value for Chi Square Test

Table 9. Testing for Statistical Significance of Interaction Term between PFGE type and all Other Variables in Final Model

Interaction	Variables	p-value
USA 300 type	Age	0.31
	White vs. Black & Other	0.59
	Chronic Liver Disease	0.98
	Alcohol	0.88
	AIDS	0.46
	Bacteremia without clinical syndrome	0.44
	Pneumonia	0.24
	Septic Shock	0.45

Table 10. Comparison of Selected Characteristics between USA 300 and USA 100 Bacteremia Cases, Atlanta, GA, 2005-2008

Characteristic	USA 100 (n=374)	USA 300 (n=414)	P-Value[a] (If <0.05)
Mean Age (years)	60.6	47.1	<.01
Mean Days in Hospital before + Culture	12.0	3.7	<.01
Mean Days in Hospital before + Culture (HAHO)	35.7	16.1	<.01
Male Sex	49.7	64.5	<.01
Race			
White	37.7	24.4	<.05
Black	58.6	73.0	<.05
Other	3.7	2.7	-
Epidemiological Type			
HAHO	31.6	17.6	<.01
Community Onset	68.2	81.4	<.01
HACO	60.7	50.7	<.01
CA	7.5	30.7	<.01
Treating Hospital			
Hospital One	19.0	16.4	-
Hospital Two	18.5	35.5	<.01
Hospital Three	14.2	11.1	-
Hospital Four	13.4	10.1	-
Comorbidities			
Alcoholism	3.2	5.1	-
Intravenous Drug Use	0.0	3.4	<.01
HIV non AIDS	1.3	4.8	<.01
AIDS	1.3	11.4	<.01
Solid Malignancy	8.3	6.0	-
Hematological Malignancy	2.1	0.7	-
Peripheral Vascular Disease	8.6	4.4	<.05
Congestive Heart Failure	17.7	10.1	<.01
Coronary Heart Disease	19.3	9.7	<.01
Cerebrovascular Accident	14.2	5.3	<.01
Chronic Pulmonary Obstructive Disease	12.3	5.6	<.01
Diabetes Mellitus	43.9	30.2	<.01
Chronic Renal Insufficiency	34.2	26.8	<.05
Hemodialysis in the Last Year	24.1	18.8	-
Hospitalized in last year	57.5	41.8	<.01
Surgery in Last year	27.5	15.2	<.01
Long Term Care Facility	26.2	10.6	<.01
Chronic Liver Disease	1.9	3.6	-
Immunosuppression	7.8	4.6	-
Clinical Characteristics			
Hospitalized	96.0	93.2	-
Persistent Disease	10.3	6.0	-

Relapse	17.7	15.5	-
Clinical Syndromes			
Endocarditis	2.7	4.1	-
Pneumonia	10.7	10.1	-
Septic Arthritis	2.7	1.9	-
Deep Abscess	0.8	2.4	-
Osteomyelitis	2.1	5.3	<.05
SSI	4.6	1.9	<.05
SSTI	4.6	20.8	<.01
No associated syndrome	71.4	51.5	<.01
Severity of Illness			
Septic Shock	2.9	3.9	-
Outcomes			
Death	20.3	16.4	-
Death within 7 days	47.4	73.5	<.01
Death within 7 days (HAHO)	33.3	58.8	<.01

[a] Chi Square P Value

Table 11. Multivariate Analysis for Predictors of Mortality in Persons with MRSA Bacteremia, Atlanta, GA 2005-8 (N=788)

Parameters	Hazard Ratio	95% CI	P-Value[b]
USA 300	**1.79**	**(1.24, 2.58)[a]**	**<.01**
Age (per increasing year)	1.04	(1.03, 1.05)	<.01
Hosptime	1.004	(1.00, 1.01)	<.10
Alcoholism	2.16	(1.10, 4.27)	<.05
Chronic Liver Disease	2.13	(1.02, 4.45)	<.05
Pneumonia	2.58	(1.32, 5.02)	<.01
No Associated Clinical Syndrome	3.18	(1.83, 5.53)	<.01
Septic Shock	6.09	(3.67,10.11)	<.01

[a] Adjusted hazard ratios

[b] Chi Square P values

Figure 1. Cox Adjusted Survival Graph for USA 300 vs. Non-USA 300 Isolates

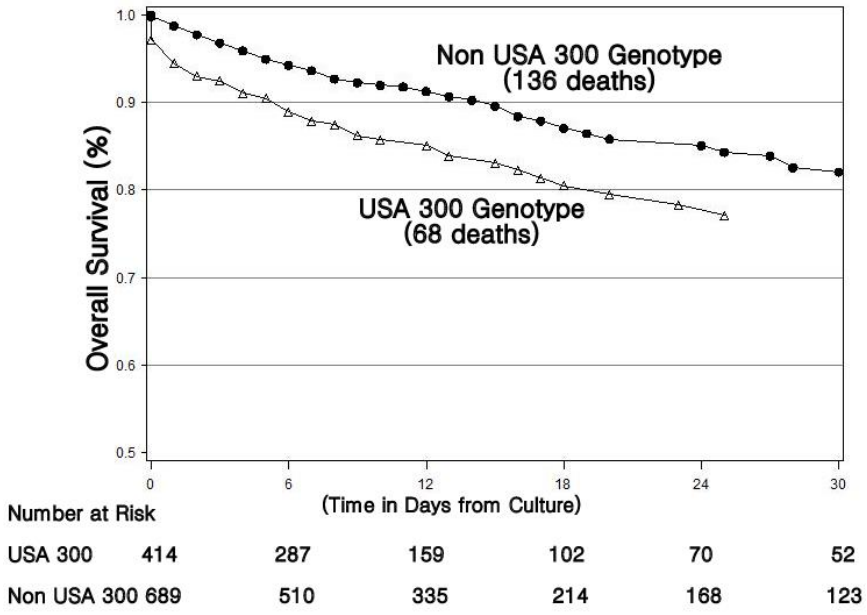


Figure 2. –Ln-Ln Survival Graph to assess Proportional Hazards Assumption for USA 300 vs. Non-USA 300

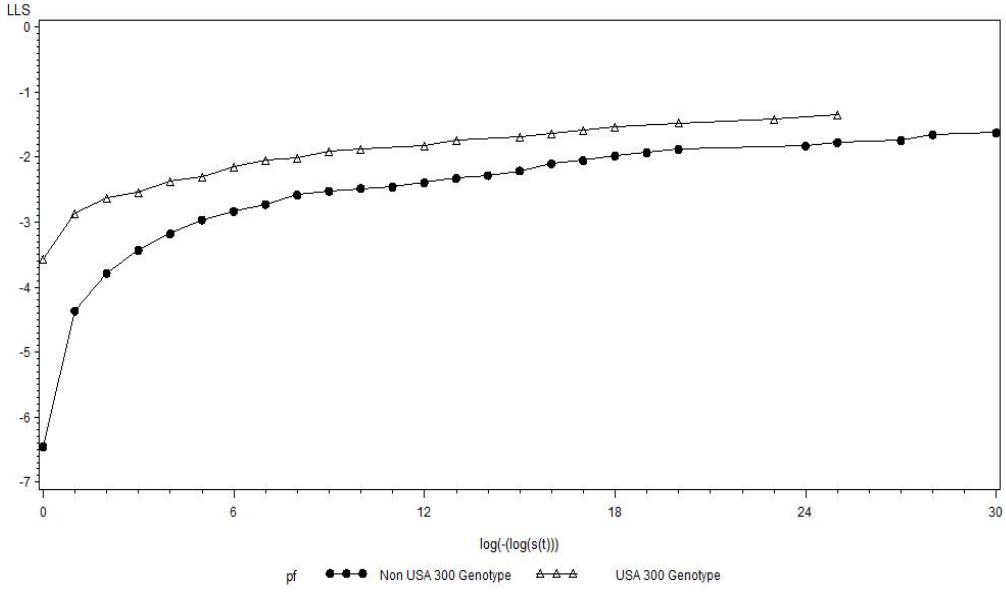
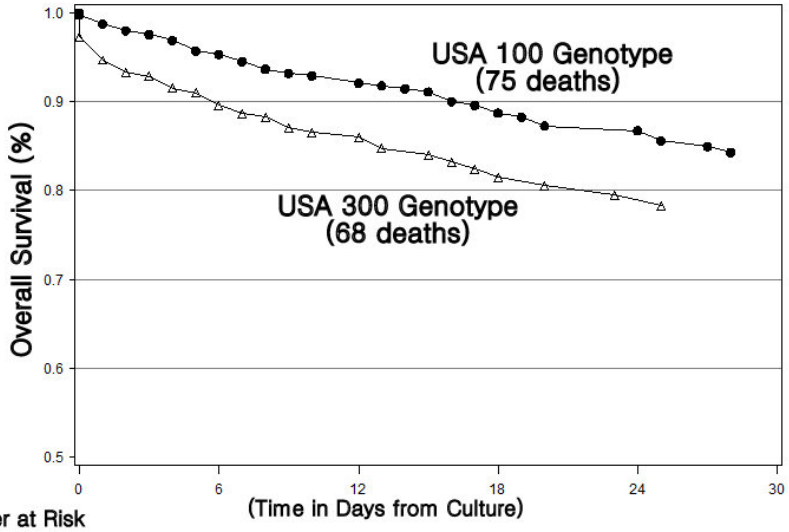


Figure 3. Cox Adjusted Survival Graph for USA 300 vs. USA 100 Isolates

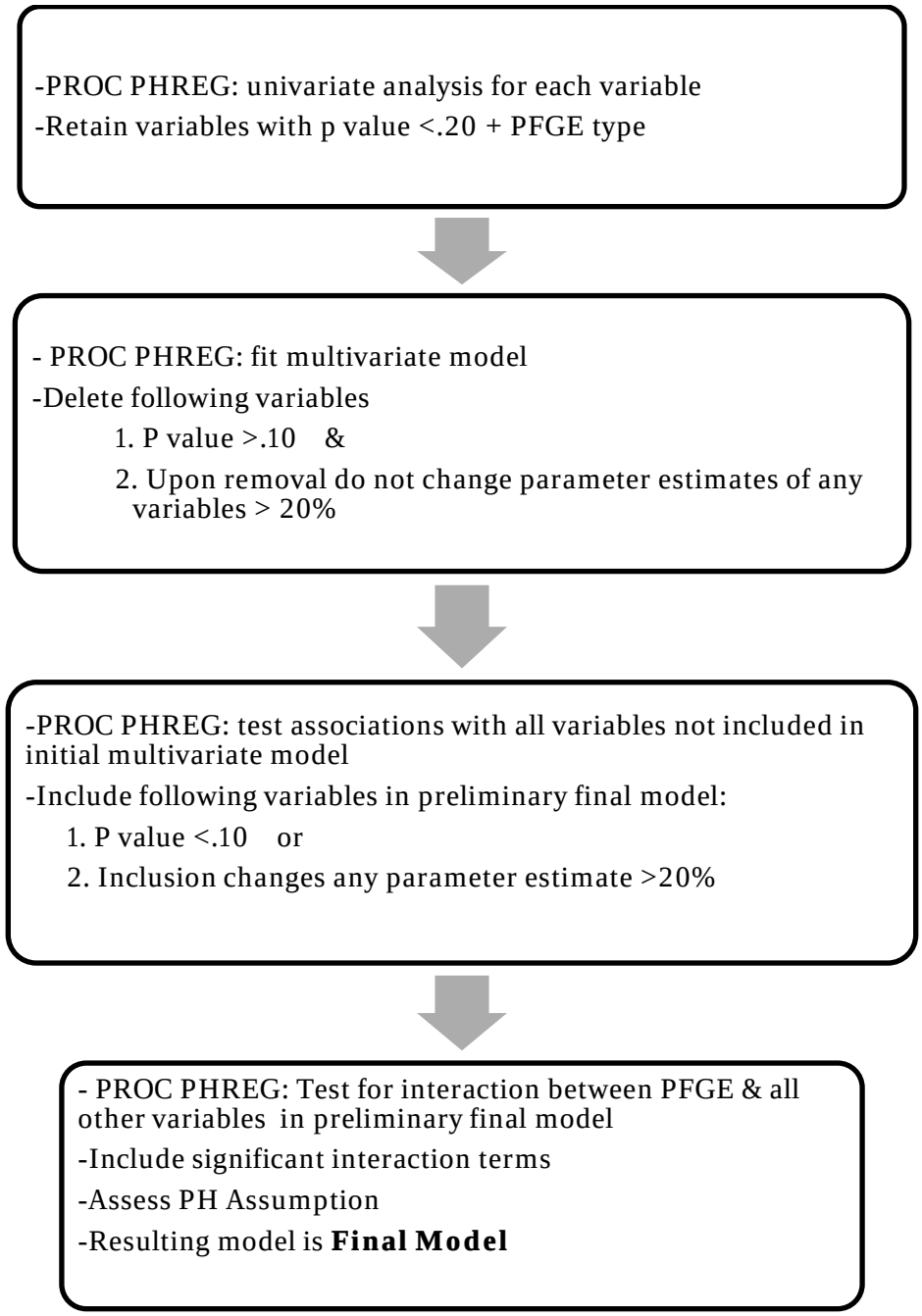


Number at Risk						
USA 300	414	287	159	102	70	52
USA 100	373	274	177	107	88	61

Appendix A. Variables Included in Survival Analysis

Laboratory	Demographics	Clinical	Comorbidities
PFGE	Age	Persistent infection	AIDS
	Gender	Relapsed infection	HIV non AIDS
	Race	Endocarditis	Solid malignancy
	Treating Hospital	Pneumonia	Hematologic malignancy
	Residence of long term care facility	Septic Arthritis	Peripheral vascular disease
		Deep Abscess	Heart failure
		Osteomyelitis	Atherosclerotic heart disease
		Surgical Site Infection	Chronic obstructive pulmonary disease
		Skins and Soft Tissue Infection	Diabetes
		No Associated clinical Syndrome	Chronic renal insufficiency
		Septic Shock	Dialysis in last year
		Immunosuppressive therapy	Chronic liver disease
		Hospitalized in last year	
		Surgery in last year	
		Days in hospital before + culture	
		Community vs. Hospital Onset	

Appendix B. Purposeful Selection of Covariates Strategy



Appendix 3. Final Multivariate Model using Purposeful Selection of Covariates Strategy

$$h(t) = h_0(t) \exp(\beta_1 * \text{USA300} + \beta_2 * \text{age} + \beta_3 * \text{race} + \beta_4 * \text{chronic\_liver\_disease} + \beta_5 * \text{alcoholism} + \beta_6 * \text{AIDS} + \beta_7 * \text{bacteremia\_without\_clinical\_syndrome} + \beta_8 * \text{pneumonia})$$