

Life After USA300: The Rise and Fall of a Superbug

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The community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) epidemic in the United States is largely attributable to the meteoric rise of a single clone, referred to as USA300. This strain not only spread across the United States in just a few years to become the predominant cause of staphylococcal disease, but it also appears to have increased the overall number of skin and soft-tissue infections (SSTIs), increasing the overall disease burden. While USA300 still constitutes a major public health burden, its prevalence may be decreasing in some parts of the United States. Other than an epidemic in South America due to a closely related strain, USA300 also seems to have been largely unable to establish itself as an endemic infection in other geographic locations. While there have been several hypotheses put forward to explain the enormous success of USA300, the reasons for its failures and its potential fall remain obscure. Far from being unique to USA300, the rise and fall of specific clones of *S. aureus* in human populations seems to be a common process that has occurred multiple times and in multiple locations. This review charts the rise of USA300 and the evidence that suggests that it may be in decline, and it considers how best to understand the future spread, containment, and possible extinction of CA-MRSA.

Keywords. MRSA; USA300; *Staphylococcus aureus*; phylogenomics; epidemic.

THE RISE OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (CA-MRSA) IN THE UNITED STATES

The epidemiology of *S. aureus* was memorably described by Chambers and DeLeo as a series of waves of resistance [1], in which strains with different antibiotic susceptibility profiles replace each other as the dominant or most clinically relevant forms and then, in turn, are replaced by a new successful strain. As an evolutionary process, this recurring pattern is reminiscent of the fixation of a successful allele or, maybe more appropriately, the spread of an invasive species. However, the factors that contribute to the fitness advantage of certain strains remain unclear, and the reasons for the decline of certain lineages are even more mysterious. Because of molecular typing strategies and, more recently, whole-genome analyses, we have been able to observe the emergence of one of these invasive species, the CA-MRSA clone USA300, at a resolution that was not possible before.

The first recorded sighting of USA300 was probably in a Mississippi jail in 1999 [2], but there were nearly simultaneous reports of this new aggressive CA-MRSA strain across the country within a very short period, starting in 2000 [3–5]. The strain appeared to get its foothold in more-crowded community contexts where people had repeated contact with each other, such as jails

and settings involving sports teams and military recruits [6–10]. However, soon USA300 appeared to be spreading more widely in the general population, and by 2005 it had become the major cause of skin and soft-tissue infections (SSTIs) [11, 12], and it was already a significant cause of invasive infections such as bacteremia and community-onset pneumonia [13–16]. By 2011, USA300 was the most common MRSA isolated from infections at all body sites [17]. Over this period, there were also increases in the numbers of serious SSTIs [18–20], suggesting that USA300 was not just replacing other *S. aureus* strains but adding to the overall burden of disease.

Attempts to find USA300 isolates from before 1999 have found no evidence that it was widely circulating in the 1990s [21], and isolates of CA-MRSA recovered before 1999 are generally thought to belong to another lineage, USA400 [1]. Because many isolates were not typed molecularly, there has been some confusion about the geographic origins and temporal trends seen in the literature. USA300 was originally defined on the basis of its pulsed-field gel electrophoresis (PFGE) pattern [22] after the initial isolates were collected. It became clear that the combined presence of several molecular markers, including multilocus sequence type 8, and the presence of Pantone-Valentine leukocidin (PVL), SAPI5, staphylococcal cassette chromosome *mec* type IV (SCC*mec* IVa) encoding *mecA*, and the arginine catabolic mobile element (ACME) could accurately identify strains of this clone [23–25]. However, isolates of USA300 have been identified that lack one or the other of the diagnostic genes/loci, suggesting loss of these elements [26–29]. With the availability of whole-genome sequencing it became clear that the strain was clonal and recently emerged [24, 30]. Indeed, whole-genome phylogenetic analysis

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appears to be the new gold standard for identification of this lineage and others.

Partly because of the rapidity of the spread of USA300 after 2000, its geographic origins have remained obscure. There is an anecdotal idea in the field that the strain started in the western part of the United States and then moved east. This is mostly based on the perception that medical centers on the East Coast did not see CA-MRSA in as high numbers as were seen in western and midwestern states. However, it is possible that this perception is based, erroneously, on the earlier emergence of USA400 in the Midwest [31–33]. Recently, Carrell et al [34] used a spatial epidemiological technique to map published reports of USA300 strains from the biomedical literature between 2000 and 2013. Interestingly, this study corroborated a pattern of emergence from west to east in the United States. It is unclear whether this reflects the true geographical pattern of spread or a bias in publication. On a larger scale, phylogenetic geographical reconstruction and comparison to closely related strains from Latin America were unable to resolve whether the strain originated in North or South America [35]. It is possible that more-complete sampling of whole genomes, combined with population genetic or phylogenetic approaches, may be able to better identify the geographic origin. In addition, studies of archival collections may also unearth earlier isolates that could add insight.

Epidemiological data showed that USA300 was very good at spreading geographically. Moreover, the dominance of USA300 as the most common strain involved in SSTIs and the overall increase in SSTIs suggested that there were specific properties of this strain that also made it better at causing disease. A rat model of pneumonia showed increased virulence as compared to USA400, which was attributed to increased production of toxins such as α -toxin and phenol-soluble modulins [36–39]. Perhaps the most well-studied, distinguishing feature of USA300 is the presence of the PVL. PVL has been implicated in the pathogenesis of bacteremia, pneumonia and osteomyelitis [40–42], but its contribution to skin infection [43, 44] and its overall contribution to virulence [45, 46] is controversial [47, 48]. It is also worth noting that the presence of PVL per se does not explain the successful spread of USA300. This is made clear by the discovery of close relatives of the USA300 epidemic isolates that are PVL positive but diverged from the lineage well before the epidemic occurred [28]. The same can be said for the acquisition of the SCCmec IVa element itself, which also appears to have been acquired significantly prior to the emergence of the epidemic [28].

Indeed, the overall virulence of USA300 itself may have nothing to do with its rapid spread, since closely related strains such as USA500, which, objectively, have been much less successful, appear to have equal or enhanced virulence phenotypes in models of infection [49, 50]. This type of observation stresses an important theme in the evolution of pathogens, which is that

increased virulence may not always be a sign of evolutionary fitness and may, in fact, be a detriment. Indeed, the way that laboratory tests of virulence (or toxicity) translate into much more complex issues of disease, invasiveness, and transmission is a new exciting area of research with sometimes counterintuitive results [51, 52].

Another genomic locus that has been implicated in the success of USA300 is the ACME, a locus that sits adjacent to the SCCmec IVa and can be mobilized by the recombinases on that element [24, 53]. The role of ACME in virulence was not initially obvious, with one study showing enhanced competitive fitness in intravenous inoculation in rabbits [53] and another showing no strong impact in rodent pneumonia and skin infection models [29]. The ACME locus encodes genes for arginine catabolism, which produces ammonia as a byproduct, allowing the strain to survive at lower pH values that might be found on skin [54]. It also encodes an *N*-acetyl transferase (SpeG) that detoxifies polyamines (spermidine/spermine) that are products of arginine degradation and present at high levels in skin [55]. The presence of *speG* has been shown to increase the pathogen burden in a skin abscess model [54] and has also been shown to influence biofilm formation and decrease antibiotic susceptibilities and killing by keratinocytes [35]. Unlike PVL, the acquisition of ACME by CA-MRSA from *Staphylococcus epidermidis* has been shown to be coincident with the beginning of the North American USA300 epidemic [35, 28], strongly implicating it in the success of this lineage. However, ACME has not yet been tied epidemiologically to more-severe disease, and some USA300 isolates do not carry it, with phylogenetic analyses suggesting that the ACME has been lost on multiple occasions [26, 27, 28].

Another important event in the rise of USA300 in North America appears to have been the acquisition of fluoroquinolone resistance through mutations in *gyrA* and *grrA*, and several publications have been able to map this particular event to a phylogenetic tree, showing that it occurred after the initial successful spread of the clone [26, 27, 56]. While this suggests that fluoroquinolone resistance is not the determining factor that spurred on the epidemic, it certainly may have helped further spread and perhaps extend the longevity of the epidemic. Demographic reconstruction of effective population size shows an inflection point and an increased rate of expansion around the time that the fluoroquinolone resistance mutations occurred [26]. Fluoroquinolone resistance has also been implicated in the rise of several other epidemic MRSA strains in other geographic locations [57–61], and multiple studies have shown a correlation between limiting fluoroquinolone prescribing and a decrease in MRSA infection [57, 62–65].

USA300 OUTSIDE THE UNITED STATES

In 2005, a strain that was very similar to USA300 from North America was isolated in Colombia [66]. This almost identical

twin sibling, named the “Latin American variant” (USA300-LV), had PVL and SAPI5. However, it did not have fluoroquinolone resistance and lacked the ACME locus. Nonetheless, it had spread rapidly through the northern part of South America, probably during the late 1990s and early 2000s [28, 66, 67]. In place of ACME, immediately adjacent to the SCCmec IVc element, many USA300-LV strains harbor a region that contains genes for copper and mercury resistance (COMER) [28]. Like ACME, the COMER locus also appears to have been acquired from *S. epidermidis* immediately prior to the rapid spread of the South American epidemic [28]. Interestingly, the only 2 genes that are shared by the ACME and COMER loci are a predicted copper exporter gene (*copB*) and an adjacent hypothetical gene predicted to encode a lipoprotein. It is tempting to speculate that these genes may play a role in the explosive spread of these epidemic clones. While it is clear that copper is quite toxic to *S. aureus* [68, 69], the role of copper toxicity in transmission and or virulence is poorly understood.

While the USA300 lineage was very successful in North America and northern South America, it has not been successful in other parts of the world. Despite multiple reported infections in other countries [70] and increasing global travel, the strain has failed to establish itself in other geographic regions. Recent phylogenomic analyses have underscored this pattern. In France [26] and England [71], USA300 strains that have been isolated are interspersed in a phylogenetic tree with North American isolates, showing multiple introductions and also multiple failures to spread. A study from Switzerland also recently used phylogenetic evidence to show that isolates of USA300 are mostly imported [72]. This study also showed evidence for the importation of South American USA300-LV strains [72, 73] with the COMER locus [73], but, as with other European studies, there was little evidence for USA300 gaining a foothold and spreading in any significant way.

It is also worth noting that a similar pattern of geographical containment is apparent within South America. USA300-LV has failed to spread outside of Colombia, Venezuela, and Ecuador [67]. The strain is only rarely isolated in countries such as Chile, Peru, Argentina, and Brazil [67, 74–77], suggesting that there may be barriers in South America that are similar to those in Europe.

IS USA300 IN DECLINE?

While USA300 is still a major problem in many places, there are hints that it may be decreasing in prevalence or, at the very least, not continuing its expansion. A study of 5 geographically dispersed centers in the United States showed marked decreases in USA300 prevalence in some locations, like Los Angeles, and significant increases in others, like New York [78]. Stable prevalence of community onset infections was observed in the 3 other centers. Interestingly, the percentage of MRSA isolates that were identified as USA300 also varied dramatically, with

higher proportions of USA300 in California and lower proportions in New York [78]. It is unclear whether these patterns simply represent local stochastic trends or different phases in the expansion and contraction of USA300. However, recent whole-genome population genetic analysis using genomes from a broad geographic distribution suggests that there has been a recent decrease in effective population size in USA300 [26]. It will be important to reproduce this result as more genomes become available.

In general, MRSA appears to be on the decline. Studies looking at MRSA infections from 2005 on show steady declines, especially in hospital-associated infections [79–82], but the same studies showed only modest changes in the rates of CA-MRSA. To the extent that CA-MRSA is represented by USA300 in the United States, these studies suggest small but detectable decreases in prevalence. Decreases in MRSA have also been seen in the pediatric population, where the large majority of cases were SSTIs [83]. It appears that this phenomenon may be occurring simultaneously in distinct parts of the world. MRSA has decreased in European countries [84–86], Australia [87], Taiwan [88], and South Africa [89]. However, data from the rest of Africa [89], parts of South America [90], and many parts of Asia [91] suggest stable or increasing MRSA prevalence.

The reasons for global decreases in MRSA are unclear. Many studies that showed decreases in MRSA have argued that aggressive infection control policies and so-called search-and-destroy strategies are largely responsible, and it is likely that these strategies have had a major impact, especially on nosocomial infections [86]. However, it is also possible that there is a larger ecological trend, and if there is indeed a decrease in the prevalence of USA300, it is likely due in large part to factors outside the hospital [78]. Hospital-associated infections can be influenced by the community ecology of colonizing strains, and whole-genome sequencing has been an important tool in establishing this point. For instance, Price et al [92] showed that only a minority of MRSA acquisitions in the intensive care unit are due to interpatient spread. Another study showed a complete absence of patient-to-patient spread [93]. These studies not only force us to examine assumptions about the differences between hospital-associated and community-associated MRSA [94], but also raise the possibility that the forces contributing to decreases in MRSA overall may also be acting on CA-MRSA.

UNDERSTANDING THE DECLINE AND PREDICTING THE NEXT WAVE

Given the hints of USA300’s possible demise, it seems like an opportune moment to understand the process of this decline and to start to pay attention to what might be coming next. It seems clear that surveillance of emerging clones will be most powerful if we use whole-genome sequencing, and the first step is to take stock of existing genomic diversity [95, 96].

However, it remains unclear exactly where we should be looking for changes in the ecology of bacterial strains.

There has been a recent new emphasis on trying to understand the dynamics of spread and colonization of MRSA in the household, which is the primary location of transmission among human populations [97, 98]. The resolution provided by whole-genome analyses for discriminating among isolates and linking them together is absolutely crucial for showing transmission events at the household level. Traditional typing methods, which lack the granularity afforded by whole genomes, fail when closely related strains are circulating at high rates, as is the case with USA300. In addition to giving insight into transmission, there is often enough information in whole genomes to apply population genetic and phylogenetic reconstruction techniques. For instance, phylogenetic divergence time analysis was recently used to suggest that specific strains may persist for very long periods, about 2–8 years, in a single household [56]. This result underscores the idea that selective pressures acting within the household likely have much larger effects on strains than pressures exerted during the relatively short time that strains are involved in disease. Understanding the dynamics of transmission in the household is even more complex than understanding pathogenesis. Reservoirs not only include other household members colonized at different body sites, but also pets [99] and environmental surfaces [100–102]. Selective pressures include not only immune responses, but also environmental toxicities and durability [103–105].

It is not clear whether there are other major environmental sources for USA300 outside of the household, but there is precedence for a broader ecological context for other MRSA strains, such as the pig-associated CC398 clone [106]. Surveillance in agriculture and animal husbandry, where there is continued use of antibiotics and bacteriocides, seems important, especially if we are interested in catching resistant organisms before they spread.

Another place to look for strains that could replace USA300 is in examples of epidemic strain invasion and replacement in other areas of the world. Examples include closely related strains, such as USA300-LV [28, 67], or more-distant relatives, such as the replacement of ST239 by ST22 (EMRSA-15) in Europe and Asia [61, 107].

Another recurring theme in strain expansion and replacement is that mobile genetic element acquisitions appear to be associated with epidemiological changes. These acquired loci (eg, COMER and ACME) can serve as important molecular markers and also might give insight into key biological changes. One interesting example of this is *sasX*, which appears to have contributed to the biological fitness of ST239 but also seems to have increased in several different sequence types in Asia [108]. This type of situation emphasizes the superiority of whole-genome analysis since it allows for the detection of mobile

genes in distinct lineages. What might look like the independent expansion of different lineages could actually be due to a beneficial trait acquired by lineages in parallel.

The ultimate goal of surveillance is to detect early phases of the expansion of pathogens and possibly forecast which lineages might spread further. New, inexpensive sequencing modalities combined with timely phylogenetic and population genetic analysis might allow us to see a small uptick in the prevalence of some strain [92, 95, 109, 110]. This type of analysis is becoming efficient and economical [109, 111–113] and also gives extra data on virulence factors and antibiotic resistance [109, 114]. It is possible that the data obtained from fine-grained surveillance could also be used in predictive forecasting. Indeed, the key factors for building predictive mathematical models of pathogen dissemination, such as duration of carriage, interaction between strains and populations, dynamics of transmission, and population heterogeneity [115], may all be addressed using a phylogenomic approach.

CONCLUSIONS

Because the rise of USA300 coincided with the widespread use of whole-genome analysis, we have a special view of the expansion of this clone. The signs that USA300 may be declining in prevalence give us an extraordinary opportunity to study its fall from the perspectives of both pattern and process. A systematic effort to understand the factors associated with the fall, the geographic variations in that pattern, and both host and environmental factors that have contributed to it, may give us clues on how to manage epidemics that present in the future. Moreover, surveillance aimed at detecting new expanding lineages will give us insight into how lineages rise to fill an open niche.

Notes

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