Trends in Antibiotic Susceptibility in Staphylococcus Aureus in Boston, Massachusetts, from 2000 to 2014

Title: Antibiotic resistance and clonal dynamics of *Staphylococcus aureus* in Boston, Massachusetts, 2000-2014

Authors: Sanjat Kanjilal, MD, MPH[1], Mohamad Sater, PhD[1], Maile Thayer, BS[1], Georgia Lagoudas, BS[2], Paul C. Blainey, PhD[2], Yonatan H. Grad, MD, PhD[1,3]

Affiliations:

1. Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA
2. MIT Department of Biological Engineering and Broad Institute of Harvard and MIT, Cambridge, MA
3. Division of Infectious Diseases, Brigham and Women's Hospital, Boston, MA

Corresponding author:

Yonatan H. Grad, MD, PhD

Building 1, Rm 715

Harvard T.H. Chan School of Public Health

665 Huntington Ave

Boston, MA 02115

ygrad@hsph.harvard.edu

617 432 2275

Word count

Abstract: 348

Main text: 2998

KEY POINTS

Question: Does the decline in rates of infection by methicillin resistant *S. aureus* (MRSA) reflect the trend for *S. aureus* overall and for methicillin- and penicillin-susceptible subpopulations?

Findings: In this study of *S. aureus* infections from 2000-2014, the rate of *S. aureus* infection declined, driven by the decrease in MRSA infection but accompanied by a rise in penicillin susceptible *S. aureus* infection. The average *S. aureus* antibiotic susceptibility has increased.

Meaning: Contrary to the expectation of an inexorable rise in resistance, *S. aureus* infections have become increasingly susceptible to antibiotics over the past decade.

ABSTRACT

Importance: Methicillin-resistant *S. aureus* (MRSA) has been declining over the past decade, but the changes in *S. aureus* overall and the implications for trends in antibiotic resistance remain unclear.

Objective: To determine whether the decline in rates of infection by MRSA has been accompanied by changes in rates of infection by methicillin-susceptible, penicillin-resistant *S. aureus* (MSSA) and penicillin-susceptible *S. aureus* (PSSA). We test if these dynamics are associated with specific genetic lineages and evaluate the gains and losses of resistance at the strain level.

Design: A retrospective observational study from January 2000 to December 2014 and whole genome sequencing of isolates obtained between January 2016 and July 2016.

Setting: The Massachusetts General Hospital and the Brigham & Women's Hospital.

Participants: All inpatients ≥18 years of age with *S. aureus* infections (n = 31589). Surveillance swabs and duplicate specimens were excluded. A sample of contemporary isolates (n = 180) underwent sequencing.

Main outcomes and measures: Changes in the annual rates of infection per 1000 inpatient admissions by *S. aureus* subtype and in the annual mean antibiotic resistance by subtype. Gain and loss of phenotypic resistance and genetic determinants of resistance were inferred from genomes of contemporary isolates.

Results: Of the 43,954 *S. aureus* infections over the study period, 21,779 were MRSA, 17,565 MSSA and 4,610 PSSA. After multivariate adjustment, annual rates of infection by *S. aureus* declined from 2003 to 2014 by 2.9% (95% CI, 1.6%-4.3%), attributable to an annual decline in MRSA of 9.1% (95% CI, 6.3%-11.9%) and in MSSA by 2.2% (95% CI, 0.4%-4.0%). PSSA increased over this time period by 4.6% (95% CI, 3.0%-6.3%) annually. Resistance in *S. aureus* decreased from 2000 to 2014 by 0.86 antibiotics (95% CI, 0.81-0.91). By phylogenetic inference, 5/35 MSSA and 2/20 PSSA isolates in the

common MRSA lineages ST5/USA100 and ST8/USA300 arose from the loss of genes conferring resistance.

Conclusions and relevance: At two large tertiary care centers in Boston, MA, *S. aureus* infections have decreased in rate and have become more susceptible to antibiotics, with a rise in PSSA making penicillin an increasingly viable and important treatment option.

INTRODUCTION

Staphylococcus aureus is one of the most common bacterial pathogens[1,2]. A commensal organism that colonizes the skin and nares of approximately 20% of the population persistently and 60% intermittently[3], it causes a range of invasive diseases, including skin and soft tissue infection (SSI), bone and joint infection, pneumonia, and bacteremia. Infections are often severe: in 2009, out of 40 million total hospitalizations, an estimated 700,000 were *S. aureus*-related, yielding a rate of 17.7 per 1000 hospitalizations[4].

Much effort has focused on characterizing the emergence and spread of antibiotic resistant *S. aureus* lineages[5]. Resistance to penicillin was documented soon after its introduction and is mediated by a plasmid-borne β-lactamase encoded by the *blaZ* gene[6]. The penicillin-resistant phage type 80/81 clone was highly prevalent in the 1950s, before mostly disappearing with the introduction of methicillin in 1960. Subsequently, methicillin resistant *S. aureus* (MRSA) emerged, with resistance conferred by the staphylococcal chromosome cassette *mec* (SCC*mec*), a genetic element that contains the methicillin resistance gene *mecA*. An "archaic" MRSA clone was prevalent until the 1970s and was superseded by the emergence of hospital-associated (HA-MRSA) lineages, in the mid to late 1970s and community-associated (CA-MRSA) lineages in the mid to late 1990s. In the United States the most widespread hospital-associated clonal complex is CC5, which contains the multi-drug resistant MRSA strain known as USA100. Infections acquired in the community are often due to the MRSA strain USA300, which belongs to CC8[7], though there is evidence for transmission of this lineage in the hospital setting[8].

The high prevalence of MRSA infections[9,10] and the increased mortality, cost, and length of hospital stay of individuals infected with MRSA as compared to methicillin susceptible

S. aureus (MSSA)[11,12] focused efforts on describing MRSA epidemiology. In the US and Europe, MRSA incidence peaked in 2005 and has been declining steadily since 2005[10,13-16]. While the factors driving the decline in MRSA infections are not fully known, infection control efforts in healthcare facilities appear to have reduced the rates of transmission and infection in colonized individuals[16,17]. Evidence from the UK suggests that intrinsic fitness differences between clonal complexes have resulted in a lineage specific decline, possibly due to alterations in selective pressures from changing antimicrobial use[15,16,18-21].

In contrast with the extensive efforts to characterize MRSA, only a small number of studies have characterized the dynamics of all *S. aureus* subtypes, possibly due to the expectation that prevalence of penicillin susceptible *S. aureus* (PSSA) is extremely low[22]. However, recent reports from diverse sites describe a rising or surprisingly high prevalence of PSSA[23-25]. Whether this trend is associated with the decline in MRSA and how it impacts overall rates of *S. aureus* infection are unclear. Furthermore, recent analyses of large datasets of *S. aureus* genome sequences have provided evidence that resistance is not permanent, but can be acquired and shed[26-28]. However, the extent to which this takes place at the local level is unclear, as studies that focus on a single resistance subtype lack the context to fully assess gain and loss of resistance at the strain level.

To determine the overall dynamics of antibiotic resistance in *S. aureus* and evaluate the hypothesis that the decline in rates of MRSA infection has been accompanied by both an absolute and relative increase in PSSA incidence, we analyzed electronic records of *S. aureus* infections in hospitalized patients from 2000-2014 at two tertiary care hospitals in Boston, MA. Through whole genome sequencing of contemporary *S. aureus* invasive

isolates, we tested the extent to which the trends are associated with specific *S. aureus* lineages and used phylogenomic methods to quantify the gains and losses of penicillin and methicillin resistance.

METHODS

Clinical data

Clinical and microbiology information was collected for all inpatients admitted to the Brigham & Women's Hospital (BWH) and Massachusetts General Hospital (MGH) between January 1 2000 and December 31 2014 who were ≥18 years of age and had at least one specimen from any site growing *S. aureus*. Clinical variables included age, gender, Charlson comorbidity index[29], and the site of infection. Surveillance swabs were excluded. To account for multiple specimens obtained from the same individual, we included the first clinical specimen and excluded specimens with identical antibiograms from the individual collected in the subsequent 2 weeks. We generated two additional datasets using 4 and 6 weeks as the cutoff. Specimens were categorized into four sites of infection: blood, lung (sputum and bronchoscopy specimens), skin and soft tissue (SSI), and other (bone / joint specimens, tissue biopsies, specimens collected from a visceral compartment, urine, and miscellaneous).

Clinical microbiology

Clinical specimens were analyzed for susceptibility to penicillin (P), oxacillin (O), erythromycin (E), clindamycin (C), levofloxacin (L), gentamicin, tetracycline, trimethoprim-sulfamethoxazole (TMP-SMX), rifampin and vancomycin. We define antibiogram-type by the set of antibiotics to which the specimen is resistant. For example, 'POE' signifies resistance to penicillin, oxacillin, and erythromycin. Phenotypes were determined using disk diffusion until 2008 for MGH and 2010 for BWH and by

automated broth microdilution (Vitek system, bioMérieux) thereafter. MGH utilized the commercial antimicrobial susceptibility testing card GP-67 from 2008 to 2014. The BWH initially utilized the GP-67 card and switched to the GP-71 card in July 2011 (see Supplementary Figure S1 for a timeline of testing protocols). Specimens reported preliminarily as penicillin susceptible by broth microdilution underwent two step testing for inducible β-lactamase activity against penicillin through the nitrocefinase disk test followed by the zone-edge test[30] since 2011 at both hospitals. Susceptibility breakpoints were per Clinical and Laboratory Standards Institute guidelines over the study period. We included data for susceptibility to clindamycin after 2010 only since this was the first full year both hospitals performed inducible clindamycin resistance testing on all specimens automatically through the Vitek machine. We categorized a specimen as PSSA if it was susceptible to penicillin and oxacillin, MSSA if it was resistant to penicillin but susceptible to oxacillin, and MRSA if it was resistant to both penicillin and oxacillin.

Statistical analyses

We analyzed the rate of infection by *S. aureus* per 1000 inpatient admissions and the mean number of antibiotics to which specimens are resistant. Annual percent changes in rates were calculated with count data adjusted for patient volume. All analyses were stratified by *S. aureus* subtype or antibiogram-type and adjusted for age, gender, Charlson comorbidity index, and site of infection. The analysis with antibiogram-type excluded clindamycin in order to examine trends for the entire study period. Analysis was performed in R version 3.2.2[31] on data pooled from both facilities. Tests of difference between subtypes or antibiogram-types comprised of t-tests for continuous variables, chi-squared tests with correction for multiple hypothesis testing for categorical variables, and the Wilcoxon-Mann-Whitney test for the Charlson comorbidity index. Linear and Poisson regression (with patient volume as offset when applicable) were

used for multivariable adjustments of rate and count data respectively and 95% confidence intervals were calculated by the profile likelihood method included in the R package MASS.

Prospective specimen collection, sequencing, and analysis

We collected non-duplicate clinical specimens from patients ≥18 years of age who had specimens submitted to the BWH clinical microbiology laboratory between January 1, 2016 and July 22, 2016. DNA was extracted and shotgun sequence ibraries constructed on a recently developed microfluidic platform using the Illumina Nextera protocol as described.[32] The microfluidic sample preparation system enabled fast turnaround and low cost per sample for the small and nonstandard batch size used in this study. Shotgun libraries were sequenced on the Illumina MiSeq platform. We mapped reads to USA300 (GenBank NC_010079.1) by BWA[33], assembled genomes with SPAdes[34], and annotated them with Prokka[35]. We used Pilon[36] to call single nucleotide polymorphisms (SNPs). Clonal complex, sequence and SCC*mec* types were assigned using eBURST[37] and online databases[38,39]. We constructed a whole-genome based maximum likelihood phylogeny using RAxML[40] and ST152 (GenBank NZ_LN854556.1) as the outgroup. We inferred by parsimony the number of acquisitions and losses of SCC*mec* within ST5 and ST8 using Mesquite[41], using N315 (GenBank NC_002745.2) as the reference genome for ST5 and USA300 (GenBank NC_010079.1) as the reference genome for ST8 isolates and with removal of Gubbins-predicted recombination blocks[41].

RESULTS

Clinical and microbiologic characteristics of *S. aureus*

Our dataset comprised records of 43,954 *S. aureus* infections, including 21,779 MRSA, 17,565 MSSA, and 4,610 PSSA (Table 1). Patients with MRSA infection were older, had

more comorbidities, and more often had lung as the site of infection compared to patients with MSSA or PSSA. Patients with PSSA were older and had more comorbidities compared to those with MSSA but were similar in terms of site of infection. Seventy-eight percent of all specimens belonged to five antibiogram-types (Table 2): two are MRSA (POEL, POE), two are MSSA (PE, P), and one is PSSA (pan-susceptible). Patients infected by POE were significantly younger, had fewer comorbidities, and a higher proportion of SSI relative to all other antibiogram-types. Patients with POEL infections were significantly older and had a higher proportion of lung infections. Resistance was rare to each of the following: gentamicin, tetracycline, TMP-SMX, rifampin, and vancomycin.

Trends in *S. aureus* infection

After adjusting for covariates including age, site of infection, and comorbidities, rates of infection from *S. aureus* were stable from 2000 to 2003, but subsequently declined annually from 2003 to 2014 by 2.9% (95% CI 1.6% to 4.3%; Figure 1A), from 30.6 to 22.0 infections per 1000 inpatients. This pattern was driven predominantly by MRSA, which declined after 2003 by 9.1% per year (95% CI 6.3% to 11.9%) from 20.5 to 5.5 infections per 1000 inpatients over this time period. In contrast, over the 2003-2014 interval PSSA increased 4.6% annually (95% CI 3.0% to 6.3%) from 2.1 to 3.6 infections per 1000 inpatients (Figure 1A). MSSA declined by 2.2% annually (95% CI 0.4% to 4.0%) from 11.7 to 9.2 infections per 1000 inpatients (Figure 1A). There was no change in trends when using 4 and 6 week duplicate exclusion criteria (Supplemental Figure S2).

Rates of infection for the drug-resistant antibiogram-type POEL declined annually by 11.3% (95% CI 9.2% to 13.3%), while rates for the POE antibiogram-type increased

annually by 6.6% (95% CI 2.1% to 11.3%; Supplementary Figure S3). For the two major MSSA antibiogram-types (PE and P), rates did not change significantly over this time period. The pan-susceptible antibiogram-type increased by 4.5% annually (95% CI 2.3% to 6.8%). The rates for the POE and pan-susceptible antibiogram-types did not significantly differ. In the analysis including clindamycin for the interval 2010-2014, we observed a significant decline in the POECL antibiogram-type (6.9%, 95% CI 3.7% to 10.2%) and a significant increase in the P (3.3%, 95% CI 0.0% to 6.6%) and pan-susceptible (10.5%, 95% CI 4.8 to 16.5%) antibiogram-types (Supplementary Figure S4).

One explanation for the decline in the MRSA POEL antibiogram-type is decreasing exposure to antibiotics, particularly fluoroquinolones. We evaluated the rates of resistance to erythromycin, clindamycin and levofloxacin and observed a significant decline for all three drugs in MRSA and a decline for levofloxacin and erythromycin in PSSA. There were no changes in resistance to these drugs in MSSA (Figure 2).

Changes in mean antibiotic resistance

Given the rise in PSSA and the decline in multidrug resistant *S. aureus*, we tested whether overall susceptibility of *S. aureus* changed during the study period. In 2000, a *S. aureus* infection was on average resistant to 3.2 antibiotics. By 2014, this decreased to 2.3 antibiotics in 2014 ($p < 0.0001$ for trend, Figure 1B). When stratifying by subtype, we found that there was a decline in the mean resistance of MRSA (4.5 antibiotics in 2000 to 3.7 antibiotics in 2014; $p < 0.0001$ for trend), but there was no change in the average resistance of MSSA and PSSA ($p = 0.52$ and $p = 0.28$ for trend, respectively). When including clindamycin for the interval 2010-2014, there was an increase in overall resistance but no change in the time trend (Supplementary Figure S5).

Population structure of contemporary *S. aureus*

Figure 3A illustrates the unrooted phylogeny for a convenience sample of 180 clinical isolates (58 MRSA, 53 MRSA, 69 PSSA), representing 15% of all clinical *S. aureus* specimens collected from the BWH between January 17, 2016 and July 22, 2016. The two most common genetic lineages in this sample were CC5 (n=62) and CC8 (n=43). Other clonal complexes with multiple specimens include CC1 (n=14), CC15 (n=11), CC30 (n=7) and CC45 (n=7). Isolates belonging to each of these CCs clustered together in the whole genome phylogeny, with the exception of a subset of ST6 (CC5) and ST72 (CC8), which were located on separate branches. Thirty-six isolates belonged to minor clonal complexes (each with ≤5 isolates per CC) and 1 isolate with three novel alleles at the MLST loci (Supplementary Figure S6). MRSA isolates were limited to CC5 (39/62) and CC8 (19/38) (Supplementary Table S7). Of POEL isolates, 97% were CC5 and 94% of these were also resistant to clindamycin (POECL). Of POE isolates, 80% were CC8 and only 20% of these were resistant to clindamycin (Supplementary Tables S8 and S9). The distribution of gender, comorbidities and race differed due to small sample size in the POE group, but otherwise the clinical characteristics of these antibiogram-types were similar between the prospective and retrospective samples (Supplementary Tables S9 and S10). MSSA and PSSA isolates and their corresponding antibiogram-types were polyclonal. CC5 and CC8 isolates exhibited a wide range of antibiotic resistance phenotypes (Figure 3B and Supplementary Figure S6). Notably, 23 of 62 isolates (37%) in the hospital-associated lineage CC5 and 24 of 43 (56%) in CC8 were MSSA or PSSA.

Based on inference from the maximum likelihood whole genome phylogeny, we identified gain and loss of *blaZ* and *mecA* (Figure 3B), indicating that penicillin and methicillin resistance are dynamic in *S. aureus* populations. In most cases, isolates with

loss of the *mecA* had complete loss of the surrounding SCC*mec* element (Supplemental Figure S11). Overall, 3/31 (10%) of PSSA and MSSA in sequence type 8 and 4/24 (17%) in sequence type 5 are inferred to represent isolates that were previously MRSA.

DISCUSSION

Over the past 15 years, the rate of MRSA infection in hospitalized patients at two tertiary care hospitals in Boston, MA declined markedly, the rate of PSSA infection increased, and the overall rate of *S. aureus* infection declined slightly (Figure 1). Combined with the decreased extent of resistance to other antibiotics in MRSA and PSSA, *S. aureus* infections on average have become more antibiotic susceptible over the past 10 years.

The observed decline in the rate of MRSA infection does not reflect a decline in all MRSA strains. As the declining MRSA POECL antibiogram-type is mostly hospital-associated CC5 and the increasing MRSA POE antibiogram-type is mostly the community-associated CC8, we note that the decline of MRSA appears to be occurring predominantly in strains adapted to the health care setting. The demographic and clinical characteristics of patients with infection from POE isolates support this hypothesis, as they are younger, healthier, and more likely to present with skin and soft tissue infections.

As shown here, studies of MRSA incidence alone fail to capture the dynamics of the overall *S. aureus* population. The observation that penicillin and methicillin resistance are gained and lost at the strain level represents a departure from the dogma that population level resistance is driven by the transmission of lineages with stable resistance phenotypes. Similar findings were noted in a recent study from France

showed *in vivo* loss of methicillin resistance in CC30, a common European lineage that has been on the decline since 2005[28].

One potential explanation for the loss of resistance across multiple lineages is shifting antibiotic pressures. The decline in use of narrow spectrum beta-lactams such as oxacillin and penicillin G since 2000[42-45] and in the inpatient use of first generation cephalosporins since 2006[44] may select against HA-MRSA and in favor of PSSA, but does not on its own explain the rise in CA-MRSA. We also noted a decline in levofloxacin resistance in MRSA and PSSA over a time period when use of levofloxacin declined significantly inpatient settings[44]. This suggests that fitness costs associated with fluoroquinolone resistance in *S. aureus* may be a factor in the decline in HA-MRSA and the increase in CA-MRSA and PSSA, which are largely quinolone susceptible. In contrast, use of TMP-SMX increased nationally over the time period of our study[44,46,47] but TMP-SMX resistance remained <5%. This raises the question whether the fitness cost incurred by TMP-SMX resistance is higher than those incurred by fluoroquinolone resistance.

There are several limitations to our study. First, testing for inducible beta-lactamase production was not routinely performed prior to 2011, raising the possibility that specimens reported as penicillin susceptible from this time period were in fact penicillin resistant. However, such a bias would augment the observed increases in rates of PSSA infection. Second, in the absence of genotyping of historical specimens, the evidence that the decline in MRSA has occurred disproportionately within CC5 relies on the consistency in the demographic and microbiologic characteristics of antibiogram-types between our retrospective and prospective cohorts. However, the over-representation of POECL isolates in CC5 and POE isolates in CC8 suggests that these antibiogram-types

may be reasonable proxies for lineage. Lastly, the generalizability of our results may be limited as the analysis is of 2 hospitals in the same geographic area. However, as the rate of decline of MRSA in our study is similar to the rate observed in a nationally representative study[13] and recent reports from geographically diverse institutions note increased rates of PSSA infection, these trends may be widespread. A major strength of this study was the unbiased analysis of the overall *S. aureus* population, whereas most prior studies have examined only the MRSA subpopulation. This has yielded a more complete picture of the clonal dynamics of this highly adaptable pathogen.

The decline in antibiotic resistance in *S. aureus* over the past 10 years runs counter to the prevailing paradigm of an inexorable rise of multidrug resistance among human pathogens. Defining the forces driving the decline will be a critical task to guide efforts to control *S. aureus* infection and antibiotic resistance. Further, as most clinical laboratories do not test for penicillin susceptibility, the increasing incidence of PSSA should prompt evaluation of broader geographic trends along with reevaluation of the cost-effectiveness of penicillin testing and treatment.

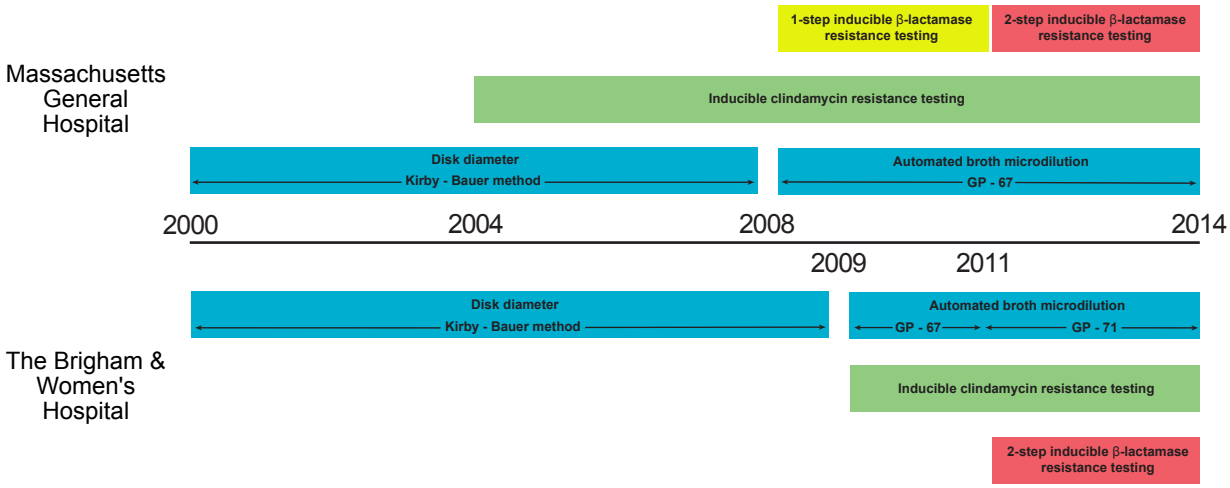
REFERENCES

1. Noskin, G. A. *et al.* The burden of Staphylococcus aureus infections on hospitals in the United States: an analysis of the 2000 and 2001 Nationwide Inpatient Sample Database. *Arch Intern Med* **165,** 1756–1761 (2005).
2. Grundmann, H., Aires-de-Sousa, M., Boyce, J. & Tiemersma, E. Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. *Lancet* **368,** 874–885 (2006).
3. Kluytmans, J., van Belkum, A. & Verbrugh, H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clinical Microbiology Reviews* **10,** 505–520 (1997).
4. Klein, E. Y., Sun, L., Smith, D. L. & Laxminarayan, R. The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: a national observational study. *Am. J. Epidemiol.* **177,** 666–674 (2013).
5. Chambers, H. F. & Deleo, F. R. Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nature Publishing Group* **7,** 629–641 (2009).
6. Lowy, F. D. Antimicrobial resistance: the example of Staphylococcus aureus. *J Clin Invest* **111,** 1265–1273 (2003).
7. David, M. Z. & Daum, R. S. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews* **23,** 616–687 (2010).
8. Seybold, U. *et al.* Emergence of community-associated methicillin-resistant Staphylococcus aureus USA300 genotype as a major cause of health care-associated blood stream infections. *CLIN INFECT DIS* **42,** 647–656 (2006).
9. Klevens, R. M. *et al.* Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *JAMA* **298,** 1763–1771 (2007).
10. Dantes, R. *et al.* National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011. *JAMA Intern Med* **173,** 1970–1978 (2013).
11. Cosgrove, S. E. *et al.* Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. *CLIN INFECT DIS* **36,** 53–59 (2003).
12. Engemann, J. J. *et al.* Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus aureus surgical site infection. *CLIN INFECT DIS* **36,** 592–598 (2003).
13. Kallen, A. J. *et al.* Health care-associated invasive MRSA infections, 2005-2008. *JAMA* **304,** 641–648 (2010).
14. Landrum, M. L. *et al.* Epidemiology of Staphylococcus aureus blood and skin and soft tissue infections in the US military health system, 2005-2010. *JAMA* **308,** 50–59 (2012).
15. Wyllie, D. H. *et al.* Decline of meticillin-resistant Staphylococcus aureus in Oxfordshire hospitals is strain-specific and preceded infection-control intensification. *BMJ Open* **1,** e000160–e000160 (2011).
16. Lawes, T. *et al.* Turning the tide or riding the waves? Impacts of antibiotic stewardship and infection control on MRSA strain dynamics in a Scottish region over 16 years: non-linear time series analysis. *BMJ Open* **5,** e006596–e006596 (2015).
17. Jain, R. *et al.* Veterans Affairs initiative to prevent methicillin-resistant Staphylococcus aureus infections. *N Engl J Med* **364,** 1419–1430 (2011).
18. Otter, J. A. & French, G. L. Molecular epidemiology of community-associated

meticillin-resistant Staphylococcus aureus in Europe. *The Lancet Infectious Diseases* **10,** 227–239 (2010).
19. Thouverez, M., Muller, A., Hocquet, D., Talon, D. & Bertrand, X. Relationship between molecular epidemiology and antibiotic susceptibility of methicillin-resistant Staphylococcus aureus (MRSA) in a French teaching hospital. *Journal of Medical Microbiology* **52,** 801–806 (2003).
20. Knight, G. M. *et al.* Shift in dominant hospital-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) clones over time. *J Antimicrob Chemother* **67,** 2514–2522 (2012).
21. Ellington, M. J. *et al.* Decline of EMRSA-16 amongst methicillin-resistant Staphylococcus aureus causing bacteraemias in the UK between 2001 and 2007. *J Antimicrob Chemother* **65,** 446–448 (2010).
22. Lowy, F. D. Staphylococcus aureus infections. *N Engl J Med* **339,** 520–532 (1998).
23. Chabot, M. R., Stefan, M. S., Friderici, J., Schimmel, J. & Larioza, J. Reappearance and treatment of penicillin-susceptible Staphylococcus aureus in a tertiary medical centre. *J Antimicrob Chemother* dkv270 (2015). doi:10.1093/jac/dkv270
24. Cheng, M. P., René, P., Cheng, A. P. & Lee, T. C. Back to the Future: Penicillin-Susceptible Staphylococcus aureus. *Am J Med* (2016). doi:10.1016/j.amjmed.2016.01.048
25. Crane, J. K. Resurgence of penicillin-susceptible Staphylococcus aureus at a hospital in New York State, USA. *J Antimicrob Chemother* **69,** 280–281 (2014).
26. Aanensen, D. M. *et al.* Whole-Genome Sequencing for Routine Pathogen Surveillance in Public Health: a Population Snapshot of Invasive Staphylococcus aureus in Europe. *MBio* **7,** (2016).
27. Gordon, N. C. *et al.* Prediction of Staphylococcus aureus antimicrobial resistance by whole-genome sequencing. *Journal of Clinical Microbiology* **52,** 1182–1191 (2014).
28. Ledda, A. *et al.* Re-emergence of methicillin susceptibility in a resistant lineage of Staphylococcus aureus. *J Antimicrob Chemother* (2017). doi:10.1093/jac/dkw570
29. Quan, H. *et al.* Coding algorithms for defining comorbidities in ICD-9-CM and ICD-10 administrative data. *Med Care* **43,** 1130–1139 (2005).
30. Gill, V. J., Manning, C. B. & Ingalls, C. M. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *Journal of Clinical Microbiology* **14,** 437–440 (1981).
31. Team, R. C. R: A language and environment for statistical computing.
32. Kim, S. *et al.* High-throughput automated microfluidic sample preparation for accurate microbial genomics. *Nat Commun* **8,** 13919 (2017).
33. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25,** 1754–1760 (2009).
34. Nurk, S. *et al.* Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* **20,** 714–737 (2013).
35. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30,** 2068–2069 (2014).
36. Walker, B. J. *et al.* Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* **9,** e112963 (2014).
37. Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P. & Spratt, B. G. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology* **186,** 1518–1530 (2004).

38. Jolley, K. A. & Maiden, M. C. J. BIGSdb: Scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **11,** 595–595 (2010).
39. SCCmec. *www.staphylococcus.net* Available at: (Accessed: 25 February 2017)
40. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30,** 1312–1313 (2014).
41. Croucher, N. J. *et al.* Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research* **43,** e15–e15 (2015).
42. ResistanceMap - Antibiotic Resistance.
43. Grijalva, C. G., Nuorti, J. P. & Griffin, M. R. Antibiotic prescription rates for acute respiratory tract infections in US ambulatory settings. *JAMA* **302,** 758–766 (2009).
44. Baggs, J., Fridkin, S. K., Pollack, L. A., Srinivasan, A. & Jernigan, J. A. Estimating National Trends in Inpatient Antibiotic Use Among US Hospitals From 2006 to 2012. *JAMA Intern Med* **176,** 1639–1648 (2016).
45. Vaz, L. E. *et al.* Recent trends in outpatient antibiotic use in children. *PEDIATRICS* **133,** 375–385 (2014).
46. Hicks, L. A., Taylor, T. H. & Hunkler, R. J. U.S. outpatient antibiotic prescribing, 2010. *N Engl J Med* **368,** 1461–1462 (2013).
47. Schweizer, M. L. *et al.* Optimizing antimicrobial prescribing: Are clinicians following national trends in methicillin-resistant staphylococcus aureus (MRSA) infections rather than local data when treating MRSA wound infections. *Antimicrobial Resistance and Infection Control* **2,** 28 (2013).

Supplemental material (in order of appearance in manuscript)



Supplementary Figure 1: Antibiotic susceptibility testing protocols for *Staphylococcus aureus* 2000 – 2014. Vitek commercial antibiotic susceptibility testing cards shown under automated broth microdilution. Two-step testing in all *S. aureus* isolates for inducible beta-lactamase activity against penicillin was performed at both hospitals after 2011 and prior to that was done upon clinician request. Inducible resistance to clindamycin was performed at both hospitals on all isolates routinely after 2010. Resistance to methicillin was inferred by testing isolates against oxacillin and cefoxitin, per Clinical and Laboratory Standards Institute (CLSI) recommendations.

Antibiogram type[a]	Dataset	n	Age Mean years (SD)	Gender % Female	CCI Median (IQR)	Type of isolate % Blood	Onset % Community
	Retrospective	16 720	63.8 (17.3)	42	2 (4)	14	54
PMEL	Prospective	36	62.0 (13.8)	36	2 (2)	11	47
	p-value[b]		0.54	0.58	0.25	0.81	0.50
	Retrospective	1715	46.2 (16.7)	43	1 (2)	14	74
PME	Prospective	15	48.7 (18.2)	73	1 (2)	13	87
	p-value[b]		0.56	**0.03**	**0.04**	1.00	0.38
	Retrospective	5041	54.3 (18.0)	42	2 (3)	23	68
PE	Prospective	17	49.4 (18.9)	76	0 (1)	12	82
	p-value[b]		0.26	**0.01**	0.05	0.39	0.30
	Retrospective	10 359	55.5 (17.9)	39	1 (3)	14	69
P	Prospective	26	52.8 (19.0)	62	0 (1)	15	88
	p-value[b]		0.44	**0.03**	**0.001**	0.77	**0.03**
	Retrospective	2889	57.0 (17.8)	38	2 (3)	14	69
Pan-susceptible	Prospective	56	55.1 (16.8)	41	1 (3)	18	80
	p-value[b]		0.44	0.69	0.40	0.43	0.13
	Retrospective	8983	58.8 (18.6)	42	2 (3)	20	59
Other	Prospective	30	57.8 (16.2)	40	2 (4)	20	77
	p-value[b]		0.76	1.00	0.44	1.00	0.06

Supplementary Table 1: Comparison of demographic and clinical characteristics of major antibiogram types between retrospective and prospective samples.

[a]Antibiogram types exclude clindamycin.

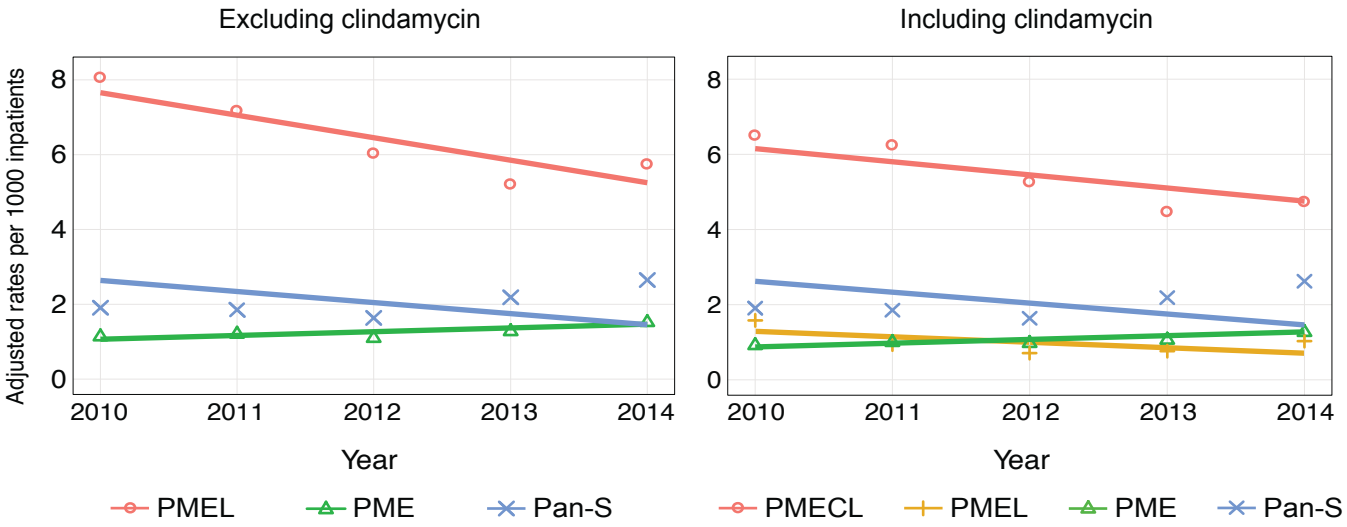
[b]Tests of difference are t test for age; Mann-Whitney test for CCI; chi-squared test for gender; Fisher's exact test for type of isolate and onset.

		Age	Gender	CCI			Site		Onset
	n	Mean (SD)	(% female)	Median (IQR)	% Blood	% Lung	% SSI	% Other	% Community
Antibiogram type[a]									
PMEL	16 720	63.8 (17.3)	42	2 (4)	14	40	23	23	54
PME	1715	46.2 (16.7)	43	1 (2)	14	14	54	18	74
PE	5041	54.3 (18.0)	42	2 (3)	23	27	30	20	68
P	10 359	55.5 (17.9)	39	1 (3)	14	32	32	23	69
Pan-susceptible	2889	57.0 (17.8)	38	2 (3)	14	32	31	23	69
Other[b]	8983	58.8 (18.6)	42	2 (3)	20	34	25	21	59

Supplementary Table 2: Demographic and microbiologic characteristics of major *S. aureus* antibiogram types.

[a]Antibiogram types exclude clindamycin.

[b]'Other' category includes all antibiograms not belonging to top 5 most common antibiogram types.

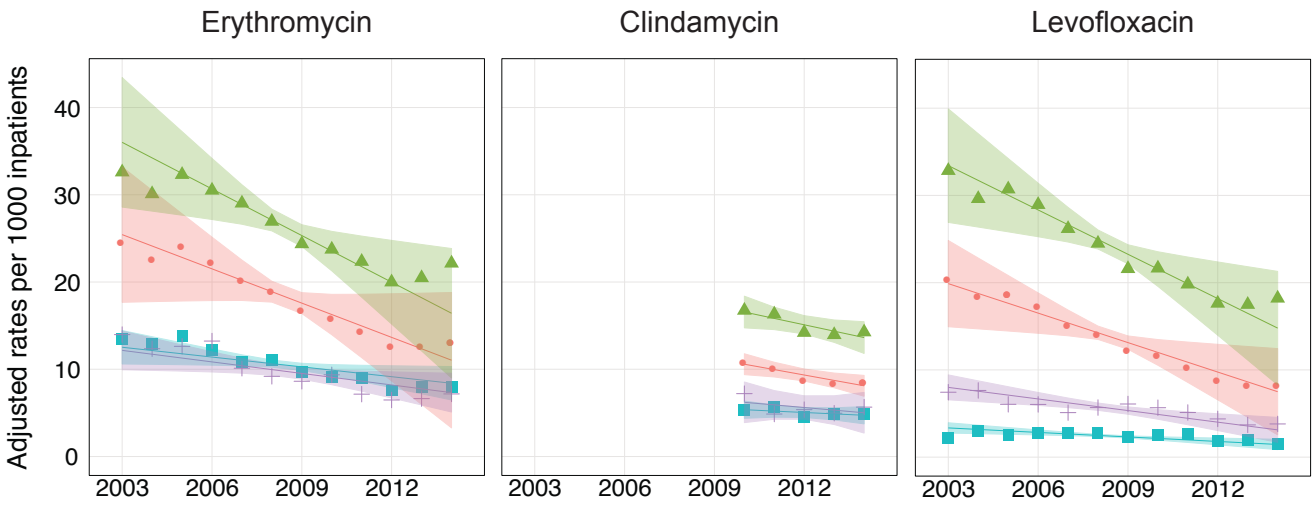


Supplementary Figure 2: Adjusted rates of *S. aureus* by major antibiogram type, excluding and including clindamycin, 2010 to 2014. Rates of inpatient infection of *S. aureus* by antibiogram type per 1000 inpatients from 2010 to 2014, excluding and including clindamycin. Estimates adjusted for year, age, and Charlson Comorbidity Index. Line represents model fit and data points represent unadjusted rates.

		Rate per 1000 inpatients		Annual % change in counts	
	n	2010 (95% CI)	2014 (95% CI)	Estimate (95% CI)	p-value
Excluding clindamycin					
PMEL	4058	7.7 (-1.1 - 16.4)	5.3 (-3.5 - 14.0)	-7.9 (-13.5 - -2.0)	0.01
PME	796	1.1 (0.2 - 1.9)	1.5 (0.6 - 2.3)	8.1 (1.6 - 15.0)	0.01
Pan-susceptible	1283	2.6 (2.5 - 2.8)	1.5 (1.3 - 1.6)	-13.9 (-24.0 - -2.5)	0.02
Including clindamycin					
PMECL	3433	6.2 (4.1 - 8.2)	4.8 (2.7 - 6.8)	-6.5 (-15.5 - 3.3)	0.19
PMEL	625	1.3 (-0.1 - 2.7)	0.7 (-0.7 - 2.1)	-12.2 (-17.1 - 7.0)	<0.0001
PMEC[a]	121	0.2 (0.1 - 0.3)	0.2 (0.1 - 0.3)	4.1 (-9.2 - 19.9)	0.57
PME	675	0.9 (0.3 - 1.4)	1.3 (0.7 - 1.8)	9.6 (2.2 - 17.6)	0.01
Pan-susceptible	1280	2.6 (2.2 - 3.1)	1.5 (1.0 - 1.9)	-13.7 (-23.8 - -2.3)	0.02

Supplementary Table 3: Adjusted rates of *S. aureus* by major antibiogram type, excluding and including clindamycin, 2010 to 2014. Rates of inpatient infection of *S. aureus* by antibiogram type per 1000 inpatients from 2010 to 2014, excluding and including clindamycin. Estimates adjusted for year, age, and Charlson Comorbidity Index.

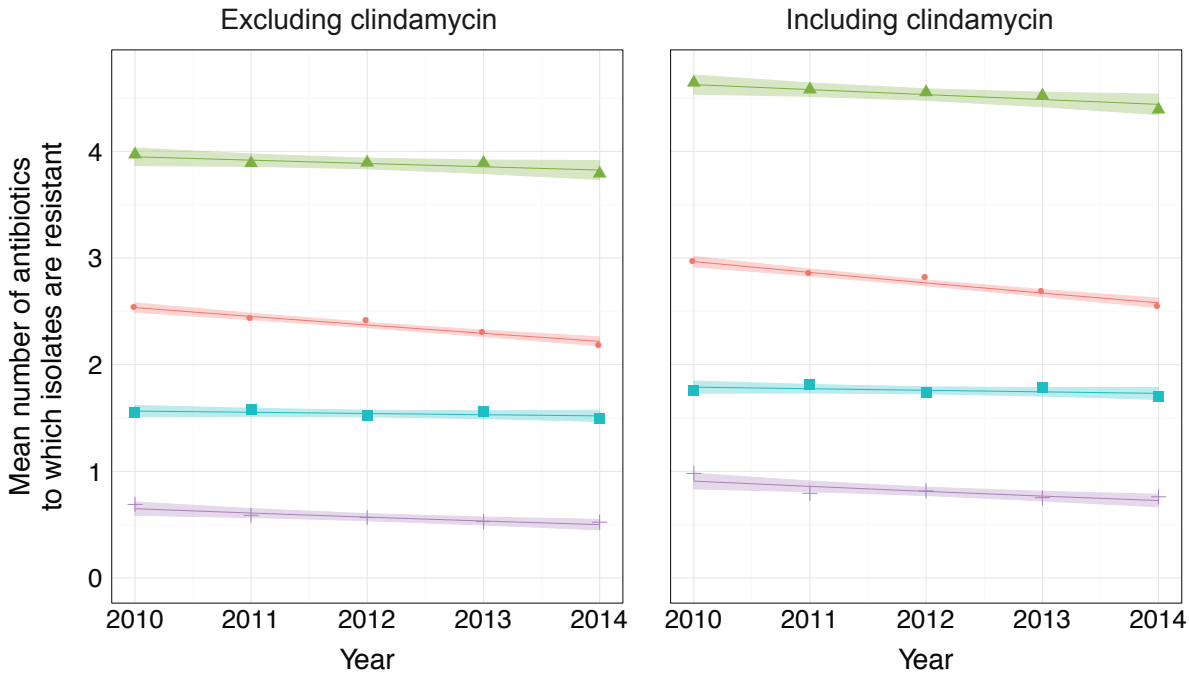
[a]The PMEC antibiogram type is omitted from plot for clarity given low rates overall with no statistically significant change over time.



Supplementary Figure 3: Adjusted rates of erythromycin, clindamycin and levofloxacin resistance in *S. aureus*. Rates of resistance of *S. aureus* per 1000 inpatients from 2003 to 2014 for erythromycin and levofloxacin and from 2010 to 2014 for clindamycin. Estimates adjusted for age, gender, Charlson Comorbidity Index, type of clinical isolate (blood versus non-blood) and onset (community versus hospital). Estimates for clindamycin represent rates adjusted for year only due to small sample size. Line represents model fit, shaded areas are 95% confidence intervals and data points represent unadjusted rates.

	Annual % change in counts	
	Estimate (95% CI)	p-value
Erythromycin		
All *S. aureus*	-8.5 (-10.2 - -6.8)	<0.0001
MRSA	-7.1 (-8.8 - -5.4)	<0.0001
MSSA	-3.9 (-6.2 - -1.6)	0.001
PSSA	-4.6 (-7.6 - -1.5)	0.004
Clindamycin		
All *S. aureus*	-6.5 (-8.2 - -4.8)	<0.0001
MRSA	-4.9 (-7.0 - -2.7)	<0.0001
MSSA	-3.2 (-6.8 - 0.6)	0.10
PSSA	-4.8 (-10.5 - 1.2	0.11
Levofloxacin		
All *S. aureus*	-11.4 (-13.4 - -9.4)	<0.0001
MRSA	-7.9 (-9.7 - -6.1)	<0.0001
MSSA	-7.5 (-12.0 - -2.8)	0.002
PSSA	-8.1 (-11.8 - -4.2)	0.0001

Supplementary Table 4: Adjusted rates of erythromycin, clindamycin and levofloxacin resistance in *S. aureus*. Rates of resistance of *S. aureus* per 1000 inpatients from 2003 to 2014 for erythromycin and levofloxacin and from 2010 to 2014 for clindamycin. Estimates adjusted for age, gender, Charlson Comorbidity Index, type of clinical isolate (blood versus non-blood) and onset (community versus hospital). Estimates for clindamycin represent rates adjusted for year only due to small sample size.

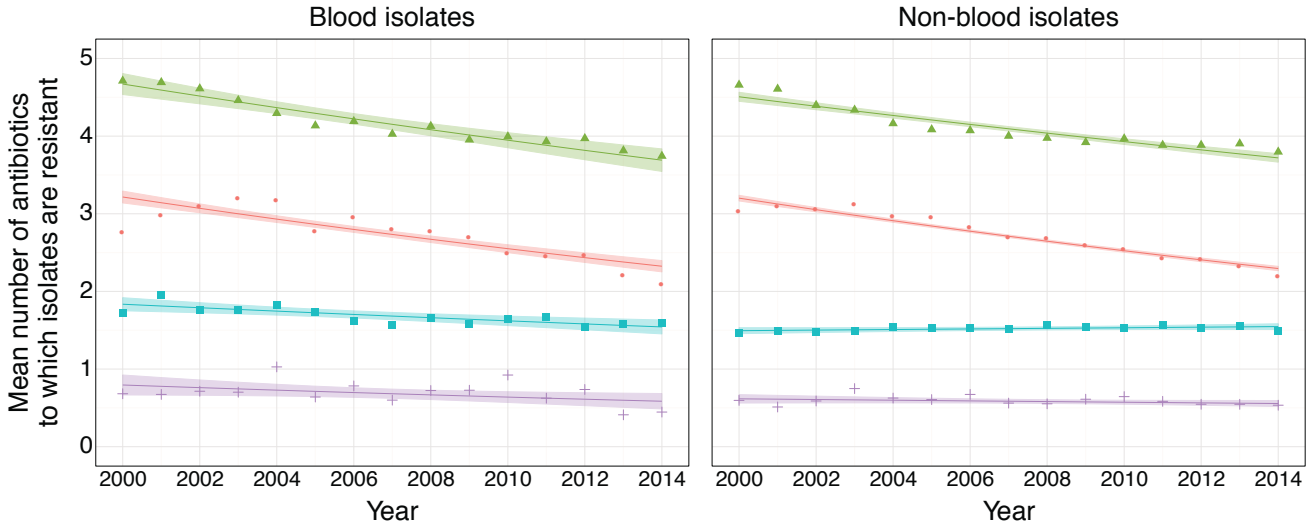


Supplementary Figure 4: Mean resistance in *S. aureus* excluding and including clindamycin. Comparison of mean resistance of a *S. aureus* isolate from 2010 to 2014 excluding and including clindamycin. Estimates adjusted for age, gender, Charlson Comorbidity Index, type of clinical isolate (blood versus non-blood) and onset (community versus hospital). Line represents model fit, shaded areas are 95% confidence intervals and data points represent unadjusted rates.

	Mean resistance		Absolute change	
	2010 (95% CI)	2014 (95% CI)	Estimate (95% CI)	p-value[a]
Excluding clindamycin				
All *S. aureus*	2.5 (2.5 - 2.6)	2.2 (2.2 - 2.3)	-0.4 (-0.5 - -0.3)	<0.0001
MRSA	3.9 (3.9 - 4.0)	3.8 (3.7 - 3.9)	**-0.2 (-0.3 - 0.0)**	0.09
MSSA	1.6 (1.5 - 1.6)	1.5 (1.5 - 1.6)	-0.1 (-0.2 - 0.1)	0.30
PSSA	0.6 (0.6 - 0.7)	0.5 (0.5 - 0.5)	-0.3 (-0.5 - -0.1)	0.001
Including clindamycin				
All *S. aureus*	3.0 (2.9 - 3.0)	2.6 (2.5 - 2.6)	-0.5 (-0.6 - -0.4)	<0.0001
MRSA	4.6 (4.5 - 4.7)	4.4 (4.3 - 4.5)	**-0.2 (-0.4 - 0.0)**	0.02
MSSA	1.8 (1.7 - 1.8)	1.7 (1.7 - 1.8)	-0.1 (-0.2 - 0.0)	0.21
PSSA	0.9 (0.8 - 1.0)	0.7 (0.7 - 0.8)	-0.4 (-0.6 - -0.2)	0.001

Supplementary Table 5: Mean resistance in *S. aureus* excluding and including clindamycin. Comparison of mean resistance of a *S. aureus* isolate from 2010 to 2014 excluding and including clindamycin. Estimates adjusted for age, gender, Charlson Comorbidity Index, type of clinical isolate (blood versus non-blood) and onset (community versus hospital). Estimates in bold indicate a significant difference in the absolute change for MRSA when excluding clindamycin versus including it.

[a] For trend

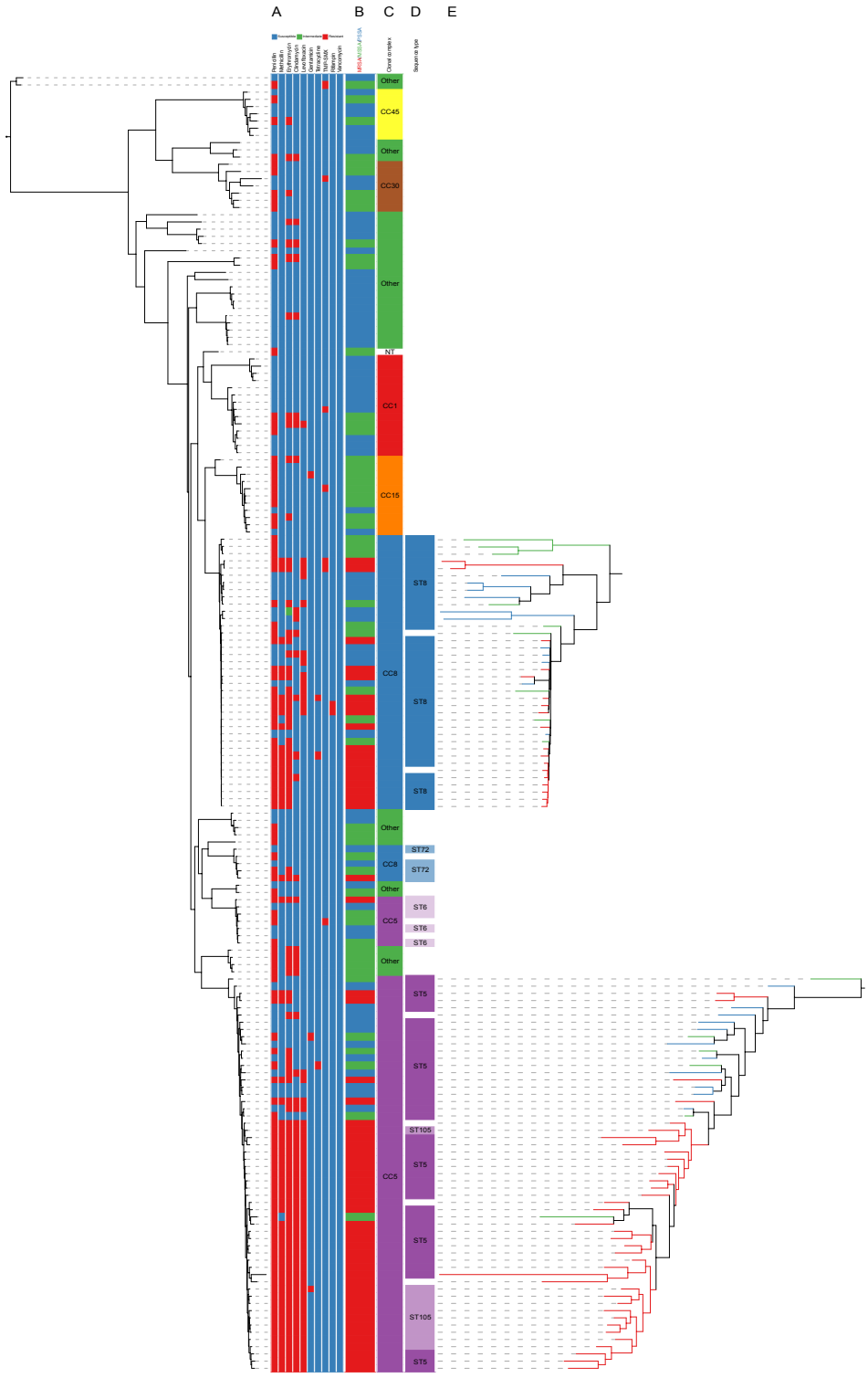


Supplementary Figure 5: Mean resistance in *S. aureus* for blood and non-blood isolates. Estimates exclude clindamycin phenotypes and are adjusted for age, gender, Charlson Comorbidity Index, and onset (community versus hospital). Line represents model fit, shaded areas are 95% confidence intervals and data points represent unadjusted rates.

	Mean resistance		Absolute change	
	2000 (95% CI)	2014 (95% CI)	Estimate (95% CI)	p-value[a]
Blood isolates				
All *S. aureus*	3.2 (3.1 - 3.2)	2.4 (2.3 - 2.4)	-0.7 (-0.8 - -0.6)	<0.0001
MRSA	4.7 (4.5 - 4.8)	3.7 (3.6 - 3.9)	-1.1 (-1.4 - -0.8)	<0.0001
MSSA	1.8 (1.7 - 1.9)	1.5 (1.5 - 1.6)	**-0.3 (-0.5 - -0.1)**	0.0002
PSSA	0.8 (0.7 - 0.9)	0.6 (0.5 - 0.7)	**-0.2 (-0.4 - 0.0)**	0.10
Non-blood isolates				
All *S. aureus*	3.1 (3.2 - 3.2)	2.3 (2.3 - 2.3)	-0.8 (-0.8 - -0.7)	<0.0001
MRSA	4.5 (4.4 - 4.6)	3.7 (3.7 - 3.8)	-0.9 (-1.0 - -0.8)	<0.0001
MSSA	1.5 (1.5 - 1.5)	1.5 (1.5 - 1.6)	**0.0 (0.0 - 0.1)**	0.17
PSSA	0.6 (0.6 - 0.7)	0.6 (0.5 - 0.6)	**0.0 (-0.1 - 0.1)**	0.91

Supplementary Table 6: Mean resistance in *S. aureus* for blood and non-blood isolates. Estimates exclude clindamycin phenotypes and are adjusted for age, gender, Charlson Comorbidity Index, and onset (community versus hospital). Estimates in bold indicate a significant difference in the absolute change between blood and non-blood isolates within the MSSA and PSSA subtypes.

[a] For trend.



Supplementary Figure 6: Complete phylogeny of contemporary *S. aureus* isolates. (A) Full resistance phenotype of sequenced *S. aureus* isolates; (B) *S. aureus* subtype; (C) Clonal complex; (D) Sequence types for CC5 and CC8; (E) Close up of ST5 (CC5), ST105 (CC5) and ST8 (CC8) isolates with branches colored by S. aureus subtype. Abbreviations: MRSA, methicillin resistant *S. aureus*; MSSA, methicillin susceptible and penicillin resistant *S. aureus*; PSSA, methicillin and penicillin susceptible *S. aureus*; CC, clonal complex; ST, sequence type, NT, non-typeable.

	n			Row %			Column %		
	MRSA	MSSA	PSSA	MRSA	MSSA	PSSA	MRSA	MSSA	PSSA
Clonal complex									
1	0	3	11	0	21	79	0	6	16
5	39	9	14	63	15	23	67	17	20
8	19	11	13	44	26	30	33	21	19
15	0	9	2	0	82	18	0	17	3
30	0	5	2	0	71	29	0	9	3
45	0	2	5	0	29	71	0	4	7
Other	0	14	22	0	39	61	0	26	32

Supplementary Table 7: Distribution of subtypes by clonal complex.

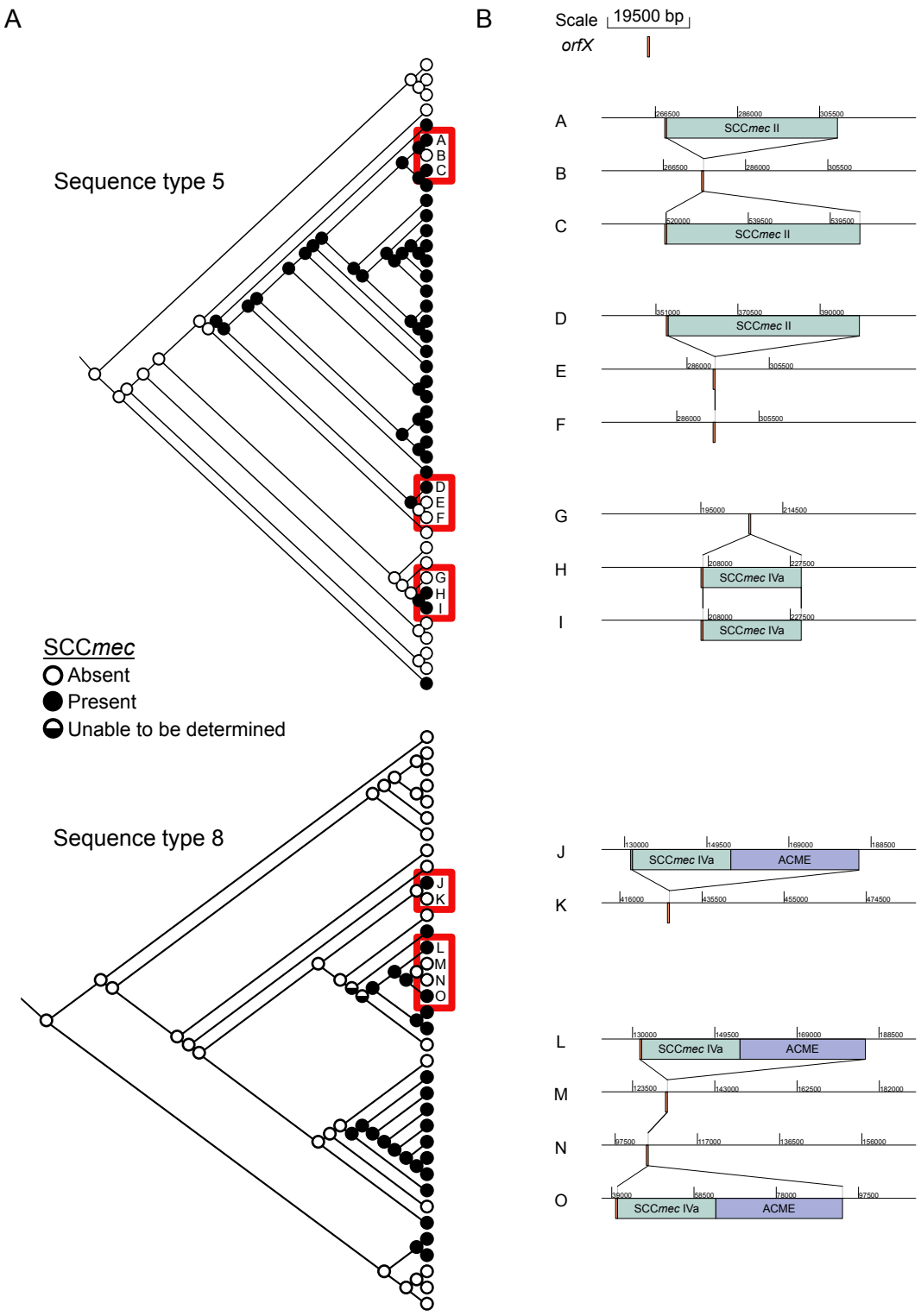
	n						Row %						Column %					
	PMEL	PME	PE	P	Pan-S	Other	PMEL	PME	PE	P	Pan-S	Other	PMEL	PME	PE	P	Pan-S	Other
Clonal complex																		
1	0	0	1	1	10	2	0	0	7	7	71	14	0	0	6	4	18	7
5	35	3	1	4	10	9	56	5	2	6	16	15	97	20	6	15	18	30
8	1	12	4	5	8	13	2	28	9	12	19	30	3	80	24	19	14	43
15	0	0	2	5	2	2	0	0	18	45	18	18	0	0	12	19	4	7
30	0	0	1	4	1	1	0	0	14	57	14	14	0	0	6	15	2	3
45	0	0	1	1	5	0	0	0	14	14	71	0	0	0	6	4	9	0
Other	0	0	7	6	20	3	0	0	19	17	56	8	0	0	41	23	36	10

Supplementary Table 8: Distribution of antibiogram types by clonal complex.

	Total n[a]		Clindamycin resistance n (%)		
	Retrospective	Prospective	Retrospective	Prospective	p-value
Antibiogram type					
PMEL	4058	36	3433 (85)	34 (94)	0.16
PME	796	15	121 (15)	3 (20)	0.88
PE	1603	17	996 (62)	10 (59)	0.98
P	3715	26	23 (1)	0 (0)	0.41
Pan-susceptible	1283	56	3 (0)	1 (2)	1.00
Other	2619	30	1303 (50)	12 (40)	0.38

Supplementary Table 9: Distribution of clindamycin resistance among antibiogram types.

[a]For years 2010 – 2014 only



Supplementary Figure 7: Genetic basis for loss of methicillin resistance in ST5 / CC5 and ST8 / CC8 (A) Maximum likelihood tree with ancestral presence or absence of SCC*mec* in ST5 and ST8, estimated using parsimony; (B) Close up of mechanism of SCC*mec* loss or gain in select isolates highlighted in panel A.

Abbreviations: ST, sequence type; CC, clonal complex; SCC, Staphylococcal cassette chromosome; ACME, arginine catabolic mobile element.

Supplementary Methods

Detailed sample preparation for microfluidics platform and bioinformatic analyses.

DNA extraction and library construction

DNA libraries were prepared from bacterial cultures using a previously described automated microfluidic sample preparation device [1]. This device was used to minimize the reagent cost and hands-on time for the 180 total samples prepared.

S. aureus isolates were obtained on frozen beads from the BWH clinical microbiology laboratory and grown in Brain-Heart infusion media for 18-24 hours at 37 degrees Celsius and shaken at 220 rpm. Cultures were spun down at 5,000 rpm for 5 minutes and the supernatant was discarded. Pellets were resuspended in 1 ml of phosphate buffered saline. The lysis enzyme mix for genomic DNA isolation was modified from Kim et al by adding 2 μL lysostaphin (2.5 mg/mL) instead of 2 μL of buffer to accommodate *S. aureus*.

The microfluidics device takes 2 μL aliquots of cells as input and subsequently performs enzymatic cellular lysis, genomic DNA purification, tagmentation to construct DNA sequencing libraries, and library cleanup, size selection, and elution steps. All mixing and elution steps are performed in the device, using valves in the two-layer microfluidic architecture. DNA capture and cleanup is achieved using solid phase reversible immobilization (SPRI) beads inside the device. The tagmentation reaction is performed following the Illumina Nextera protocol using the Tagment DNA Enzyme (Illumina), as described in Kim et al [1].

Amplification and barcoding PCR

The DNA library was eluted from the device (8 μL) and combined with 10 μL of NEBNext High Fidelity 2X PCR MasterMix, 1 μL forward primer, and 1 μL reverse primer (obtained from the Broad Institute Genomics Platform). We performed PCR barcoding and amplification using the following protocol: 72C for 3 minutes, 98C for 30 seconds, 17 cycles of [98C for 10 seconds, 60C for 30 seconds, 72C for 30 seconds], and 72C for 5 minutes. The barcoded DNA was purified suing SPRI beads.

Sample pooling and sequencing

Barcoded libraries were quantified with Quant-iT (ThermoFisher) and pooled at equal concentrations. DNA sequencing was performed on the Illlumina MiSeq (2x150 and 2x75 cycle runs). Samples with less than 20X coverage and contigs less than 500bp in length were discarded.

Genomic analysis

Genome assemblies were generated with SPAdes v3.9 [2]. Isolate sequence type (ST) and the corresponding clonal complex (CC) was determined using the PubMLST database (https://pubmlst.org/saureus/) [3] and eBURST (http://saureus.mlst.net/eburst/) [4]. Assembled contigs from ST5 and ST8 isolates were re-ordered using ABACAS [5] with respect to reference genomes N315 (NC_002745.2) and TCH1516 (NC_010079.1), depending on isolate sequence types. Genome annotations were generated using Prokka v1.11 [6]. Whole genome alignments were produced using ProgressiveMauve [7]

and pairwise BlastN [8]. The SCC*mec* cassette was visualized with the Artemis Comparison Tool [9] and typed using in-silico primers obtained from http://www.staphylococcus.net/.

SNP calling and phylogenetic analysis

Paired end reads were mapped against the chromosome of *S. aureus* strain USA300-TCH1516 (NC_010079.1) [10] and N315 (NC_002745.2) using BWA v0.7.13 [11]. Duplicate reads were identified using Picard Tools (https://broadinstitute.github.io/picard/) and ignored. SNP calling was performed using Pilon (https://www.broadinstitute.org/gaag/pilon) [12]. Reads with a low quality score (<30), low coverage (<5 reads) or ambiguous SNPs from heterogeneous mappings were discarded. A maximum-likelihood tree was generated using RAxML v8.2.2 [13] assuming a General Time Reversible model under the gamma model of rate heterogeneity and with 1000 bootstrap replicates. A *S. aureus* strain from ST152 (NZ_LN854556.1) was utilized as a divergent outgroup to root the tree.

The Mesquite v3.2 algorithm [14] was used to estimate acquisition or loss of the SCC*mec* cassette by parsimony. To obtain accurate ancestry sorting, recombination blocks within each ST group were removed based on elevated SNP densities as per the gubbins algorithm [15]. Whole genome maximum likelihood trees were built for each ST5 and ST8 using RAxML as described above.

Supplementary references

1. Kim S, De Jonghe J, Kulesa AB, et al. High-throughput automated microfluidic sample preparation for accurate microbial genomics. Nat Commun **2017**; 8:13919.

2. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **2012**; 19:455–477.

3. Jolley KA, Maiden MCJ. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics **2010**; 11:595–595.

4. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. Journal of Bacteriology **2004**; 186:1518–1530.

5. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. ABACAS: algorithm-based automatic contiguation of assembled sequences. Bioinformatics **2009**; 25:1968–1969.

6. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics **2014**; 30:2068–2069.

7. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS ONE **2010**; 5:e11147.

8. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J. Mol. Biol. **1990**; 215:403–410.

9. Carver TJ, Rutherford KM, Berriman M, Rajandream M-A, Barrell BG, Parkhill J. ACT: the Artemis Comparison Tool. Bioinformatics **2005**; 21:3422–3423.

10. Highlander SK, Hultén KG, Qin X, et al. Subtle genetic changes enhance virulence of methicillin resistant and sensitive Staphylococcus aureus. BMC Microbiol. **2007**; 7:99.

11. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **2009**; 25:1754–1760.

12. Walker BJ, Abeel T, Shea T, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS ONE **2014**; 9:e112963.

13. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics **2014**; 30:1312–1313.

14. Maddison, W. P. and D.R. Maddison. 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75. http://mesquiteproject.org.

15. Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Research **2015**; 43:e15–e15.