J Antimicrob Chemother 2013; **68**: 2453–2457
doi:10.1093/jac/dkt213 Advance Access publication 13 June 2013

Journal of Antimicrobial Chemotherapy

Molecular and phenotypic evidence for the spread of three major methicillin-resistant *Staphylococcus aureus* clones associated with two characteristic antimicrobial resistance profiles in China

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Received 13 February 2013; returned 5 April 2013; revised 15 April 2013; accepted 3 May 2013

Objectives: The distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) clones is dynamic and geographically unique. To understand the changing epidemiology of MRSA infections in China, we performed a prospective, multicity surveillance study with molecular typing and phenotypic analysis to determine the association of major prevalent clones with their antimicrobial resistance profiles.

Methods: A total of 517 *S. aureus* isolates collected between January 2009 and March 2012 from six cities in China were subjected to antibiogram analysis and molecular typing, including staphylococcal cassette chromosome *mec* typing, multilocus sequence typing, staphylococcal protein A gene typing and PFGE typing.

Results: Among the isolates collected, 309 were characterized as MRSA, with a prevalence of 59.8%. Three major clones were found to be prevalent in China: ST239-MRSA-III-t030, ST239-MRSA-III-t037 and ST5-MRSA-II-t002. These three clones were associated with two characteristic resistance profiles, namely, gentamicin/ciprofloxacin/rifampicin/levofloxacin for the first clone and gentamicin/ciprofloxacin/clindamycin/erythromycin/tetracycline/levofloxacin/trimethoprim/sulfamethoxazole for the latter two. Several geographically unique minor clones were also identified.

Conclusions: The predominant MRSA clones in China were associated with characteristic antimicrobial resistance profiles. Antibiotics for treating patients with MRSA infections can be selected based on the strain typing data.

Keywords: molecular typing, antibiogram analysis, clonal complexes, drug resistance profiles

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), the most representative nosocomial pathogen since *S. aureus* isolates acquired a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*)[1] from other staphylococcal isolates, imposes a high burden on healthcare resources and significantly contributes to morbidity and mortality.[2] The distribution of MRSA clones is dynamic and geographically unique. One way to control the spread of MRSA is by determining the genotypic characteristics of MRSA clones and the genetic relatedness of strains in different geographic regions. Several molecular methods have been developed to type MRSA isolates, including PFGE,[3] multilocus sequence typing (MLST)[4] and typing based on unique fragments of the genetic loci SCC*mec*[5] and the polymorphisms of the X region in the staphylococcal protein A gene (*spa*).[6] With these typing methods, several MRSA clones have been identified worldwide, including New York and Japan (ST5-SCC*mec* II), Hungary (ST239-SCC*mec* III) and the USA (ST8-SCC*mec* IV).[7,8] MRSA isolates also harbour numerous determinants associated with antibiotic resistance.[9–12] The association of MRSA antimicrobial resistance profiles (ARPs) with their molecular characteristics can provide useful information for the clinical treatment of MRSA infections.

MRSA was originally detected in China in the 1970s, and the proportion of MRSA amongst *S. aureus* isolates increased to ~20% after 1980 and continued to increase at this level until 2000. In the 21st century, MRSA prevalence rapidly increased, comprising 60% of *S. aureus* in hospital isolates in 2008.[13] Two major clones, ST239-MRSA-SCC*mec* type III and ST5-MRSA-SCC*mec* type II, were shown to be prevalent in China during 2005 and 2006.[14] To determine the dynamic status of the predominant MRSA types in China, 517 *S. aureus* isolates (with 309 MRSA isolates) collected from nine teaching hospitals in six cities were subjected to antimicrobial susceptibility testing and molecular typing. The correlation between the MRSA molecular types and their antibiotic resistance phenotypes was also investigated.

Materials and methods

Bacterial isolates

A total of 517 *S. aureus* isolates from nine teaching hospitals in six cities collected between January 2009 and March 2012 were included in the present study. The isolates were recovered from the respiratory tract secretions, blood, drainage, pus, wound and abdominal fluid of 496 patients. Among the 517 isolates, there were 35 isolates collected from 14 patients at different collection times, from different samples or with different resistance phenotypes. The isolates were kept frozen at −80°C in tryptic soy broth supplemented with 40% glycerol. DNA was extracted as described by Unal *et al*.[15] To confirm the MRSA isolates, the *mecA* and *femB* genes were detected by multiplex PCR as previously described.[16]

SCC*mec* typing

SCC*mec* typing of MRSA isolates was performed using four unique and specific pairs of primers for SCC*mec* types I, II, III, IV and V as previously described.[5] The MRSA isolates with unanticipated fragments or lacking fragments by multiplex PCR were defined as non-typeable (NT). MRSA NCTC10442 (SCC*mec* I), MRSA N315 (SCC*mec* II), MRSA85/2082 (SCC*mec* III), MRSA JCSC 4744 (SCC*mec* IV) and MRSA WZ153 (SCC*mec* V) were provided by Dr Fangyou Yu (Wenzhou Medical College, China).[17]

MLST, spa typing and PFGE

A previous study demonstrated that ST239-MRSA-SCC*mec* type III is the most predominant MRSA clone in China.[14] Two sets of primers designed by Feil *et al*.[18] were used to screen ST239, and the remaining MRSA isolates were subjected to MLST analysis by PCR amplification and sequencing of the seven housekeeping genes (*arc*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL*) as described.[4] Allelic profiles and sequence types (STs) were assigned according to the MLST web site (http://saureus.mlst.net/). STs sharing at least five of seven identical alleles were grouped into a single clonal complex (CC).[19] The X region of the *spa* gene in *S. aureus* contains variable numbers of 21–27 bp repeats, with the 24 bp repeat being the most common.[6] The X region of each MRSA isolate was amplified by PCR as described by Shopsin *et al*.[20] The amplified products were sequenced and analysed based on the *spa* database web site (http://www.ridom.de/spaserver/), and each isolate was assigned a specific *spa* type.[21] The SmaI-digested genomic DNA of each MRSA isolate was separated by PFGE as previously described.[22] A type of MRSA strain N315 DNA PFGE molecular size standard was included in each gel. The PFGE patterns were analysed with BioNumerics version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) according to the unweighted pair-group matching analysis clustering algorithm. The strains with >90% identity were categorized into the same group.

Antimicrobial susceptibility testing

Antibiogram testing of the MRSA isolates was performed by broth dilution tests, according to the guidelines set by the CLSI. The MICs of 3 β-lactam antimicrobial agents (oxacillin, penicillin and ampicillin) and 14 non-β-lactam antimicrobial agents (gentamicin, ciprofloxacin, clindamycin, erythromycin, rifampicin, tetracycline, vancomycin, teicoplanin, levofloxacin, moxifloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, linezolid and quinupristin/dalfopristin) were determined. CLSI breakpoints were used for MIC interpretation.

Definitions

The clone comprising >10% of the isolates was considered the major prevalent clone. An isolate was considered multidrug resistant (MDR) when the isolate was resistant to three or more classes of non-β-lactam antimicrobial agents.[23] Resistance rates >70% of MRSA isolates to the non-β-lactam antimicrobial agents comprise the ARPs.

Results

MRSA prevalence and SCC*mec* types

Genetic detection of the *mecA* and *femB* genes revealed that 59.8% (309/517) of *S. aureus* isolates were MRSA. MRSA prevalence exceeded 60% in four coastal cities: Beijing (100%), Guangzhou (78.5%), Shanghai (69.5%) and Shenyang (68.3%) (Table 1). Meanwhile, MRSA prevalence was <60% in the inland cities of Chongqing (45.0%) and Urumchi (25.8%) (Table 1). In addition to the number of strains recovered for analysis, the spread of MRSA may be related to local geographical conditions such as climate, hygiene, population and available healthcare.

A total of 309 MRSA isolates were analysed by SCC*mec* typing. Five SCC*mec* types were found: I, II, III, IV and V (Table 1). The most common SCC*mec* type was type III, which was present in 178 isolates (57.6%; 178/309) and distributed in all cities, especially in Urumqi (87.0%) and Beijing (75.0%). SCC*mec* type II was the second most predominant type, present in 22.0% (68/309) of the isolates and in four cities, being most prevalent in Shenyang (60.7%) and Shanghai (48.8%). The third predominant type was SCC*mec* type IV, present in 8.7% (27/309) of the isolates and present in five cities, being most prevalent in Beijing (12.5%), Guangzhou (11.3%) and Chongqing (11.9%). Nineteen of 309 (6.2%) isolates belonged to SCC*mec* type I, which were distributed in four cities. SCC*mec* type V was found in 13 isolates (4.2%). Four of 309 isolates (1.3%) were defined as NT by multiplex SCC*mec* typing.

Characterization of MRSA with MLST, spa typing and PFGE

The STs of the 309 MRSA isolates were characterized. Fifteen STs that could be clustered into 12 CCs and 4 new STs were found (Table S1, available as Supplementary data at JAC Online). Four major CCs (CC239, CC5, CC59 and CC45) comprising 93.2% of the MRSA isolates were found to be prevalent in China. ST239 (CC239) was the most predominant ST (51.5%, 159/309), and was distributed in all six cities. By *spa* typing, ST239 included *spa* types t030, t037, t138, t459, t632 and t3167. The most predominant *spa* type in ST239 isolates was t030 (56.6%, 90/159), followed by t037 (30.2%, 48/159). ST5 (CC5) was determined to be the second most common ST (25.2%, 78/309), and the most predominant ST for SCC*mec* II isolates. ST5 was the most common ST identified in Shenyang (78.6%) and Shanghai (51.2%), whereas ST239

Table 1. Distribution of MRSA isolates in six cities

City	No. (%) of MRSA/*S. aureus*	No. (%) of MRSA of the following SCC*mec* types					
		I	II	III	IV	V	NT
Beijing	16/16 (100)	0	2 (12.5)	12 (75.0)	2 (12.5)	0	0
Chongqing	59/131 (45.0)	12 (20.3)	0	34 (57.6)	7 (11.9)	6 (10.2)	0
Guangzhou	142/181 (78.5)	5 (3.5)	29 (20.4)	87 (61.3)	16 (11.3)	4 (2.8)	1 (0.7)
Shanghai	41/59 (69.5)	0	20 (48.8)	18 (43.9)	1 (2.4)	0	2 (4.9)
Shenyang	28/41 (68.3)	1 (3.6)	17 (60.7)	7 (25.0)	1 (3.6)	2 (7.1)	0
Urumqi	23/89 (25.8)	1 (4.4)	0	20 (87.0)	0	1 (4.4)	1 (4.4)
Total	309/517 (59.8)	19 (6.2)	68 (22.0)	178 (57.6)	27 (8.7)	13 (4.2)	4 (1.3)

was similarly identified in the other four cities. ST5 included *spa* types t002, t010, t570, t2460 and t045. The most predominant *spa* type in ST5 isolates was t002 (62.8%, 49/78). Other than ST5, CC5 also included ST105 and ST267 isolates, which were recovered from Guangzhou and Shanghai, respectively. ST59 (CC59) comprised 10.7% of the tested isolates and was the third most common ST. ST59 was the most predominant ST in SCC*mec* I, IV and V MRSA isolates and was mainly prevalent in Chongqing and Guangzhou. ST59 included *spa* types t437, t138, t441 and t7281. The most predominant *spa* type in ST59 isolates was t437. ST45 (CC45) was a small-scale epidemic ST (5.2%, 16/309) that was prevalent in Guangzhou.

The distribution of *spa* types varied among the cities: the most common *spa* type in Guangzhou, t030, was distributed among Guangzhou (50%), Urumqi (21.4%) and Beijing (13.1%); t002 was predominant in Guangzhou (60%) and Shanghai (30%); t037 was predominant in Guangzhou (50%) and Chongqing (37%); and t570 was most common in Shenyang.

All 309 MRSA strains were subjected to PFGE analysis: 17 major groups (more than five strains per group), 6 minor groups (fewer than four strains per group) and 37 singletons with unique patterns (data not listed) were found.

Antimicrobial resistance of MRSA

Antibiotic susceptibility testing showed high rates of resistance to antimicrobial agents (Table S2, available as Supplementary data at *JAC* Online). Excluding intermediate resistance, 97.6% of the MRSA isolates were resistant to oxacillin, 100% to penicillin, 99.4% to ampicillin, 77.3% to gentamicin, 80.2% to ciprofloxacin, 80.9% to clindamycin, 86.2% to erythromycin and 81.7% to levofloxacin. The results also showed that 74.8% (231/309) of the MRSA were MDR isolates, with 36.8% (7/19), 70.6% (48/68), 87.6% (156/178), 40.7% (11/27) and 61.5% (8/13) for SCC*mec* types I, II, III, IV and V MRSA isolates, respectively. One MDR isolate was defined as NT. Interestingly, the SCC*mec* type II and III MRSA isolates shared the same ARPs, they showed high resistance rates (>70%) to seven non-β-lactam antimicrobial agents (gentamicin, ciprofloxacin, clindamycin, erythromycin, tetracycline, levofloxacin, trimethoprim/sulfamethoxazole). The SCC*mec* type I and V isolates exhibited high rates of resistance to three non-β-lactam antimicrobial agents (clindamycin, erythromycin, levofloxacin). The SCC*mec* type IV MRSA isolates shared ARPs of clindamycin and erythromycin. Moreover, one linezolid-resistant isolate (ST239-MRSA-III-t037) from Chongqing and no vancomycin-resistant isolates were found.

Discussion

The accumulated epidemiological data have revealed the mechanism by which MRSA strains spread geographically. Phenotypic typing (such as phage typing and antibiogram profile analysis) and molecular typing (including SCC*mec*, PFGE, MLST, and *spa* typing) techniques are widely performed to type MRSA.[3–6] Each typing method has its own strengths and weaknesses, and no one method meets all demands. Previous studies demonstrated that the MRSA prevalence in China from 1998 to 1999 was 27.5% and rapidly reached 60.7% in 2009.[24,25] Coastal cities such as Shanghai have reported an MRSA prevalence >80%.[26] This study indicated that the prevalence of MRSA was 59.8%, with 69.5% in Shanghai and >80% in Beijing and Guangzhou. Meanwhile, MRSA prevalence in inland cities was lower: 45.0% and 25.8% in Chongqing and Urumchi, respectively. By SCC*mec* typing, SCC*mec* III (57.6%) was still the most common type prevalent in China, followed by SCC*mec* II (22.0%) and IV (8.7%) (Table 1). This result is similar to that of a very recent report that found SCC*mec* III is the main documented genotype (81.3%, 87/107) of MRSA from the lower respiratory tract.[27]

Our study also reported other findings. The first finding was that three major clones (ST239-MRSA-III-t030, ST239-MRSA-III-t037 and ST5-MRSA-II-t002) comprised 57.0% (176/309) of the tested MRSA isolates in China (Table 2); ST239-MRSA-III-t037 prevailed before 2000 and was then replaced by ST239-MRSA-III-t030, which continues to be the most predominant clone.[28] The results suggest that ST239-MRSA-III-t030 has a stronger survival strategy than ST239-MRSA-III-t037 for easy transmission in Chinese hospitals. A similar finding was obtained in London, where a higher relative fitness clone (CC22) allowed it outcompete with CC30 and became dominant from 2006 onwards.[29] By mapping genome-wide single-nucleotide polymorphisms (SNPs) to the reference sequence, Harris *et al.*[30] found that t037 represented the ancestral ST239-MRSA-III spa type, whereas our findings suggest that this type continues to be the second most common MRSA clone prevalent in China. ST5-MRSA-II was initially described as the main clone in the USA[31,32] and Japan[33] and was subsequently detected in

Table 2. Major clones and their ARPs

Clone	No. (%)	Distribution by city (no. of isolates)	ARPs
ST239-MRSA-III-t030	87 (28.2)	BJ (11), CQ (6), GZ (45), SH (5), SY (3), Ur (18)	GEN, CIP, RIF, LVX
ST239-MRSA-III-t037	46 (14.9)	BJ (1), CQ (17), GZ (23), SH (5)	GEN, CIP, CLI, ERY, TET, LVX, SXT
ST5-MRSA-II-t002	43 (13.9)	GZ (27), SH (13), SY (3)	GEN, CIP, CLI, ERY, TET, LVX, SXT

BJ, Beijing; CQ, Chongqing; GZ, Guangzhou; SH, Shanghai; SY, Shenyang; Ur, Urumqi; GEN, gentamicin; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; RIF, rifampicin; TET, tetracycline; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole.

several European[34] and Asian countries.[35,36] In China, ST5-MRSA-II was reportedly prevalent in Shenyang and Dalian.[14] The evolutionary history of the ST5-MRSA clone investigated by Nubel *et al.*[37] showed that the geographically associated clades ascertained by SNPs were not concordant with previously described groupings based on *spa* typing. The geographic spread of ST5-MRSA over a large distance rarely occurred, and the progeny of the recombinant strains resided locally rather than globally.[37] In the present study, ST5-MRSA-II-t002 was the third most common clone, constituting 13.9% (43/309) of the MRSA isolates, and this clone was mostly found in Shanghai.

The second finding of our study was that the three major prevalent clones were associated with two characteristic ARPs (Table 2). Despite having different ST and SCC*mec* types, ST239-MRSA-III-t037 and ST5-MRSA-II-t002 clones shared the same resistance profile (gentamicin, ciprofloxacin, clindamycin, erythromycin, tetracycline, levofloxacin, trimethoprim/sulfamethoxazole). Most isolates of these two clones were resistant to clindamycin, tetracycline and trimethoprim/sulfamethoxazole, compared with that of the ST239-MRSA-III-t030 clone. By contrast, the MRSA isolates of the ST239-MRSA-III-t030 clone were more resistant to rifampicin, which was concordant with a previous report.[28] This finding suggests that the clinical selection of antibiotics based on typing information is advantageous for the treatment of patients with MRSA infections.

The third finding was that six small-scale epidemic clones of MRSA had spread in China and were accompanied by the major clones. Among MRSA-III isolates, ST239-MRSA-III-t138 (3.6%, 11/309) with the same resistance profile as t037 was found in Chongqing, whereas ST239-MRSA-III-t1081 (3.6%, 11/309) with the resistance profile of gentamicin, clindamycin, erythromycin and tetracycline was found in Guangzhou. Among MRSA-II isolates, ST5-MRSA-II-t570 with the same resistance profile as t002 was the major clone found in Shenyang (46.4%, 13/28). In addition, the isolates of ST59-MRSA-I-t437 (2.6%, 8/309), ST59-MRSA-IV-t437 (3.6%, 11/309) and ST59-MRSA-V-t437 (1.6%, 5/309) clones belonging to CC59 with different resistance profiles were found.

In conclusion, we found that three major clones and six minor clones of MRSA currently prevail in China. These epidemic clones formed 76.1% (235/309) of the MRSA isolates that were spread in Chinese hospitals or the community. The major clones have broadly geographic distributions, whereas the six minor clones seem geographically unique to China. The epidemic clones were associated with characteristic ARPs, suggesting that antibiotics for treating patients with MRSA infections should be selected based on the typing information of infected strains.

Acknowledgements

We would like to sincerely express our thanks to Dr Fangyou Yu (Wenzhou Medical College, China) for critical reading and revising our manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (grant numbers 81171622 and 31270979) and the New Drug Development Project of China (2012ZX09103301-038).

Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J Clin Invest* 2009; **119**: 2464–74.

2 Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2008; **46** Suppl 5: S344–9.

3 Tenover FC, McDougal LK, Goering RV *et al*. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. *J Clin Microbiol* 2006; **44**: 108–18.

4 Enright MC, Day NP, Davies CE *et al*. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 1008–15.

5 Kondo Y, Ito T, Ma XX *et al*. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007; **51**: 264–74.

6 Frenay HM, Bunschoten AE, Schouls LM *et al*. Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 60–4.

7 DeLeo FR, Otto M, Kreiswirth BN *et al*. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* 2010; **375**: 1557–68.

8 Tristan A, Ferry T, Durand G *et al*. Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2007; **65** Suppl 2: 105–9.

9 Moise-Broder PA, Sakoulas G, Eliopoulos GM *et al*. Accessory gene regulator group II polymorphism in methicillin-resistant *Staphylococcus aureus* is predictive of failure of vancomycin therapy. *Clin Infect Dis* 2004; **38**: 1700–5.

10 Sakoulas G, Moise-Broder PA, Schentag J *et al*. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004; **42**: 2398–402.

11 Yuan W, Hu Q, Cheng H *et al*. Cell wall thickening is associated with adaptive resistance to amikacin in methicillin-resistant *Staphylococcus aureus* clinical isolates. *J Antimicrob Chemother* 2013; **68**: 1089–96.

12 Wendlandt S, Lozano C, Kadlec K *et al*. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2013; **68**: 473–5.

13 Zou MX, Zhou RR, Wu WJ *et al*. Antimicrobial resistance and molecular epidemiological characteristics of clinical isolates of *Staphylococcus aureus* in Changsha area. *Chin Med J (Engl)* 2012; **125**: 2289–94.

14 Liu Y, Wang H, Du N *et al*. Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. *Antimicrob Agents Chemother* 2009; **53**: 512–8.

15 Unal S, Hoskins J, Flokowitsch JE *et al*. Detection of methicillin-resistant staphylococci by using the polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 1685–91.

16 Kobayashi N, Wu H, Kojima K *et al*. Detection of *mecA*, *femA*, and *femB* genes in clinical strains of staphylococci using polymerase chain reaction. *Epidemiol Infect* 1994; **113**: 259–66.

17 Du J, Chen C, Ding B *et al*. Molecular characterization and antimicrobial susceptibility of nasal *Staphylococcus aureus* isolates from a Chinese medical college campus. *PLoS One* 2011; **6**: e27328.

18 Feil EJ, Nickerson EK, Chantratita N *et al*. Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239, a dominant strain in Asian hospitals. *J Clin Microbiol* 2008; **46**: 1520–2.

19 Feil EJ, Li BC, Aanensen DM *et al*. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004; **186**: 1518–30.

20 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.

21 Harmsen D, Claus H, Witte W *et al*. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol* 2003; **41**: 5442–8.

22 Bannerman TL, Hancock GA, Tenover FC *et al*. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* 1995; **33**: 551–5.

23 Pillar CM, Draghi DC, Sheehan DJ *et al*. Prevalence of multidrug-resistant, methicillin-resistant *Staphylococcus aureus* in the United States: findings of the stratified analysis of the 2004 to 2005 LEADER Surveillance Programs. *Diagn Microbiol Infect Dis* 2008; **60**: 221–4.

24 Li J, Weinstein AJ, Yang M. Surveillance of bacterial resistance in China (1998–1999). *Zhonghua Yi Xue Za Zhi* 2001; **81**: 8–16.

25 Xiao YH, Giske CG, Wei ZQ *et al*. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resist Updat* 2011; **14**: 236–50.

26 Wang H, Liu Y, Sun H *et al*. In vitro activity of ceftobiprole, linezolid, tigecycline, and 23 other antimicrobial agents against *Staphylococcus aureus* isolates in China. *Diagn Microbiol Infect Dis* 2008; **62**: 226–9.

27 Chu H, Zhao L, Zhang Z *et al*. Antibiotic resistance and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* from lower respiratory tract: multi-resistance and high prevalence of SCC*mec* III type. *Cell Biochem Biophys* 2013; .

28 Chen H, Liu Y, Jiang X *et al*. Rapid change of methicillin-resistant *Staphylococcus aureus* clones in a Chinese tertiary care hospital over a 15-year period. *Antimicrob Agents Chemother* 2010; **54**: 1842–7.

29 Knight GM, Budd EL, Whitney L *et al*. Shift in dominant hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) clones over time. *J Antimicrob Chemother* 2012; **67**: 2514–22.

30 Harris SR, Feil EJ, Holden MT *et al*. Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 2010; **327**: 469–74.

31 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.

32 Tenover FC, Goering RV. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. *J Antimicrob Chemother* 2009; **64**: 441–6.

33 Aires de Sousa M, de Lencastre H, Santos Sanches I *et al*. Similarity of antibiotic resistance patterns and molecular typing properties of methicillin-resistant *Staphylococcus aureus* isolates widely spread in hospitals in New York City and in a hospital in Tokyo, Japan. *Microb Drug Resist* 2000; **6**: 253–8.

34 Baba T, Takeuchi F, Kuroda M *et al*. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 2002; **359**: 1819–27.

35 Ko KS, Lee JY, Suh JY *et al*. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *J Clin Microbiol* 2005; **43**: 421–6.

36 Song JH, Hsueh PR, Chung DR *et al*. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *J Antimicrob Chemother* 2011; **66**: 1061–9.

37 Nubel U, Roumagnac P, Feldkamp M *et al*. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 2008; **105**: 14130–5.