Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology

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Methicillin-resistant *Staphylococcus aureus* (MRSA) PFGE strain type USA300 (multilocus sequence type 8, clonal complex 8, staphylococcal cassette chromosome *mec* type IV) was first reported in the USA as a cause of skin and soft tissue infection among college football players in Pennsylvania and among prisoners in Missouri in 2000. Over the next 5 years, USA300 became the predominant community-associated MRSA strain in the USA. It was the most common PFGE type recovered from skin and soft tissue infections in persons presenting to 11 emergency departments across the USA, and caused outbreaks in Native American populations, children in daycare centres, military recruits, prison inmates and among men who have sex with men. Although predominantly a cause of skin and soft tissue infection, USA300 isolates also have been recovered from cases of invasive disease including bacteraemia, endocarditis, severe necrotizing pneumonia and osteomyelitis. Isolates of USA300 usually carry the genes encoding the Panton–Valentine leucocidin and the arginine catabolic mobile element, but rarely carry staphylococcal enterotoxin genes. USA300 isolates are becoming more resistant to antimicrobial agents, including erythromycin, levofloxacin, mupirocin and tetracycline, and have spread to Europe, South America and Australia. The emergence of the MRSA USA300 strain type represents a unique biological success story.

Keywords: MRSA, staphylococci, antimicrobial resistance, strain typing, virulence

Background

In the 1950s, microbiologists and epidemiologists interested in *Staphylococcus aureus* infections used bacteriophage typing (or ‘phage typing’ for short) to distinguish among the various strains of *S. aureus*. Referring to phage types became a common way of communicating the characteristics of specific strains of *S. aureus* among clinicians, epidemiologists and researchers. Even today, some bacteriophage types, such as the 80/81 strains, remain in the vernacular of many clinical researchers (even though some of these individuals do not understand the derivation of the term ‘80/81’). Yet, the characteristics of this epidemic strain, which was the scourge of neonatal intensive care units around the world for many years, are well known. Clearly certain bacterial strains are associated with specific disease entities and thus become a sort of shorthand for communicating the key characteristics of the organisms. For example, the mention of *Escherichia coli* O157:H7 usually conjures up associations with bloody diarrhoea and haemolytic–uraemic syndrome. In much the same way, USA300 is becoming synonymous with severe community-associated staphylococcal disease, especially in the USA.

In the late 1990s, before multilocus sequence typing (MLST), staphylococcus protein A (spa) typing, and staphylococcal cassette chromosome *mec* (SSCmec) typing were used to characterize *S. aureus* isolates, PFGE was the typing method of choice for *S. aureus* isolates and particularly for isolates of methicillin-resistant *S. aureus* (MRSA). While the technique was highly successful in clustering epidemiologically related isolates together while simultaneously differentiating among epidemiologically unrelated isolates, there was considerable variation among laboratories in the interpretation of the banding patterns and the naming of strain types. For example, while names such as the Archaic, Brazilian, Berlin, Iberian and New York–Tokyo clones were becoming common and gaining acceptance in the literature, it was still possible to see multiple papers, sometimes in the same journal, reporting *S. aureus* strains as ‘PFGE type A’, even though the ‘type A’ strains of one journal article bore little resemblance to ‘type A’ strains of another article. In the USA, those laboratories that adopted the European nomenclature for classifying their PFGE patterns had difficulty explaining to local physicians (e.g. in Oklahoma) how a patient that had never left the state managed to contract an infection with the ‘Berlin’ clone. In addition, there were
a number of MRSA isolates recovered from human infections in the USA that had PFGE patterns that were different from those in Europe, Asia and South America. Thus, the CDC established a novel system of *S. aureus* strain nomenclature based on the PFGE patterns (for both MRSA and methicillin-susceptible *S. aureus*) that were common in the USA, i.e. the USA types (Figure 1).10

**Origins of strain USA300**

The MRSA PFGE type USA300 emerged within *S. aureus* MLST clonal complex (CC) 8. As shown in Figure 2, methicillin-susceptible *S. aureus* of CC8, sequence type (ST) 8, is the presumptive ancestor of the first MRSA strain, which belonged to ST250 and carried SCCmec type I.11–13 Li et al.12 have proposed a parallel line of evolution in which acquisition of SCCmec type IV by this same progenitor strain led to USA500, which has typically been linked with healthcare-associated infections. MRSA PFGE type USA300 subsequently appeared, differing from USA500 by at least 20 genes, a significant number of which are potentially mobile elements.14 Li et al.12 argued, however, that the virulence of USA300 strains is primarily due to high expression of core genomic virulence determinants, not mobile genetic elements (see below).

The epithet ‘USA300’ was first reported in 2003 by McDougal et al.10 as one of the eight initial MRSA ‘USA’ strain types (USA100–USA800). Of these eight strains, USA100 (i.e. the New York–Tokyo clone, ST5-SCCmec II) was the most commonly isolated MRSA strain in the USA and was recovered exclusively from healthcare-associated infections. USA300, which did not have a clearly identifiable European counterpart, carried the genes encoding the Panton–Valentine leucocidin (PVL; i.e. lukF-PV and lukS-PV), contained the *msr(A)* erythromycin resistance gene and carried SCCmec type IV. Isolates of this lineage had several closely related PFGE banding patterns, indicating that USA300 was not a single clone, but rather a family of isolates with related PFGE patterns all showing >80% similarity when analysed by BioNumerics software using DICE coefficients and UPGMA clustering. However, isolates exhibiting one unique PFGE pattern, designated USA300-0114, were repeatedly implicated in unrelated outbreaks of community-associated MRSA (CA-MRSA) infections across the USA.15,16

USA300 first came to the attention of the CDC in 2000 during an investigation of an outbreak of community MRSA infections among football players in Pennsylvania.17 This was followed by an investigation of an outbreak of MRSA skin and soft tissue infections in a state prison in Mississippi where 59 inmates were infected.18 In retrospect, however, the infections were first noted in the prison in November 1999. USA300-0114
was not the only strain of MRSA isolated during this outbreak, but it was among the strains most frequently recovered from the prisoners. Investigations of MRSA infections among athletes in Colorado, Indiana and Los Angeles County followed, with all of the MRSA isolates showing highly related PFGE profiles, the majority of which were USA300-0114.

During the next 2 years, the CDC investigated a further series of MRSA outbreaks in which the infected individuals had no link to healthcare systems. These outbreaks occurred among prisoners in California, Texas and Georgia, children in Texas, Illinois and Arkansas, and athletes in Colorado and Indiana. Although there were no ascertainable links among the various outbreaks across the country that the CDC investigated, the PFGE patterns of the isolates recovered from all of the involved individuals were, for the most part, indistinguishable and were type USA300-0114. What was also remarkable was that not only were patients infected with the same strain but that they comprised distinct populations (i.e. athletes, children in daycare and inmates in prisons and jails) not traditionally considered as being at risk of MRSA infection. It was clear that the CDC had to follow this strain more closely. That proved to be easy because USA300 became the most commonly isolated strain type other than those from the CDC, and it became increasingly apparent that USA300 strains were starting to appear in publications and by various surveillance systems. By 2005, references to the USA300 strain type were starting to appear in publications other than those from the CDC, and it became increasingly clear that USA300 was showing up frequently in community settings among otherwise healthy young people.

The next major risk group for acquiring CA-MRSA infections that emerged after athletes, prisoners and children was military recruits. Outbreaks primarily of skin infections among army and navy recruits in basic training identified risk factors such as crowding, lack of cleanliness and poor wound management in the spread of MRSA infection in this population. Although skin infections were common, some soldiers developed more life-threatening illnesses including sepsis. USA300 continues to be a problem among military recruits.

While investigations of CA-MRSA outbreaks in prisons, daycare centres and among military recruits all contributed to our knowledge of the development and spread of CA-MRSA infections, it was the occurrence of MRSA infections in a professional football team in the USA that really brought all the information together. Investigation of this outbreak identified a number of critical risk factors including sharing towels, razors and other personal items; frequent skin to skin contact; massive skin abrasions that went unattended during football games; and lack of attention to cleaning of environmental surfaces and equipment. In addition, of particular note as a selective factor for MRSA disease was the issue of antimicrobial use among the athletes, which was 13 times the rate of that of the general population. This investigation thus helped shape a strategy for a series of interventions to interrupt transmission of CA-MRSA in a variety of community settings. The CDC worked with the National Collegiate Athletic Association, several professional athletic associations, the Department of Corrections and a number of state health departments to draft guidance for various groups to address the CA-MRSA issue. Even so, reports of outbreaks of CA-MRSA due to sharing towels and other personal items, poor hygiene and inadequate wound management continue to appear.

The colonization story

MRSA-infected patients and their contacts were typically screened for nasal carriage of USA300 strains during many of the early outbreak investigations of community-associated disease, yet most proved negative. At first, it was speculated that USA300 strains were not capable of colonizing the nares, but in retrospect it is now appreciated that many of the people tested had already received antimicrobial agents, which may have prevented recovery of the organisms. Moreover, the National Health and Nutrition Examination Survey (NHANES), which collected nasal swabs from ~5000 non-institutionalized people (i.e. not hospitalized or in long-term care facilities) each year for 4 years, revealed a statistically significant increase in USA300 during the 2001–2004 study period, and an even more statistically significant increase specifically in USA300-0114. Thus, USA300 found an ecological niche in the human nose. It is likely that USA300 can also colonize other body sites and that this ability to colonize aids in the dissemination of the strain.

The spread of USA300 internationally

USA300 isolates have been recognized in Australia, Austria, Colombia, Denmark, Germany, Italy, Japan, Switzerland, the UK and Hawaii. While several anecdotal cases of MRSA in European travellers have linked infection with USA300 to incidents that occurred while vacationing in the USA, it is likely that USA300 strains are now resident and causing infection in many regions around the world and are not simply imported strains. Given the demonstrated ability of this organism to colonize the nares and potentially other body sites, USA300 is likely to cause a global epidemic of skin and soft tissue infections at the least, and possibly more serious disease such as necrotizing pneumonia.

The development of antimicrobial resistance

Although USA300 isolates were initially resistant only to semi-synthetic penicillins (mediated by mecA) and macrolides [mediated by msr(A)], they have broadened their resistance profiles considerably over the last 5 years. This includes the addition of clindamycin resistance due to the acquisition of erm(A) and erm(C), and tetracycline resistance due to the acquisition of tet(K) and tet(M). Most of the resistance mediated by these three genes is associated with plasmid carriage. In addition, high-level mupirocin resistance due to the acquisition of mupA-encoding plasmids has been associated with multidrug-resistant strains of USA300 isolated during a study of infections among men who have sex with men. The USA300 isolates recovered during a study of infections in the emergency department reported by Moran et al. showed considerable resistance to the fluoroquinolones, which is presumed to be due to chromosomal mutation as was observed with other multidrug-resistant isolates. Several USA300 isolates have developed reduced susceptibility to vancomycin and, in some cases, to daptomycin, in addition to occasional resistance to gentamicin and trimethoprim–sulfamethoxazole. Thus, USA300 is fast beginning to look like its multiresistant cousin, USA100.
Pathogenicity and virulence

Most USA300 isolates contain a variety of virulence factors, such as PVL and the arginine catabolic mobile element (ACME), which are primarily located on mobile genetic elements. However, Li et al. have speculated that the virulence of USA300 isolates is primarily a function of core genomic virulence determinants rather than these acquired elements. These core determinants include α-toxin and phenol-soluble modulators (PSMs), such as those that lyse human neutrophils. In a study of a series of CC8 MRSA isolates, which included representatives of the Archaic and Iberian clones, USA300 and USA500, the latter of which is devoid of the mobile genetic elements, isolates had essentially the same high levels of production of PSMs. The USA300 and USA500 isolates also showed high levels of secreted proteases. These changes cannot be ascribed solely to changes in the global regulator, agr, although agr is likely to be involved.

A study of the genomic sequences of 10 unique USA300 isolates recovered from invasive MRSA infections from all across the USA from patients ranging in age from 1 to 86 years showed remarkable homogeneity, especially among the 8 isolates that carried the SCCmecIVa cassette as opposed to the SCCmecIVb cassette. The SCCmecIVa-containing isolates showed <44 single nucleotide polymorphisms (SNPs) among their genomes even though the isolates came from both healthcare-associated and community-associated infections and spanned the clinical spectrum of diseases from uncomplicated bacteremia to endocarditis, necrotizing pneumonia and osteomyelitis. Interestingly, among the SNPs identified, the ratio of non-synonymous to synonymous mutations was 3 to 1, suggesting that these were, in fact, significant changes from an evolutionary standpoint. These data emphasize that USA300 represents a highly successful clone that is widely disseminated across the USA and capable of causing a large spectrum of illnesses without the need of acquiring additional virulence factors.

One of the intriguing aspects of USA300 infections in the USA has been the observed increase in necrotizing pneumonia cases following influenza. Most of these cases of infection occurred in otherwise healthy individuals, many of whom were young adults. Although staphyloococal pneumonia after influenza or an influenza-like illness is not a new disease, the fact that so many of the cases were caused by USA300 isolates indicates that this strain is not simply a skin pathogen.

Spread among animals

Animals are also at risk of acquiring USA300 infections, although they are more likely to be the unfortunate recipients of these isolates from their human handlers rather than a significant reservoir for disease. USA300 isolates have been reported from a variety of animals, most recently including an elephant calf in a municipal zoo. Usually, these animals do not require antimicrobial therapy; the wounds typically resolve on their own, although family members should observe strict hand washing practices when handling an infected animal to prevent further dissemination within a household.

Discussion

The emergence of USA300 isolates in the USA and their continuing spread around the world represents a remarkable biological success story of a human pathogen. Our mobile society, when coupled with the cramped and unsanitary living conditions of various populations, and the reluctance to culture wounds in emergency departments in the late 1990s and early 2000s probably led to the dramatic spread of this organism before it was recognized as a medical and public health problem. It is now epidemic in the USA and is gaining ground around the world. A recent mathematical model developed by D’Agata et al. suggests that USA300 will become the predominant strain in US hospitals in the future and that it will probably manifest increased virulence relative to the USA100 strain it will be displacing. Ultimately, the way to halt the spread of this pathogen is through: improved hygiene in homes, communities and healthcare settings; judicious antimicrobial use in all venues; and common sense. While vaccines may play a role in curtailing the spread of USA300 isolates in the future, the time to act is now. Go wash your hands.

Transparency declarations

F. C. T. is employed by Cepheid, which markets diagnostic products for MRSA, but owns no stock at present. R. V. G. has no conflicts of interest to declare.

References


