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Approval Sheet

USA500 Methicillin-Resistant *Staphylococcus aureus*: An Evaluation of Clinical Virulence in Bacteremia

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Abstract Cover Page

USA500 Methicillin-Resistant *Staphylococcus aureus*: An Evaluation of Clinical Virulence in Bacteremia

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B.S., B.A., Indiana University, 2004
M.D., Indiana University School of Medicine, 2008

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

USA500 Methicillin-Resistant *Staphylococcus aureus*: An Evaluation of Clinical Virulence in Bacteremia

By Andre Gerardo Melendez

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common pathogen in healthcare-associated infections in the US. USA500 and USA100 are the predominant molecular subtypes causing healthcare-associated MRSA (HA-MRSA) infections. These strains possess different microbiologic characteristics and several *in vitro* and animal studies have suggested that USA500 is more virulent than USA100. However, it is unknown whether individuals with USA500 MRSA infections have worse outcomes compared to USA100. The main objectives of this study were to identify the epidemiologic, molecular, and clinical characteristics of USA500 bacteremia and determine whether bacteremia due to USA500 is associated with greater attributable mortality compared to USA100.

Methods: Population based-surveillance for MRSA bacteremia was conducted in the 8-county metropolitan Atlanta area from 2005-2011. The analysis included patients with MRSA bloodstream infections due to USA500 or USA100 strains. Bivariate analyses were performed to compare characteristics of USA500 and USA100. Multivariable logistic regression models were used to evaluate the association of USA500 strain type and other factors predictive of attributable mortality.

Results: A total of 107 USA500 and 608 USA100 cases of MRSA bacteremia were included in the study cohort. USA500 MRSA cases were more likely to occur in blacks (72.4% vs 56.3%, P=0.005), have HIV/AIDS (21.5% vs 2.6%, P<0.0001), and have resistance to trimethoprim-sulfamethoxazole (90.7% vs 1.2%, P<0.0001) compared to USA100. In multivariable analysis, USA500 was not associated with increased attributable mortality compared to USA100 (aOR 0.57, 95% CI 0.26-1.22). Septic shock (aOR 7.36, 95% CI 3.88-13.97), ICU admission prior to index culture (aOR 3.21, 95% CI 1.39-7.40), age >55 years (aOR 1.99, 95% CI 1.11-3.53), and central line-associated bloodstream infection (aOR 0.46, 95% CI 0.26-0.82) were factors associated with attributable mortality.

Conclusions: USA500 has a strong association with HIV/AIDS and trimethoprim-sulfamethoxazole resistance compared to its HA-MRSA counterpart, USA100. Contrary to *in vitro* and animal studies, USA500 MRSA was not more virulent than USA100 in individuals with bacteremia as measured by attributable mortality in this study population.

Cover Page

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961 and has historically been an important pathogen causing infections with significant morbidity and mortality. Over the last three decades the incidence of MRSA infection in the United States and abroad has dramatically increased [1, 2]. Different MRSA strains have been epidemiologically linked with either healthcare-associated MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA) infections and molecular typing by pulsed-field gel electrophoresis (PFGE) has been the reference standard in the United States used to discriminate among the strains. Strains causing HA-MRSA disease have been PFGE-typed as USA100, USA200, and USA500 and the majority typically carry the large staphylococcal chromosomal antibiotic resistance cassette *mec* (SCC*mec*) types I, II, III, whereas USA500 carries a smaller type IV cassette [3-5]. USA100 is the predominant strain, accounting for 53.6% of invasive MRSA infections in a national, population-based analysis, while USA500 is the third leading strain at 3.7% [3]. However, there is significant geographic variability and USA500 compromises approximately 15% of MRSA isolates in the Atlanta metropolitan area [6].

Multiple studies have investigated whether certain HA-MRSA and CA-MRSA strains cause more severe disease or lead to worse patient outcomes. Studies by Li et al demonstrated that USA500 MRSA is more virulent than USA100 MRSA in *in vitro* and animal studies [7, 8]. However, it is unknown whether USA500 MRSA is clinically more virulent (i.e. causes more severe infections leading to higher mortality) than USA100 in humans.

The main purpose of this study was to evaluate whether bacteremia due to USA500 MRSA is associated with greater mortality compared to USA100. In addition, further characterization of USA500 MRSA isolates was performed and the epidemiologic, microbiologic, and clinical characteristics of USA500 MRSA bacteremia were compared to those of USA100.

The nested cohort includes a convenience sample from the overall cohort of MRSA bacteremia cases for which an isolate was available for further characterization. A total of 107 cases of USA500 and 608 cases of USA100 were identified from January 1, 2005 to December 30, 2011. The results of this study are expected to elucidate whether USA500 is more virulent than its healthcare-associated counterpart, USA100. Establishing whether specific HA-MRSA strains lead to worse patient outcomes is of great importance because it can direct efforts for targeted interventions to reduce the mortality associated with MRSA infections.

BACKGROUND

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been an important nosocomial pathogen associated with significant morbidity and mortality since its initial description in the 1960s. The healthcare environment continues to be the primary setting where MRSA exposure occurs and the incidence of MRSA infection in the United States and abroad rose steadily in the in the last three decades [1]. Despite a decline in invasive infections since 2005, MRSA remains the most common pathogen in healthcare-associated infections, comprising 16% of 69,475 infections during a study period in 2009-2010 [2, 9].

Bacteremia is the most common invasive infection caused by MRSA and is associated with increased mortality, longer hospital stays, and higher costs compared to bacteremia from other microorganisms [2, 10-12]. Certain patient characteristics and underlying comorbidities have been associated with increased risk of death in individuals with MRSA bacteremia. Age >55 years old [13, 14], female gender [13], HIV/AIDS [6, 13], chronic liver disease [6, 15], cancer [15], a high Charlson Comorbidity Index, a composite score of the presence and degree of severity of comorbidities [14], and ICU admission prior to index culture [13, 14] are predictive of mortality. Clinical syndromes that are associated with increased mortality in bacteremia include pneumonia [6, 16], bacteremia without identified clinical syndrome [6], infective endocarditis [17] and septic shock [6, 16, 17].

Different MRSA strains have been epidemiologically linked with either healthcare-associated MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA) infections. Molecular typing by pulsed-field gel electrophoresis (PFGE) has been the reference standard in the United States to differentiate strains. Strains causing HA-MRSA disease have been PFGE-typed as USA100, USA200, and USA500 [3, 5]. In a national, population-based analysis of invasive MRSA infections in 2005-2006, USA100 was the predominant strain accounting for 53.6% of infections.

USA500, on the other hand, was the third leading strain at 3.7% [3]. In contrast to USA100, which is evenly distributed across the United States, USA500 exhibits significant geographic variability. In Atlanta, USA500 compromises a much larger proportion of infections. In a study by Kempker et al, USA500 accounted for approximately 15% of all invasive MRSA isolates in the Atlanta metropolitan area [6].

USA500 is distinct among HA-MRSA strains in that its genotypic features share more in common with the predominant strain causing CA-MRSA infections, USA300. It carries the smaller SCC*mec* type IV cassette and falls within the same clonal complex (CC8, as determined by multilocus sequence typing) as USA300. In contrast, USA100 contains the larger SCC*mec* type II cassette and belongs to clonal complex 5 [4]. Both USA500 and USA300 express higher levels of α-type phenol soluble modulin, an important virulence factor, than USA100 [8, 18]. From an evolutionary standpoint, USA500 has been postulated to be the progenitor of the USA300 strain [8].

Several studies have investigated whether there is a difference in virulence among MRSA strains. In studies by Li et al, USA500 strains demonstrated greater virulence than USA100 in the ability to lyse human neutrophils in *in vitro* assays and resulted in larger abscesses and higher levels of inflammatory marker release in a rabbit skin infection model [7, 8]. The authors postulated that the underlying mechanism may be increased expression of core genome-encoded pathogenic determinants, such as alpha-type phenol soluble modulin and alpha-toxin and not dependent upon the presence of PVL toxin or other exogenous toxins. A study by Rudkin et al demonstrated in an animal model that strains with SCC*mec* type IV, such as USA500, produced a smaller quantity of the gene product, PBP2a, which lead to increased expression of other virulence genes as compared to *SCCmec* type II containing HA-MRSA strains (USA100) in the animal model [19].

The effect of MRSA strain type on clinical outcomes, such as mortality, has been studied with conflicting results. Kempker et al found that patients with USA300 MRSA bacteremia had an increased risk of in-hospital mortality compared to USA100 (aHR 1.79; 95% CI 1.24 – 2.58) [6]. However, Lessa et al demonstrated that mortality did not differ between USA300 and USA100 in individuals with MRSA central line-associated blood stream infections [14]. In a study by Hota et al, all USA300 MRSA infectious clinical syndromes had lower mortality compared to USA100 and other MRSA strains (aOR 0.37; 95% CI 0.15 – 0.91), with the exception of embolic pneumonia [20]. No studies have been performed that specifically compare the mortality among the two most common HA-MRSA strains, USA500 and USA100, in individuals with bacteremia. The purpose of this study is to evaluate the association of USA500 and USA100 with attributable mortality in individuals with bacteremia and to compare the epidemiologic, microbiologic, and clinical characteristics of USA500 with those of USA100. Identifying whether certain MRSA strains are more virulent than others is important because it can lead to the development of targeted preventive and therapeutic strategies to reduce the morbidity and mortality of MRSA bacteremia.

METHODS

The overall objective of this study is to determine the association of USA500 MRSA and USA100 MRSA with attributable mortality in individuals with bacteremia.

Specific Aims

1. Identify the epidemiologic, microbiologic, and clinical characteristics of USA500 MRSA bacteremia and compare with those of USA100
2. Estimate association of MRSA strain type (USA500 or USA100) with attributable mortality
3. Evaluate predictors of attributable mortality among patients with USA500 MRSA and USA100 bacteremia

Hypothesis

Bacteremia due to USA500 MRSA is associated with greater attributable mortality compared to USA100 MRSA.

Study Design

This study is a secondary analysis of a prospective observational population-based surveillance cohort.

Data Source

Cases of invasive MRSA infection were identified from January 1, 2005 to December 31, 2011 by the Georgia Emerging Infections Program (GA EIP) as part of the Centers for Disease Control and Prevention's (CDC) Active Bacterial Core Surveillance (ABCs) system for invasive

MRSA disease. The ABCs methodology for surveillance of invasive MRSA disease has been described elsewhere [2, 3]. Briefly, according to ABCs methodology, a case of invasive MRSA disease was defined as identification of a MRSA strain isolated from a normally sterile body site in a resident of surveillance catchment area. A MRSA culture from a normally sterile site obtained within 30-days of an initial MRSA culture was considered part of the same episode, whereas an invasive MRSA culture obtained more than 30-days after an initial culture was considered to represent a recurrent episode. The GA EIP population area for invasive MRSA infections was Health District 3 (HD3), which represents 8 counties in metropolitan Atlanta. The population under surveillance was approximately 3.5 million people.

For this analysis, the inclusion criteria were patients with MRSA isolated from blood culture (bacteremia) for whom an isolate was available and classified as either USA500 or USA100 by PFGE. Patients with MRSA bacteremia due to other strain types were excluded. This nested cohort represents a convenience sample from the overall bacteremia cohort, as approximately 30% of the cases have an isolate available for further testing. Case finding was both active and laboratory-based and included review of clinical microbiological laboratory printouts from all HD3 hospitals and reference laboratories. Laboratory audits were performed approximately every two weeks to evaluate reporting accuracy and identify cases not originally reported.

Microbiologic and Molecular Characterization of MRSA

All laboratories in the Atlanta EIP catchment area were asked to submit the initial MRSA isolate to the GA EIP laboratory. Isolates were typed by PFGE as previously described [5, 21]. Further characterization was performed to determine the SCC*mec* type, staphylococcal protein A (*spa* type), and presence of staphylococcal enterotoxin A or B (*sea* and/or *seb*) for all PFGE

type USA500 isolates. The SCC*mec* type was determined by multiplex PCR described by Milheirico et al [22]. *Spa* typing was performed as previously described [23]. *Spa* DNA fragments were sequenced by Beckman Coulter Genomics (Danvers, MA) and analyzed by Bionumerics v. 5.10 (Applied Maths; Austin, TX). The presence of staphylococcal enterotoxin A or B and SCC*mec* IVa was determined by conventional PCR with primers previously described by Limbago et al [3]. Further laboratory characterization of USA100, including PFGE and SCC*mec* typing, was performed at the CDC [3]. USA500 was defined as SCC*mec* IV (not IVa), *spa* type belonging to clonal complex 8, and staphylococcal enterotoxin A and B negative [8]. USA100 was defined by PFGE typing and/or by the presence of SCC*mec* II [24].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed at the CDC using the reference broth microdilution method [25] for susceptibility to vancomycin, trimethoprim-sulfamethoxazole (TMP-SMX), clindamycin, doxycycline, and mupirocin using MIC plates prepared in-house at the CDC [3].

Detection of Staphylococcal Virulence Toxins

The presence of genes encoding Panton-Valentine leukocidin (PVL), a pore-forming toxin that leads to neutrophil destruction, toxic shock syndrome toxin 1 (TSST-1), and staphylococcal enterotoxins A, B, C, D, E, and H (SEA-SHE) was assessed by conventional PCR [3].

Demographic, Clinical, and Outcome Variables

A standardized medical record chart abstraction was performed by trained epidemiologists at the GA EIP using a case report form to record demographic data,

comorbidities, clinical syndromes, and outcome data. 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 reports. Demographic data included age, race, and epidemiologic classification. Cases were classified epidemiologically as hospital-onset (HO), healthcare-associated community-onset (HACO) or community-associated (CA) based on abstracted information and predefined criteria. HO was defined as MRSA isolated from a culture obtained more than 3 calendar days after admission. HACO was defined as a positive culture obtained in the outpatient setting or within 3 calendar days of hospital admission and the case had documented healthcare exposures. Healthcare exposures were defined as having one or more of the following: presence of a central venous catheter (CVC) at presentation, history of MRSA infection or colonization, surgery, dialysis, or residence in a long-term care facility within a year before index culture date, or culture obtained > 48 hours after admission to hospital. CA cases lacked any of the above healthcare risk factors and the illness onset occurred in the community or <48 hours after admission.

The presence of the following comorbidities was based on their inclusion in the medical chart: alcohol use, intravenous drug use (IVDU), tobacco use, diabetes mellitus, and HIV/AIDS. Coronary artery disease included history of myocardial infarction. Chronic renal insufficiency was defined as a creatinine level of 1.6mg/dl or higher and included end stage renal disease on dialysis but excluding acute renal failure/insufficiency. Chronic pulmonary disease included chronic obstructive pulmonary disease, chronic bronchitis, asthma or cystic fibrosis. Chronic skin breakdown was defined as individuals with decubitus ulcers, eczema, psoriasis, diabetic ulcers, or chronic wounds. Congestive heart failure also included cardiomyopathy of any cause. Malignancy included presence of solid organ or hematologic malignancy. Cerebral vascular accident included history of strokes and transient ischemic attack. Peripheral vascular disease

was defined as disease of blood vessels outside the brain and heart. Chronic liver disease included cirrhosis, chronic liver failure, or chronic hepatitis from hepatitis B or hepatitis C. Connective tissue disorder was defined as presence of systemic lupus erythematous, rheumatoid arthritis, polymyositis, polymyalgia rheumatica, dermatomyositis, systemic sclerosis, or mixed connective-tissue disease.

All cases in this study had a diagnosis of bacteremia, defined as an individual with a positive blood culture for MRSA. Bacteremia can be associated with a variety of clinical syndromes and these were recorded based on their documentation in the medical record. Cases could be designated as having more than one clinical syndrome when appropriate. The clinical syndrome of pulmonary infection included pneumonia, tracheitis or empyema. Central line-associated bloodstream infections were defined as a case of primary MRSA bacteremia (i.e. no evidence of infection at a site other than blood) in a patient with a central venous catheter in place at the time of or within two calendar days before collection of initial MRSA culture. Endocarditis included all cases of prosthetic and native valve endocarditis. The clinical syndrome of bone or joint infection included all cases of septic arthritis, bursitis, tenosynovitis, discitis, or osteomyelitis of any bony structure. The clinical syndrome of deep tissue abscess included all cases with an internal body site abscess. Surgical site infection included cases marked as either internal surgical site infection or surgical incision site infection. The clinical syndrome of skin and soft tissue infection (SSTI) included all cases of cellulitis, traumatic wound infection, pressure ulcer-related infections or any infection involving soft tissue structures. Urinary tract infection (UTI) included pyelonephritis and subsequent documentation of a positive urine culture for MRSA. All cases without any associated clinical syndrome or evidence of infection at another site were grouped together into a category named bacteremia without focus. The clinical syndromes were organized into primary and secondary importance. Clinical

syndromes of primary importance were those that have previously been associated with mortality in MRSA bacteremia, which included pulmonary infection, central line-associated bloodstream infection, and bacteremia without associated clinical syndrome. Skin or soft tissue infection, bone or joint infection, endocarditis, urinary tract infection, deep tissue abscess, and surgical site infection were of secondary importance.

Measurement of severity of illness included surrogates such as septic shock and ICU admission prior to index culture. Septic shock was defined by the recording of septic shock or symptoms associated with septic shock in the medical chart. ICU admission prior to index culture was defined by the location of the patient at the time of the first positive blood culture for MRSA.

The primary outcome was attributable death, defined as death within 7-days of index culture or death with a persistent focus of infection (death within 7-days of a positive MRSA culture from other sterile site). Individuals not meeting these criteria, including those who survived, died more than 7-days from index culture, or died more than 7-days after a positive MRSA culture from another sterile site, served as the comparison group. In-hospital mortality was the secondary outcome, defined as death occurring during index hospitalization.

Causal Pathway

The exposure of interest was MRSA strain type (USA500 vs USA00) and the primary outcome was attributable mortality (Figure 1). There were several intermediate variables that were placed within the causal pathway of importance: bacteremic clinical syndromes, antibiotic resistance, and presence of select virulence toxins. Certain clinical syndromes have been associated with increased mortality [6, 16] and other studies have suggested that antibiotic resistance (elevated vancomycin MICs) [26, 27] and specific virulence toxins lead to increased

mortality [28]. These variables are can exert an effect on mortality and therefore it was important to determine the distribution of the intermediate variables among USA500 and USA100 MRSA bacteremia. Potential confounders are variables that were associated with both MRSA strain type (USA500 vs USA100) and mortality.

Sample Size Calculation

Kempker et al demonstrated a 20% difference in mortality within 7-days between USA300 and non-USA300 strains in cases of bacteremia [6]. The assumption was made that USA500 will behave similarly to USA300 given data demonstrating similar virulence of USA500 and USA300 in *in vitro* and animal models. In order to detect a more conservative 15% difference between USA500 and USA100, to achieve a power of 80% and α=0.05, and with a ratio of 6:1 of USA100 to USA500 cases based on prior literature, 75 cases of USA500 and 453 cases of USA100 are needed.

Database Management

Data was entered into a Microsoft Access 2007 database (Microsoft Corp., Redmond, WA) and statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

Analytic Plan

Descriptive statistics were used to compare clinical characteristics (age, gender, epidemiologic classification, and outcomes) among the cases of MRSA bacteremia where isolates were available for strain typing to cases where an isolate was unavailable. The purpose of this comparison was to determine if bacteremia cases with strain typing were representative of the overall cohort of bacteremia cases.

To compare differences between bacteremia due to USA500 and USA100, a bivariate analysis was performed to compare the association of MRSA strain type with covariates (including demographics, comorbidities, clinical syndromes, antimicrobial susceptibility, virulence toxins, and outcomes). Other bivariate analyses were performed to determine the association of the primary outcome (attributable mortality) with covariates (including MRSA strain type, demographics, comorbidities, clinical syndromes, antimicrobial susceptibility). Differences in proportions of categorical variables were analyzed using chi-square test. Normality assumptions were evaluated for the continuous variable of age using histograms, normality plot, skewness and kurtosis values between -1.0 and 1.0. A two sample pooled t-test was used to evaluate the difference in mean age between the two groups. All reported p-values are two-sided, those with values <0.05 were considered significant.

Effect modification of the association of MRSA strain type (USA500 vs USA100) with attributable mortality was evaluated for a select number of plausible variables (age, race, healthcare-associated, HIV/AIDS, and malignancy). Interaction was defined by Breslow-Day test for homogeneity p-value <0.1.

Collinearity analysis was performed on all eligible variables for multivariable models using SAS Macro for diagnostics of nonlinear models using condition indices and variance decomposition proportions [29].

Logistic regression models were built based on purposeful selection of covariates and not on statistical algorithms. For the first model, the purpose was to determine the adjusted association of MRSA strain (USA500 versus USA100) with attributable mortality. Eligible variables for inclusion were those with p-value <0.25 in the bivariate analysis, excluding intermediate variables that are in the causal pathway (MRSA strain types may exert their effect on mortality through these variables). Confounding variables were defined by a ≥ 10% change in

the coefficient estimate of the exposure of interest, and were included in the multivariable model regardless of p-values. A second logistic regression model to evaluate predictors of attributable mortality in MRSA bacteremia was built in similar fashion as described above, with the exception that the intermediate variables were eligible for inclusion.

IRB Approval

The population-based MRSA surveillance study has been approved by the Emory University and the Georgia Department of Public Health institutional review boards, the Atlanta VA Research and Development Committee and the Grady Memorial Hospital Research Oversight Committee.

RESULTS

Comparison of Nested Cohort with MRSA Strain Typing to Overall Cohort

Among the overall MRSA bacteremia cohort, 26.5% (1,904/7181) had isolates sent for further characterization from January 1, 2005 to December 31, 2011. The collection of isolates was performed by a convenient sample. Appendix A contains a table comparing selected variables between MRSA bacteremia cases where an isolate was available for typing and cases without an isolate. Age and gender were not significantly different between the two groups; however, epidemiologic classification differed: hospital onset comprised 21.0% of the strain typing cohort compared to 0.4% of the other cohort. There were 227 (11.9%) attributable deaths in cases with strain typing and 629 (11.9%) attributable deaths in cases without strain typing (P-value = 1.0).

Comparison of USA500 versus USA100

A total of 107 USA500 and 608 USA100 cases of bacteremia were identified during the study period. Patients with USA500 were more likely to be black (72.4% versus 56.3%, P=0.005) and have a differing epidemiologic classification than USA100. USA500 were more often HACO than HO (70.1% versus 62.3%, P=0.02) when compared to USA100 (Table 1). There were no significant differences in age and gender between the two strain types.

HIV/AIDS was present in 21.5% of USA500 cases compared to only 2.6% of USA100 (OR 10.13, 95% CI 5.14 – 19.95, P<0.0001). There were no other statistically significant differences in comorbidities between USA500 and USA100, though chronic liver disease (6.5% versus 3.1%, P= 0.09) and IVDU (1.9% versus 0.2%, P=0.06) tended to be more common in those with USA500 infection (Table 1).

Bacteremia without focus and central line-associated bloodstream infection were the most common clinical syndromes for USA500 and USA100 MRSA cases. Other than surgical site infection, which was more frequently associated with USA100 than USA500 (5.9% versus 0.0%, P=0.004), the remainder of the clinical syndromes did not differ between the two strains (Table 2). There were no significant differences in severity of disease as measured by the proportion of cases with septic shock and ICU admission prior to index culture between USA500 and USA100.

Ninety-seven of the 107 USA500 isolates were available for antimicrobial susceptibility testing. Figure 2 shows the proportion of USA500 and USA100 isolates with antimicrobial resistance to selected antibiotics used for treatment of MRSA infections. Vancomycin is the antibiotic of choice for treatment of MRSA bacteremia. Overall, USA500 isolates had lower vancomycin MICs than USA100: 30.9%, 68.0%, and 1.0% of USA500 isolates had MICs of 0.5 μg/ml, 1 μg/ml, and 2 μg/ml, respectively, compared to 12.2%, 84.1%, and 3.8% of USA100 isolates. A higher proportion of USA100 isolates had vancomycin MICs ≥1 μg/ml than USA500 (87.8% versus 69.1%, P<0.0001) and clindamycin resistance (55.1% versus 22.7%, P<0.0001). Trimethoprim-sulfamethoxazole resistance was much more common in USA500 than USA100 (90.7% versus 1.2%, P<0.0001). A mupirocin MIC of >4 was more likely in USA500 isolates than USA100 (20.6% versus 5.6%, P<0.0001).

The isolates were evaluated for the presence of selected toxins (Table 3). Only 1 USA500 isolate contained the PVL gene and 2 isolates had TSST-1; neither was present in USA100 isolates. The only staphylococcal enterotoxin identified in this cohort was SED, which was present in 3.5% of USA100 isolates and none in USA500 (P=0.04).

There were a total of 87 attributable deaths in the cohort with no significant difference between USA500 and USA100 (8.4% versus 12.8%, P=0.20). Table 4 compares mortality in patients with USA500 and USA100 MRSA bacteremia by categories. For attributable mortality,

death within 7-days was the primary driver for both USA500 and USA100. Overall in-hospital mortality was not significantly different between the two strains (16.0% versus 20.9%, P=0.25).

Multivariable Analysis

A bivariate analysis was performed to calculate unadjusted odds ratios for the association of covariates with attributable mortality. The results are summarized on Tables 5 and 6. The main exposure variable, MRSA strain type (USA500 versus USA100) was not significantly associated with attributable mortality (OR 0.62, 95% CI 0.30-1.29). The following factors were significantly associated with attributable mortality: age > 55 years (OR 2.14, 95% CI 1.23-3.73), residence in LTCF facility in the prior year (OR 1.84, 95% CI 1.17-2.91), malignancy (OR 1.84, 95% CI 1.04-3.25), chronic pulmonary disease (OR 1.66, 95% CI 1.00-2.77), bacteremia without focus (OR 1.90, 95% CI 1.21-2.98), septic shock (OR 7.25, 95% CI 3.92-13.41), and ICU admission prior to index culture (OR 3.27, 95% CI 1.51 – 7.09). The association of pulmonary infection (OR 1.80, 95% CI 0.99-3.26) with attributable mortality trended towards being significant. The only factor found to be protective against attributable mortality was presence of a central line-associated bloodstream infection (OR 0.46, 95% CI 0.27-0.81).

No evidence of effect modification was observed regarding the association of MRSA strain (USA500 versus USA100) with attributable mortality for the following biologically plausible factors: age > 55 years, black race, healthcare-associated versus community-associated, HIV/AIDS, or malignancy (Table 7).

A multivariable logistic regression model was constructed to estimate the association of MRSA strain type (USA500 versus USA100) with attributable mortality to adjust for confounders using a purposeful selection strategy. Candidate variables are listed in Appendix B. Diagnostics were performed to detect collinearity among eligible variables (Table 8). No variables were

found to be collinear, including age > 55 and residence in LTCF in the prior year. After adjusting for confounding variables, USA500 was not associated with increased risk of attributable mortality compared to USA100 (aOR 0.57, 95% CI 0.26-1.22). Table 9 lists the results of the other variables included in the model.

In order to evaluate the most important predictors of attributable mortality, a second multivariable logistic model was constructed that included the intermediate variables of clinical syndromes, antimicrobial resistance, and presence of selected virulence toxins in the evaluation. MRSA strain type (USA500 versus USA100) was also included in the model. Candidate variables are listed in Appendix C. Collinearity diagnostics of eligible variables for the predictive model were performed and no variables were found to be collinear, including septic shock and ICU admission (Table 10). Important predictors that increased the risk of attributable mortality were septic shock (aOR 7.36, 95% CI 3.88-13.97), ICU admission prior to index culture (aOR 3.21, 95% CI 1.39-7.40), and age > 55 years (aOR 1.99, 95% CI 1.11-3.53). Central line-associated bloodstream infection was found to be protective against attributable mortality (aOR 0.46, 95% CI 0.26-0.82). MRSA strain USA500 was not found to be associated with attributable mortality (aOR 0.67, 95% CI 0.31-1.42) (Table 11).

DISCUSSION

We did not detect a significant difference in attributable mortality between USA500 and USA100 MRSA strains in individuals with bacteremia in both bivariate and multivariable analyses. To our knowledge, this is the first study to evaluate clinical outcomes of the two most common strains causing HA-MRSA infections in the US. Additionally, we described the epidemiologic, microbiologic, and clinical characteristics of bacteremia due to USA500 MRSA and compared it with USA100.

The results of this study suggest that there may not be a difference in clinical virulence between these two strains, contrary to *in vitro* and animal studies. Li et al previously demonstrated that USA500 MRSA caused a higher degree of *in vitro* neutrophil lysis, larger skin abscess and higher blood levels of TNF-α and IL-8 in a rabbit abscess model compared to USA100 [7]. Our surrogate for clinical virulence was the measurement of attributable mortality, defined as death within 7-days of index culture or death with a persistent focus of infection (death within 7-days of a positive MRSA culture from other sterile site). Attributable mortality was selected as the primary outcome instead of in-hospital mortality to account for other competing causes of death that may occur during a hospitalization that are likely unrelated to MRSA infection. Similar definitions of attributable mortality have been used in other publications investigating outcomes in MRSA infections. Time-to-death was used as the primary outcome in a study of MRSA bacteremia and death within 7-days was also reported [6]. The definition of severe illness due to MRSA in a study by Hota et al included death within 1-week of admission [20]. Lastly, in a study of complicated *Staphylococcus aureus* bacteremia, the definition of attributable mortality included patients who died with persistent signs and symptoms of systemic infection, positive blood culture results, or persistent focus of infection [30]. In our study, the majority (>75%) of patients met the definition of attributable death

because they died within 7-days of index culture and the remainder were due to persistent focus of infection. In-hospital mortality was used as a secondary outcome for a sensitivity analysis of our definition of attributable death. There was not a significant difference for in-hospital mortality between USA500 and USA100 MRSA bacteremia in bivariate analysis.

Two multivariable logistic regression models were built to assess the association of MRSA strain type (USA500 versus USA100) with attributable mortality in bacteremia. Table 9 shows the results of the model based on the causal diagram (Figure 1), in which the intermediate variables are not eligible for inclusion in the model. Although ICU admission prior to index culture may function as an intermediate variable in the causal pathway, meaning that individuals are in the ICU due to a severe MRSA bacteremia, it may also function as a confounder where sicker individuals are already in the ICU for other reasons and subsequently develop MRSA bacteremia. Therefore it was included in the multivariable model to estimate the adjusted association of USA500 and USA100 with attributable mortality. In this model, USA500 was not significantly associated with attributable mortality and the direction of the adjusted odds ratio was opposite of expected. The results suggest that USA100 tends to have higher attributable mortality than USA500 MRSA. The second model (Table 11) is a predictive model of attributable mortality in MRSA bacteremia that utilizes the eligible intermediate variables. MRSA strain type was kept in the model to determine if the addition of the intermediate variables changed its association with the outcome. With the addition of strong predictors, the adjusted odds ratio for USA500 moved toward the null, however it was not statistically significant.

Several risk factors in the predictive multivariable model were associated with higher risk of attributable death in individuals with MRSA bacteremia. Septic shock was the strongest predictor for attributable mortality, a finding that has been demonstrated in multiple studies [6,

16, 27, 31, 32]. ICU admission prior to index culture and age > 55 years were also important predictors in our study as were previously demonstrated in other studies [13, 14].

The clinical syndrome of central line-associated bloodstream infection was found to be protective in MRSA bacteremia. Chen et al also found similar results in a multivariable analysis of community onset *Staphylococcus aureus* (SA) bacteremia, where the presence of vascular device-related infection was associated with a decreased risk of death [33]. A likely explanation is that central line-associated infections involve an easily removable focus. Removal of infectious foci in *Staphylococcus aureus* bacteremia is important and it has been demonstrated that the individuals with eradicable foci have improved outcomes [15, 32].

Because USA500 and USA100 are both predominantly healthcare-associated MRSA strains, it is not surprising that they shared many demographic and clinical characteristics in common. The few notable exceptions are that USA500 MRSA cases of bacteremia were more likely to occur in blacks, in those with HIV/AIDS, and those that were healthcare-associated community onset infections. Both strains caused similar bacteremic clinical syndromes, with the only difference being that surgical site infection was more common in USA100. Of these differences, the most striking was the high proportion of USA500 with HIV/AIDS (21.5% versus 2.6%).

Antimicrobial susceptibilities for numerous antibiotics differed between USA500 and USA100 isolates. Vancomycin is the treatment of choice for MRSA bacteremia and overall USA500 had lower MICs than USA100. A recent systematic review demonstrated that elevated vancomycin MICs were associated with worse patient outcomes including increased mortality [26]. Wi et al found that at a vancomycin MIC ≥1 μg/ml was an independent predictor of 30-day mortality in patients with MRSA bacteremia [27]. However, we did not find an increased risk of attributable mortality in individuals with vancomycin MIC ≥1 μg/ml on bivariate analysis.

Clindamycin, doxycycline, and trimethoprim-sulfamethoxazole (TMP-SMX), are common antibiotics used to treat other types of MRSA non-invasive infections, such as isolated skin and soft tissue infections. Clindamycin resistance was more common in USA100 and there was not a significant difference in doxycycline resistance between the two strains. A striking difference in TMP-SMX resistance was noted between USA500 and USA100 MRSA. Approximately 91% of USA500 isolates were resistant to TMP-SMX, compared to only 1.2% of USA100 isolates. Given the high prevalence of HIV/AIDS in USA500 MRSA infections, the finding of near universal TMP-SMX resistance among USA500 strains suggests a potentially interesting association. Patients with HIV/AIDS are often prescribed TMP-SMX for prevention of pneumocystis pneumonia if they have CD4 count ≤ 200 cells/μL, CD4 percentage < 14%, history of oropharyngeal candidiasis, or history of AIDS-defining illness [34] and they may be on TMP-SMX for prolonged periods of time. It raises the question of whether prolonged TMP-SMX use in HIV/AIDS patients may increase the risk for already TMP-SMX resistant USA500 MRSA infections in these patients. Alternatively, patients with HIV/AIDS may become colonized or infected with USA500 for other unknown reasons and prolonged exposure to TMP-SMX may lead to the development of resistance in USA500. The data suggest the former as the high prevalence of TMP-SMX resistance in USA500 MRSA does not appear to be completely explained by the 21.5% of HIV/AIDS infected individuals with USA500 MRSA bacteremia.

Mupirocin is a topical antibiotic applied intranasally that is often used in MRSA decolonization strategies [35]. It is unclear at this time why an elevated mupirocin MIC >4, consistent with low-level mupirocin resistance [36], is more common in USA500 MRSA isolates than USA100. It is unknown whether patients with HIV/AIDS are prescribed topical mupirocin more often for MRSA decolonization due to their increased risk of infection.

The presence of selected toxins was uncommon for both USA500 and USA100 MRSA and therefore unlikely to be important determinants of virulence in these infections. TSST-1, the toxin responsible for toxic shock syndrome that carries high mortality in adults, was found in only two USA500 isolates. Staphylococcal enteroxin D (SED), an exoprotein with emetic properties that contributes to staphylococcal food poisoning [37], was only found in a small number of USA100 isolates. In a study by Descachy et al, SED was not associated with mortality in *Staphylococcus aureus* bacteremia [38].

Our study has the strength of analyzing population-based data that has been collected prospectively, via active systematic methods, of a large cohort under surveillance in metropolitan Atlanta. Due to the high proportion of USA500 MRSA bacteremia cases in Atlanta, this was a unique opportunity to investigate the second leading HA-MRSA strain in the US. We also performed a detailed characterization and categorization of USA500 MRSA isolates using multiple molecular typing methods not available in clinical laboratories.

The study does have several limitations to note. First, the study population of USA500 and USA100 arises from a nested cohort of all cases of MRSA bacteremia where an isolate was available for further strain typing. Convenience sampling of cases was used and may introduce selection bias as the nested cohort may be different from the overall cohort. We compared the nested cohort of cases with MRSA strain typing to the cases without strain typing in the overall cohort. There were no significant differences in important variables such as age, gender, or attributable mortality between the two groups. A significant difference was found in the epidemiologic classification of hospital onset, where it was more common in the group with MRSA strain typing. A likely explanation for this finding is that retrieval of MRSA isolates by EIP staff is easier in hospital onset cases because the index blood culture is readily available in the hospital microbiology lab. Overall, based on this comparison, the study population is generally

representative of the overall cohort. A second limitation is that the findings of this study are applicable only to MRSA bacteremia, and not to other types of infections caused by MRSA. However, it is unlikely that an evaluation of all MRSA clinical syndromes would produce different results as we did not detect a difference in clinical virulence between USA500 and USA100 MRSA in patients with bacteremia, an invasive and common form of infection. Third, our sample size calculations were based on minimal data regarding the differences in mortality in MRSA bacteremia among MRSA strains and therefore our study was powered to detect a 15% difference in mortality at 7-days. We did not detect a difference in attributable mortality between USA500 and USA100; however it remains possible that a smaller difference may exist.

Future Directions

As highlighted previously, USA500 MRSA varies geographically and it comprises a significant proportion of MRSA infections in the metropolitan Atlanta area [6]. It is currently unknown why USA500 is a predominant strain in this geographic area. The association with HIV/AIDS may be a contributing factor, however future studies are needed to further investigate this issue. Full genomic analysis of USA500 MRSA strains from the Atlanta area and comparison to USA500 strains from other EIP sites may help elucidate the origins of USA500. Additionally, full genomic analysis would allow for a complete evaluation of the presence of virulence factors and identification of all resistance genes in USA500 MRSA.

Low-level mupirocin resistance in approximately 20% of USA500 MRSA isolates was an unexpected finding in our study. Given that MRSA decolonization strategies using topical mupirocin are becoming more common in healthcare-settings, a better understanding of the trends, mechanisms, and outcome of resistance is needed.

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TABLES AND FIGURES

Figure 1. Causal pathway

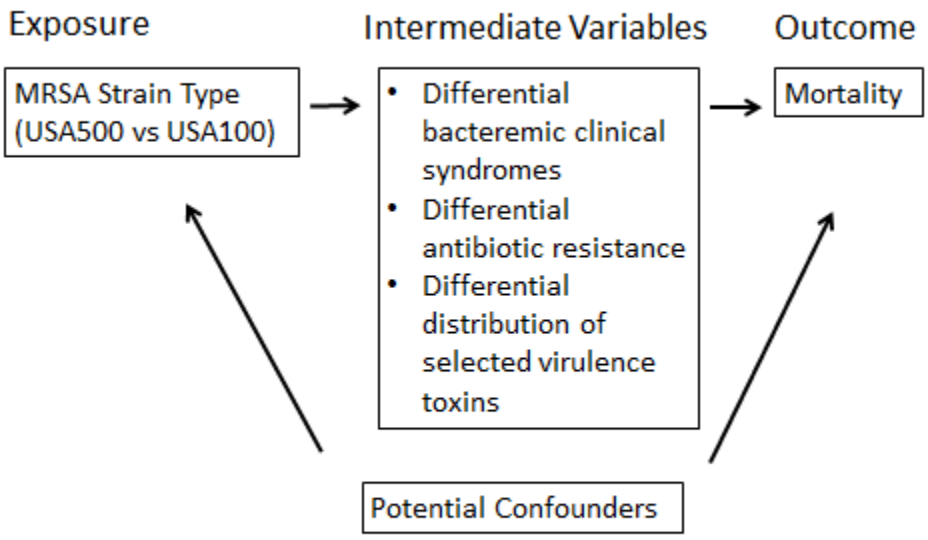


Table 1. Comparison of demographics, epidemiologic and comorbidities in patients with USA500 and USA100 methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia

Characteristic	USA500 (n=107)[a]	USA100 (n=608)[a]	P-value[b]
Age, in years, mean (SD)	60.8 (16.1)	62.2 (17.7)	0.46[c]
Male	62 (57.9)	319 (52.5)	0.30
Race[e]			0.005
White	29 (27.6)	255 (43.7)	-
Black	76 (72.4)	329 (56.3)	-
Residence in LTCF in year prior to infection	33 (30.8)	191 (31.4)	0.91
Epidemiologic Classification			0.02
Community-associated	13 (12.1)	48 (7.9)	-
Healthcare-associated community onset	75 (70.1)	379 (62.3)	-
Hospital onset	19 (17.8)	181 (29.8)	-
Comorbidities			
Diabetes mellitus	46 (43.0)	290 (47.7)	0.37
Chronic renal insufficiency	38 (35.5)	244 (40.1)	0.37
Chronic pulmonary disease	26 (24.3)	115 (18.9)	0.20
HIV/AIDS	23 (21.5)	16 (2.6)	<0.0001
Chronic skin breakdown	20 (18.7)	101 (16.6)	0.60
Congestive heart failure	19 (17.8)	129 (21.2)	0.42
Malignancy	13 (12.2)	83 (13.7)	0.67
Coronary artery disease	13 (12.2)	107 (17.6)	0.16
Cerebrovascular accident	11 (10.3)	85 (14.0)	0.30
Tobacco Use	10 (9.4)	56 (9.2)	0.96
Peripheral vascular disease	9 (8.4)	64 (10.5)	0.51
Chronic liver disease	7 (6.5)	19 (3.1)	0.09[d]
Alcohol	3 (2.8)	13 (2.1)	0.72[d]
IVDU	2 (1.9)	1 (0.2)	0.06[d]
Connective tissue disorder	2 (1.9)	10 (1.6)	0.70[d]

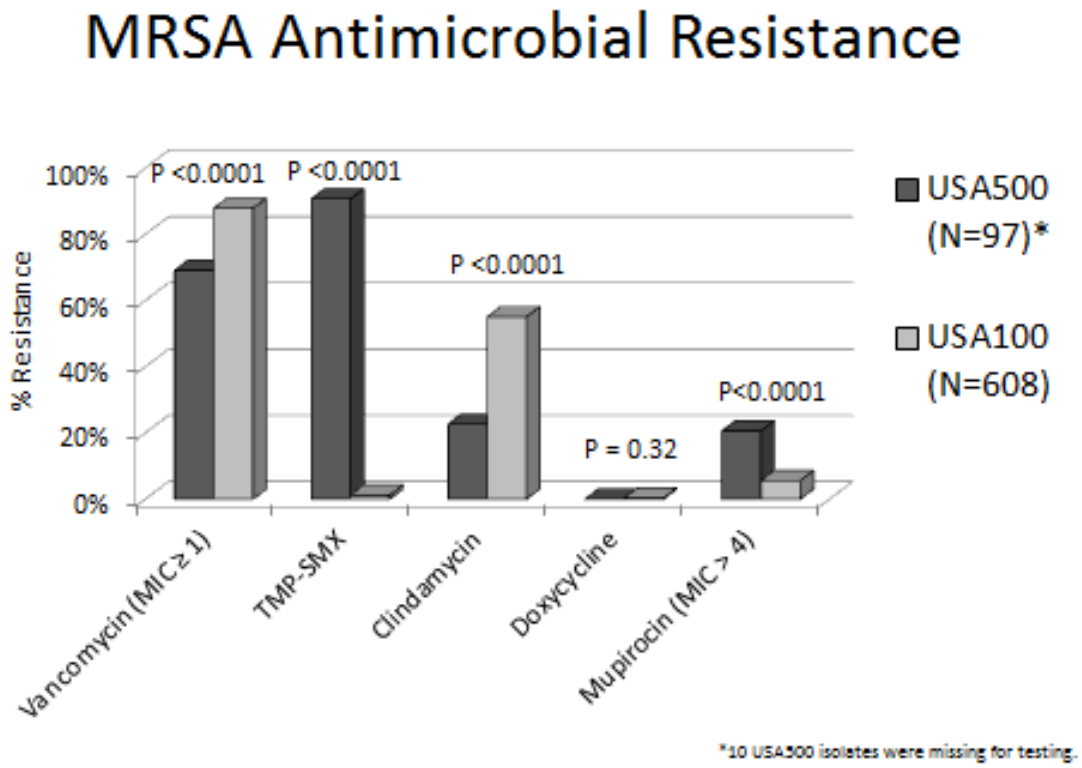
Abbreviations: SD, standard deviation; LTCF, long-term care facility; IVDU, intravenous drug use.
[a] Data are No. (%) of participants unless otherwise indicated.
[b] Chi-square Test; α=0.05.
[c] Two-sided pooled T-test.
[d] Fisher's Exact Test, two-tailed p-value.
[e] 3.6% of overall population was of other race (Asian, American Indian, or Unknown).

Table 2. Comparison of clinical syndromes and severity of illness in patients with USA500 and USA100 MRSA bacteremia

Characteristic	USA500 (n=107)[a]	USA100 (n=608)[a]	P-value[b]
Clinical syndromes			
Bacteremia without focus	41 (38.3)	208 (34.2)	0.41
Central line-associated bloodstream infection	35 (32.7)	198 (32.6)	0.98
Urinary tract infection	11 (10.3)	52 (8.6)	0.56
Pulmonary infection	10 (9.3)	76 (12.5)	0.36
Skin and soft tissue infection	10 (9.3)	63 (10.4)	0.75
Bone or joint infection	7 (6.5)	39 (6.4)	0.96
Endocarditis	5 (4.7)	30 (4.9)	0.91
Deep tissue abscess	3 (2.8)	8 (1.3)	0.22[c]
Surgical site infection	0 (0.0)	36 (5.9)	0.004[c]
Severity of illness			
Septic shock	5 (4.7)	45 (7.5)	0.31
ICU admission prior to index culture	4 (3.8)	30 (5.0)	0.59

Abbreviations: ICU, intensive care unit.
[a] Data are No. (%) of participants.
[b] Chi-square Test; α=0.05.
[c] Fisher's Exact Test, two-tailed p-value.

Figure 2. Antimicrobial resistance among USA500 and USA100 MRSA isolates in patients with bacteremia



Abbreviations: MIC, minimum inhibitory concentration; TMP-SMX, trimethoprim-sulfamethoxazole

Table 3. Toxin distribution among USA500 and USA100 MRSA isolates in patients with bacteremia

Toxin	USA500 (n=97[a])[b]	USA100 (n=608)[b]	P-value[c]
PVL	1 (0.9)	0 (0)	0.15
TSST-1	2 (2.0)	0 (0)	0.02
SEA	0 (0)	0 (0)	-
SEB	0 (0)	0 (0)	-
SEC	0 (0)	0 (0)	-
SED	0 (0)	21 (3.5)	0.04
SEE	0 (0)	0 (0)	-

Abbreviations: PVL, Panton-Valentin leukocidin; TSST-1, toxic shock syndrome toxin 1; SEA-SEE, Staphylococcal enterotoxin A-E.

[a] 10 USA500 isolates were missing for testing

[b] Data are No. (%) of participants.

[c] Fisher's Exact Test, two-tailed p-value.

Table 4. Comparison of outcomes in patients with USA500 and USA100 MRSA bacteremia

Outcome	USA500 (n=107)[a]	USA100 (n=608)[a]	P-value[b]
Attributable mortality[c]	9 (8.4)	78 (12.8)	0.20
Death within 7-days	9 (8.4)	58 (9.5)	-
MRSA + culture from other sterile site within 7-days of death	0 (0.0)	20 (3.3)	-
In-hospital mortality[d]	17 (16.0)	126 (20.9)	0.25

[a] Data are No. (%) of participants.

[b] Chi-square Test; α=0.05.

[c] Defined as death within 7-days of index culture or MRSA cultured from other sterile site within 7-days of death.

[d] Missing data on 6 patients for in-hospital mortality: USA500 (n=106); USA100 (n=603).

Table 5. Bivariate analysis of strain type, demographics, antimicrobial susceptibility, and comorbidities for attributable mortality in patients with MRSA bacteremia

Risk Factor	Attributable Death[a] (N=87)[c]	Comparison[b] (N=628)[c]	P-value[d]	OR (95% CI)[e]
USA500 vs USA100	9 (4.1)	98 (6.1)	0.20	0.62 (0.30-1.29)
Age > 55 years	70 (80.5)	413 (65.8)	0.006	2.14 (1.23-3.73)
Female	43 (49.4)	291 (46.3)	0.59	1.13 (0.72-1.77)
Race – Black[g]	45 (51.7)	360 (57.3)	0.32	0.80 (0.51-1.25)
Epidemiologic Classification				
HA vs CA	82 (94.3)	572 (91.1)	0.32	1.61 (0.62-4.13)
Residence in LTCF in the prior year	38 (43.7)	186 (29.6)	0.008	1.84 (1.17-2.91)
Vancomycin susceptibility (MIC ≥1 vs 0.5)	74 (87.1)	527 (85.0)	0.62	1.19 (0.61-2.32)
Comorbidities				
Malignancy	18 (20.7)	78 (12.4)	0.03	1.84 (1.04-3.25)
Alcohol	3 (3.5)	13 (2.1)	0.43[f]	1.69 (0.47-6.05)
Chronic pulmonary disease	24 (27.6)	117 (18.6)	0.049	1.66 (1.00-2.77)
Congestive heart failure	24 (27.6)	124 (19.8)	0.09	1.55 (0.93-2.58)
Coronary artery disease	19 (21.8)	101 (16.1)	0.18	1.46 (0.84-2.53)
Chronic liver disease	4 (4.6)	22 (3.5)	0.54[f]	1.33 (0.45-3.95)
Peripheral vascular disease	11 (12.6)	62 (9.9)	0.42	1.32 (0.67-2.62)
Cerebrovascular accident	11 (12.6)	85 (13.5)	0.82	0.93 (0.47-1.81)
Chronic renal insufficiency	32 (36.8)	250 (39.8)	0.59	0.88 (0.55-1.40)
Chronic skin breakdown	13 (14.9)	108 (17.2)	0.60	0.85 (0.45-1.58)
HIV/AIDS	4 (4.6)	35 (5.6)	0.71	0.82 (0.28-2.36)
Diabetes mellitus	36 (41.4)	300 (47.8)	0.26	0.77 (0.49-1.22)
Tobacco Use	4 (4.6)	62 (9.9)	0.11	0.44 (0.16-1.24)
IVDU	0 (0)	3 (0.5)	1.00[f]	-
Connective tissue disorder	0 (0)	12 (1.91)	0.38[f]	-

Abbreviations: MIC, minimum inhibitory concentration; LTCF, long-term care facility; HA, healthcare-associated; CA, community-associated; IVDU, intravenous drug use.

[a] Death within 7-days of initial culture or MRSA positive culture from other sterile site within 7-days of death.

[b] Survive or death occurring after 7-days of index or other sterile site MRSA culture.

[c] Data are No. (%) of participants.

[d] Chi-square Test; α=0.05.

[e] Mantel-Haenzel odds ratio, 95% confidence interval.

[f] Fisher's Exact Test, two-tailed p-value.

[g] Comparison to Other (includes White, Asian, American Indian, and Unknown).

Table 6. Bivariate analysis of clinical syndromes and severity of illness for attributable mortality in individuals with MRSA bacteremia

Risk Factor	Attributable Death[a] (N=87)[c]	Comparison[b] (N=628)[c]	P-value[d]	OR (95% CI)[e]
Clinical syndromes				
Bacteremia without focus	42 (48.3)	207 (33.0)	0.005	1.90 (1.21 – 2.98)
Central line-associated bloodstream infection	17 (19.5)	216 (34.4)	0.006	0.46 (0.27 – 0.81)
Urinary tract infection	9 (10.3)	54 (8.6)	0.59	1.23 (0.58 – 2.58)
Pulmonary infection	16 (18.4)	70 (11.2)	0.05	1.80 (0.99 – 3.26)
Skin and soft tissue infection	6 (6.9)	67 (10.7)	0.28	0.62 (0.26 – 1.48)
Bone or joint infection	2 (2.3)	44 (7.0)	0.09	0.31 (0.07 – 1.31)
Endocarditis	4 (4.6)	31 (4.9)	1.00[f]	0.93 (0.32 – 2.70)
Deep tissue abscess	1 (1.2)	10 (1.6)	1.00[f]	0.72 (0.09 – 5.68)
Surgical site infection	3 (3.5)	33 (5.3)	0.61	0.64 (0.19 – 2.15)
Severity of Illness				
Septic shock	22 (25.3)	28 (4.5)	<0.0001	7.25 (3.92 – 13.41)
ICU admission prior to index culture	10 (11.5)	24 (3.8)	0.005[f]	3.27 (1.51 – 7.09)

Abbreviations: ICU, intensive care unit

[a] Death within 7-days of initial culture or MRSA positive culture from other sterile site within 7-days of death.

[b] Survive or death occurring after 7-days of index or other sterile site MRSA culture.

[c] Data are No. (%) of participants.

[d] Chi-square Test; α=0.05.

[e] Mantel-Haenzel odds ratio, 95% confidence interval.

[f] Fisher's Exact Test, two-tailed p-value.

Table 7. Association of attributable mortality and MRSA strain type (USA500 vs USA100) controlling for selected covariates

Variable	Stratum Specific OR		Adjusted OR[a]	95% CI	P-value for interaction[b]
	OR_1	OR_2			
Age > 55 years	0.72	0.32	0.63	0.30 - 1.30	0.46
Race – Black[c]	0.64	0.66	0.64	0.31 - 1.34	0.97
HA vs CA	0.71	NA	0.64	0.31 - 1.31	0.31
HIV/AIDS	0.67	0.62	0.62	0.29 - 1.32	0.95
Malignancy	0.32	0.71	0.63	0.31 - 1.30	0.49

Crude OR (95% CI): 0.62 (0.30-1.29).

Abbreviations: HA, healthcare-associated; CA, community-associated.

OR_1 is stratum specific odds ratio for those with covariate, OR_0 for those without covariate.

[a] Adjusted OR by Mantel-Haenszel method.

[b] Breslow-Day Test, α=0.05.

[c] Comparison to Other (includes White, Asian, American Indian, and Unknown).

Table 8. Collinearity diagnostics for multivariable logistic regression model for the association of MRSA strain type and attributable mortality in MRSA bacteremia

VARIABLE	VDP 1	VDP 2	VDP 3	VDP 4	VDP 5	VDP 6	VDP 7	VDP 8	VDP 9	VDP 10
EIGENVAL	0.11	0.33	0.61	0.63	0.67	0.85	0.89	0.96	1.01	3.93
CONDINDX	6.05	3.45	2.54	2.49	2.42	2.15	2.10	2.02	1.97	1.00
	.	.	.	.	.	.	.	.	.	.
Intercept	0.87	0.12	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
USA500	0.01	0.03	0.00	0.04	0.04	0.01	0.77	0.10	0.00	0.01
Age > 55	0.81	0.17	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
LTCF Last Year	0.01	0.48	0.00	0.42	0.03	0.00	0.00	0.02	0.01	0.02
ICU Collect	0.01	0.05	0.00	0.02	0.04	0.22	0.15	0.24	0.26	0.01
Smoker	0.01	0.03	0.06	0.07	0.03	0.00	0.03	0.28	0.48	0.00
Malignancy	0.00	0.15	0.04	0.07	0.00	0.64	0.02	0.04	0.03	0.01
Pulmonary Dz	0.00	0.02	0.67	0.13	0.11	0.00	0.00	0.00	0.04	0.02
CHF	0.00	0.09	0.37	0.20	0.19	0.07	0.00	0.05	0.01	0.02
CAD	0.00	0.09	0.01	0.04	0.66	0.01	0.01	0.16	0.02	0.02

Abbreviations: LTCF, long-term care facility; ICU, intensive care unit Dz, disease; PVD, peripheral vascular disease; CHF, congestive heart failure; CAD, coronary artery disease.

Table 9. Multivariable analysis of attributable mortality in individuals with USA500 and USA100 MRSA bacteremia

USA500 and USA100 Bacteremia Cohort (n=715)

Risk Factor	Adjusted OR	95% CI	P-value[a]
USA500 vs USA100	0.57	0.26 – 1.22	0.15
Age > 55 years	1.83	1.01 – 3.33	0.046
Residence in LTCF in the prior year	1.71	1.07 – 2.75	0.03
ICU admission prior to index culture	3.32	1.50 – 7.38	0.03
HIV/AIDS	1.78	0.56 – 5.65	0.33
Malignancy	1.74	0.97 – 3.12	0.07
Chronic pulmonary disease	1.46	0.86 – 2.49	0.16

USA500 vs USA100 Crude OR (95% CI): 0.62 (0.30 - 1.29).
Abbreviations: LTCF, long-term care facility; ICU, intensive care unit
All variables coded (1) for presence of covariate, (0) for absence.
Hosmer and Lemeshow Goodness-of-Fit: p=0.94.
[a] α=0.05.

Table 10. Collinearity diagnostics for multivariable logistic regression predictive model of attributable mortality in MRSA bacteremia

VARIABLE	VDP 1	VDP 2	VDP 3	VDP 4	VDP 5	VDP 6	VDP 7	VDP 8	VDP 9	VDP 10	VDP 11	VDP 12	VDP 13	VDP 14
EIGENVAL	0.07	0.19	0.42	0.57	0.63	0.65	0.76	0.81	0.87	0.89	1.01	1.13	1.17	4.82
CONDINDX	8.12	5.02	3.38	2.90	2.76	2.71	2.52	2.44	2.35	2.33	2.19	2.06	2.03	1.00
Intercept	0.97	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
USA500	0.01	0.00	0.01	0.05	0.00	0.05	0.09	0.05	0.33	0.30	0.02	0.00	0.09	0.01
Age > 55	0.32	0.64	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
LTCF Last Year	0.00	0.06	0.53	0.15	0.14	0.01	0.03	0.04	0.01	0.01	0.00	0.00	0.00	0.01
ICU collect	0.02	0.00	0.06	0.01	0.06	0.01	0.17	0.03	0.09	0.02	0.53	0.01	0.00	0.00
Malignancy	0.00	0.00	0.09	0.06	0.07	0.00	0.29	0.07	0.35	0.00	0.01	0.04	0.01	0.01
Pulmonary Dz	0.00	0.02	0.05	0.18	0.47	0.02	0.16	0.00	0.00	0.07	0.00	0.01	0.00	0.01
CHF	0.01	0.01	0.04	0.47	0.00	0.25	0.01	0.01	0.05	0.10	0.00	0.04	0.00	0.01
CAD	0.00	0.07	0.05	0.03	0.00	0.64	0.01	0.02	0.00	0.03	0.03	0.10	0.02	0.01
Line infection	0.29	0.17	0.07	0.03	0.01	0.04	0.02	0.20	0.00	0.04	0.04	0.01	0.10	0.01
Pulmonary infection	0.17	0.14	0.14	0.01	0.05	0.02	0.12	0.08	0.02	0.02	0.07	0.03	0.12	0.01
Bone/Joint infection	0.06	0.03	0.02	0.00	0.00	0.05	0.02	0.11	0.07	0.24	0.00	0.37	0.01	0.00
Bacteremia without focus	0.47	0.31	0.09	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.09	0.00
Septic shock	0.00	0.01	0.01	0.19	0.27	0.00	0.04	0.23	0.01	0.05	0.09	0.10	0.00	0.01

Abbreviations: LTCF, long-term care facility; ICU, intensive care unit Dz, disease; PVD, peripheral vascular disease; CHF, congestive heart failure; CAD, coronary artery disease.

Table 11. Predictors of attributable mortality in individuals with MRSA bacteremia

USA500 and USA100 Bacteremia Cohort (n=715)

Risk Factor	Adjusted OR	95% CI	P-value[a]
USA500 vs USA100	0.67	0.31 – 1.42	0.32
Septic Shock	7.36	3.88 – 13.97	<0.001
ICU admission prior to index culture	3.21	1.39 – 7.40	0.006
Age > 55 years	1.99	1.12 – 3.53	0.02
Central line-associated bloodstream infection	0.46	0.26 – 0.82	0.008

USA500 vs USA100 Crude OR (95% CI): 0.62 (0.30 - 1.29).
All variables coded (1) for presence of covariate, (0) for absence.
Hosmer and Lemeshow Goodness-of-Fit: p=0.91.
[a] α=0.05.

APPENDIX

Appendix A. Comparison of characteristics between MRSA bacteremia cases with and without strain typing

Characteristic	Strain Typing Cohort (n=1904)[a]	No Strain Typing Cohort (n=5272)[a]	P-value[b]
Age, in years, mean (SD)	55.7 (19.1)	56.5 (20.7)	0.14[c]
Male	1114 (58.5)	2958 (56.1)	0.07
Race[d]			<0.0001
White	590 (31.0)	2022 (38.4)	
Black	1251 (65.7)	2989 (56.7)	
Epidemiologic Classification			<0.0001
Community-associated	348 (18.3)	1124 (21.3)	
Healthcare-associated community onset	1156 (60.7)	4134 (78.3)	
Hospital onset	400 (21.0)	19 (0.4)	
Outcomes			
Attributable mortality[e]	227 (11.9)	629 (11.9)	1.00
In-hospital mortality[f]	341 (18.1)	843 (16.1)	0.15

Abbreviations: SD, standard deviation; LTCF, long-term care facility; IVDU, intravenous drug use.
[a] Data are No. (%) of participants unless otherwise indicated.
[b] Chi-square Test; α=0.05.
[c] Two-sided pooled T-test.
[d] 8.3% of overall population was of other race (Asian, American Indian, or Unknown).
[e] Defined as death within 7-days of index culture or MRSA cultured from other sterile site within 7-days of death.
[f] Missing data on 59 patients for in-hospital mortality: MRSA Strain Typing (n=1888); No Strain Typing (n=5229).

Appendix B. Candidate variables for multivariable analysis of the association of MRSA strain type and attributable mortality in MRSA bacteremia

Laboratory	Demographics	Comorbidities	Severity of Illness
MRSA strain (USA500 versus USA100)	Age > 55 years	HIV/AIDS	ICU admission prior to index culture
	Residence in LTCF in the prior year	Malignancy	
		Chronic pulmonary disease	
		Tobacco Use	
		Congestive Heart Failure	
		Coronary Artery Disease	

Appendix C. Candidate variables for multivariable analysis of predictors of attributable mortality in MRSA bacteremia

Laboratory	Demographics	Comorbidities	Clinical Syndromes	Severity of Illness
MRSA strain (USA500 versus USA100)	Age > 55 years	HIV/AIDS	Septic shock	ICU admission prior to index culture
	Residence in LTCF in the prior year	Malignancy	Central line-associated bloodstream infection	
		Chronic pulmonary disease	Bone or joint infection	
		Tobacco Use	Bacteremia without focus	
		Congestive Heart Failure		
		Coronary Artery Disease		