Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*

Duarte C Oliveira, Alexander Tomasz, and Hermínia de Lencastre

The first European isolate of meticillin-resistant *Staphylococcus aureus* (MRSA) was detected in 1960. Since then MRSA has become a leading cause of nosocomial infections worldwide. Using molecular typing techniques—primarily pulsed-field gel electrophoresis (PFGE)—we identified five major MRSA clones that accounted for almost 70% of the over 3000 MRSA isolates recovered in hospitals mainly in southern and eastern Europe, South America, and the USA. Most of our surveillance studies were done in these areas. Multilocus sequencing typing (MLST) of representative isolates of this collection showed that these five pandemic MRSA clones have evolved from only two distinct ancestral genetic backgrounds, one of which can be traced back to the very first European MRSA isolates and also to meticillin susceptible *S aureus* strains circulating in Danish hospitals during the mid to late 1950s—ie, shortly before the introduction of meticillin into therapy. The second lineage with a completely different MLST profile included MRSA frequently recovered in the USA, Japan, and among paediatric isolates from several parts of the world. A few isolates with a third distinct MLST type corresponding to that of EMRSA-16 were also detected in the early Danish isolates. The four structural types of *mec* element, the heterologous DNA segment containing the meticillin resistance determinant *mecA*, were present in unique combinations with the MRSA clonal types. Our findings establish evolutionary associations in the most widely spread pandemic clones of MRSA. The epidemiological factors that contributed to the massive dissemination of a few MRSA clones are not well understood. We suggest, however, that the secrets of effectiveness of MRSA could be hidden in the unique genetic background of a surprisingly few lineages of *S aureus* particularly well able to cope with the contemporary clinical environment.

Lancet Infectious Diseases 2002; **2:** 180–89

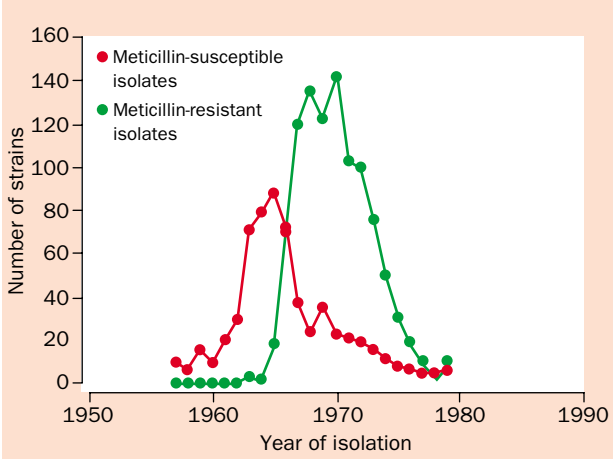

Figure 1. Sequential appearance of meticillin-susceptible and meticillin-resistant blood isolates of S aureus *belonging to phage group III and the related 83A complex in Denmark. The numbers plotted represent all* S aureus *blood isolates identified in Denmark during the particular period (adapted from reference 33).*

The adaptive power of *Staphylococcus aureus*: emergence of antibiotic-resistant strains

Staphylococcus aureus has long been recognised as one of the major human pathogens responsible for a wide range of afflictions from minor infections of the skin to wound infections, bacteraemia, infections of the central nervous system, respiratory and urinary tracts, and infections associated with intravascular devices and foreign bodies.[1,2] Most *S aureus* strains are opportunistic pathogens that can colonise individuals, without symptoms, for either short or extended periods of time, causing disease when the immune system becomes compromised. Indications are that this bacterial pathogen has affected human beings since prehistoric times. Before the antibiotic era *S aureus* diseases had high mortality rates. In 1941 the mortality rate of *S aureus* bacteraemia at the Boston City hospital was reported to be 82%.[3] In versatility of pathogenic strategies, numbers of virulence factors, and capacity to survive and multiply in a wide range of environments, *S aureus* is unsurpassed by any other human pathogen. The immense genetic repertoire of this bacterium for adapting to rapidly changing and

DCO and HdL are at the Instituto de Tecnologia Química e Biológica, Oeiras, Portugal; and all authors are at Rockefeller University, New York, USA

Correspondence: Dr Hermínia de Lencastre, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA. Tel 212 327 8278; fax 212 327 8688; email lencash@mail.rockefeller.edu

uniformly hostile environments was repeatedly shown by the emergence of *S aureus* strains that acquired resistance mechanisms to virtually all antimicrobial agents shortly after the introduction of these drugs into clinical practice.

The introduction of benzylpenicillin into chemotherapy in the early 1940s found *S aureus* fully susceptible and several of the first successes of penicillin therapy were related to the cure of formerly untreatable staphylococcal diseases.[4] But by the mid 1950s the number of *S aureus* clinical isolates showing high-level resistance to penicillin increased rapidly, to such an extent that penicillin ceased to be a useful therapeutic agent against staphylococcal infections. The mechanism of penicillin resistance involved the acquisition of a plasmid-borne penicillinase capable of degrading the antibiotic before it had reached its cellular targets. The effects of this "plasmid epidemic" were first seen on hospital isolates of *S aureus* but soon afterwards the penicillinase-based mechanism found its way into community isolates as well.[5] Currently, most *S aureus* isolates that cause disease or simply colonise healthy individuals are resistant to penicillin. A study of the *S aureus* colonising flora of 1001 healthy volunteers showed that 97% of the *S aureus* isolates recovered carried the resistant trait to penicillin.[6]

The power of *S aureus* to mount counter-strategies to additional products of the antibiotic era besides penicillin is also well documented. Records of the Danish Health Board registered the years of introduction of various antimicrobials into clinical practice, beginning with penicillin in 1945–1946, streptomycin in 1948, tetracycline in 1950, and erythromycin in 1953.[5] The same records indicate that *S aureus* bloodstream isolates resistant to penicillin, streptomycin, tetracycline, and erythromycin were recovered as early as 1957. The introduction of meticillin in clinical practice in 1960 was followed by the appearance of the first bloodstream isolate of *S aureus* that was resistant not only to penicillin, streptomycin, and tetracycline (and occasionally to erythromycin), but to meticillin as well.

Meticillin, originally called celbenine, was the first mechanism-based antimicrobial agent: it is a semisynthetic derivative of penicillin chemically modified to withstand the degradative action of penicillinase.[7] The drug was introduced into therapy in Europe in 1959–1960. 1 year later, the first meticillin-resistant *S aureus* (MRSA) strains were detected,[8] and the first clinical failure by an MRSA strain was described,[9] followed by a report on the first MRSA outbreak in 1963.[10] Since the 1960s, MRSA strains have spread among hospital isolates in several waves, which eventually disseminated these strains worldwide. Recent surveillance studies in hospitals in various parts of the world indicate a varying incidence of MRSA strains depending on the country and the hospital. In the USA, the National Nosocomial Infections Surveillance System (NNISS) recorded an increase of MRSA in large US hospitals, from 4% in the 1980s to 50% in the late 1990s. In some hospitals resistance frequencies as high as 80% have been recorded.[11]

Antibiotic resistance in an evolutionary context

The introduction of vast quantities of structurally diverse antimicrobial agents into the human environment during the past 60 years has presented a new set of challenges to bacterial pathogens such as *S aureus*. Effective lineages of contemporary pathogens must excel in several capacities: they must be able to acquire resistant genes and to construct regulatory mechanisms that can adjust resistance levels to increasing concentrations of the antimicrobial agent. In the environment, the resistance-related determinants must find their way into genetic backgrounds that assure the capacity to compete with other bacteria. Pathogens must be able to spread, establish ecological reservoirs, colonise, and cause disease.

Detection and elucidation of the mechanisms of drug resistance requires the collection of *S aureus* isolates from the clinical environment. Strain collection in increasingly sophisticated epidemiological contexts and analysis of the strains by molecular typing techniques has led to the emergence of the new discipline, molecular epidemiology, which has greatly improved the capacity of hospitals to track the source and transmission routes of bacterial pathogens during hospital outbreaks. Beyond producing sophisticated tools for epidemiological tracking, molecular epidemiology has become an alliance of clinical and molecular microbiologists that began to tackle issues of the population structure of microbial pathogens in their native environment.[12–17]

Evolutionary zero time in the emergence of MRSA

The emergence and dissemination of meticillin-resistant *S aureus* is a case of accelerated evolution in which these bacteria are propelled by the selective pressure of vast quantities of antimicrobial-related agents in the planetary environment.[18] The evolutionary zero time of this process is the acquisition of the central genetic determinant of MRSA, the *mecA* gene, a 2·1 kb stretch of DNA that is not native to the species *S aureus* and which is embedded in a larger block—up to 60kb—of additional "foreign" DNA called the *mec* element[19] or staphylococcal chromosomal cassette (SCC*mec*),[20] which together with the *mecA* is incorporated into the *S aureus* chromosome at a site-specific location.[21,22] The *mecA* gene encodes for a 78 kDa penicillin-binding protein (PBP2A), which has very low affinity for beta-lactam antibiotics.[23–25] Recent evidence indicates that PBP2A is a transpeptidase[26] that, assisted by the transglycosidase domain

Table 1. Resolution of three molecular typing methods (adapted from reference 30)

Typing method(s)*	Number of patterns
mecA + Tn*554* + PFGE subtype	97
mecA + Tn*554* + PFGE	42
mecA + Tn*554*	23
mecA + PFGE subtype	86
mecA + PFGE	29
mecA	9
Tn*554* +PFGE subtype	92
Tn*554* +PFGE	34
Tn*554*	9
PFGE subtype	80
PFGE	20

*Cla*I-*mecA* vicinity polymorphisms (*mecA*); *Cla*I-Tn*554* insertion patterns; PFGE major patterns and subtypes.

Table 2. Properties of historically early strains of MSSA recovered in Denmark (adapted from reference 33)

Strain	Date	Origin*	Phage group	PFGE pattern	Antibiogram†	*spaA* type	MLST profile	Genetic background
E213	1957	ND	83A	A19	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E298	1958	ND	83A	A19	PST	YHGFMBQBLO		
E306	1958	ND	83A	A19	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E712	1959	1	83A	A19	PST	YO		
E803	1959	2	83A	A22	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E1038	1960	3	83A	A23	PST	YHGFMBQBLO		
E1045	1960	4	83A	A19	PST	YHGFMBQBLO		
E1210	1961	5	83A	A19	PST	YHGFMBQBLO		
E1215	1961	6	83A	A24	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E1404	1962	7	83A	A19	PST	YHGFMBQBLO		
E1600	1963	9	83A	A19	PST	YHGGFMBQBLO		
E1611	1963	10	83A	A25	PST	YHGFMBQBLO		
E1907	1964	11	83A	A20	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E2251	1965	14	83A	A19	PST	YHGFMBQBLO	3 3 1 1 4 4 16	A1
E2260	1965	15	83A	A20	PST	YHFGFMBQBLO	3 3 1 1 4 4 16	A1
E2310	1965	16	83A	A26	PST	YHGFMBQBLO		
E2611	1966	18	83A	A21	PST	YHGFMBQBLO		
E228	1957	ND	83A	f	PS	YHGFMBQBLO	3 3 1 1 4 4 3	A3
E1410	1962	8	NT	g	PS	WGKAKAOMQ	2 2 2 2 6 3 2	C
E2104	1964	4	III	e1	P	TJMBMDMGMK	1 4 1 4 12 1 10	B
E3001	1967	7	83A	e2	P	TJMBMDMMK	1 4 1 4 12 1 10	B
E3008	1967	19	III	A27	P	YHGFMBQBLO	3 3 1 1 4 4 3	A3
E2615	1966	17	III	d	P	UJFKBPE		
E3410	1968	20	83A	d	Susceptible	UJFKBPE	1 1 1 1 1 1 1	
E3445	1968	7	83A	d	Susceptible	UJFKBPE		

*Numbers represent different cities; †P=penicillin, S=streptomycin, T=tetracycline. ND=not determined, NT=not typable.

of the native PBP2 of *S aureus*,[27] takes over the function of cell wall biosynthesis in the presence of beta-lactam antibiotics in the medium.

Molecular typing of MRSA

The purpose of this overview is not to detail the molecular mechanism of meticillin resistance but to survey recent information that has allowed insights into the evolutionary process that has led to the emergence of a relatively few pandemic clones of MRSA. Critical for obtaining this information were the combination of several molecular typing techniques, the elucidation of the structure of the *mec* elements,[22] and the availability of large collections of MRSA in our laboratories. These collections, from various parts of the world, include the strain collection of the Danish surveillance system that preserved every *S aureus* blood-stream isolate recovered in Denmark since the mid 1950s.[5]

A particularly successful strategy for MRSA typing has been the combination of three molecular techniques with different discriminative characteristics:[28] (i) macrorestriction pattern of chromosomal DNA after *Sma*I digestion and separation of the fragments by pulsed-field gel electrophoresis (PFGE); (ii) polymorphisms in the vicinity of the *mecA* gene detected by probing *Cla*I-digested DNAs with a *mecA* probe;[29] and (iii) transposon Tn*554* insertion patterns detected by probing *Cla*I-digested DNAs with a specific probe.[29] The individual and combined resolving ability of these methods is shown in table 1, where analysis of a group of MRSA recovered in a surveillance study in 12 hospitals in New York showed that the highest resolution was achieved by the PFGE patterns.[30] PFGE has been recognised as a highly discriminatory technique and suggested to be the gold standard for outbreak investigations.[31] Two sequencing-based techniques, multilocus-sequencing type (MLST) and *spaA* typing, have been developed recently, with great potential for global epidemiology studies.[13,15,32] MLST is based on the DNA sequencing of the internal fragments of seven unlinked housekeeping genes (*arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) and *spaA* typing is based on the DNA sequencing of the polymorphic region of protein A, a superantigen of *S aureus*.

The nature of early MRSA isolates

The preservation of all *S aureus* bacteraemic isolates in Denmark since the mid 1950s has allowed us to look at the epidemiological context of the appearance of MRSA in that country. Among 3704 *S aureus* bacteraemic isolates recovered between 1957 and 1970, 646 MRSA isolates were identified and all but nine of these were resistant not only to meticillin but to penicillin, streptomycin, tetracycline, and often to erythromycin. Furthermore, 616 of these 646 isolates (95%) belonged to unique phage groups called phage group III or the related 83A complex.[5] Data from the Danish surveillance system indicate that almost half of all *S aureus* bacteraemic isolates—both MRSA and meticillin-

Figure 2. International spread of the pandemic MSRA clones.

susceptible *S aureus* (MSSA)—recovered in Denmark during this period belonged to these phage groups. The very first European MRSA isolates identified in the UK also belonged to the same phage group and were resistant to penicillin, streptomycin, tetracycline (PST), and occasionally to erythromycin (PSTE) as well.[8,10] These observations strongly suggested that we might be able to identify among these Danish MSSA isolates one of the first *S aureus* lineages that received the *mecA* gene. Additional evidence pointing in this direction was the sequential appearance of MSSA strains belonging to phage group III and/or 83A with the PST antibiotype, and the appearance of MRSA strains belonging to the same phage group and sharing the same PST or PSTE multiresistance pattern in addition to being meticillin resistant—PSTM and PSTEM (figure 1). Molecular typing with MLST, PFGE, and *spaA* typing fully confirmed this point.[33] The overwhelming majority of historically early MSSA and MRSA isolates from

Table 3. Pandemic MRSA clones prevalence (adapted from reference 37)

Country	Isolation dates	Number of cities	Number of hospitals	Number of isolates studied	% of MRSA pandemic Clones				
					Ib	Br	Hun	NY/Jp	Ped
Argentina	1994–1998	3	13	237	..	71	..	..	10
Brazil	1992–1998	3	11	187	..	89	..	..	..
Chile	1996–1998	2	7	118	..	53	..	..	..
Colombia	1996–1998	2	5	76	..	..	..	..	98
Czech Rep.	1996–1997	7	7	59	12	80	..	..	..
Hungary	1993–1998	10	24	285	..	..	67	..	..
Italy	1993–1995	1	12	53	47	..	..	..	..
Japan	1997–1998	1	1	143	..	..	..	76	..
Poland	1990–1998	8	18	270	10	..	..	..	32
Portugal	1990–1997	5	8	504	49	22	..	..	6
Spain	1989–1993	1	1	189	83	..	..	..	..
UK	1990–1993	1	10	13	62	..	..	..	..
Uruguay	1996–1998	2	5	102	..	100	..	..	..
USA	1994–1998	4 states	42	831	14	..	..	35	6
Total				3067	19	21	6	13	9
							68%		

Ib=Iberian; Br=Brazilian; NY/Jp=New York/Japan; Paed=Pediatric; Hun=Hungarian.

Denmark and the earliest MRSA isolates from the UK shared identical or very similar molecular profiles (table 2). Characterisation of these earliest, archaic, strains of MRSA showed remarkably uniform properties in individual isolates:[34] low meticillin minimum inhibitory concentration (MIC) value (6–25 μg/ml) for most cells; heterogeneous expression of resistance; a single, common *Cla*I-*mecA* polymorph II; lack of the regulatory gene *mecI*; lack of Tn*554* in most isolates; and a closely related PFGE profile. The epidemic capacity of these archaic MRSA strains was shown by their recovery from as many as 18 hospitals in Denmark.

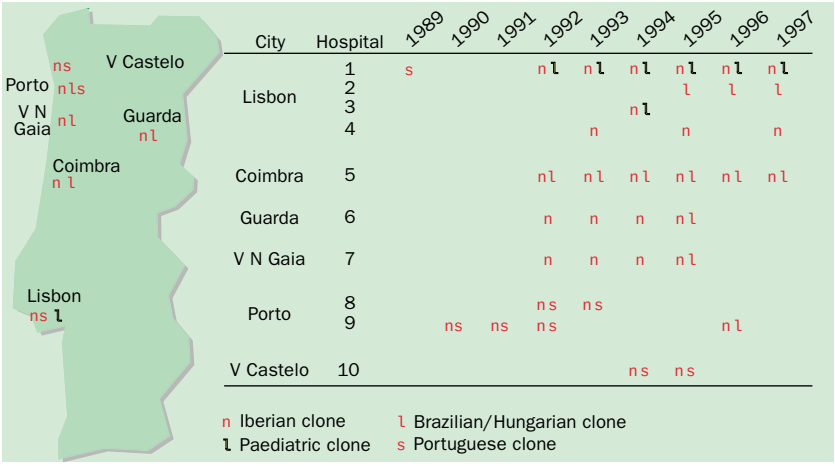

Figure 3. MRSA clonal distribution in hospitals in six cities in Portugal.

These results identify the progeny that must have been one of the first *S aureus* receiving the heterologous *mec* element from an unknown bacterial donor—ie, the first stage in the evolutionary process of emergence of MRSA. The genetic background of early MSSA and MRSA isolates was very similar or identical to the genetic background of one of the most widely spread contemporary multidrug-resistant clones of MRSA, the Iberian clone.[33,34] Although the contemporary Iberian clone shares the same genetic background as the "archaic" MRSA isolates, this clone seems to be a further step ahead in MRSA evolution because it has acquired extra resistance determinants, some of them resident in mobile elements such as plasmids (pUB110) and transposons (Tn*554*). Thus, by contrast with the limited antibiotic resistance pattern of the archaic MRSA (PSTEM), most isolates of the Iberian clone are resistant to most commonly used antimicrobial agents, with the exception of the co-trimoxazole (sulfamethoxazole-trimethoprim) and the glycopeptides.[35] The integration of the linearised plasmid pUB110 in the downstream vicinity of the *mecA* gene explains the differences in the *mecA* polymorphism detected between the archaic strains (*mecA* polymorph II) and the contemporary Iberian MRSA strains (*mecA* polymorph I).[36]

Identification of five pandemic MRSA clones

In recent years, the combination of *mecA* polymorphisms, Tn*554* insertion patterns, and PFGE profiles was applied to characterise more than 3000 MRSA isolates collected in surveillance studies and outbreak investigations in southern and eastern Europe, Latin America, and the USA between 1994 and 2000, through the international CEMNET initiative.[17] Five major clonal lineages were defined, which are widely disseminated in those areas and accounted for a large proportion—68%—of the isolates, indicating that they represented successful lineages in terms of ability to cause infection, to persist, and to spread from one geographic site to another, including across continents (table 3). The names assigned to these five MRSA—Iberian, Brazilian, Hungarian, New York/Japan, and paediatric pandemic MRSA clones—reflect the geographic area in which they were first identified and/or indicate some unique epidemiological property. In addition to their distinct PFGE patterns, the prototypes of these five clonal types had distinct properties. The Iberian clone carried a *Cla*I::*mecA* polymorph I and *Cla*I::Tn*554* pattern E (clonal type I::E). The corresponding clonal types in the other MRSA clones were as follows: Brazilian clone XI::B; Hungarian clone III::B; New York/Japan clone I::A; and paediatric clone II::NH. All strains were tested for susceptibility to nine antibiotics (oxacillin, penicillin, clindamycin, erythromycin, gentamicin, spectinomycin, co-trimoxazole, tetracycline, and vancomycin). The Iberian, Hungarian, and New York/Japan clones were only susceptible to co-trimoxazole and the Brazilian clone was only susceptible to spectinomycin. The paediatric clone was only resistant to oxacillin, penicillin, gentamycin, and occasionally erythromycin. All isolates were susceptible to vancomycin.

Figure 2 documents the spread of these five pandemic MRSA clones. The Iberian clone was first reported in Spain in 1989 and since then has been reported in Portugal, Italy, the UK, Germany, Belgium, Switzerland, France, Czech Republic, Poland, and the USA. The Brazilian clone was first

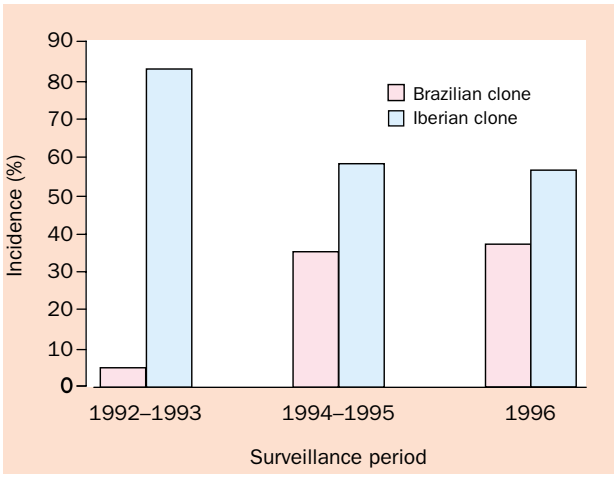

Figure 4. Variation of the incidence of two pandemic MRSA clones during three consecutive surveillance periods in the largest Portuguese hospital, located in Coimbra (adapted from reference 38).

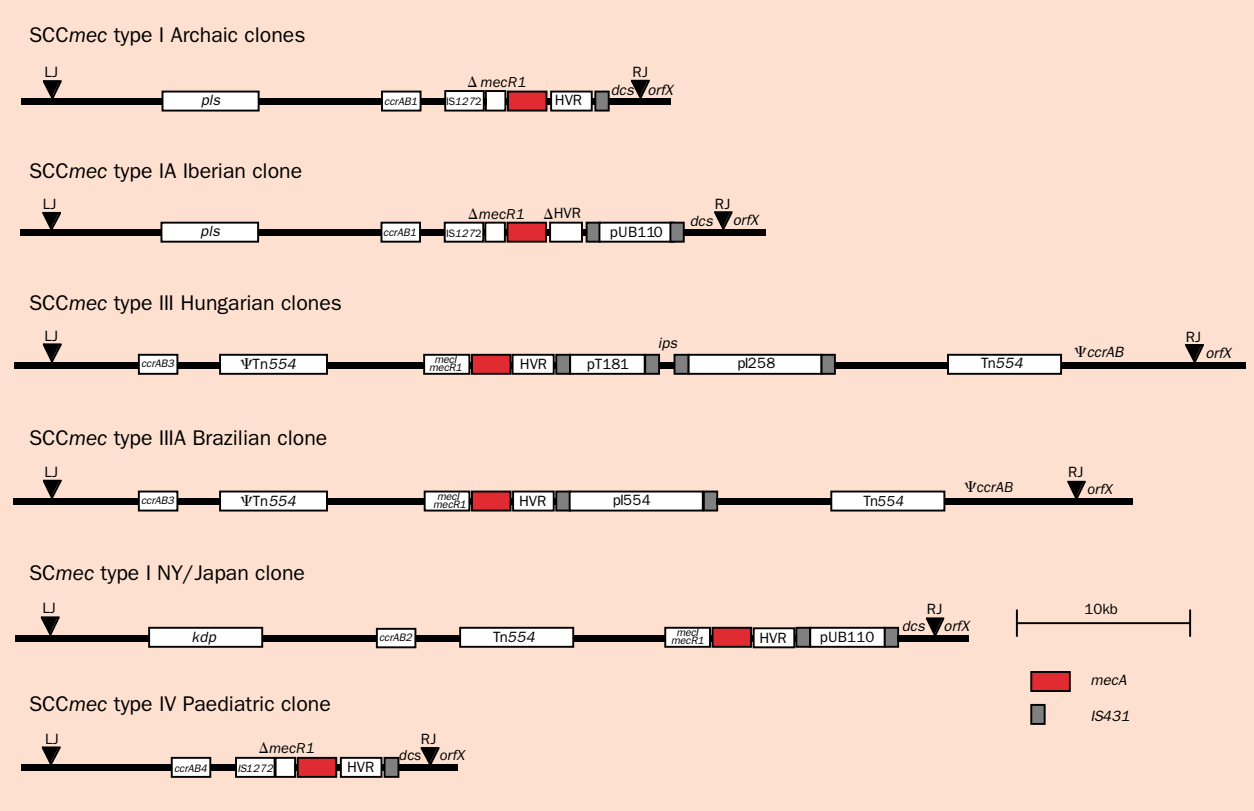

Figure 5. SCCmec element structures and variants. The basic structures of SCCmec types I, II III and IV are depicted, as well as its variants SCCmec IA and IIIA.[21,22,38,39] *LJ=chromosomal left junction, RJ= chromosomal right junction.*

described in 1992 in Brazil and then in Portugal, Argentina, Uruguay, Chile, and the Czech Republic. The Hungarian clone was extensively disseminated among hospitals in that country and was most recently also identified in Taiwan. The New York/Japan clone was identified as the dominant MRSA in hospitals in metropolitan New York, New Jersey, Pennsylvania, and Connecticut, and also in one hospital in Tokyo, Japan. The paediatric clone was first reported in a paediatric hospital in Portugal in 1992 and was also reported in Poland, USA, Argentina, and Colombia (for a list of references concerning the spread of the MSRA pandemic clones see table 1 of reference 37).

Molecular typing techniques have not only allowed the identification of pandemic clones of MRSA, but have also enabled us to monitor MRSA clones circulating in different hospitals and at different time intervals in a country providing evidence for the stability of epidemic clones over time. Figure 3 shows the MRSA clonal distribution in ten hospitals in six Portuguese cities during 9 years. Figure 4 shows that in the largest Portuguese teaching hospital located in Coimbra, during three distinct surveillance periods, the Iberian and Brazilian clones were present in different proportions but together accounted for virtually all the MRSA isolates. In this case, molecular typing techniques were able to document the introduction of the Brazilian clone during the first surveillance period. During the following surveillance periods, the prevalence of this clone increased from 5% to 38%, whereas the previous endemic Iberian MRSA clone decreased from 89% to 55%.[38]

Table 4. Genetic backgrounds and *SCCmec* types of the pandemic MRSA clones.

Clonal Type	*spaA* type motif	MLST Profile	Genetic Background	*SCCmec* type
Archaic	MBQBLO	3-3-1-1/12-4-4-16	A1	I
Iberian	MBQBLO	3-3-1-1/12-4-4-16	A2	IA
Clone V	MBQBLO	3-3-1-1-4-4-3	A3	IV
Brazilian	KAOMQ	2-3-1-1-4-4-3	A4	IIIA
Hungarian	KAOMQ	2-3-1-1-4-4-3	A4	III
NY/Japan	DMGMK	1-4-1-4-12-1-10	B	II
Pediatric	DMGMK	1-4-1-4-12-1-10	B	IV

Identification of two distinct ancestral lineages among the five pandemic MRSA clones

The primary criterion used for the initial classification of MRSA isolates into the five pandemic clonal types was their PFGE pattern. PFGE is a high-resolution technique capable of registering DNA rearrangements and acquisition or loss of genetic determinants—ie, genetic events that can happen on a short time scale. While providing a high degree of resolution, this technique could blur long-range evolutionary associations in MRSA strains. By contrast, MLST variations accumulate slower than variations in PFGE, since they arise from neutral mutations in housekeeping genes. Thus, the same MLST allelic profile could include temporally distant strains that have

accumulated differences in PFGE patterns.[13]

Using MLST we identified two major genetic backgrounds, A and B, among representative isolates of the five pandemic MRSA clones.[37] Background A has four closely related variants (single-locus variants). Backgrounds A1 and A2 were both identified among members of the "archaic" and Iberian clones, background A3 was detected in a minor USA clone (clone V), and background A4 characterises both the Brazilian and Hungarian clones (table 4). The variant alleles in backgrounds A2 and A3 each differ from A1 in a single nucleotide at one of the alleles, suggesting evolution through single point mutations.[14] Backgrounds A1, A2, and A3 also share the same *spaA* type motif. Background A4 is a single locus variant of background A3, but it differs in three nucleotides in the *arcC* allele, suggesting evolution by recombinational events.[14] Background A4 is also characterised by a distinct *spaA* type. Together, these findings suggest that the Iberian, Brazilian and Hungarian clones, and clone V, sharing the closely related genetic backgrounds A1–A4, have evolved from a common ancestor with a genotype very similar to that of the archaic MRSA. A second and completely different chromosomal background B was identified in MRSA strains originally recognised in the New York/Japan and paediatric MRSA clones.

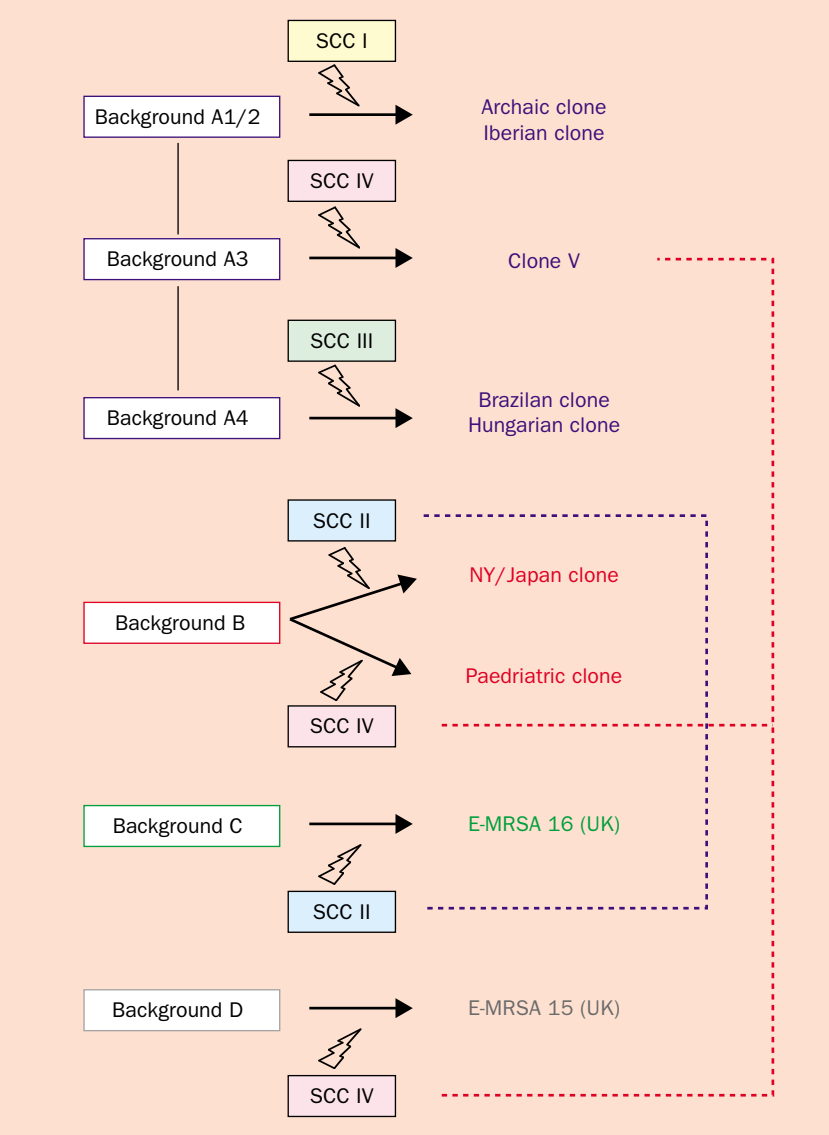

Figure 6. Proposed evolutionary pathways of the contemporary pandemic MRSA clones. Letter colors represent different genetic backgrounds (MLST), box colours represent different SCCmec types and dash lines represent possible horizontal transfers of the mec element.

The *mec* element as an evolutionary marker

The initial genetic event marking the emergence of an MRSA lineage is the acquisition of the *mec* element, introducing the meticillin-resistance determinant *mecA* into a MSSA lineage. Four types of *mec* elements or SCC*mec* have been identified so far. Type I (34 kb) was detected in the first MRSA strain isolated in 1961 in the UK (strain NCTC10442); type II (52 kb) was identified in an MRSA strain isolated in 1982 in Japan (strain N315); type III (66 kb) was identified in an MRSA strain isolated in 1985 in New Zealand (strain 82/2082);[22] and type IV (20–24 kb) was identified in two community-acquired MRSA strains,[39] as well as in representatives of the paediatric clone from Poland and from Portugal.[37]

The *mec* element structures resident in representative isolates of the five pandemic MRSA clones were recently characterised, and specific patterns of association between clonal types and SCC*mec* types were recorded[38] (table 4). MRSA strains with the genetic background A1 and A2 (archaic and Iberian clone) carried SCC*mec* types I and IA; strains with genetic background A4 (Brazilian and Hungarian clone) carried SCC*mec* types III and IIIA. SCC*mec* type IA (Iberian clone) differs from type I by the presence of the linearised plasmid pUB110 in the *mecA* downstream vicinity; whereas SCC*mec* type IIIA (Brazilian clone) varies from type III by the absence of the linearised plasmid pT181 in the *mecA* downstream vicinity. Strains representing the New York/Japan clone (genetic background B) carried SCC*mec* type II. The SCC*mec* type IV was found in strains representing the paediatric clone (genetic background B) and also in the minor clone V (background A3) (figure 5).

A search of the MLST database identified two additional important genetic backgrounds characteristic of E-MRSA 15

(allelic profile 7-6-1-5-8-8-6, background D) and E-MRSA 16 (allelic profile 2-2-2-2-3-3-2, background C), two MRSA clones that currently represent the most frequent MRSA in UK hospitals.[40,41] E-MRSA 15 was also detected in Germany.[42] An E-MRSA 16 representative strain is being sequenced. Examination of its preliminary genome sequence data indicates that it harbours a SCC*mec* type II, the same *mec* element shown to be resident in a completely different genetic background: background B characteristic of the New York/Japan clone. The presence of SCC*mec* type II in both of these distinct genetic backgrounds (B and C) could represent either an independent acquisition event or horizontal transfer of the *mec* element from one *S aureus* strain to another.

MLST studies of MSSA strains uncovered a great diversity of allelic profiles,[12,13] which contrasts with the clonal structure of the highly epidemic MRSA. The worldwide spread of MRSA seems to be primarily driven by the dissemination of a few pandemic clones in which the *mec* element found genetic backgrounds that provided appropriate fitness for virulence and epidemic spread of the bacteria. One of these genetic backgrounds—A1—has recently been identified in MSSA strains that were highly prevalent in the late 1950s in Denmark.[33] MSSA with genetic backgrounds A3 (clone V), B (Brazilian and Hungarian clones), and C (E-MRSA 16) were also detected in a few Danish isolates from this era (table 2).[33] In a recent communication by Enright,[43] the same backgrounds were detected among an independent collection of 394 epidemic MRSA isolates from 22 different countries characterised by MLST. The most frequent clonal complex I described by this author appears to correspond to genetic background A and its variants described in this communication. While MRSA with either ST22 or ST36 were not detected among pandemic isolates in our collection, a few MSSA isolates with ST36—corresponding to our genetic background C—were detected among historically early MSSA from Denmark.[33] What Enright[43] describes as a fourth type of MRSA detected among isolates from the USA and Europe presumably corresponds to our genetic background B (MLST 1-4-1-4-12-1-10) characteristic of both the New York/Japan and the paediatric clones of MRSA, which share a common MLST but may be distinguished by the distinct SCC*mec* type they carry (see table 4).

MRSA lineages and the acquisition of *mec* elements

Putting together all data one might propose important inferences as to the evolutionary pathways through which MRSA lineages could have emerged. MRSA strains have a strong clonal structure, by contrast with MSSA strains, probably due to their shorter existence (four decades). The *mec* element, containing the meticillin-resistance determinant, seems to have been imported into the species of *S aureus* at multiple, yet restricted and independent, occasions, as shown by the specific pattern of association between clonal types and SCC*mec* types. Horizontal transfer of the *mec* element between different *S aureus* lineages could also have happened as suggested by the presence of SCC*mec* type IV in backgrounds A, B, and D, and SCC*mec* type II in backgrounds B and C. However, horizontal transfer seems to be a rare event and the spread of meticillin-resistance in *S aureus* seems to be predominantly due to the clonal expansion of very few lineages. A tentative scheme outlining steps in the evolution of MRSA clonal families and the acquisition of SCC*mec* elements is presented in figure 6. The proposed evolutionary associations are primarily based on the properties of MRSA strains analysed and, in our case, these came mainly from sources in southern and eastern Europe, Latin America, and the USA. Important findings were also made possible by the availability of the Danish MRSA collection and by the information available in the MLST database. Analysis of isolates from other geographic areas would be of great interest.

The origin of the *mec* element

The origin of the *mec* element is not known. The assembly of the several *mec* element structures may have involved multiple hosts, possibly among coagulase-negative staphylococci.[44] A close homologue of the *S aureus mecA* gene was recently identified as a gene native to the animal commensal species *Staphylococcus sciuri*, most strains of which are fully susceptible to meticillin and all beta-lactam antibiotics.[45,46] The insertion sequence IS*1272* present in SCC*mec* types I and IV appears in several copies in the *Staphylococcus haemolyticus* genome.[47]

The distribution of SCC*mec* type IV provides some interesting clues concerning possible evolutionary associations. In MRSA this *mec* element structure is seen in three different genetic backgrounds, and SSC*mec* type IV was also seen in community-acquired MRSA[6,39] and also in coagulase-negative staphylococci (D C Oliveira, unpublished data).[39] These findings suggest an enhanced mobility of this *mec* element, perhaps explained by its smaller size. SCC*mec* type IV has the same downstream structure as the SCC*mec* type I. A high degree of similarity is also apparent upstream of *mecA* where both SCC*mec* I and IV have a deleted *mecR1*, an IS*1272*, and *ccrAB* flanked by two homologous sequences. However, the region between the chromosomal left junction and the upstream region of *ccrAB*—ie, the gene *pls* and its flanking regions, close to 18 kb in size—is missing in SCC*mec* IV and was replaced by a variable DNA segment.[37] The close association of SCC*mec* type IV to SCC*mec* type I, which is present in the earliest MRSA isolates, together with its presence in hospital-acquired and community-acquired MRSA strains as well as in coagulase-negative staphylococci, suggests that this *mec* element structure might be closely related to a putative precursor of the *mec* element.

Search strategies and selection criteria

Four types of references are quoted to provide context and background information to the observations and hypotheses described in this communication: historical references to MRSA, MRSA in disease, and the changing epidemiology of MRSA; references to novel molecular typing techniques such as MLST and *spaA* typing; references to the origin, structure and putative functions of *mec* elements (SCC*mec* types); and references related to the origins, epidemiological features and initial molecular characterisation of the large strain collection which was the source of epidemic MRSA clones described in our paper.

New trends in MRSA epidemiology

The specific pattern of association of SCC*mec* types and MRSA clones opens the door to some speculations about possible contributions of the SCC*mec* itself to the pathogenic potential of MRSA strains. It has been proposed that the *mec* element might be a pathogenic and/or antibiotic-resistance island,[22,48] capable of autonomous excision provided by the chromosomal cassette recombinase genes *ccrA* and *ccrB* present in every *mec* element.[22] The *mec* element, besides being the carrier of *mecA* and its regulatory genes (*mecI* and *mecR1*), also contains additional resistance determinants in the form of integrated mobile elements (pUB110, pT181, pI258, Tn*554*, and ψTn*554*). In this respect the SCC*mec* structure clearly represents an antibiotic-resistance island. Other regions of the SCC*mec* might be more directly contributing to the pathogenic potential of the *S aureus* cell. Such regions could be the *pls* gene present in SCC*mec* type I and the *kdp* operon present in SCC*mec* type II. The *pls* gene, which codes for a large surface protein, could suppress adhesion of the bacteria to their host at some stages during infection.[49] The *kdp* operon is an extra copy of the potassium-transporter operon present elsewhere in the genome,[48] which could improve the colonisation and invasion processes of the host tissues. A possible association should be considered between the SCC*mec* structure and the epidemiology of some MRSA clones. For instance, SCC*mec* type IV, which differs mainly from SCC*mec* type I by the absence of the *pls* gene and its flanking regions, characterises both the paediatric MRSA clone and the USA clone V, which have completely different genetic backgrounds. However, both clones have low-level and heterogeneous meticillin resistance and are frequently isolated from patients with immature or compromised immune systems (children and AIDS patients).[30,50–52] In a recent study, 22 of 28 patients with AIDS had MRSA infections that were seen to belong to the USA Clone V.[30] It is tempting to consider the possibility that the *pls* deletion contributes to the affinity of these strains for patients with weak immune systems.

Several recent findings suggest that MRSA might be emerging as a community pathogen[53–57] following perhaps the historic precedent of penicillin-resistant *S aureus*.[58] A screening of 1001 *S aureus* isolates recovered from colonisation sites of young and healthy people in a community showed that more than 95% of the isolates were resistant to penicillin but only seven were MRSA.[6] However, five of these seven MRSA isolates were representatives of the pandemic Iberian, Brazilian, and paediatric clones, giving another reminder of the evolutionary process of these staphylococcal lineages.

Acknowledgments

Some findings were made possible by the information available at the MLST database (www.mlst.net) and the E-MSRA 16 genome-sequencing project (www.sanger.ac.uk). Partial support for this study was provided by project POCTI/1999/ESP/34872 from Fundação para a Ciência e Tecnologia, Lisbon, Portugal, awarded to HL, and by a grant from the US Public Health Service awarded to AT, project RO1 AI37275. DCO was supported by a grant from Fundação para a Ciência e Tecnologia, Lisbon, Portugal, and also from July 2001 by a doctoral grant from Fundação Calouste Gulbenkian, Lisbon, Portugal.

References

1 Crossley KB, Archer GL, eds. The staphylococci in human disease. New York: Churchill Livingstone, 1997.
2 Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993; **6:** 428–42.
3 Skinner D, Keefer CS. Significance of bacteremia caused by *Staphylococcus aureus*. *Arch Intern Med* 1941; **68:**851–75.
4 Abraham EP, Gardner AD, Chain E, et al. Further observations on penicillin. *Lancet* 1941; **ii:**177–89.
5 Jessen O, Rosendal K, Bulow P, Faber V, Eriksen KR. Changing staphylococci and staphylococcal infections. A ten-year study of bacteria and cases of bacteremia. *N Engl J Med* 1969; **281:** 627–35.
6 Sa-Leao R, Sanches IS, Couto I, Alves CR, de Lencastre H. Low prevalence of methicillin-resistant strains among *Staphylococcus aureus* colonizing young and healthy members of the community in Portugal. *Microb Drug Resist* 2001; **7:** 237–45.
7 Rolinson GN. Letter. *BMJ* 1961. **1:** 125–26.
8 Jevons MP. "Celebenin"-resistant staphylococci. *BMJ* 1961; **1:** 124–25.
9 Dowling HF. The new penicillins. *Clin Pharmacol Ther* 1961; **2:** 572–80.
10 Stewart GT, Holt RJ. Evolution of natural resistance to the newer penicillin. *BMJ* 1963; **1:** 308–11.
11 Wenzel RP, Nettleman MD, Jones RN, Pfaller MA. Methicillin-resistant *Staphylococcus aureus*: implications for the 1990s and effective control measures. *Am J Med* 1991; **91:** 221S–27S.
12 Day NP, Moore CE, Enright MC, et al. A link between virulence and ecological abundance in natural populations of *Staphylococcus aureus*. *Science* 2001; **292:** 114–16.
13 Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38:** 1008–15.
14 Feil EJ, Smith JM, Enright MC, Spratt BG. Estimating recombinational parameters in *Streptococcus pneumoniae* from multilocus sequence typing data. *Genetics* 2000; **154:** 1439–50.
15 Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA* 1998; **95:** 3140–45.
16 Smith JM, Feil EJ, Smith NH. Population structure and evolutionary dynamics of pathogenic bacteria. *Bioessays* 2000; **22:** 1115–22.
17 Tomasz A, de Lencastre H. Molecular microbiology and epidemiology: coexistance or alliance? In: Wenzel RP, ed. Prevention and control of nosocomial infections. Baltimore, MD: Williams & Wilkins, 1997: 309–21.
18 Tomasz A. Accelerated evolution: emergence of multidrug resistant gram-positive bacterial pathogens in the 1990's. *Neth J Med* 1998; **52:** 219–27.
19 Beck WD, Berger-Bachi B, Kayser FH. Additional DNA in methicillin-resistant *Staphylococcus aureus* and molecular cloning of mec-specific DNA. *J Bacteriol* 1986; **165:** 373–78.
20 Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2000; **44:** 1549–55.
21 Ito T, Katayama Y, Hiramatsu K. Cloning and nucleotide sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus* N315. *Antimicrob Agents Chemother* 1999; **43:** 1449–58.
22 Ito T, Katayama Y, Asada K, et al. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; **45:** 1323–36.
23 Hartman B, Tomasz A. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1981; **19:** 726–35.
24 Reynolds PE, Brown DF. Penicillin-binding proteins of beta-lactam-resistant strains of *Staphylococcus aureus*: effect of growth conditions. *FEBS Lett* 1985; **192:** 28–32.
25 Ubukata K, Nonoguchi R, Matsuhashi M, Konno M. Expression and inducibility in *Staphylococcus aureus* of the *mecA* gene, which encodes a methicillin-resistant *S aureus*-specific penicillin-binding protein. *J Bacteriol* 1989; **171:** 2882–85.
26 Pinho MG, Filipe SR, de Lencastre H, Tomasz A. Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance protein PBP2A in *Staphylococcus aureus*. *J Bacteriol* 2001; **183:** 6525–31.
27 Pinho MG, de Lencastre H, Tomasz A. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. *Proc Natl Acad Sci USA* 2001; **98:** 10886–91.
28 de Lencastre H, Couto I, Santos I, Melo-Cristino J, Torres-Pereira A, Tomasz A. Methicillin-resistant *Staphylococcus aureus* disease in a Portuguese hospital: characterization of clonal types by a combination of DNA typing methods. *Eur J Clin Microbiol Infect Dis* 1994; **13:** 64–73.
29 Kreiswirth B, Kornblum J, Arbeit RD, et al. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. *Science* 1993; **259:** 227–30.
30 Roberts RB, de Lencastre A, Eisner W, et al. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in 12 New York hospitals. MRSA Collaborative Study Group. *J Infect Dis* 1998; **178:** 164–71.
31 Tenover FC, Arbeit R, Archer G, et al. Comparison of traditional and molecular methods of typing isolates of *Staphylococcus aureus*. *J Clin Microbiol* 1994; **32:** 407–15.
32 Shopsin B, Gomez M, Montgomery SO, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999; **37:** 3556–63.
33 Crisostomo MI, Westh H, Tomasz A, Chung M, Oliveira DC, de Lencastre H. The evolution of methicillin resistance in *Staphylococcus aureus*: similarity of genetic backgrounds in historically early methicillin susceptible and resistant isolates and contemporary epidemic clones. *Proc Nat Acad Sci USA* 2001; **98:** 9865–70.
34 de Lencastre H, Chung M, Westh H. Archaic strains of methicillin-resistant *Staphylococcus aureus*: molecular and microbiological properties of isolates from the 1960s in Denmark. *Microb Drug Resist*

2000; **6:** 1–10.
35 Dominguez MA, de Lencastre H, Linares J, Tomasz A. Spread and maintenance of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone during an outbreak of MRSA disease in a Spanish hospital. *J Clin Microbiol* 1994; **32:** 2081–87.
36 Oliveira DC, Wu SW, de Lencastre H. Genetic organization of the downstream region of the *mecA* element in methicillin-resistant *Staphylococcus aureus* isolates carrying different polymorphisms of this region. *Antimicrob Agents Chemother* 2000; **44:** 1906–10.
37 Oliveira DC, Tomasz A, de Lencastre H. The evolution of pandemic clones of methicillin resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. *Microb Drug Resist* 2001; **7:** 349–61.
38 Oliveira D, Sanches IS, Tamayo M, et al. Virtually all MRSA infections in the largest Portuguese hospital are caused by two internationally spread multiresistant strains: the "Iberian" and the "Brazilian" clones of MRSA. *Clin Microbiol Infect* 1998; **4:** 373–84.
39 Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol* 2001; **9:** 486–93.
40 Cox RA, Conquest C, Mallaghan C, Marples RR. A major outbreak of methicillin-resistant *Staphylococcus aureus* caused by a new phage-type (EMRSA-16). *J Hosp Infect* 1995; **29:** 87–106.
41 Richardson JF, Reith S. Characterization of a strain of methicillin-resistant *Staphylococcus aureus* (EMRSA-15) by conventional and molecular methods. *J Hosp Infect* 1993; **25:** 45–52.
42 Witte W, Enright M, Schmitz FJ, Cuny C, Braulke C, Heuck D. Characteristics of a new epidemic MRSA in Germany ancestral to United Kingdom EMRSA 15. *Int J Med Microbiol* 2001; **290:** 677–82.
43 Enright M. Global epidemiology of MRSA using multilocus sequencing. 2001. 41st ICAAC; Chicago, IL; Dec 16–19, 2001. Abstr 1989.
44 Archer GL, Niemeyer DM. Original evolution of DNA associated with resistance to methicillin in staphylococci. *Trends Microbiol* 1994; **2:** 343–47.
45 Wu S, Piscitelli C, de Lencastre H, Tomasz A. Tracking the evolutionary origin of the methicillin resistance gene: cloning and sequencing of a homologue of *mecA* from a methicillin susceptible strain of *Staphylococcus sciuri*. *Microb Drug Resist* 1996; **2:** 435–41.
46 Couto I, de Lencastre H, Severina E, et al. Ubiquitous presence of a *mecA* homologue in natural isolates of *Staphylococcus sciuri*. *Microb Drug Resist* 1996; **2:** 377–91.
47 Archer GL, Thanassi JA, Niemeyer DM, Pucci MJ. Characterization of IS*1272*, an insertion sequence-like element from *Staphylococcus haemolyticus*. *Antimicrob Agents Chemother* 1996; **40:** 924–29.
48 Kuroda M, Ohta T, Uchiyama I, et al. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet* 2001; **357:** 1225–40.
49 Savolainen K, Paulin L, Westerlund-Wikstrom B, Foster TJ, Korhonen TK, Kuusela P. Expression of *pls*, a gene closely associated with the *mecA* gene of methicillin-resistant *Staphylococcus aureus*, prevents bacterial adhesion in vitro. *Infect Immun* 2001; **69:** 3013–20.
50 Shopsin B, Mathema B, Zhao X, Martinez J, Kornblum J, Kreiswirth BN. Resistance rather than virulence selects for the clonal spread of methicillin-resistant *Staphylococcus aureus*: implications for MRSA transmission. *Microb Drug Resist* 2000; **6:** 239–44.
51 Gomes AR, Sanches IS, Aires de Sousa M, Castaneda E, de Lencastre H. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Colombian hospitals: dominance of a single unique multidrug- resistant clone. *Microb Drug Resist* 2001; **7:** 23–32.
52 Sa-Leao R, Santos Sanches I, Dias D, Peres I, Barros RM, de Lencastre H. Detection of an archaic clone of *Staphylococcus aureus* with low-level resistance to methicillin in a pediatric hospital in Portugal and in international samples: relics of a formerly widely disseminated strain? *J Clin Microbiol* 1999; **37:** 1913–20.
53 Adcock PM, Pastor P, Medley F, Patterson JE, Murphy TV. Methicillin-Resistant *Staphylococcus aureus* in two child care centers. *J Infect Dis* 1998; **178:** 577–80.
54 CDC. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus* - Minnesota and North Dakota, 1997–1999. *MMWR Morb Mortal Wkly Rep* 1999; **48:** 707–10.
55 Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998; **279:** 593–98.
56 Lindenmayer JM, Schoenfeld S, O'Grady R, Carney JK. Methicillin-resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding community. *Arch Intern Med* 1998; **158:** 895–99.
57 O'Brien FG, Pearman JW, Gracey M, Riley TV, Grubb WB. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. *J Clin Microbiol* 1999; **37:** 2858–62.
58 Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* 2001; **7:** 178–82.