Waves of resistance: *Staphylococcus aureus* in the antibiotic era

Henry F. Chambers and Frank R. DeLeo‡*

Abstract | *Staphylococcus aureus* is notorious for its ability to become resistant to antibiotics. Infections that are caused by antibiotic-resistant strains often occur in epidemic waves that are initiated by one or a few successful clones. Methicillin-resistant *S. aureus* (MRSA) features prominently in these epidemics. Historically associated with hospitals and other health care settings, MRSA has now emerged as a widespread cause of community infections. Community or community-associated MRSA (CA-MRSA) can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infections have been reported worldwide, and CA-MRSA strains are now epidemic in the United States. Here, we review the molecular epidemiology of the epidemic waves of penicillin- and methicillin-resistant strains of *S. aureus* that have occurred since 1940, with a focus on the clinical and molecular epidemiology of CA-MRSA.

Necrotizing fasciitis
A rapidly progressive, tissue-destructive infection of the deep soft tissue and muscle, which spreads along the fibrous connective tissue that separates and binds muscles.

Necrotizing pneumonia
An infection of the lung, usually caused by bacteria, that produces death and destruction of the lung tissue and is often accompanied by abscess formation.

**Division of Infectious Diseases, Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California 94110, USA.*
‡Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 903 South 4th Street, Hamilton, Montana 59840, USA.
Correspondence to H.F.C.
e-mail: hchambers@medsfgh.ucsf.edu.
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Staphylococcus aureus is naturally susceptible to virtually every antibiotic that has ever been developed. Resistance to antibiotics is often acquired by the horizontal transfer of genes from outside sources, although chromosomal mutation and antibiotic selection are also important. This exquisite susceptibility of *S. aureus* led to Alexander Fleming's discovery of penicillin, which ushered in the 'antibiotic era'. Penicillin was truly a miracle drug: uniformly fatal infections could now be cured. However, by the mid 1940s, only a few years after its introduction into clinical practice, penicillin resistance was encountered in hospitals, and within a decade it had become a notable problem in the community.

A fundamental biological property of *S. aureus* is its ability to asymptomatically colonize healthy individuals. Approximately 30% of humans are asymptomatic nasal carriers of *S. aureus*[1,2], such that in these individuals *S. aureus* is part of the normal flora. *S. aureus* carriers are at higher risk of infection and they are presumed to be an important source of the *S. aureus* strains that spread among individuals. The primary mode of transmission of *S. aureus* is by direct contact, usually skin-to-skin contact with a colonized or infected individual, although contact with contaminated objects and surfaces might also have a role[3–6]. Various host factors can predispose individuals to infection, including the loss of the normal skin barrier, the presence of underlying diseases such as diabetes or AIDS and defects in neutrophil function.

Infections that are caused by antibiotic-resistant strains of *S. aureus* have reached epidemic proportions globally[7]. The overall burden of staphylococcal disease, particularly disease caused by methicillin-resistant *S. aureus* (MRSA) strains, is increasing in many countries in both health care and community settings[8–13]. In the United States, the emergence of community-associated MRSA (CA-MRSA) strains accounts for much of this increase, as it is a major cause of skin and soft-tissue infections[14,15]. The rapidity and extent of the spread of CA-MRSA strains has been remarkable. In addition to the United States, CA-MRSA strains have been reported in Canada, Asia, South America and Australia as well as throughout Europe, including in countries that historically have a low prevalence of MRSA, such as Norway, the Netherlands, Denmark and Finland[12,16–29]. Globally, CA-MRSA strains have shown considerable diversity in the number of different clones that have been identified.

In addition to their increasing prevalence and incidence, CA-MRSA strains seem to be particularly virulent. Overwhelming and tissue-destructive infections, such as necrotizing fasciitis and fulminant, necrotizing pneumonia[30–32], were rarely seen before the emergence of CA-MRSA strains. The factor (or factors) that is responsible for this hypervirulent behaviour is not known, but Panton–Valentine leukocidin (PVL), which has been epidemiologically associated with severe skin infections and pneumonia that are caused by methicillin-susceptible *S. aureus* (MSSA) strains[33], is a leading candidate.

Antibiotics arguably constitute the most concentrated selective pressure on *S. aureus* in its long co-evolutionary history with mankind. The consequences of this selective pressure, in conjunction with horizontal

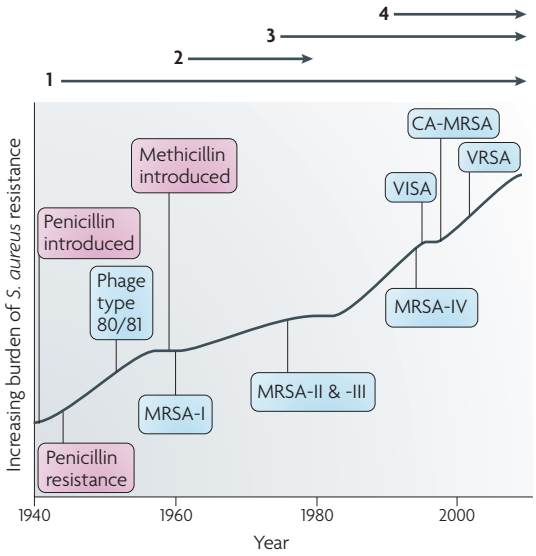


Figure 1 | **The four waves of antibiotic resistance in *Staphylococcus aureus*.** Wave 1 (indicated above the graph), which continues today, began shortly after the introduction of penicillin into clinical practice in the 1940s. The first pandemic antibiotic-resistant strains, from the lineage known as phage type 80/81, were penicillin-resistant and produced Panton–Valentine leukocidin (PVL). Wave 2 began almost immediately following the introduction of methicillin into clinical practice with the isolation of the first MRSA strain (an archaic clone), which contained staphylococcal chromosome cassette *mec* I (SCC*mec*I) (indicated on the graph as MRSA-I); this wave extended into the 1970s in the form of the Iberian clone. Wave 3 began in the mid to late 1970s with the emergence of new MRSA strains that contained the new SCC*mec* allotypes, SCC*mec*II and SCC*mec*III (MRSA-II and MRSA-III), marking the ongoing worldwide pandemic of MRSA in hospitals and health care facilities. The increase in vancomycin use for the treatment of MRSA infections eventually led to the emergence of vancomycin-intermediate *S. aureus* (VISA) strains. Wave 4, which began in the mid to late 1990s, marks the emergence of MRSA strains in the community. Community-associated MRSA (CA-MRSA) strains were susceptible to most antibiotics other than β-lactams, were unrelated to hospital strains and contained a new, smaller, more mobile SCC*mec* allotype, SCC*mec*IV (MRSA-IV) and various virulence factors, including PVL. Vancomycin-resistant *S. aureus* (VRSA) strains, ten or so of which have been isolated exclusively in health care settings, were first identified in 2002.

and vertical gene transfer, are discussed in this Review. Given their crucial importance as therapeutic agents, we focus on resistance to penicillins and the structurally related β-lactam antibiotics.

Epidemic waves of resistance

The emergence of antibiotic resistance in *S. aureus* can be visualized as a series of waves (FIG. 1). The first wave began in the mid 1940s as the proportion of infections caused by penicillin-resistant strains of *S. aureus* increased in hospitals[34,35]. These strains produced a plasmid-encoded penicillinase, which hydrolyses the β-lactam ring of penicillin that is essential for its antimicrobial activity. Penicillin-resistant strains soon began to cause community infections, and by the early 1950s they had become pandemic[36]. These infections, both in hospitals and in the community, were frequently caused by an *S. aureus* clone known as phage type 80/81 (REFS 36–39). Pandemic phage type 80/81 *S. aureus* infections largely disappeared after the introduction of methicillin[40], but the prevalence of penicillinase-producing strains from other *S. aureus* lineages has remained high.

The introduction of methicillin marks the onset of the second wave of resistance (FIG. 1). The first reports of a *S. aureus* strain that was resistant to methicillin were published in 1961 (REFS 41,42). Although the specific gene responsible for methicillin resistance (*mecA*, which encodes the low-affinity penicillin-binding protein PBP2a (also known as PBP2′)) was not identified until over 20 years later, it was appreciated early on that the resistance mechanism involved was different from penicillinase-mediated resistance because drug inactivation did not occur. Unlike penicillinase-mediated resistance, which is narrow in its spectrum of activity, methicillin resistance is broad, conferring resistance to the entire β-lactam class of antibiotics, which include penicillins, cephalosporins and carbapenems. Among the earliest MRSA clinical isolates was the archetypal MRSA strain COL, a member of the 'archaic' clone of MRSA and perhaps the most studied MRSA strain, which was isolated from a patient in Colindale, UK, in 1960 (REF. 42). COL is a member of the most successful MRSA lineage, which includes both hospital and community-associated strains.

Archaic MRSA strains circulated in hospitals throughout Europe until the 1970s[43]. There were also isolated reports of MRSA in hospitals in the United States[44,45], but the rest of the world was largely unaffected, and these early MRSA strains never gained a foothold in the community. By the 1980s, for reasons that remain unclear, the archaic MRSA clone had largely disappeared from European hospitals, marking the end of the second and the beginning of the third wave of antibiotic resistance. Descendants of the archaic MRSA clone (for example, the Iberian and Rome clones[46]) and other, highly successful MRSA lineages emerged[47–49] (TABLE 1). Outbreaks of infections caused by MRSA strains were reported in hospitals in the United States in the late 1970s, and by the mid 1980s these strains were endemic[50,51], leading to the worldwide pandemic of MRSA in hospitals that continues to the present time. Although global in its distribution and impact, MRSA was still confined mainly to hospitals and other institutional health care settings, such as long-term care facilities. The ever-increasing burden of MRSA infections in hospitals led to the increased use of vancomycin, the last remaining antibiotic to which MRSA strains were reliably susceptible. This intensive selective pressure resulted in the emergence of vancomycin-intermediate *S. aureus* (VISA) strains, which are not inhibited *in vitro* at vancomycin concentrations below 4–8 μg ml^{-1} (REF. 52), and vancomcyin-resistant *S. aureus* (VRSA) strains, which are inhibited only at concentrations of 16 μg ml^{-1} or more[53].

Panton–Valentine leukocidin
A bacteriophage-encoded, two-component, β-pore-forming toxin that integrates into the membranes of macrophages, monocytes and neutrophils and is cytolytic for these cells.

Phage type
An intraspecies strain, clone or type of bacterium that is differentiated and defined on the basis of its susceptibility to lysis by one or a panel of species-specific bacteriophages (viruses that propagate in bacterial cells).

Table 1 | **Lineages of common nosocomial MRSA strains**

Clonal complex	Sequence type	Common name(s)	Comment and SCC*mec* allotype
CC5	ST5	USA100, NewYork or Japan clone	The most common health care-associated MRSA strain in the United States; SCC*mec*II
	ST5	EMRSA-3	SCC*mec*I
	ST5	USA800 or paediatric clone	Prevalent in Argentina, Colombia and the United States; SCC*mec*IV
	ST5	HDE288 or paediatric clone (in Portugal)	SCC*mec*VI
CC8	ST250	Archaic	The first MRSA clone to be identified, includes the COL strain; SCC*mec*I
	ST247	Iberian clone or EMRSA-5	A descendant of COL-type strains; SCC*mec*I
	ST239	Brazilian or Hungarian clone	SCC*mec*III
	ST239	EMRSA- 1	An Eastern Australian epidemic clone of the 1980s; SCC*mec*III
	ST239	AUS-2 and AUS-3	Common Australian multidrug-resistant clones of the early 2000s; SCC*mec*III
	ST8	Irish-1	Common hospital-acquired isolate in the 1990s in Europe and the United States; SCC*mec*II
	ST8	USA500, EMRSA-2 or EMRSA-6	SCC*mec*IV
CC22	ST22	EMRSA-15	An international clone that is prominent in Europe and Australia; SCC*mec*IV
CC30	ST36	USA200 or EMRSA-16	The single most abundant cause of MRSA infections in UK hospitals and the second most common cause of MRSA infections in US hospitals in 2003; SCC*mec*II
CC45	ST45	USA600	SCC*mec*II
	ST45	Berlin clone	SCC*mec*IV

CC, clonal complex; MRSA, methicillin-resistant *Staphylococcus aureus*; SCC*mec*, staphylococcal chromosome cassette *mec*; ST, sequence type.

The MRSA invasion of the community constitutes the fourth and most recent wave of antibiotic resistance (FIG. 1). Some of the earliest cases of CA-MRSA infection occurred in indigenous populations in Western Australia in the early 1990s[54–56]. These MRSA strains were distinguishable from the contemporary clones or genotypes that were circulating in Australian hospitals by their pulsed-field gel electrophoresis patterns and their susceptibility to most antibiotics other than β-lactams, suggesting that they were either remote, 'feral' descendants of hospital strains or community strains that had acquired *mecA* by horizontal gene transfer. In the United States, the first well-documented cases of MRSA infection that were truly community associated occurred in otherwise healthy children from 1997 to 1999 (REF. 57). These children had no risk factors for developing MRSA and all died with overwhelming infection, suggesting that these CA-MRSA strains were especially virulent. Like their Australian counterparts, these CA-MRSA isolates were unrelated to hospital-associated clones and were susceptible to most antibiotics. The CA-MRSA epidemic in the United States can be traced back to the early 1990s on the basis of retrospective data from 1993 to 1995, which show a dramatic increase in MRSA infections in Chicago among children who lacked risk factors for hospital-associated MRSA exposure[58]. CA-MRSA has since been reported in numerous populations, including American Indians and Alaskan natives[59], Pacific Islanders[60], athletes[4], jail and prison inmates[61], men who have sex with men[62], contacts of patients with CA-MRSA infection[63], military personnel[61], adult emergency room patients[14] and children in day care centres[64]. CA-MRSA clones have also gained a foothold in hospitals and are increasingly being identified as a cause of hospital-onset and heath care-associated infections[10,12,25,65,66].

The epidemic wave of CA-MRSA in the United States and Canada[67,68] is actually two overlapping epidemics. The USA400 clone, which was isolated from the paediatric cases described above, was most prevalent before 2001 (REFS 3,57,69) and remains a common cause of community-onset disease among indigenous populations in Alaska and the Pacific Northwest[70]. A second epidemic clone, MRSA strain USA300, which is unrelated to USA400 and has largely displaced it in most other locations, emerged between 1999 and 2001 and now causes most of the CA-MRSA infections in the United States[3,4,71–74].

Outbreaks and epidemics of CA-MRSA now occur worldwide and have a similar epidemiology, although the specific clones that have emerged vary with geographical location. CA-MRSA strains are not merely escapees from health care facilities; their genotypes indicate that they are not closely related to endemic

Pulsed-field gel electrophoresis
A method for the separation of large fragments of DNA that is used in molecular epidemiology to visualize the bacterial strain-specific genome fingerprints that are generated by restriction digestion of whole genomes.

Box 1 | ***Staphylococcus aureus* genotyping**

Multilocus sequence typing
Multilocus sequence typing (MLST) is a sequence-based genotyping method based on single nucleotide variations (each variant is termed an allele) of seven housekeeping genes in *Staphylococcus aureus*, providing a discriminatory allelic profile known as a sequence type (ST)[75] for each bacterial isolate. Because it indexes variations that accumulate slowly over time, MLST can be used to measure long periods of evolution among *S. aureus* lineages, and the results obtained are highly reproducible. *S. aureus* isolates that have identity at five or more of the seven housekeeping genes as determined by MLST are known as a clonal complex (CC)[76,79].

Pulsed-field gel electrophoresis
Pulsed-field gel electrophoresis (PFGE) has a more rapid clock speed than MLST and is suitable for the evaluation of more recent evolution among groups of strains. The method relies on the separation of *Sma*I-digested *S. aureus* genomic DNA fragments in an agarose gel according to size. Related strains are clustered according to an 80% similarity coefficient[91]. The CDC has developed a national PFGE database for *S. aureus*, which uses the 'USA' designation; for example, USA300 refers to an ST8, Panton–Valentine leukocidin-positive community-associated MRSA strain[91].

***spa* typing**
spa typing[78] is based on the sequence analysis of variable-number tandem repeats in the gene that encodes protein A (*spa*). *spa* typing takes into account point mutations in the repeat region as well as the number of repeat variations. This method is suitable for the investigation of local or global *S. aureus* outbreaks. This sequence-based analysis of a single target locus is an inexpensive way of acquiring robust data that can be used to determine both epidemiological and phylogenetic relationships.

hospital clones and they are susceptible to numerous antibiotics to which hospital strains are routinely resistant. Two molecular markers that are not found in typical hospital MRSA strains are strongly associated with the emergence of CA-MRSA regardless of geographical origin: a specific cassette element encoding *mecA* and genes encoding PVL. These markers are discussed in detail below.

Molecular epidemiology of *S. aureus*

S. aureus *clonal complexes*. Robust, sequence-based molecular methods for genotyping strains of *S. aureus*, and multilocus sequence typing (MLST)[75] in particular, have made it possible to study the evolutionary history of this pathogen (BOX 1). MLST is carried out by sequence analysis of ~450 bp internal fragments of seven housekeeping genes (FIG. 2). Isolates that have identical sequences at all seven loci are considered to be a clone and are assigned a unique sequence type (ST). STs that differ by single nucleotide polymorphisms (SNPs) at fewer than three loci are thought to be closely related and are grouped into clonal complexes (CCs). This grouping is accomplished by the eBURST algorithm, which uses MLST data to group closely related strains into a CC. It also predicts the probable founding clone, or ST, of each group and the recent evolutionary descent of all other strains in the CC from the founder[76,77]. The analysis can be further refined to identify specific subclones by the addition of other methods, such as *spa* typing[78] or pulsed-field gel electrophoresis of genomic DNA (BOX 1), or by the presence of other genetic markers (for example, toxin genes or specific plasmids).

Studies of MSSA strains, carriage isolates and hospital and community isolates causing disease that were collected worldwide between 1961 and 2004 show that 88% of the collected strains can be assigned to one of 11 clonal complexes (CC1, CC5, CC8, CC9, CC12, CC15, CC22, CC25, CC30, CC45 and CC51/121)[47,75,76,79–85] (FIG. 3a). For ten of these CCs, the percentage of isolates in each complex ranges from 2% to 9%; CC30 is an outlier, accounting for 21% of isolates.

The CCs for contemporary isolates are almost certainly the same as those of strains that were circulating before 1940. For example, the ST5 lineage (the founder of CC5) is estimated to have existed for over 2,000 years[86]. Gomes and colleagues[87] genotyped 22 penicillin-susceptible and 67 penicillin-resistant MSSA blood culture isolates that were collected between 1957 and 1973 by the Statens Serum Institute in Copenhagen, which has collected and maintained every blood culture isolate from patients in Denmark from 1957 to the present. They found that 86% of the isolates fell into seven CCs, the most common being CC8 and CC30, which together accounted for 46% of the isolates (FIG. 3b). The distributions of penicillin-sensitive and penicillin-resistant isolates were similar. In this analysis, only a few isolates were tested and they all originated from a single country, which probably accounts for the absence of isolates from CC9, CC12, CC15 and CC22.

CC8 and CC30 have given rise to epidemics during each of the four waves of antibiotic resistance. The first well-characterized pandemic of antibiotic-resistant *S. aureus* that is attributable to a single clone was caused by phage type 80/81 strains, which belong to CC30 (REF. 88). Phage type 80/81 strains were originally isolated in Australia in 1953 (REF. 39). They are penicillin resistant and have caused both hospital and community outbreaks on a global scale[88]. These strains are prevalent in collections that date back to 1927; they were thought to be highly transmissible and particularly virulent and were also among the first to be identified as penicillin resistant[37]. Almost all of the phage type 80/81 isolates in a collection dating to the 1950s and 1960s encode PVL[88], which is reminiscent of the association between PVL and resistance to methicillin in the contemporary epidemic CA-MRSA strains. For unknown reasons, phage type 80/81 strains virtually disappeared in the early 1960s, and this coincided with the first use of semi-synthetic penicillins, which are resistant to penicillinase. Modern descendents of the CC30 lineage include the PVL-positive southwest Pacific (SWP) clone of CA-MRSA in Australia and the hospital-associated ST36 EMRSA-16 clone, a major cause of nosocomial infections and bacteraemia in both Australia and the United Kingdom[88–90].

MRSA CCs. The first MRSA clinical isolates, of which COL is an example, were ST250 and members of CC8. ST250 MRSA strains circulated in the United Kingdom and the rest of Europe before the 1970s but did not become established in the United States and had largely disappeared by the 1980s. However, other highly successful clones emerged, including the ST247 Iberian or EMRSA-5 clone, which is closely related to ST250. No fewer than nine other endemic nosocomial clones

Multilocus sequence typing
An unambiguous procedure for characterizing isolates of bacterial species using the sequences of internal fragments of (usually) seven housekeeping genes. Approximately 450–500 bp internal fragments of each gene are used, as these can be accurately sequenced on both strands using an automated DNA sequencer.

are descendants of the ST8 founder of this lineage. The CA-MRSA strain USA300 (which is PVL positive) that is prevalent in the United States is also ST8 (REF. 91).

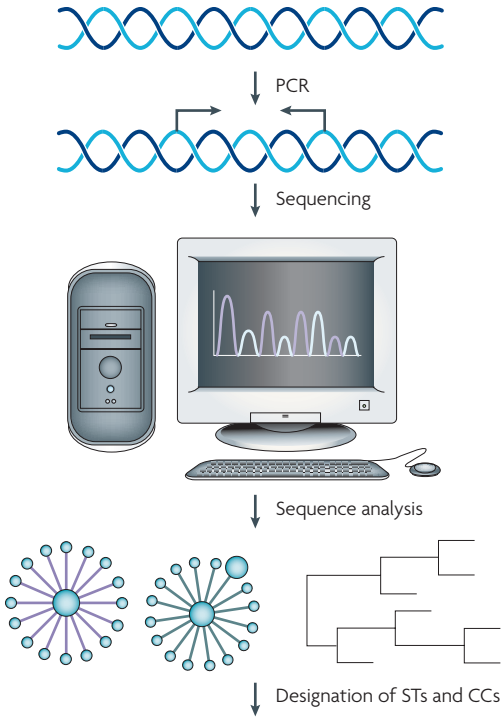
MRSA strains have generally been found to be members of a subset of *S. aureus* CCs, including CC1, CC5, CC8, CC22, CC30 and CC45, although CA-MRSA strains have exhibited some diversity (discussed below). These CCs were widespread before the emergence of methicillin resistance[43,87], indicating that superior epidemicity preceded the acquisition of drug resistance and that the adaptations and innovations that make *S. aureus* clones successful can also favour their adaptation to antibiotic selective pressures.

Staphylococcal chromosome cassette *mec*

The discovery by Hiramatsu and colleagues[92] that *mecA* is always found in a mobile cassette element was a great advance for our understanding of the biology of methicillin resistance and provided an additional tool for determining the evolutionary relationships among MRSA strains. Staphylococcal chromosome cassette *mec* (SCC*mec*) is integrated into *orfX*, an *S. aureus* gene of unknown function (FIG. 4). To date, eight SCC*mec* allotypes, designated SCC*mec*I–SCC*mec*VIII[49,92–96], have been described (TABLE 2), along with numerous subtypes, and more will probably be identified as sequence data become available for more MRSA strains (see the SCC*mec* website for additional descriptions and information). Similar elements are present in coagulase-negative staphylococci, which are commensal organisms that are part of the normal skin flora of humans and other mammals[97]. Two gene complexes, *mec* and *ccr* (the recombination and excision locus encoding the gene or genes that mediate the integration and excision of the whole cassette into and out of *orfX*), are used to classify the SCC*mec* allotypes (TABLE 2). There are also other differences among the various SCC*mec* allotypes, particularly in terms of insertion sequences and antimicrobial resistance genes. However, as these are themselves mobile elements, they have not proved useful for the classification of the main allotypes, although they are useful for defining subtypes.

The class A *mec* gene complex is the prototype complex and is found in SCC*mec*II (FIG. 4a), SCC*mec*III and SCC*mec*VIII. It contains *mecA*, the complete *mecR1* and *mecI* regulatory genes upstream of *mecA*, and the hypervariable region (HVR) and insertion sequence 431 (IS*431*) downstream of *mecA*. The class B *mec* gene complex is found in SCC*mec*I, SCC*mec*IV (FIG. 4b) and SCC*mec*VI and is composed of *mecA*, a truncated *mecR1* (resulting from the insertion of IS*1272*) upstream of *mecA*, and the HVR and IS*431* downstream of *mecA*. There are two distinct class C *mec* gene complexes, both of which contain *mecA*, a truncated *mecR1* (resulting from the insertion of IS*431*) upstream of *mecA*, and the HVR and IS*431* downstream of *mecA*. In the class C1 *mec* gene complex, the IS*431* elements upstream and downstream of *mecA* are in the same orientation, whereas in the class C2 *mec* gene complex, which is found in SCC*mec*V and SCC*mec*VII, the orientation of the IS*431* upstream of *mecA* is reversed. C1 and C2

SCC*mec* allotype
A variant of the chromosomal cassette (a mobile element in staphylococci) that encodes the gene (*mecA*) that is responsible for resistance to β-lactam antibiotics; specific allotypes are defined according to differences in the sequence or genetic organization of two regions, *mecA* and the *ccr*, which encodes the recombinase function that excises and integrates the cassette at a specific location in the staphylococcal chromosome.



Chromosomal genes and allelic designation							ST	CC
arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpiA*	*yqiL*		
1	1	1	1	1	1	1	1	1
1	4	1	4	12	1	10	5	5
3	3	1	1	4	4	3	8	8
3	3	1	1	4	4	16	250	8
3	3	1	12	4	4	16	247	8

Figure 2 | **An example of a multilocus sequence typing scheme and the designation of clonal complexes.** Multilocus sequence typing in *Staphylococcus aureus* involves PCR amplification and sequencing of approximately 450 nucleotides of seven chromosomal 'housekeeping' genes that were selected for their presumed absence of selective pressure and their moderately stable nucleotide sequences (carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triose phosphate isomerase (*tpiA*) and acetyl-CoA acetyltransferase (*yqiL*)). Each unique sequence within a gene locus is assigned a number. The numbers are concatenated left-to-right in the order shown to provide a seven-integer series of numbers, which is then assigned a sequence type (ST). Strains that are identical at all seven loci are classified as the same ST. Strains differing at one or two loci are related but, as they are not identical, they are assigned different STs. Closely related STs are grouped into a clonal complex (CC). In the example shown, ST1, ST5 and ST8 differ at most loci and so are not closely related; ST250 and ST247 differ from each other at one locus (*gmk*) and from ST8 at one (*yqiL*) and two loci (*gmk*, *yqiL*), respectively. Therefore, ST8, ST250 and ST247 are closely related and form CC8, so designated because the analysis of sequence identities and differences in a large collection of strains indicates that ST8 is the founder of this CC and the ancestor of both ST247 and ST250, and that ST247 is a descendant of ST250.

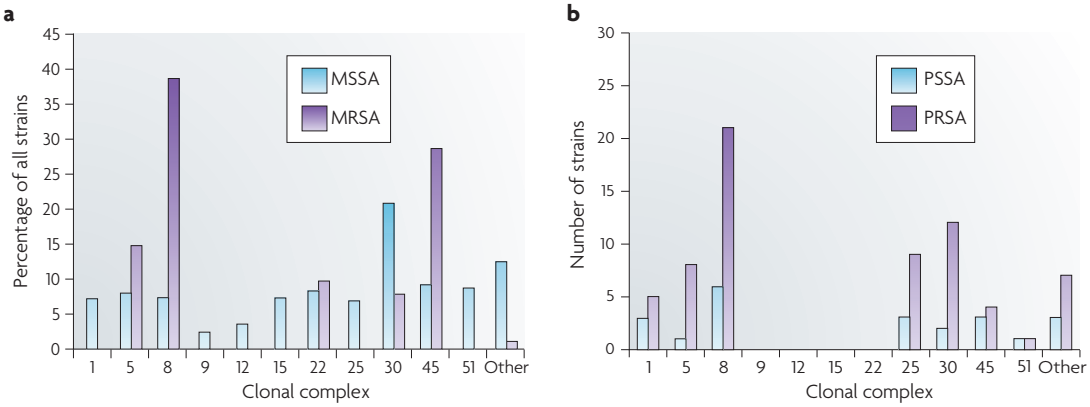


Figure 3 | **Distribution of antibiotic-susceptible and -resistant *Staphylococcus aureus* among clonal complexes.** **a** | The distribution of methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) among the various clonal complexes. These data were collected from six continents between 1961 and 2004. **b** | The distribution of penicillin-susceptible *S. aureus* (PSSA) and penicillin-resistant *S. aureus* (PRSA) among the various clonal complexes. These data are from a single study of 89 isolates that were collected in Copenhagen from 1957 to 1973. See main text for details.

are regarded as different *mec* gene complexes, as they have probably evolved independently. The *mecA*, *mecR1* and *mecI* sequences are highly conserved, with >99% nucleotide sequence identity.

The *ccr* gene complex consists of two adjacent genes, *ccrA* and *ccrB*, in SCC*mec*I–SCC*mec*IV, SCC*mec*VI and SCC*mec*VIII and one gene, *ccrC*, in SCC*mec*V and SCC*mec*VII. MRSA strains that were isolated before 1990, which were all nosocomial isolates, contained predominantly SCC*mec*I–SCC*mec*III. CA-MRSA isolates most frequently contain variants of the SCC*mec*IV or SCC*mec*IV allotypes; less commonly, they contain SCC*mec*V[28,98]. SCC*mec*IV is also increasingly identified in contemporary hospital MRSA strains.

The three epidemic waves of MRSA correspond to evolutionary changes in SCC*mec*. The early MRSA strains (COL and other CC8 strains that circulated in the United Kingdom and Denmark in the early 1960s) all carried SCC*mec*I. They were replaced in the 1980s by new and arguably more successful lineages that eventually became established in hospitals throughout the world. These clones, which were predominantly CC5 and CC8, carried SCC*mec*II or SCC*mec*III (for example, New York/Japan EMRSA, EMRSA-16 in Australia and the United Kingdom, the Brazilian clone and the Hungarian clone), or the type IA variant of the archaic SCC*mec*I (the Iberian clone). Why SCC*mec*II and SCC*mec*III were more successful than SCC*mec*I is not known, but it could be that the recombinase genes, which are defective in SCC*mec*I but functional in SCC*mec*II and SCC*mec*III[92], limited the potential for horizontal gene transfer of SCC*mec*I into new genomes.

SCC*mec*IV, which seems to have evolved from SCC*mec*I (although it has the *ccrA* and *ccrB* genes of SCC*mec*II[99]), gave rise to the most recent worldwide epidemic wave of CA-MRSA. Originally identified in the community-associated USA400 strain, MRSA strain MW2, the first occurrence of SCC*mec*IV in *S. aureus* might have been in the ST5 'paediatric' clone that was circulating in hospitals in the late 1980s and the 1990s[100]. The ultimate origins of *mecA* and SCC*mec* elements might never be known, but there is good evidence suggesting that coagulase-negative staphylococci are the sources[101–103].

The success of SCC*mec*IV is borne out by two observations. First, it is the most widely distributed SCC*mec* among *S. aureus* isolates. It has been found in nine distinct MRSA CCs or STs, whereas there are only two such lineages for SCC*mec*I, three for SCC*mec*II and two for SCC*mec*III[99]. Second, CA-MRSA strains containing SCC*mec*IV have faster growth rates than hospital MRSA strains carrying other SCC*mec* allotypes, and these growth rates are no different from MSSA isolates[98]. In a rabbit bacteraemia model the fitness and virulence of USA300, which carries SCC*mec*IVA, were indistinguishable from those of its isogenic MSSA variant[104]. Thus, the SCC*mec*IV seems to confer little or no cost in fitness on the organism.

The epidemiology of CA-MRSA

As mentioned above, the earliest reported cases of CA-MRSA infection in the United States were caused by a USA400 strain, MW2 (REF. 57). MW2 is closely related to the PVL-negative clone WA-1, which is an important CA-MRSA clone in Australia, and to the MSSA476 strain in the United Kingdom[55]. USA400 has been supplanted by USA300, which is currently by far the most frequent cause of CA-MRSA infections in the United States[105]. The USA300 clone seems to be well adapted to the community, and there are reports of CA-MRSA infections caused by USA300 or its close relatives in Australia, Denmark and Colombia[106–108]. USA300 strains can also cause health care-associated infections[65,66,109,110].

Although there is evidence for the international spread of USA300 and USA400 (REFS 18,23,111,112), CA-MRSA strains that are not related to either USA300 or USA400 have been responsible for infections outside of the United States. ST80 is the predominant clone circulating in Europe, ST59 is the main clone in Taiwan

and ST30 is the most frequent in Eastern Australia, demonstrating that CA-MRSA strains have evolved in separate geographical regions[21–23]. There can also be considerable diversity in CA-MRSA strains from country to country. For example, in Australia 45 distinct clones of CA-MRSA have been identified[23]; many of these are related to well-known MRSA lineages, but others seem to be new. The diversity of CA-MRSA isolates has also been noted by other studies[18,27,106,111,112]. In the United Kingdom, most CA-MRSA infections are caused by EMRSA-15 (ST22) and EMRSA-16 (ST36), which are also important hospital-acquired clones[113]; ST80 is also present, but accounts for only a small proportion of isolates[114]. A CA-MRSA strain of swine origin that is transmissible to humans, ST398, has also been described[115,116].

The epidemiology of CA-MRSA is similar regardless of the country of origin. Isolates tend not to be resistant to multiple drugs, SCC*mec*IV or SCC*mec*V is typically present, and infections of skin and soft tissue are the most common. The presence of PVL among CA-MRSA isolates is more variable. For example, in Australia and the United Kingdom most CA-MRSA clones do not produce PVL[23,113], and the prevalence of PVL among the more common CA-MRSA isolates from Denmark ranges from 17% to 100% (REF. 112). Conversely, isolates of clones that typically do not carry PVL genes (for example EMRSA-15 and EMRSA-16) have occasionally been found to be PVL-positive[113].

Nasal carriage of MRSA has increased in parallel with the emergence of MRSA as a community pathogen, which is not unexpected given that approximately 30% of individuals are asymptomatic nasal carriers of *S. aureus*. Between 2001 and 2004, carriage of MRSA strains in a US population-based study approximately doubled from 0.8% to 1.5% (REF. 2), and the percentage of CA-MRSA genotypes increased from 7% to 24.2% (REF. 80). Although the sites of carriage (for example, nares versus groin versus other sites) and the relationship between the carriage of CA-MRSA strains and disease are not entirely clear, CA-MRSA strains, especially USA300, seem to be more easily transmitted than other strains[117], which could account for the increasing carriage rates in the community. Thus, no individual or group can be considered not to be at risk for CA-MRSA infection.

The virulence of CA-MRSA

CA-MRSA infections have been associated with fulminant and lethal infections and worse clinical outcomes than are seen with infections caused by health care-associated MRSA strains and community MSSA[30,77,118], giving rise to the impression that CA-MRSA strains, especially USA300, are more virulent than other strains. Much of our understanding of the unique virulence properties of CA-MRSA is based on studies of USA300 strains, the most extensively investigated clone. The USA300 core genome (the chromosome, excluding any mobile genetic elements) is similar to that of the early MRSA strain COL[119]. However, studies in animal models indicate that USA300 is more virulent than COL[120,121]. The expression of virulence factors by USA300 is high, and this and other closely related strains are more lethal than their more distant relatives and cause more extensive disease in animal models of infection[121–123]. The main difference between the COL and USA300 genomes is in their mobile genetic elements, which include prophages, plasmids, pathogenicity islands and transposons that have been acquired through horizontal gene transfer. These elements encode factors that can affect transmission, antibiotic resistance and virulence. Prophages ΦSA2 and ΦSA3, which are present in USA300 strains but not in COL, could contribute to the noted differences in virulence between these two lineages. Prophage ΦSA2 contains *lukS–PV* and *lukF–PV*, which encode PVL. Prophage ΦSA3 is present in strains other than CA-MRSA and encodes staphylokinase, staphylococcal complement inhibitor (SCIN) and *S. aureus* chemotaxis inhibitory protein (CHIPS), all of which are modulators of the innate immune system[124,125]. In addition, USA300 contains the pathogenicity island SaPI5, which is similar to the island that is present in COL. SaPI5 encodes two superantigens that are not present in COL, staphylococcal enterotoxin Q (SEQ) and staphylococcal enterotoxin K (SEK), which are also found in other MRSA and MSSA lineages. *S. aureus* produces many other molecules that promote host colonization, facilitate evasion of the innate immune system and alter immune responses[123,126,127] (see Supplementary information S1 (table)). Most of these molecules are not unique to CA-MRSA. The virulence factors that are found more commonly in CA-MRSA than in other strains, that are linked by epidemiology to CA-MRSA infections or that have been studied in animal models of CA-MRSA infection are discussed below.

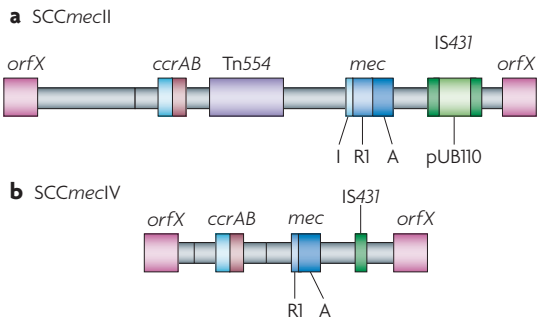


Figure 4 | **Comparison of the methicillin resistance cassettes that are typical of hospital- or community-acquired methicillin-resistant *Staphylococcus aureus*.** Staphylococcal chromosome cassette *mec* II (SCC*mec*II) is most abundant in hospitals, whereas SCC*mec*IV is present in the most abundant community-acquired methicillin-resistant *Staphylococcus aureus* strains. The *mecR1* gene (R1) in SCC*mec*IV is truncated, whereas the copy in SCC*mec*II is full-length. Transposon Tn*554*, which is present in SCC*mec*II but not in SCC*mec*IV, encodes resistance to macrolide–lincosomide–streptogramin B antibiotics and spectinomycin. pUB110 is an integrated plasmid that encodes a tobramycin resistance gene. SCC*mec*II therefore encodes resistance to multiple antibiotics, whereas SCC*mec*IV encodes resistance to methicillin alone. A, *mecA*; I, *mecI*; IS*431*, insertion sequence 431.

Staphylokinase
A secreted 15.5 kDa fibrin-specific protein produced by *S. aureus* that forms a complex with plasminogen to generate plasmin, a proteolytic enzyme that cleaves fibrin.

Staphylococcal complement inhibitor
A secreted 9.8 kDa protein that inhibits the activation of human complement, thereby interfering with the phagocytosis and killing of staphylococci by neutrophils.

Staphylococcus aureus chemotaxis inhibitory protein
A secreted 14.1 kDa protein that inhibits the recruitment of neutrophils and the inflammatory response by blocking the C5a receptor and the *N*-formyl-methionyl-leucyl-phenylalanine receptor.

Superantigen
A bacterial protein that non-specifically activates T cells, resulting in an inappropriate and massive release of cytokines and chemokines.

Table 2 | **Comparison of staphylococcal chromosome cassette *mec* allotypes**

Feature*	SCC*mec* allotype							
	I	II	III	IV	V	VI	VII	VIII
Size (kb)	34	53	67	21–24	28	24	41–49	32
mec complex	B	A	A	B	C2	B	C1 or C2	A
ccr complex	A1 and B1	A2 and B2	A3 and B3	A2 and B2	C	A4 and B4	C2 and C8	A4 and B4
IS*431* (n)	1	2	4	1	2	1	1	1
Tn*554* (n)	0	1	2	0	0	0	0	1
pUB110	–	+	–	–	–	–	–	–
pT181	–	–	+	–	–	–	–	–
pI258	–	–	+	–	–	–	–	–
Other resistance genes	None	*erm*, *spc* and *tobra*	*erm*, *tet* and Hg++	None	None	None	None	*erm* and *spc*

**mec* complex A has intact regulatory genes, *mecR1* and *mecI*, upstream of *mecA*; *mec* complex B has regulatory gene deletions resulting from the insertion sequence 1272 (IS*1272*) insertion; *mec* complexes C1 and C2 have regulatory gene deletions resulting from the IS*431* insertion; the *ccr* complex is the recombinase locus; pUB110, pT181 and pI258 are plasmids integrated at insertion sequences. *erm*, erythromycin resistance gene; Hg++, mercury resistance gene; IS*431*, insertion sequence 431; n, number of copies; *spc*, spectinomycin resistance gene; *tet*, tetracycline resistance gene; Tn*554*, transposon 554; *tobra*, tobramycin resistance gene.

PVL. PVL has been studied extensively since its discovery by Panton and Valentine 70 years ago[128] **.** The role of PVL in the marked epidemicity and enhanced virulence of CA-MRSA is a subject of debate. PVL is composed of two subunits, LukS-PV and LukF-PV[129], which are encoded by the horizontally acquired prophage ΦSA2 (REF. 130) and are secreted by the bacterium. These subunits bind to specific membrane receptors, which have yet to be identified, and associate to form pores in the membrane of host leukocytes[131,132]. At high concentrations (for example, 200 nM) PVL causes lytic cell death, but at sublytic concentrations (for example, 5 nM) it seems to partially activate neutrophils in a phenomenon known as priming, as they secrete potent mediators of inflammation, such as leukotriene B4 and interleukin 8, and also cause the release of neutrophil granule contents through exocytosis[133–135]. In addition, PVL primes neutrophils for the enhanced production of reactive oxygen species on stimulation with the widely used neutrophil agonist fMLP (*N*-formyl-methionyl-leucyl-phenylalanine)[136]. Therefore, PVL could contribute to pathogenesis by causing an exaggerated inflammatory response and injury to the host. Several lines of evidence that are largely circumstantial indicate that PVL is associated with severe skin infections and severe necrotic haemorrhagic pneumonia[33,137,138]. Both USA300, which is now the leading cause of skin and soft tissue infections in the United States and a cause of extremely severe infections, and the penicillin-resistant phage type 80/81 strains that were associated with numerous outbreaks and severe disease in the 1950s produce PVL. The epidemiological association between PVL and the emergence of genetically unrelated CA-MRSA strains (that is, different and unrelated STs) that are geographically dispersed is striking.

There are other observations that call into question the presumption that PVL is driving the CA-MRSA epidemic. First, PVL is found infrequently in other common, successful community strains. For example, the genes encoding PVL are present in only ~1–10% of MSSA clinical isolates[81,139,140]. Second, although both USA300 and USA400 express PVL, USA300 has become the predominant CA-MRSA clone in the United States. This suggests that factors other than PVL are important for the recent emergence of CA-MRSA.

The experimental evidence does not provide a clear picture either. Voyich *et al.*[141] found that USA300 and USA400 wild-type and isogenic PVL-deficient (Δ*pvl*) strains caused virtually identical courses of infection in mouse abscess and sepsis models. Furthermore, there was no difference in neutrophil phagocytosis or lysis after uptake of the bacteria. However, because these experiments were carried out using culture supernatants, the results could reflect the action of multiple lytic factors. Similar results from a rat pneumonia model were reported by Montgomery and Daum[142]. Bubeck Wardenburg *et al.*[143,144] also showed that USA300 and USA400 wild-type and isogenic Δ*pvl* strains were equally virulent in mouse abscess and pneumonia models. Diep *et al.*[145] used two rabbit bacteraemia models to compare the haematogenous dissemination of wild-type and Δ*pvl* CA-MRSA strains to major organs: although PVL did not promote seeding of lungs, spleen or blood by USA300, there was a modest, transient contribution of PVL to colonization of the kidneys. In a series of experiments that used the same USA300 wild-type and mutant (Δ*pvl*) strain pair as Voyich *et al.*[141], Brown *et al.*[146] found that the parent strain was more virulent than the Δ*pvl* mutant in mouse pneumonia and abscess models and that the disease caused by the wild-type strain was attenuated by immunization with recombinant LukF-PV or LukS-PV. In addition, Labandeira-Rey *et al.*[147]

found evidence to suggest that PVL might have a role in disease development in a mouse model of staphylococcal pneumonia: direct instillation of high doses of purified toxin provoked an inflammatory response in the lung and reduced survival. The authors used a laboratory strain of *S. aureus* that had been transduced with PVL-encoding bacteriophage to establish infection, and reported more severe disease in mice infected with this PVL-producing variant than in those infected with the PVL-negative parent. However, in addition to the presence of PVL, this transduced laboratory strain has substantial alterations in global gene expression that confounded the interpretation of the data. As PVL has no impact on protein or gene expression in USA300 or USA400 (REF. 145), it is possible that factors other than PVL accounted for the experimental results. Taken together, the data suggest that the contribution of PVL to CA-MRSA pathogenesis could be minor or perhaps dependent on an as-yet-unidentified bacterial factor or host susceptibility component.

α-haemolysin. The pore-forming toxin α-haemolysin (also known as Hla or α-toxin) causes the destruction of a wide range of host cells, including epithelial cells, erythrocytes, fibroblasts and monocytes, and is lethal in animal models when injected in purified form[148]. α-haemolysin is ubiquitous among clinical isolates, although some strains lack an active α-toxin. Recent studies by Bubeck Wardenburg *et al.*[143] showed that α-haemolysin is essential for USA300 and USA400 to cause lethal pneumonia in a mouse model of the disease. The amount of this toxin that is produced by these strains *in vitro* correlates with the severity of the resultant lung disease[122,143,149].

α-type phenol-soluble modulins. α-type phenol-soluble modulins (PSMαs) are a newly discovered group of peptides in *S. aureus* that are similar to the PSMs of *Staphylococcus epidermidis*[123]. High expression of PSMαs might contribute to the enhanced virulence of CA-MRSA; PSMs are produced at higher levels *in vitro* by prominent CA-MRSA strains, including USA300 and USA400, than by hospital-acquired MRSA strains[123]. PSMα peptides recruit, activate and ultimately lyse human neutrophils, thereby promoting *S. aureus* pathogenesis, and greatly contribute to the virulence of USA300 and USA400 in mouse abscess and sepsis models. The study by Wang *et al.*[123] was the first to identify molecules from CA-MRSA that could account at least in part for the enhanced virulence of USA300 and USA400.

Arginine catabolic mobile element. The arginine catabolic mobile element (ACME) is a 30.9 kb segment of DNA that seems to be unique to USA300 (REF. 104). This element is adjacent to SCC*mec*IV and is mobilized by the recombinases that are encoded by SCC*mec*. It contains two potential virulence factors, a cluster of arginine catabolism (*arc*) genes that encode an arginine deiminase pathway and *opp3*, which encodes an oligopeptide permease[150,151]. Deletion of ACME but not SCC*mec* has been shown to decrease the fitness of USA300 in a rabbit bacteraemia model[104]. Therefore, ACME might contribute to the fitness and epidemic spread of USA300.

Although mobile genetic elements such as ACME are likely to play a part in the transmission of CA-MRSA, there are differences in virulence potential and human disease manifestation even among similar USA300 isolates. For example, Kennedy *et al.*[105] used comparative whole-genome sequencing to determine whether USA300 arose by convergent evolution towards a hypervirulent phenotype or from a recent common ancestor of high virulence potential. Ten USA300 isolates, including some from a wide range of clinical syndromes and from different geographical locations in the United States, were examined. The strains differed from the USA300 reference strain FPR3757 genome by only a few SNPs, ranging from 11 to 408 in number. Phylogenetic analysis indicated that 8 of the strains, differing on average by 32 SNPs from the reference strain and 50 SNPs from each other, clustered with the reference strain and had descended from a recent common ancestor. These nine closely related isolates constitute the epidemic USA300 clone. Eight of the nine strains were ACME positive and all nine contained the same SCC*mec*IVA subtype. The two other strains were outliers, both lacking ACME and carrying a different SCC*mec* subtype, type IVB. Unexpectedly, the virulence of the more closely related isolates was variable in animal infection models. Some of these isolates had caused dramatically different disease syndromes in humans (for example, necrotizing pneumonia versus abscesses were caused by isolates that differed by only 23 SNPs), which serves to highlight the importance of host factors in disease presentation and severity.

Treatment in the era of CA-MRSA

CA-MRSA has had a marked impact on empirical therapy of suspected staphylococcal infection. Most β-lactam antibiotics, including all orally available agents, can no longer be assumed to be effective for a range of common staphylococcal infections, in particular for skin and soft-tissue infections. In regions where CA-MRSA is prevalent, antimicrobial therapy should be active against MRSA strains. However, there are few clinical data to support the use of agents other than vancomycin, daptomycin or linezolid. Despite a lack of rigorous clinical studies, the oral agents that are recommended for the treatment of CA-MRSA skin and soft-tissue infections include clindamycin, long-acting tetracyclines (doxycycline and minocycline) and trimethoprim–sulphamethoxazole, as well as rifampin and fusidic acid as adjunctive agents to be used in combination[152–154].

Surgical incision and drainage is the treatment of choice for cutaneous abscesses; adjunctive antimicrobial therapy is of little or no benefit in most of these cases[14,15,155,156]. Antibiotic therapy after drainage of CA-MRSA abscesses is not routinely recommended unless the patient has severe or extensive disease, has

Lysostaphin
A zinc metalloenzyme produced by *Staphylococcus simulans* that specifically lyses the *S. aureus* cell wall.

rapid progression in the presence of associated cellulitis, has symptoms of systemic illness, is very old or very young, has another illness or immune suppression (for example, type I diabetes, HIV infection or neoplastic disease), has an abscess in an area that is difficult to drain or has an abscess that is associated with septic phlebitis[152].

Vancomycin is still the preferred drug for the treatment of serious MRSA infections. However, its effectiveness is limited by prolonged, persistent or recurrent bacteraemia during therapy[157,158], high rates of microbiological and clinical failures[159], nephrotoxicity[160] and the increasing prevalence of non-susceptible strains[161,162]. Randomized clinical trials of alternative agents, such as linezolid and daptomycin, show that they are comparable or, more precisely, neither inferior nor superior to standard therapy[163–168]. Resistance and drug toxicity will remain concerns regardless of the choice of agent.

One or more new compounds that are currently being developed are likely to become available for the treatment of MRSA infections in the near future[169,170]. Telavancin, dalbavancin and oritavancin are vancomycin derivatives that rapidly kill *S. aureus* in a concentration-dependent manner *in vitro*. Whether more rapid killing will translate into an improved efficacy over vancomycin for more serious infections, such as endocarditis or bacteraemia, remains to be determined. Carbapenems and cephalosporins that bind PBP2a, the penicillin-binding protein that mediates methicillin resistance, with much higher affinity than the currently available β-lactams have been developed[171]. Two cephalosporins, ceftobiprole and ceftaroline, were shown to be clinically effective for the treatment of MRSA skin and soft-tissue infections[172,173]. One drawback with these and the other anti-MRSA β-lactams under development is that they are broad-spectrum antibiotics and are therefore not narrowly targeted treatments of MRSA infection. Further studies are needed to define their eventual role in the therapy of MRSA infections. Moreover, the vancomycin derivatives and anti-MRSA β-lactams, which can only be administered intravenously, do not address the need for orally administered agents. Orally bioavailable oxazolidinones that are active against MRSA are in the early stages of development[174].

Several non-traditional approaches to the treatment and prevention of MRSA infections have been or are still being investigated. These include lysostaphin[175], antimicrobial peptides[176] and other natural products (for example, tea tree oil)[177], as well as anti-staphylococcal vaccines[178]. There are considerable challenges to be faced in the development of these agents, including prohibitively expensive costs, the potential for patient hypersensitivity (caused by the repeated administration of protein products), the short half-lives that are associated with systemic administration and the short-lived or only partially protective immunity that is gained from vaccines, as was the case with an anti-capsular vaccine that proved to be ineffective[179]. These approaches are years away from being available in the clinic, if they make it at all. Prudent use of the agents that are now available is essential to avoid further erosion of the antimicrobial armamentarium.

Concluding remarks

S. aureus is an extraordinarily adaptable pathogen with a proven ability to develop resistance. The steady erosion of the effectiveness of β-lactam antibiotics since their first use only 60 years ago is particularly worrying. As we have described, there have been four waves of resistance over the past 60 years. Although the details vary, the basic themes of each successive wave of antibiotic resistance are similar. Often occurring as a consequence of horizontal gene transfer, resistance is initially encountered in hospitals and health care institutions, where the selective pressures for resistance are greatest. Resistant strains are temporarily contained in hospitals but eventually, through a series of modifications and adjustments, they find their way into or arise from within the community to emerge as fully fit and virulent pathogens. Our understanding of the forces that direct the evolution of virulent and drug-resistant organisms is not perfect, but the overuse and misuse of antibiotics is clearly a contributing factor. The discovery and development of new antimicrobials, although necessary, is unlikely to solve the problem of drug resistance for long. New technologies that lead to improved and more rapid diagnostics, a better understanding of the pathogenesis of staphylococcal disease and non-antimicrobial approaches to the prevention and treatment of infection will also be needed to forestall the coming of the post-antibiotic era.

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FURTHER INFORMATION

Henry F. Chambers' homepage: http://id.medicine.ucsf.edu/about/facpages/chambers.html
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