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Clinical and molecular epidemiology of invasive *Staphylococcus aureus* infection in Utah children; continued dominance of MSSA over MRSA

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Abstract

Background

Invasive *Staphylococcus aureus* infections are a common cause of morbidity and mortality in children. In the early 2000's the proportion of infections due the methicillin-resistant *S. aureus* (MRSA) increased rapidly. We described the clinical and molecular epidemiology of invasive *S. aureus* disease in a pediatric population.

Methods

We prospectively identified children in Utah with invasive *S. aureus* infections. Medical records were reviewed to determine diagnosis and clinical characteristics. Isolates were genotyped using multi-locus sequence typing. The presence of genes encoding the Panton-Valentine leukocidin (PVL) was determined using polymerase chain reaction.

Results

Over a 4-year period between January 2009 and December 2012, we identified 357 children, hospitalized at Primary Children's Hospital, with invasive *S. aureus* infections and isolates available for the study. Methicillin-susceptible *S. aureus* (MSSA) caused 79% of disease, while MRSA caused only 21% of disease. Mortality associated with invasive *S. aureus* infection was 3.6%. The most common diagnoses were osteoarticular infections (38%) followed by central line associated blood stream infections (19%) and pneumonia (12%). We identified 41 multi-locus sequence types. The majority of isolates belonged to 6

predominant clonal complexes (CC5, CC8, CC15, CC30, CC45, CC59). PVL was present in a minority (16%) of isolates, of which most were ST8 MRSA.

Conclusions

MSSA was the primary cause of invasive *S. aureus* infections at our institution throughout the study period. A limited number of predominant strains accounted for the majority of invasive disease. The classic virulence factor PVL was uncommon in MSSA isolates. Further study is needed to improve our understanding of *S. aureus* virulence and disease pathogenesis.

Introduction

Invasive infections with *Staphylococcus aureus* are a significant cause of morbidity and mortality in children [1, 2]. Disease caused by *S. aureus* can range from minor skin and soft tissue infections to severe life-threatening infections with invasion into almost any anatomic site [3, 4]. Epidemiologic studies beginning in the early 2000s reported an increasing burden of invasive methicillin-resistant *S. aureus* (MRSA) infections in both healthy children as well as children with chronic illness [2, 5, 6]. Community acquired (CA)-MRSA has since become an established pathogen worldwide [2, 3, 6]. Several clonal lineages of CA-MRSA have been described and one virulent clone, designated USA300 by pulse-field gel electrophoresis and belonging to multilocus sequence type (ST) 8, is the dominant strain in many communities in the United States [7–9]. Some virulent strains, including USA300, carry genes for the Panton-Valentine leukocidin (PVL). Although PVL has been associated with severe disease, the role of PVL in pathogenesis remains unclear [10–15]. While there has always been considerable geographic variation in overall rates of community acquired *S. aureus* disease and clone types causing disease, recent epidemiologic reports in both adults and children suggest that national burden of CA-MRSA infection is declining, while rates of invasive CA-MSSA infection remain relatively unchanged [16–18]. Among children in Utah, rates of invasive *S. aureus* disease have remained high and relatively stable over time.

In this study we investigate the clinical and molecular epidemiology of invasive *S. aureus* disease at our institution, over a 4-year period, almost two decades after the emergence of CA-MRSA.

Materials and methods

Setting and study population

We prospectively identified all children younger than 18 years with culture-documented invasive *S. aureus* infection hospitalized at Primary Children's Hospital (PCH, Salt Lake City, UT) between January 1, 2009 and December 31, 2012. PCH is a 289 bed free-standing children's hospital that serves as both the pediatric community hospital for Salt Lake County and the only pediatric tertiary care center in the intermountain west region. PCH receives referrals from Utah, Arizona, Idaho, Wyoming, Nevada, and Montana.

Demographic and clinical information was obtained from the Intermountain Healthcare Enterprise Data Warehouse (IHC EDW). Manual review of the medical record was performed to confirm diagnosis and validate electronic data for all patients. The Institutional Review

Boards of the University of Utah and Primary Children's Hospital approved this study with a waiver of informed consent (IRB#00027819) as the data have been analyzed anonymously.

Definitions

Invasive infection.   Isolation of *S. aureus* from a normally sterile, with clinical evidence of disease at that site constituted an invasive infection. ICD-9 codes and chart review were used to determine infection site(s). Invasive infections included bacteremia, osteoarticular infections (OI), pneumonia, myositis or pyomyositis, necrotizing fasciitis, and meningitis or ventriculitis. Pneumonia cases were defined as chest imaging supportive of pneumonia and growth of *S. aureus* from a normally sterile (pleural fluid and/or blood) or near sterile (protected brush or bronchioalveolar lavage). Isolated skin and soft tissue infections were excluded unless associated with bacteremia.

Central line associated blood stream infection (CLABSI).   Children with a *S. aureus* blood stream infection and a central line or intravascular catheter in place >48 hours and without an infection at another site were assigned a diagnosis of CLABSI [19].

Toxic shock syndrome (TSS).   We defined TSS using the Centers for Disease Control and Prevention 2011 case definition: children with growth of *S. aureus* and fever >38.9˚C, rash, post-infectious desquamation, hypotension and multisystem involvement [20].

Severe sepsis.   Children with an invasive *S. aureus* infection and evidence of tissue hypoperfusion or organ dysfunction due to the infection were categorized as having severe sepsis [21].

Complex chronic conditions.   Children with complex chronic conditions (CCC) were identified and classified using *International Classification of Diseases Ninth Revision* (ICD-9) diagnosis codes according to the schema proposed by Feudtner et al [22].

***S. aureus*-associated mortality.**   We defined *S. aureus*-associated mortality as a death within 30 days attributable to invasive *S. aureus* infection based on chart review. Patients who experienced an invasive *S. aureus* infection and survived the acute episode but died at a later date were not included in *S. aureus*-associated mortality.

Community acquired (CA), community onset healthcare associated (CO-HCA) versus hospital acquired (HA) [23].   Community acquired (CA) infections were those diagnosed within 48 hours of admission to a hospital in an otherwise healthy child. Community-onset healthcare associated (CO-HCA) infections were those diagnosed within 48 hours of admission to a hospital and occurring within one year of a previous hospitalization or in a patient with an underlying condition requiring frequent healthcare encounters. Hospital-acquired (HA) infections were those that developed greater than 48 hours after admission to the hospital.

Identification and typing of bacterial isolates

S. aureus isolates were identified in the clinical microbiology laboratory by morphology and traditional microbiologic methods. All *S. aureus* isolates underwent routine antibiotic susceptibility testing by broth microdilution for oxacillin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin, clindamycin, erythromycin and penicillin using Clinical and Laboratory Standards Institute methods and interpretation guidelines. Isolates were stored at -80˚C until further analysis.

Isolation of DNA and molecular typing

S. aureus isolates were inoculated into brain heart infusion broth and incubated at 37˚C with 5% CO_2 for 20 hours. Bacterial DNA was extracted using the DNeasy Blood and Tissue Kit

(Qiagen, Venlo, Netherlands) for Gram-positive bacteria, per protocol, with the addition of 250 U/ml of Lysostaphin (Sigma-Aldrich, St. Louis, Missouri) during the initial lysis step. We performed next-generation sequencing of all isolates using the Ion Torrent Proton (Thermo-Fisher, Waltham, Massachusetts).

Multilocus sequence type (MLST) of isolates was determined using total genome sequencing data as previously described [24]. Sequence reads were assembled and extracted for all seven MLST loci and the arginine catabolic mobile element (ACME) using Geneious 8 (Biomatters, Auckland, New Zealand) [25]. Allelic sequences were then used to batch query the *S. aureus* MLST database and each isolate was assigned a sequence type and clonal complex [26]. Polymerase chain reaction (PCR) was used to detect PVL genes (*lukSF*-PV) [11]. The staphycoccal cassette chromosome *mec* (SCC*mec*) type was determined for MRSA isolates using the SCC*mec*Finder [27].

Statistical analysis

Categorical data were compared using χ^2 or Fisher exact test as appropriate. Continuous variables are reported as the median and interquartile range (IQR) and compared using Wilcoxon-Mann-Whitney rank-sum test. Rates and proportions were compared using χ^2 or Fisher exact tests as appropriate. Statistical significance was set at $P = .05$, and all reported comparisons are 2-tailed. Statistical analyses were performed in STATA15 (Stata Corp, College Station, Texas).

Results

Patients demographics

We identified 363 children with invasive *S. aureus* infection treated at PCH during the 4-year study period. The rate of invasive *S. aureus* infection was similar across the four complete study years, with 94, 85, 103, and 81 in 2009–2012 respectively, and approximately 15 cases per 1000 inpatient admissions annually (**Fig 1**). The majority of invasive infections (283/357, 79%) were due to MSSA; the proportion was stable over the study period and has remained similar in subsequent years. Six *S. aureus* isolates were unavailable or failed to regrow from frozen stock, leaving 357 patient and isolate pairs included in the study.

The mean age of children with invasive *S. aureus* disease in was 6.7 years and did not differ between those with MSSA and those with MRSA (**Table 1**). Thirty percent of children had two or more complex chronic conditions. There was a slight male predominance overall, however, MRSA infection was associated with female gender (57% (42/73) vs. 40% (113/283); OR 2; 95%; CI 1.2–3.4; $P = .007$). There were no differences in ethnicity, or the presence of complex chronic conditions between those with MSSA and MRSA infection.

Overall, 163/357 (45.7%) of infections were community-acquired, of these 126 (77%) were MSSA and 37 (23%) were MRSA. Community-onset healthcare associated infections accounted for 32% of cases while only 22% of cases were hospital-acquired (**Table 1**). Patients with hospital-acquired infections were significantly younger than the overall study population with an average age of 2.7 ± 4.23 years ($P = <0.001$). Children with two or more complex chronic conditions accounted for 73% (79/115) of CO-HCA and 27% (29/79) of HA infections.

Clinical manifestations and diagnose(s)

The median length of hospital stay for children with invasive *S. aureus* infection was seven days. (**Table 2**) More than one-third (36%) of patients required admission to an intensive care
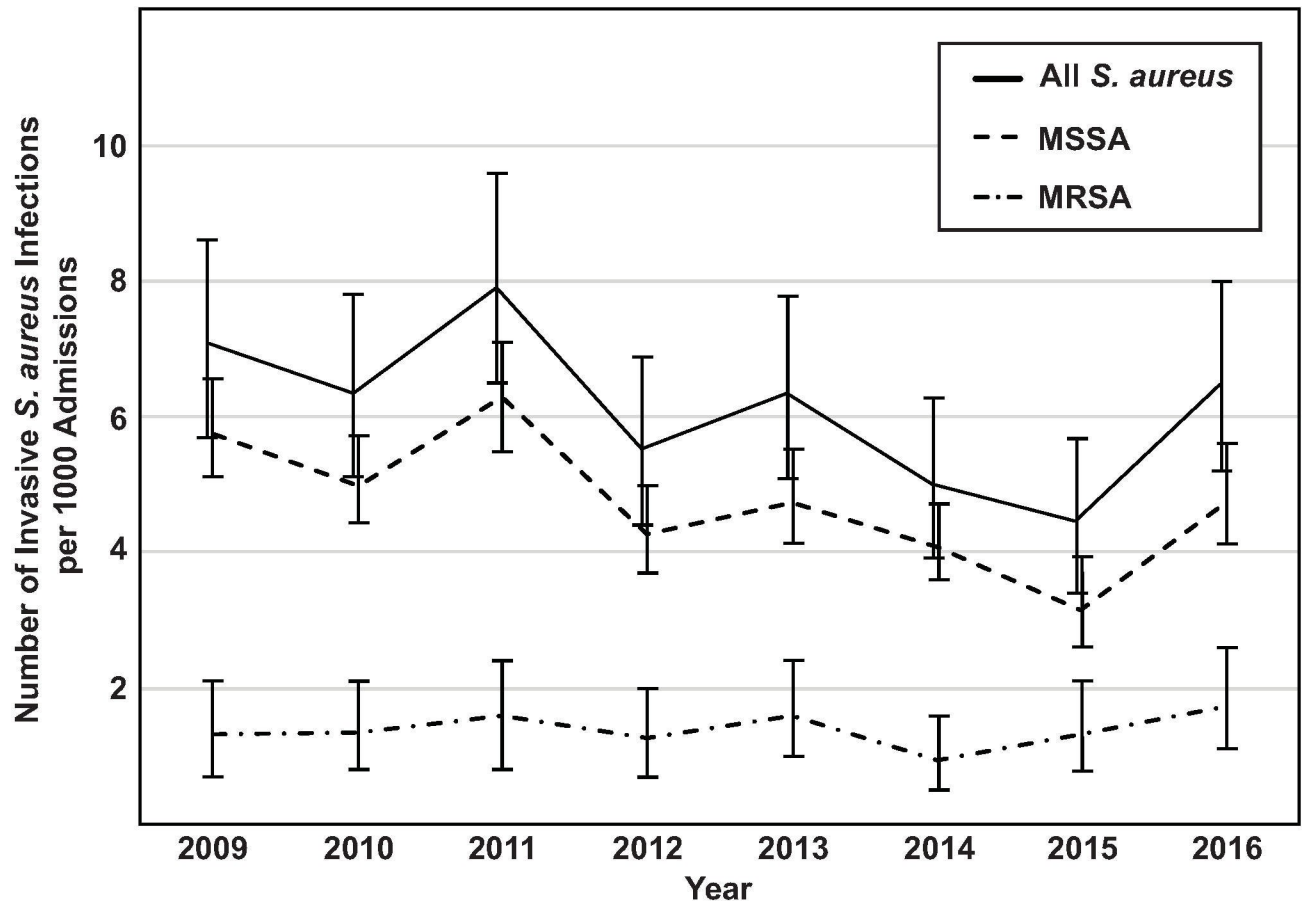
Fig 1. Rate of invasive *S. aureus* infections at Primary Children's Hospital, 2009–2016 per 1000 inpatient admissions per year, error bars show the 95% CI.

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unit (ICU). Patients with MRSA has longer hospital LOS than those with MSSA ($P = 0.008$), hospital LOS was similar between the groups. Severe sepsis developed in 18% of children. Neutropenia (ANC <1500) was present, either as a pre-existing condition or during the course of infection, in 17% of children. Thirteen children (3.6%) died as a result of invasive *S. aureus* infection. The majority of deaths (7/13) occurred in children with one CCC, while four children who died had greater than two CCC and two children were previously healthy and had no CCC. Mortality in neonates (< 30 days of age) was 12% (4/34), accounting for 31% (4/13) of all deaths. Disease severity characteristics including length of stay, intensive care unit admission and neutropenia did not differ between those with invasive infection due to MSSA or MRSA. However, patients with MRSA had significantly greater elevation of inflammatory markers (C-reactive protein ($P = 0.03$) and erythrocyte sedimentation rate ($P = 0.04$)).

Osteoarticular infections (OI) were the most common type of invasive *S. aureus* infection, accounting for 38% all infections. (Table 3) The majority (114/137) of patients with OI were previously healthy. Isolated central line associated bloodstream infection (CLABSI) accounted for 18% of infections. Pneumonia (11%) and endocarditis (3%) were less common. Infections of the central nervous system (meningitis or ventriculitis) accounted for 5.3% of invasive infections; all were associated with either a medical device (e.g. ventriculoperitoneal shunt or intrathecal pump) or trauma (e.g. basilar skull fracture). While, overall, 21% of invasive *S. aureus* infections were due to MRSA, MRSA was responsible for only 27 of 117 (14.6%) cases of OI.

Table 1. Demographic characteristics of children with invasive *S. aureus* infection.

Characteristic	All (n = 357)	MSSA (n = 283)	MRSA (n = 74)	*P*-value
Age (years), mean ± s.d.	6.7 ± 5.7	6.8 ± 5.8	6.6 ± 5.3	0.95
Gender male, n (%)	201 (56.5)	170 (60.1)	31 (42.5)	0.007
Race/Ethnicity, n (%)				0.1
White	255 (71.4)	209 (73.8)	46 (62.2)	
Black	5 (1.4)	5 (1.8)	0	
Hispanic	45 (12.6)	32 (11.3)	13 (17.6)	
Asian	5 (1.4)	5 (1.8)	0	
American Indian Alaska Native	3 (0.84)	1 (0.35)	2 (2.7)	
Hawaiian or Pacific Islander	11 (3.1)	10 (3.5)	1 (1.4)	
Other or Unknown	33 (9.2)	21 (7.4)	12 (16.2)	
2 or more CCC, n (%)	108 (30.3)	86 (30.4)	22 (29.7)	0.53
Community Acquired	163 (45.7)	126 (44.5)	37 (50)	0.14
CO-HCA	115 (32.2)	98 (34.6)	17 (23)	
Hospital Acquired	79 (22.1)	59 (20.9)	20 (27)	

Abbreviations: MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; CCC, complex chronic conditions [22]; CoHCA, community-onset healthcare associated.

In contrast, 21 of 41 (51%) cases of staphylococcal pneumonia were due to MRSA, the majority (13/21) of which were ST8. Most children with staphylococcal pneumonia (31/41, 75%) required ICU admission. ICU admission rates were higher (*P* = 0.036) among patients with MSSA (18/20) vs. MRSA (13/21) pneumonia.

Molecular characteristics

We identified 41 *S. aureus* MLST clones amongst our 357 isolates. Six distinct clonal complexes (CC), including CC5, CC8, CC15, CC30, CC45 and CC59 accounted for 68.6% of isolates causing invasive *S. aureus* disease (**Fig 2**). The majority of MRSA infections (40/74;

Table 2. Clinical and laboratory characteristics of children with invasive *S. aureus* infection.

	All (n = 357)	MSSA (n = 283)	MRSA (n = 74)	*P*-value
Severity Characteristics				
Hospital LOS (days), median (IQR)	7 (4–17)	6 (4–16)	10 (5–19)	0.008
ICU admission, n (%)	128 (35.9)	97 (34.3)	31 (41.9)	0.22
ICU LOS (days), median (IQR)	6.9 (2.3–19)	5.6 (2–21.3)	9.6 (3–19)	0.39
Severe Sepsis, n (%)	65 (18.2)	46 (16.3)	19 (25.7)	0.06
ANC <1500, n (%)	61 (17.1)	52 (18.4)	9 (12.2)	0.2
Mortality[c], n (%)	13 (3.6)	9 (3.2)	5 (5.4)	0.48
Laboratory Parameters				
Peak WBC, 10^3 cells/mm^3 ± SD[a]	16.7 ± 14.4	16.5 ± 15.6	17.5 ± 8.1	0.46
Peak CRP, mg/dL ± SD[a]	16.1 ± 10.9	15.3 ± 10.6	18.9 ± 11.9	0.03
Peak ESR, mm/hour ± SD[a]	53.3 ± 35.3	50.5 ± 34.1	63 ± 38.2	0.04

Abbreviations: MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; LOS, length of stay; ICU, intensive care unit; ANC, absolute neutrophil count; WBC, white blood cell (WBC); SD, standard deviation; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
[a]Laboratory values were not obtained on all patients, for WBC n = 350 (MSSA n = 277, MRSA n = 73), CRP n = 299 (MSSA n = 234, MRSA n = 65), ESR n = 219 (MSSA n = 171, MRSA n = 48).

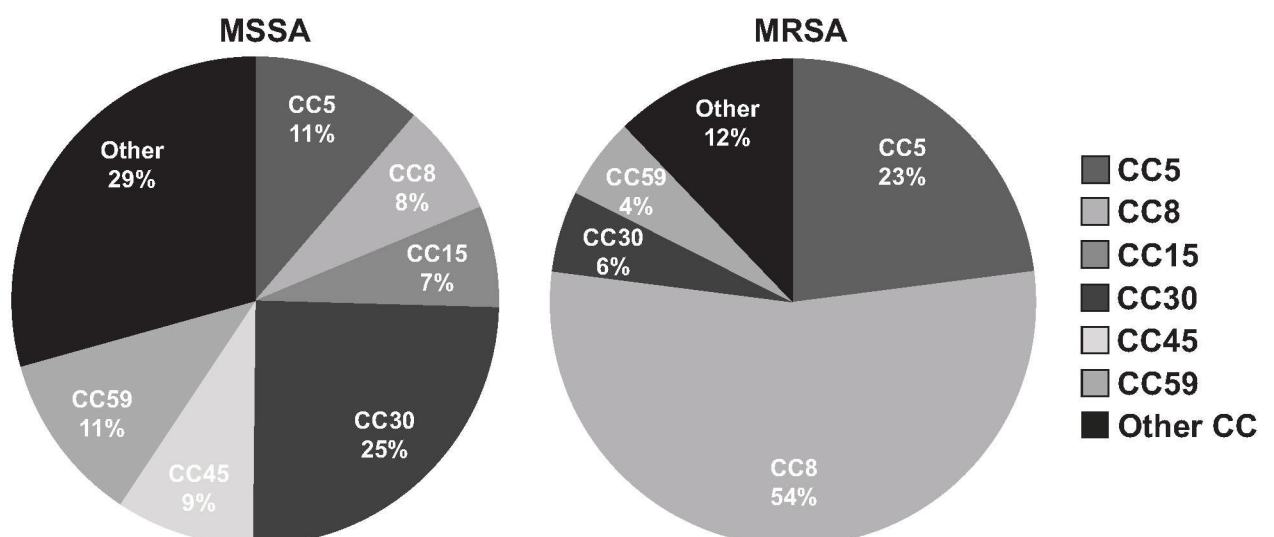**Table 3. Type of invasive *S. aureus* infection by anatomic site or syndrome.**

Type of Infection, n (%)	All (n = 357)	MSSA (n = 283)	MRSA (n = 74)	*P*-value
Osteoarticular infection	137 (38.4)	117 (41.3)	20 (27)	0.024
Osteoarticular infection with bacteremia	85 (23.8)	71 (25.1)	14 (18.9)	0.27
CLABSI	66 (18.5)	56 (19.8)	10 (13.5)	0.22
Pneumonia	41 (11.5)	20 (7.1)	21 (28.4)	<0.001
Pneumonia with bacteremia	21 (12.3)	14 (12.2)	5 (12.8)	0.92
Meningitis or ventriculitis	19 (5.3)	16 (5.7)	3 (4.1)	
SSTI with bacteremia	13 (3.6)	8 (2.8)	5 (6.8)	
Endocarditis	10 (2.8)	7 (2.5)	3 (4.1)	
Mediastinitis	9 (2.5)	7 (2.5)	2 (2.7)	
Toxic shock syndrome	6 (1.7)	6 (2.1)	0	
Thrombophlebitis	5 (1.4)	3 (1.1)	2 (2.7)	
Necrotizing fasciitis	2 (0.7)	2 (0.7)	0	
Bacteremia, all sources	222 (62.2)	182 (64.3)	40 (54.1)	0.11

Abbreviations: MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; central line-associated blood stream infection, CLABSI; skin and soft tissue infection, SSTI.

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54.1%) were caused by isolates belonging to CC8, all of which were categorized as ST8. An additional large subset of the MRSA isolates (17/74; 23%) belonged to CC5. MSSA infections were predominantly caused by CC5, CC15, CC30, CC45 and CC59 isolates. ST8 MRSA isolates were more commonly associated with pneumonia (21.3% vs. 9.5%, OR = 2.6, 95% CI 1.26–5.3, *P* = .008) (**Table 4**). CC5 was significantly less commonly associated with OI than other clonal complexes (22.4% vs. 40.9%; OR = 0.42, 95% CI 0.21–0.84, *P* = .01). There were no other statistically significant associations between sequence types and specific disease manifestation(s).

The putative virulence factor PVL was only present in 16% of our isolates (56/357) (**Table 4**). Only 6% (17/283) of MSSA isolates harbored PVL, compared to 53% of MRSA isolates (39/74, *P* < .001). PVL was present in 67.2% (41/61) of ST8 isolates and 85% (34/40) of

Fig 2. Proportional distribution of the most common *S. aureus* clonal complex types for MSSA and MRSA.

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Table 4. Clinical Presentations, outcomes and molecular characteristics of invasive *S. aureus* infections.

	All (n = 357)	CC5 (n = 49)	CC8 (n = 61)	CC15 (n = 19)	CC30 (n = 74)	CC45 (n = 27)	CC59 (n = 35)	Other (n = 92)
Type of Infection, n (% of CC)								
Osteoarticular infection	137 (37.9)	11 (22.4)	21 (34.3)	4 (21.1)	32 (43.2)	14 (51.9)	17 (48.6)	38 (41.3)
CLABSI	66 (18.5)	12 (24.5)	5 (8.2)	4 (21.1)	18 (24.3)	4 (14.8)	4 (11.4)	19 (20.1)
Pneumonia	41 (11.5)	5 (10.2)	13 (21.3)	2 (10.5)	8 (10.8)	1 (3.7)	1 (2.9)	11 (12)
Endocarditis	10 (2.8)	1 (2.0)	3 (4.9)	1 (5.3)	2 (2.7)	0	2 (5.7)	1 (1.1)
Bacteremia, all sources	222 (62.2)	29 (59.2)	38 (62.3)	10 (52.6)	49 (66.2)	13 (48.2)	24 (68.6)	59 (64.1)
Clinical Outcomes, n (% of CC)								
Severe sepsis	65 (18.2)	10 (20.4)	15 (24.6)	4 (21.1)	13 (17.6)	1 (3.7)	6 (17.1)	16 (17.4)
ICU admission	128 (35.9)	22 (44.9)	23 (36.1)	9 (47.4)	28 (37.8)	6 (22.2)	13 (37.1)	28 (30.4)
Mortality	13 (3.6)	3 (6.1)	1 (1.6)	1 (5.3)	3 (4.1)	1 (3.7)	1 (2.9)	3 (3.3)
Genetic Factors, n (% of CC)								
PVL+	56 (15.8)	0	41 (67.2)	0	1 (1.3)	0	0	14 (15.4)
MRSA	74 (20.7)	17 (34.7)	40 (65.6)	0	4 (5.4)	1 (3.7)	3 (8.6)	9 (9.8)
ACME	36 (10.2)	0	33 (55)	0	1 (1.4)	0	0	2 (2.2)

Abbreviations: CC, clonal complex; central line-associated blood stream infection, CLABSI; skin and soft tissue infection, SSTI; ICU, intensive care unit; PVL, Panton-Valentine leukocidin; MRSA, methicillin-resistant *S. aureus*; ACME, arginine catabolic mobile element.

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ST8 MRSA isolates. The presence of PVL was not associated with increased risk for ICU admission (19/128 vs. 37/228; OR 0.9, 95% CI 0.5–1.63, *P* = 0.73) or the development of severe sepsis (14/65 vs. 42/291, RR 1.63, 95% CI 0.84–3.2, *P* = 0.15). ACME was present in 10.2% of all isolates, the majority of which (33/36) were ST8. The presence of ACME was not associated with increased risk for ICU admission (12/127 vs. 24/227, OR 0.88, 95% CI 0.43–1.81, *P* = 0.74) or the development of severe sepsis (14/65 vs. 42/291, OR 1.89, 95% CI 0.87–4.1, *P* = 0.11). PVL and ACME were both significantly more likely to be present in ST8 isolates (P<0.001) and were rare in other STs (Table 4).

Antibiotic resistance to commonly used antistaphylococcal antibiotics was unusual among MRSA isolates and did not appear to change over the study period. MRSA isolates most commonly harbored SCC*mec* types IV (2B) and II (2A) (Table 5). Clindamycin resistance occurred in 14.9% of MRSA isolates and was associated with CC5 isolates (*P* = 0.001) but not healthcare associated infections, while resistance to trimethoprim-sulfamethoxazole was present in only 5.4% of MRSA isolates (Table 5).

Table 5. Antibiotic resistance patterns in invasive methicillin resistant *S. aureus* infections.

	All (n = 74)	CC5 (n = 17)	CC8 (n = 40)	CC30 (n = 4)	CC45 (n = 1)	CC59 (n = 3)	Other (n = 9)
SCC*mec*, n (% of CC)							
Type II (2A)	13 (17.6)	10 (58.8)	0	0	1 (100)	0	2 (22.2)
Type IV (2B)	57 (77)	7 (41.2)	39 (97.5)	2 (50)	0	3 (100)	6 (66.6)
Type V (5C2&5)	1 (1.3)	0	1 (2.5)	0	0	0	0
Unknown	3 (4.0)	0	0	2 (50)	0	0	1 (1.1)
Other Antibiotics, n (% of CC)							
Clindamycin	11 (14.9)	6 (35.3)	1 (2.5)	1 (25)	1 (100)	0	2 (2.2)
Trimethoprim-Sulfamethoxazole	4 (5.4)	0	1 (2.5)	0	0	1 (33.3)	2 (2.2)

Abbreviations: CC, clonal complex; SCC, staphylococcal cassette chromosome.

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Discussion

In contrast to reports from the earlier decade, we observed stable rates of invasive disease due to both MSSA and MRSA at our institution; only 21% of invasive disease was caused by MRSA [28–32]. Invasive disease due to *S. aureus* was severe; median length of stay was seven days, 35% of patients required ICU admission, and 4% of children died. Surprisingly, the severity of disease cause by MSSA was generally comparable to disease caused by MRSA. The majority of MRSA isolates were ST8 (USA300) and carried genes for PVL and ACME, two previously identified putative virulence factors [25, 33, 34]. In contrast, our MSSA isolates were over-whelmingly negative for PVL and ACME, and were associated with a limited number of domi-nant clonal types, suggesting that specific genetic factors present in these clones contribute to virulence and disease pathogenesis.

Studies in the early 2000s described the emergence of CA-MRSA as a dominant cause of invasive staphylococcal disease in certain geographic areas [2, 35]. In many communities in the US an increase in severe invasive disease was associated with specific lineages of CA-MRSA, such as ST8 (USA300) strains [8, 31, 36]. Recent reports describe significant varia-tion in rates of MRSA disease across US medical centers, and in some areas CA-MRSA disease may be declining [9, 17, 37]. In addition, epidemiologic studies from some of these locations have reported an increasing incidence of severe invasive CA-MSSA disease [38]. For example Hulten et al., recently reported that at Texas Children's Hospital, where USA300 CA-MRSA previously dominated, the rate of USA300 CA-MRSA-associated disease has declined accom-panied by an modest increase in non-USA300 CA-MSSA disease [16]. In our patient popula-tion we observed relatively modest and stable rates of severe invasive disease caused by ST8 strains and a sustained and high rate of severe invasive MSSA infections. The severity of inva-sive MSSA infections in our population were generally similar to those associated with ST8 MRSA.

To understand the clonal origins of our invasive *S. aureus* disease we characterized isolates by MLST. Similar to reports from other locations, the majority of CA-MRSA disease in our population was caused by ST8 (USA300) isolates [8, 35, 39]. These ST8 strains had similar genetic profiles to previous reports of USA300 isolates, harboring PVL, ACME and SCC*mec* type IV [25, 40]. We identified ST8 isolates more frequently in patients with pneumonia. This is consistent with several studies that have identified ST8 (USA300) as a frequent cause of severe pneumonia indicating there may be specific genetic factors present in ST8 isolates that contribute to both virulence and tropism for the respiratory tract [29, 33]. Interestingly, although ST8 strains account for the majority of MRSA infections, they did not emerge as the predominant strain type causing invasive infection at any point in the study period. We hypothesize that this epidemiologic pattern may be due to a predominance of highly patho-genic MSSA isolates. The re-emergence of MSSA across many regions of the US suggests the need to identify factors contributing to re-emergence and virulence of MSSA not associated with methicillin-resistance or the ST8 genotype.

In our population, MSSA sorted into six predominant clonal types dominated by CC30. Several of the dominant clonal types in our study (CC30, CC45, and CC59) have been previ-ously described as both MSSA and MRSA clones [41, 42]. In our study, CC30, CC45 and CC59 isolates were almost exclusively MSSA and were predominantly associated with OI. Although CC30 and CC45 have been reported in association with OI, an association between CC59 with OI has not previously been reported [43, 44]. Select CC30 lineages have been asso-ciated with increased rates of invasive disease, OI, and endocarditis [45]. For example, Niena-ber *et al.* found that MSSA isolates causing infective endocarditis were significantly more likely to be CC30 and possess a unique set of potential virulence genes. Luedicke *et al.* found

an increased proportion of CC45 isolates among patients with OI [43, 46]. Cassat *et al*. associated the presence the *cna* gene with hypervirulent OI strains in their population [47]. In contrast, Perez-Montarelo *et al*. noted increased frequency of *msrA* and *hla* in OI strains as well as *sed*, *splE* and *fib* in endocarditis strains [48]. Our study, in conjunction with these and other previous reports, suggests that there are specific genetic factors that may impact both the likelihood of hematogenous spread as well as tropism of certain *S. aureus* isolates for connective tissue, bone and joints.

Multiple factors may contribute to the molecular basis of *S. aureus* pathogenesis and virulence. Among these, the bicomponent leukotoxin PVL is perhaps the most intensely investigated. PVL has been associated with both ST8 (USA300) isolates and severe invasive infections [11, 49]. Consistent with previous reports, PVL was present in the majority of ST8 isolates in our cohort and was rare in other isolates [8, 10]. The majority of invasive infections in our population were caused by PVL-negative isolates, consistent with complex and multifactorial determinants of virulence. Other investigators have described a number of possible non-PVL *S. aureus* virulence factors [50]. In future investigations, we will use the full sequences of this large collection of invasive isolates from a well characterized cohort to investigate the multifactorial virulence of *S. aureus*.

This study has several limitations. Our study is limited to a single center, and the distribution of *S. aureus* clones and their genetic composition may be different in other regions. Our study is limited to children; epidemiology may differ in adults due to differences in colonization patterns and immunity. A strength of our study is its size and completeness, as our hospital cares for the vast majority of invasive *S. aureus* disease in children in our region.

Our study along with other recent studies highlight the complexity of changing patterns of S. aureus epidemiology and the challenges of dissecting determinants of virulence. Continued study of the genetic commonalities and differences of these isolates as well as associations with disease phenotypes will lead to a better understanding of *S. aureus* virulence.

Supporting information

S1 File.
(CSV)

S2 File.
(XLSX)

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References

1. Crawford SE, Daum RS. Epidemic community-associated methicillin-resistant Staphylococcus aureus: modern times for an ancient pathogen. Pediatr Infect Dis J. 2005; 24(5):459–60. Epub 2005/05/07. https://doi.org/10.1097/01.inf.0000164170.67897.97 PMID: 15876949.

2. Kaplan SL, Hulten KG, Gonzalez BE, Hammerman WA, Lamberth L, Versalovic J, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. Clin Infect Dis. 2005; 40 (12):1785–91. Epub 2005/05/24. https://doi.org/10.1086/430312 PMID: 15909267.

3. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA. 2007; 298(15):1763–71. Epub 2007/10/18. https://doi.org/10.1001/jama.298.15.1763 PMID: 17940231.

4. Prevention CfDCa. Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus—Minnesota and North Dakota, 1997–1999. MMWR Morb Mortal Wkly Rep. 1999; 48 (32):707–10. Epub 1999/08/20. PMID: 21033181.

5. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA. 1998; 279(8):593–8. Epub 1998/03/05. https://doi.org/10.1001/jama.279.8.593 PMID: 9486753.

6. Iwamoto M, Mu Y, Lynfield R, Bulens SN, Nadle J, Aragon D, et al. Trends in invasive methicillin-resistant *Staphylococcus aureus* infections. Pediatrics. 2013; 132(4):e817–24. Epub 2013/09/26. https://doi.org/10.1542/peds.2013-1112 PMID: 24062373.

7. Kennedy AD, Otto M, Braughton KR, Whitney AR, Chen L, Mathema B, et al. Epidemic community-associated methicillin-resistant *Staphylococcus aureus*: recent clonal expansion and diversification. Proc Natl Acad Sci U S A. 2008; 105(4):1327–32. https://doi.org/10.1073/pnas.0710217105 PMID: 18216255; PubMed Central PMCID: PMC2234137.

8. McCaskill ML, Mason EO Jr., Kaplan SL, Hammerman W, Lamberth LB, Hulten KG. Increase of the USA300 clone among community-acquired methicillin-susceptible Staphylococcus aureus causing invasive infections. Pediatr Infect Dis J. 2007; 26(12):1122–7. Epub 2007/11/29. https://doi.org/10.1097/INF.0b013e31814536e0 PMID: 18043449.

9. David MZ, Daum RS, Bayer AS, Chambers HF, Fowler VG Jr., Miller LG, et al. *Staphylococcus aureus* bacteremia at 5 US academic medical centers, 2008–2011: significant geographic variation in community-onset infections. Clin Infect Dis. 2014; 59(6):798–807. Epub 2014/06/01. https://doi.org/10.1093/cid/ciu410 PMID: 24879783; PubMed Central PMCID: PMC4200044.

10. Bocchini CE, Hulten KG, Mason EO Jr., Gonzalez BE, Hammerman WA, Kaplan SL. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. Pediatrics. 2006; 117(2):433–40. Epub 2006/02/03. https://doi.org/10.1542/peds.2005-0566 PMID: 16452363.

11. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 1999; 29(5):1128–32. Epub 1999/10/19. https://doi.org/10.1086/313461 PMID: 10524952.

12. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Panton-Valentine leukocidin-positive Staphylococcus aureus in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing

multiresistance. J Clin Microbiol. 2014; 52(3):859–70. Epub 2013/12/29. https://doi.org/10.1128/JCM. 02799-13 PMID: 24371244; PubMed Central PMCID: PMC3957793.

13. Voyich JM, Otto M, Mathema B, Braughton KR, Whitney AR, Welty D, et al. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus* aureus disease? J Infect Dis. 2006; 194(12):1761–70. https://doi.org/10.1086/509506 PMID: 17109350.

14. Bubeck Wardenburg J, Palazzolo-Ballance AM, Otto M, Schneewind O, DeLeo FR. Panton-Valentine leukocidin is not a virulence determinant in murine models of community-associated methicillin-resistant Staphylococcus aureus disease. J Infect Dis. 2008; 198(8):1166–70. https://doi.org/10.1086/592053 PMID: 18729780; PubMed Central PMCID: PMC2574921.

15. Kobayashi SD, Malachowa N, Whitney AR, Braughton KR, Gardner DJ, Long D, et al. Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. J Infect Dis. 2011; 204(6):937–41. Epub 2011/08/19. https://doi.org/10.1093/infdis/jir441 PMID: 21849291; PubMed Central PMCID: PMC3156927.

16. Hulten KG, Mason EO, Lamberth LB, Forbes AR, Revell PA, Kaplan SL. Analysis of Invasive Community-Acquired Methicillin-Susceptible Staphylococcus aureus Infections During a Period of Declining Community Acquired Methicillin-Resistant Staphylococcus aureus Infections at a Large Children's Hospital. Pediatr Infect Dis J. 2018; 37(3):235–41. Epub 2017/09/01. https://doi.org/10.1097/INF. 0000000000001753 PMID: 28859018.

17. Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. JAMA Intern Med. 2013; 173(21):1970–8. Epub 2013/09/18. https://doi.org/10.1001/jamainternmed.2013.10423 PMID: 24043270.

18. Sutter DE, Milburn E, Chukwuma U, Dzialowy N, Maranich AM, Hospenthal DR. Changing Susceptibility of *Staphylococcus aureus* in a US Pediatric Population. Pediatrics. 2016; 137(4). https://doi.org/10. 1542/peds.2015-3099 PMID: 26933211.

19. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008; 36 (5):309–32. Epub 2008/06/10. https://doi.org/10.1016/j.ajic.2008.03.002 PMID: 18538699.

20. Prevention CfDCa. Toxic Shock Syndrome (Other Than Streptococcal) (TSS) 2011 Case Definition 2011. Available from: https://wwwn.cdc.gov/nndss/conditions/toxic-shock-syndrome-other-than-streptococcal/case-definition/2011/.

21. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med. 2013; 41(2):580–637. Epub 2013/01/29. https://doi.org/10.1097/CCM.0b013e31827e83af PMID: 23353941.

22. Feudtner C, Silveira MJ, Christakis DA. Where do children with complex chronic conditions die? Patterns in Washington State, 1980–1998. Pediatrics. 2002; 109(4):656–60. Epub 2002/04/03. https://doi. org/10.1542/peds.109.4.656 PMID: 11927711.

23. Hulten KG, Kaplan SL, Gonzalez BE, Hammerman WA, Lamberth LB, Versalovic J, et al. Three-year surveillance of community onset health care-associated Staphylococcus aureus infections in children. Pediatr Infect Dis J. 2006; 25(4):349–53. Epub 2006/03/29. https://doi.org/10.1097/01.inf.0000207404. 50143.1e PMID: 16567988.

24. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012; 50(4):1355–61. Epub 2012/01/13. https://doi.org/10.1128/JCM.06094-11 PMID: 22238442; PubMed Central PMCID: PMC3318499.

25. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, 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):1523–30. Epub 2008/08/14. https://doi.org/10.1086/587907 PMID: 18700257.

26. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol. 2000; 38(3):1008–15. Epub 2000/03/04. https://doi.org/10.1128/JCM.38.3.1008-1015.2000 PMID: 10698988; PubMed Central PMCID: PMC86325.

27. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesoe RL, et al. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. mSphere. 2018; 3(1). Epub 2018/02/23. https://doi.org/10.1128/ mSphere.00612-17 PMID: 29468193; PubMed Central PMCID: PMC5812897.

28. Buckingham SC, McDougal LK, Cathey LD, Comeaux K, Craig AS, Fridkin SK, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus at a Memphis, Tennessee Children's Hospital. Pediatr Infect Dis J. 2004; 23(7):619–24. Epub 2004/07/13. https://doi.org/10.1097/01. inf.0000131981.67342.c4 PMID: 15247599.

29. Carrillo-Marquez MA, Hulten KG, Hammerman W, Lamberth L, Mason EO, Kaplan SL. Staphylococcus aureus pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. Pediatr Infect Dis J. 2011; 30(7):545–50. Epub 2011/03/17. https://doi.org/10.1097/INF.0b013e31821618be PMID: 21407143.

30. Carrillo-Marquez MA, Hulten KG, Hammerman W, Mason EO, Kaplan SL. USA300 is the predominant genotype causing Staphylococcus aureus septic arthritis in children. Pediatr Infect Dis J. 2009; 28 (12):1076–80. Epub 2009/10/13. https://doi.org/10.1097/INF.0b013e3181adbcfe PMID: 19820424.

31. Gonzalez BE, Martinez-Aguilar G, Hulten KG, Hammerman WA, Coss-Bu J, Avalos-Mishaan A, et al. Severe Staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus*. Pediatrics. 2005; 115(3):642–8. Epub 2005/03/03. https://doi.org/10.1542/peds.2004-2300 PMID: 15741366.

32. Hulten KG, Mason EO, Lamberth LB, Forbes AR, Revell PA, Kaplan SL. Analysis of Invasive Community-Acquired Methicillin-Susceptible Staphylococcus aureus Infections during a Period of Declining CA-MRSA Infections at a Large Children's Hospital. Pediatr Infect Dis J. 2017. https://doi.org/10.1097/INF.0000000000001753 PMID: 28859018.

33. Pasquale TR, Jabrocki B, Salstrom SJ, Wiemken TL, Peyrani P, Haque NZ, et al. Emergence of methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of late-onset nosocomial pneumonia in intensive care patients in the USA. Int J Infect Dis. 2013; 17(6):e398–403. Epub 2013/02/05. https://doi.org/10.1016/j.ijid.2012.12.013 PMID: 23375542.

34. Diep BA, Palazzolo-Ballance AM, Tattevin P, Basuino L, Braughton KR, Whitney AR, et al. Contribution of Panton-Valentine leukocidin in community-associated methicillin-resistant Staphylococcus aureus pathogenesis. PLoS One. 2008; 3(9):e3198. https://doi.org/10.1371/journal.pone.0003198 PMID: 18787708; PubMed Central PMCID: PMC2527530.

35. Carrel M, Perencevich EN, David MZ. USA300 Methicillin-Resistant *Staphylococcus aureus*, United States, 2000–2013. Emerg Infect Dis. 2015; 21(11):1973–80. Epub 2015/10/21. https://doi.org/10.3201/eid2111.150452 PMID: 26484389; PubMed Central PMCID: PMC4622244.

36. Day CT, Kaplan SL, Mason EO, Hulten KG. Community-associated Staphylococcus aureus infections in otherwise healthy infants less than 60 days old. Pediatr Infect Dis J. 2014; 33(1):98–100. Epub 2013/08/13. https://doi.org/10.1097/INF.0b013e3182a5f9a8 PMID: 23934207.

37. David MZ, Acree ME, Sieth JJ, Boxrud DJ, Dobbins G, Lynfield R, et al. Pediatric *Staphylococcus aureus* Isolate Genotypes and Infections from the Dawn of the Community-Associated Methicillin-Resistant *S. aureus* Epidemic Era in Chicago, 1994 to 1997. J Clin Microbiol. 2015; 53(8):2486–91. Epub 2015/05/29. https://doi.org/10.1128/JCM.00096-15 PMID: 26019202; PubMed Central PMCID: PMC4508449.

38. David MZ, Daum RS. Treatment of *Staphylococcus aureus* Infections. Curr Top Microbiol Immunol. 2017; 409:325–83. Epub 2017/09/14. https://doi.org/10.1007/82_2017_42 PMID: 28900682.

39. Tenover FC, Goering RV. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J Antimicrob Chemother. 2009; 64(3):441–6. Epub 2009/07/18. https://doi.org/10.1093/jac/dkp241 PMID: 19608582.

40. Strauss L, Stegger M, Akpaka PE, Alabi A, Breurec S, Coombs G, et al. Origin, evolution, and global transmission of community-acquired Staphylococcus aureus ST8. Proc Natl Acad Sci U S A. 2017; 114 (49):E10596–E604. Epub 2017/11/22. https://doi.org/10.1073/pnas.1702472114 PMID: 29158405; PubMed Central PMCID: PMC5724248.

41. McAdam PR, Templeton KE, Edwards GF, Holden MT, Feil EJ, Aanensen DM, et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant *Staphylococcus aureus*. Proc Natl Acad Sci U S A. 2012; 109(23):9107–12. Epub 2012/05/16. https://doi.org/10.1073/pnas.1202869109 PMID: 22586109; PubMed Central PMCID: PMC3384211.

42. Crisostomo MI, Westh H, Tomasz A, Chung M, Oliveira DC, de Lencastre H. The evolution of methicillin resistance in *Staphylococcus aureus*: similarity of genetic backgrounds in historically early methicillin-susceptible and -resistant isolates and contemporary epidemic clones. Proc Natl Acad Sci U S A. 2001; 98(17):9865–70. Epub 2001/08/02. https://doi.org/10.1073/pnas.161272898 PMID: 11481426; PubMed Central PMCID: PMC55544.

43. Luedicke C, Slickers P, Ehricht R, Monecke S. Molecular fingerprinting of *Staphylococcus aureus* from bone and joint infections. Eur J Clin Microbiol Infect Dis. 2010; 29(4):457–63. Epub 2010/02/27. https://doi.org/10.1007/s10096-010-0884-4 PMID: 20186451.

44. Kechrid A, Perez-Vazquez M, Smaoui H, Hariga D, Rodriguez-Banos M, Vindel A, et al. Molecular analysis of community-acquired methicillin-susceptible and resistant *Staphylococcus aureus* isolates recovered from bacteraemic and osteomyelitis infections in children from Tunisia. Clin Microbiol Infect. 2011; 17(7):1020–6. Epub 2010/10/28. https://doi.org/10.1111/j.1469-0691.2010.03367.x PMID: 20977540.

45. McGavin MJ, Arsic B, Nickerson NN. Evolutionary blueprint for host- and niche-adaptation in *Staphylococcus aureus* clonal complex CC30. Front Cell Infect Microbiol. 2012; 2:48. Epub 2012/08/25. https://doi.org/10.3389/fcimb.2012.00048 PMID: 22919639; PubMed Central PMCID: PMC3417553.

46. Nienaber JJ, Sharma Kuinkel BK, Clarke-Pearson M, Lamlertthon S, Park L, Rude TH, et al. Methicillin-susceptible *Staphylococcus aureus* endocarditis isolates are associated with clonal complex 30 genotype and a distinct repertoire of enterotoxins and adhesins. J Infect Dis. 2011; 204(5):704–13. Epub 2011/08/17. https://doi.org/10.1093/infdis/jir389 PMID: 21844296; PubMed Central PMCID: PMC3156104.

47. Cassat JE, Dunman PM, McAleese F, Murphy E, Projan SJ, Smeltzer MS. Comparative genomics of *Staphylococcus aureus* musculoskeletal isolates. J Bacteriol. 2005; 187(2):576–92. Epub 2005/01/05. https://doi.org/10.1128/JB.187.2.576-592.2005 PMID: 15629929; PubMed Central PMCID: PMC543526.

48. Perez-Montarelo D, Viedma E, Larrosa N, Gomez-Gonzalez C, Ruiz de Gopegui E, Munoz-Gallego I, et al. Molecular Epidemiology of Staphylococcus aureus Bacteremia: Association of Molecular Factors With the Source of Infection. Front Microbiol. 2018; 9:2210. Epub 2018/10/16. https://doi.org/10.3389/fmicb.2018.02210 PMID: 30319561; PubMed Central PMCID: PMC6167439.

49. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet. 2002; 359(9308):753–9. Epub 2002/03/13. https://doi.org/10.1016/S0140-6736(02)07877-7 PMID: 11888586.

50. DeLeo FR, Diep BA, Otto M. Host defense and pathogenesis in Staphylococcus aureus infections. Infect Dis Clin North Am. 2009; 23(1):17–34. Epub 2009/01/13. https://doi.org/10.1016/j.idc.2008.10.003 PMID: 19135914; PubMed Central PMCID: PMC2748223.

51. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 2018; 3:124. Epub 2018/10/23. https://doi.org/10.12688/wellcomeopenres.14826.1 PMID: 30345391; PubMed Central PMCID: PMC6192448.