Journal of Microbiology, Immunology and Infection (2015) **48**, 335–339

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-jmii.com

CASE REPORT

Elderly infection in the community due to ST5/SCC*mec*II methicillin-resistant *Staphylococcus aureus* (the New York/Japan clone) in Japan: Panton–Valentine leukocidin-negative necrotizing pneumonia

Olga Khokhlova [a], Yusuke Tomita [b], Wei-Chun Hung [a], Tomomi Takano [a], Yasuhisa Iwao [a], Wataru Higuchi [a], Akihito Nishiyama [a], Ivan Reva [a], Tatsuo Yamamoto [a,*]

[a] *Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University, Graduate School of Medical and Dental Sciences, Niigata, Japan*
[b] *Department of Respiratory Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan*

Received 23 May 2012; received in revised form 1 September 2012; accepted 17 September 2012
Available online 30 November 2012

KEYWORDS
Community-acquired necrotizing pneumonia;
Elderly infection;
Methicillin-resistant *Staphylococcus aureus* (MRSA);
New York/Japan clone

An 89-year-old man suffered from and died of necrotizing pneumonia with rapid progression and cavity formation due to methicillin-resistant *Staphylococcus aureus* (MRSA). He was at no risk for hospital-acquired MRSA infection. His MRSA exhibited genotype ST5/*spa*2(t002)/*agr*2/SCC*mec*II/coagulaseII and was negative for Panton–Valentine leukocidin, indicating the New York/Japan clone (the predominant epidemic hospital-acquired MRSA clone in Japan). However, this strain expressed the cytolytic peptide (phenol-soluble modulin or δ-hemolysin) genes at high level, similar to USA300 (the most common community-acquired MRSA in the United States), indicating a variant of the New York/Japan clone with an important feature of community-acquired MRSA.

* Corresponding author. Division of Bacteriology, Department of Infectious Disease Control and International Medicine, Niigata University, Graduate School of Medical and Dental Sciences, 757 Ichibanchou, Asahimachidori, Niigata 951-8510, Japan.
E-mail address: tatsuoy@med.niigata-u.ac.jp (T. Yamamoto).

1684-1182/$36
http://dx.doi.org/10.1016/j.jmii.2012.09.004

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a common nosocomial pathogen since 1961[1]; this class of MRSA is now called hospital-acquired MRSA (HA-MRSA). HA-MRSA generally possesses staphylococcal cassette chromosome *mec* (SCC*mec*) type I, II, or III and is multidrug-resistant.[1,2] The traditional healthcare-associated risk for acquisition of MRSA includes surgery, residence in a long-term care facility, dialysis, indwelling percutaneous medical devices and catheters, and age (50—60 years and older).[3—6]

Previous studies[7—12] and our unpublished data (in 2006—2011) suggested that multilocus sequence type (ST) 5/SCC*mec*II MRSA (the epidemic New York/Japan clone) is currently the predominant clone in hospitals in Japan; this clone in Japan is also characterized by the carriage of SaPIm1/n1 (with the *tst*, *sec*, and *sel* genes) and enterotoxin gene cluster (*egc*; with the *seg*, *sei*, *sem*, *sen*, and *seo* genes) and multiple-drug resistance (including levofloxacin or fosfomycin resistance). The New York/Japan clone (Japanese type with SaPIm1/n1) has also been isolated from Taiwan.[13]

Another class of MRSA, designated community-acquired MRSA (CA-MRSA), emerged in the community from 1997 to 1999.[1,5,14] CA-MRSA generally carries SCC*mec* type IV or V, is resistant to β-lactam agents only or to some agents belonging to limited classes, and often produces Panton—Valentine leukocidin (PVL),[1,5,14] which causes apoptosis and necrosis in human polymorphonuclear cells or monocytes.[15] CA-MRSA infections are associated mainly with skin and soft tissue infections (SSTIs), but occasionally with invasive infections such as bacteremia (and sepsis) and necrotizing pneumonia in healthy individuals, especially children and adolescents (such as athletes) or even the elderly[1,3—5,14]; median ages of CA-MRSA and HA-MRSA patients are 23 and 68 years, respectively.[3]

ST8/SCC*mec*IVa MRSA USA300, the most common CA-MRSA clone in the United States, is one of the most well-characterized CA-MRSA[2,16]; USA300 is positive for PVL and the arginine catabolic mobile element (ACME), and produced a greater amount of cytolytic peptide [phenol-soluble modulins (PSMs) or δ-hemolysin (Hld)] than HA-MRSA.[16]

In Japan, the New York/Japan clone (HA-MRSA) has also been spreading in the community, among healthy children and pediatric outpatients,[17] and even on public transport[18]; however, the association of this New York/Japan clone (nasal or public transport MRSA) with diseases in the community has not been reported. In this report, we describe the first necrotizing pneumonia case caused by the New York/Japan MRSA clone in the community in Japan. In this study, CA-MRSA and HA-MRSA were classified according to a previous definition[4]; CA-MRSA is defined as MRSA isolated from outpatients with no history of hospitalization within at least the past year and who presented with no other established risk factors for HA-MRSA infections (except age).

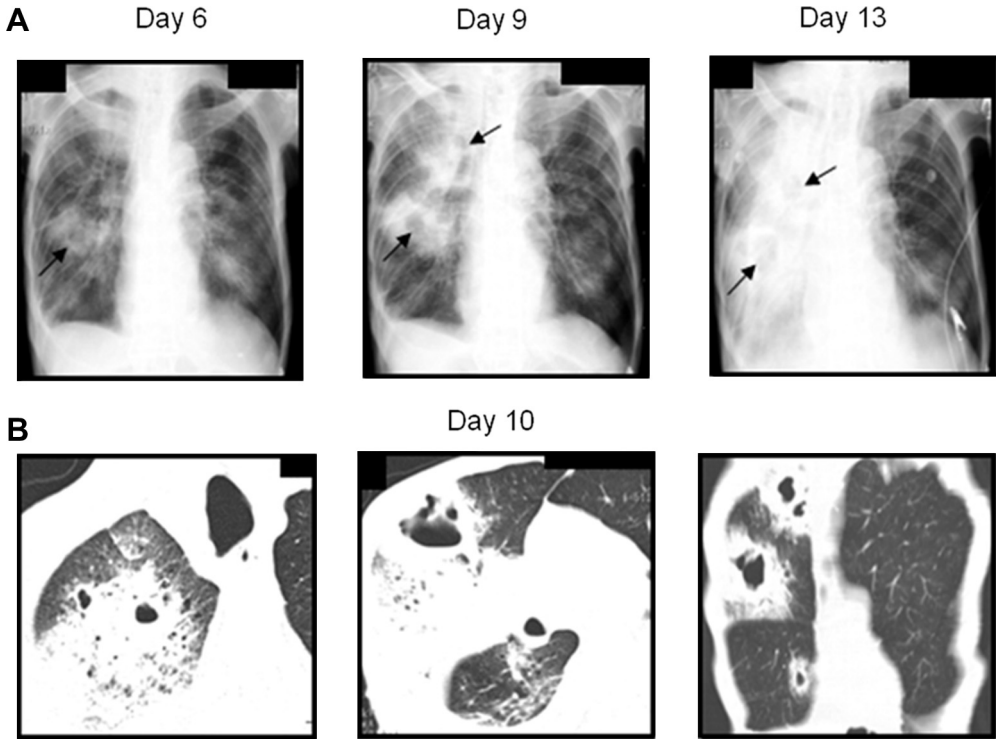

Figure 1. (A) Chest X-ray on days 6—13 and the (B) transaxial and coronal sections of chest CT on day 10 of a patient with community-acquired necrotizing pneumonia. In (A), arrows indicate pulmonary infiltrates with multiple cavity lesions in the right middle lung field; they showed fast radiological progression (days 6—13). Bilateral pulmonary infiltrates in the lungs are also seen. In (B), multiple cavity lesions within the consolidation are seen in the right upper and middle lobes.

Case report

An 89-year-old man was admitted to a hospital on March 14, 2007 (day 1), because of progressive dyspnea. White blood cell count and C-reactive protein were 9300/μL and 11.5 mg/dL, respectively. Chest computed tomography revealed centrilobular nodules mainly in the right lung, with a small volume of right pleural effusion. He had hemoptysis on day 4 and disseminated intravascular coagulation on day 6. On the same day, chest radiography revealed multiple cavities in the right lung (Fig. 1A). Since MRSA was detected upon sputum culture (including day 1), necrotizing pneumonia by MRSA was suspected (blood culture examination was negative for MRSA). Although he was treated with teicoplanin (800 or 400 mg/day), clindamycin (1.2 g/day), and pazufloxacin (1 g/day), increased multiple cavities and bilateral pleural effusion were observed on day 10 (Fig. 1B), and multiple organ failure developed. He died on day 15. He had had no risk factors for HA-MRSA in the past year and no previous MRSA infections. His MRSA, epidemiologically classified as CA-MRSA, was named NPk1.

Next, we investigated the molecular characteristics of MRSA strain NPk1. Molecular typing and virulence gene analysis were performed as described previously.[13,19] Susceptibility testing of MRSA was carried out using the agar dilution method according to previous procedures described by the Clinical and Laboratory Standards Institute.[20] Breakpoints for drug resistance were those described by the Clinical and Laboratory Standards Institute.[20] MRSA strain NPk1 shared the same genotype (ST5/*spa*2[t002]/*agr*2/SCC*mec*II.1.1.1[II]/CoaII) and the same virulence genes (including SaPIm1/n1 and *egc*) as the epidemic New York/Japan clone of HA-MRSA (reference strains Mu50 and N315), as shown in Table 1. Strain NPk1 was multidrug-resistant, but to fewer classes of agents than the reference strains (e.g., strain NPk1 was susceptible to fosfomycin).

Since staphylococcal superantigens, such as toxic shock syndrome toxin-1 (TSST-1) and staphylococcal enterotoxin B, are associated with pulmonary disease in an animal model[21] and ST8 CA-MRSA/J (a major CA-MRSA in Japan, which was associated with invasive infections including necrotizing pneumonia) produced TSST-1 at a high level,[19] TSST-1 production levels (the amount of TSST-1 in the supernatant of bacterial cultures at $2.0 \times l0^9$ cfu/ml) were examined using a TST-RPLA kit (Denka Seiken, Tokyo, Japan). The TSST-1 production level of strain NPk1 was

Table 1 Molecular characteristics of the patient's MRSA strain NPk1 compared to reference strains of the New York/Japan clone (in Japan)

Type, virulence gene, drug resistance	MRSA strain		
	NPk1	New York/Japan[a]	
		Mu50	N315
Type			
ST	5	5	5
spa	2 (t002)	2 (t002)	2 (t002)
agr	2	2	2
SCC*mec* type	II.1.1.1	II.1.1.1	II.1.1.1
Coagulase type	II	II	II
Virulence gene			
Leukocidin			
lukE—lukD	+	+	+
Hemolysin			
hla	+	+	+
hlg, hlg-v	+	+	+
hlb	(+)[b]	(+)[b]	(+)[b]
Peptide cytolysin			
psmα, hld	+	+	+
Enterotoxin			
SaPIm1/n1 (*tst, sec, sel*)	+	+	+
egc (*seg, sei, sem, sen, seo*)	+	+	+
Adhesin			
c12ag[c]	+	+	+
Drug resistance (non-β lactams)[d]	ERY, CLI, KAN, LVX	ERY, CLI, GEN, KAN, TET, FOF, LVX, VAN	ERY, CLI, GEN, KAN, TET, FOF, LVX

[a] Reference strains (Mu50 and N315) of the New York/Japan clone (in Japan) were kindly provided by K. Hiramatsu.
[b] *hlb* (+), split *hlb* gene due to insertion of bacteriophage.
[c] *c12ag*, core 12 adhesin genes shared by all strains: *icaA*, *icaD* (for biofilm formation); *eno* (for laminin—adhesin); *fnbA, fnbB* (for fibronectin—adhesin); *ebpS* (for elastin—adhesin); *clfA, clfB, fib, sdrC, sdrD, sdrE* (for fibrinogen—adhesin).
[d] CLI = clindamycin; ERY = erythromycin; FOF = fosfomycin; GEN = gentamicin; KAN = kanamycin; LVX = levofloxacin; TET = tetracycline; VAN = vancomycin.

200 μg/ml, and was relatively similar to those of the New York/Japan clone, including reference strains [Mu50 (800 μg/ml) and N315 (50 μg/ml)] and clinical isolates from TSS patients [I6 (200 μg/ml) and I8 (200 μg/ml)], although the TSST-1 production level of strain NPk1 was lower than that of ST8 CA-MRSA/J (6400 μg/ml).[19]

Finally, the mRNA expression levels of the cytolytic peptide genes [*psmα* (encoding PSMα) and *hld* (encoding Hld)], *hla* (encoding α-hemolysin, Hla; alternatively named α-toxin), and 16S rRNA genes were examined by reverse transcription-polymerase chain reaction (RT-PCR) assay, as described previously.[19] Briefly, total RNA was extracted and purified from the bacterial cells, cultured at 37 °C for 8 h, using the RNAprotect Bacteria Reagent and RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. RT of the RNA sample was achieved using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The cDNA products of the *psmα*, *hld*, *hla*, and 16S rRNA genes were amplified by PCR using the reported PCR primers.[13,22,23] PCR products were electrophoresed on 2% agarose gel and then stained with ethidium bromide. After visualization of PCR products by ultraviolet illumination, band intensity was determined using image processing and analyzing software (NIH Image; NIH, Bethesda, MD, USA), and *psmα*, *hld*, or *hla* expression was normalized by 16S rRNA expression. For statistical analysis, data were evaluated by Student's *t*-test. The level of significance was defined as $p < 0.05$. As shown in Fig. 2, for either the *psmα* or *hld* gene, mRNA expression levels of community isolates of the New York/Japan clone (including strain NPk1) were similar to those of ST8 CA-MRSA USA300, but significantly higher than those of inpatient isolates of the New York/Japan clone ($p < 0.05$). In contrast, the mRNA expression levels of the *hla* gene were similar among the three groups (community and inpatient isolates of the New York/Japan clone and USA300) (Fig. 2).

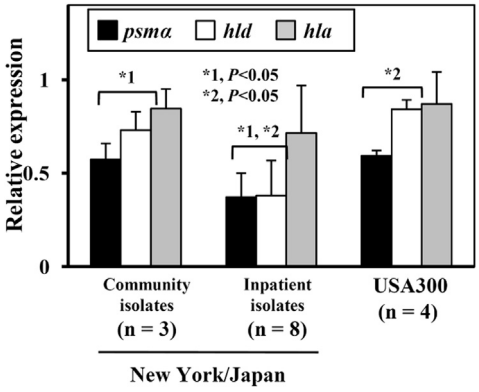

Figure 2. The levels of mRNA expression for cytolytic peptide genes (*psmα* and *hld*) and *hla* of MRSA strain NPk1, compared to those of the New York/Japan clone (Japanese type) and USA300. Community isolates of the New York/Japan clone included strain NPk1 (Table 1) and two isolates from a child and public transport. Inpatient isolates of the New York/Japan clone ($n = 8$) included reference strains Mu50 and N315 (Table 1), two MRSA isolates from patients with toxic shock syndrome, and four isolates from sputum and blood, which were randomly selected. USA300 included USA300-0114, a type strain of USA300 (it was kindly provided by L.K. McDougal and L.L. McDonald), and three clinical isolates in Japan. *psmα*, *hld*, or *hla* expression was normalized by 16S rRNA expression. Data (mean ± SD) were obtained from three experiments.

Discussion

This study represents the first case of fatal community-acquired pneumonia (CAP), with rapid progression and cavity formation (with pleural effusion), caused by the New York/Japan clone (strain NPk1). Although pleural infiltrates with multiple nodular and cavity lesions have been noted especially for necrotizing pneumonia caused by PVL-positive CA-MRSA,[24] this study was a PVL-negative case. Moreover, cavity formation may be observed in some cases of CAP from methicillin-susceptible *S. aureus* and *Klebsiella pneumoniae* and in septic pulmonary embolism, but it is not usually observed in HA-MRSA infections in Japan; therefore, this case indicates the involvement of a strong virulence factor(s) (other than PVL) in the pathogenesis of fatal necrotizing pneumonia.

In the case of USA300, remarkable virulence markers involve PVL (possibly related to pneumonia, albeit with controversial data in animal models),[15] ACME (possibly related to colonization),[16] Hla (possibly related to necrotizing pneumonia),[16] and enhanced production levels of cytolytic peptides PSMs and Hld (possibly related to invasive infections)[16]; the *hla*, *psm*, and *hld* genes are generally present in all *S. aureus* strains. PSMα (20—22 amino acids) and Hld (26 amino acids) cause cell lysis of human polymorphonuclear neutrophils and are also a potent inducer of chemotaxis and interleukin-8 production of polymorphonuclear neutrophils.[25]

Strain NPk1 was negative for the PVL gene and ACME; however, it expressed the cytolytic peptide (PSMα and Hld) genes at higher levels than HA-MRSA (New York/Japan clone); the expression levels were similar to USA300 and a successful CA-MRSA clone (ST8 CA-MRSA/J) in Japan, which we recently characterized as a cause of CAP,[19] indicating that strain NPk1 is a variant of the New York/Japan clone with an important feature of CA-MRSA.

Strain NPk1 also produced Hla and TSST-1; the expression levels of the *hla* gene were similar among HA- and CA-MRSA strains examined, and the TSST-1 production level of strain NPk1 was similar to those of HA-MRSA inpatient strains. We speculate that the combination (in synergy) of a high amount of cytolytic peptides (PSMα and Hld), Hla, superantigens (such as TSST-1), and others contributed to the pathogenesis of severe MRSA CAP in Japan (although the patient was elderly in this case).

In Japan, the New York/Japan clone has mainly been isolated in hospitals, but it has also been isolated from children (mainly 5- to 9-year-old) in the community (albeit at low frequencies: 0.4% [1/274]); no nasal MRSA has been isolated from university students (<0.2%; 0/526).[17] Children are frequently treated as outpatients at hospitals near their homes, so it is conceivable that some children carry the New York/Japan clone from hospitals, and transmission of such MRSA occurs among their family members, because

MRSA colonizing the nares could also be detected on their hands.[2,23] This may be the case even for elderly people. Most probably reflecting these situations, the New York/Japan clone is also detected from the straps and handrails of trains (public transport) in Japan[18]; of 15 MRSA isolates, two were the New York/Japan clone (13.3%).

These community isolates of the New York/Japan clone expressed the cytolytic peptide (PSMα and Hld) genes at high levels (similar to NPk1), in contrast to inpatient isolates of the New York/Japan clone. There is a possibility that the New York/Japan clone with enhanced expression of the cytolytic peptide genes has been selected in the community and caused fatal CAP in an elderly individual. This case may pose a threat, since the elderly population has been increasing. Further detailed analysis with more strains is needed.

References

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006;**368**:874—85.
2. Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2008;**197**:1523—30.
3. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003;**290**:2976—84.
4. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007;**298**:1763—71.
5. Yamamoto T, Nishiyama A, Takano T, Yabe S, Higuchi W, Razvina O, et al. Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance. *J Infect Chemother* 2010;**16**:225—54.
6. Harinstein L, Schafer J, D'Amico F. Risk factors associated with the conversion of methicillin-resistant *Staphylococcus aureus* colonisation to healthcare-associated infection. *J Hosp Infect* 2011;**79**:194—7.
7. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, et al. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 2001;**357**:1225—40.
8. Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002;**2**:180—9.
9. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, et al. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. *Antimicrob Agents Chemother* 2006;**50**:1001—12.
10. Zaraket H, Otsuka T, Saito K, Dohmae S, Takano T, Higuchi W, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* in hospitals in Niigata, Japan: divergence and transmission. *Microbiol Immunol* 2007;**51**:171—6.
11. Ohkura T, Yamada K, Okamoto A, Baba H, Ike Y, Arakawa Y, et al. Nationwide epidemiological study revealed the dissemination of methicillin-resistant *Staphylococcus aureus* carrying a specific set of virulence-associated genes in Japanese hospitals. *J Med Microbiol* 2009;**58**:1329—36.
12. Yanagihara K, Araki N, Watanabe S, Kinebuchi T, Kaku M, Maesaki S, et al. Antimicrobial susceptibility and molecular characteristics of 857 methicillin-resistant *Staphylococcus aureus* isolates from 16 medical centers in Japan (2008—2009): nationwide survey of community-acquired and nosocomial MRSA. *Diagn Microbiol Infect Dis* 2012;**72**:253—7.
13. Takano T, Higuchi W, Otsuka T, Baranovich T, Enany S, Saito K, et al. Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* strains belonging to multilocus sequence type 59 in Taiwan. *Antimicrob Agents Chemother* 2008;**52**:837—45.
14. Zetola N, Francis JS, Nuermberger EL, Bishai WR. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect Dis* 2005;**5**:275—86.
15. Löffler B, Hussain M, Grundmeier M, Brück M, Holzinger D, Varga G, et al. *Staphylococcus aureus* Panton—Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog* 2010;**6**:e1000715.
16. Diep BA, Otto M. The role of virulence determinants in community-associated MRSA pathogenesis. *Trends Microbiol* 2008;**16**:361—9.
17. Ozaki K, Takano M, Higuchi W, Takano T, Yabe S, Nitahara Y, et al. Genotypes, intrafamilial transmission, and virulence potential of nasal methicillin-resistant *Staphylococcus aureus* from children in the community. *J Infect Chemother* 2009;**15**:84—91.
18. Iwao Y, Yabe S, Takano T, Higuchi W, Nishiyama A, Yamamoto T. Isolation and molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from public transport. *Microbiol Immunol* 2012;**56**:76—82.
19. Iwao Y, Ishii R, Tomita Y, Shibuya Y, Takano T, Hung WC, et al. The emerging ST8 methicillin-resistant *Staphylococcus aureus* clone in the community in Japan: associated infections genetic diversity, and comparative genomics. *J Infect Chemother* 2012;**18**:228—40.
20. Clinical and Laboratory Standards Institute. *Performance standard for antimicrobial susceptibility testing; 21st informational supplement. Approved standard M100—MS21*. Wayne, PA: CLSI; 2011.
21. Strandberg KL, Rotschafer JH, Vetter SM, Buonpane RA, Kranz DM, Schlievert PM. Staphylococcal superantigens cause lethal pulmonary disease in rabbits. *J Infect Dis* 2010;**202**:1690—7.
22. Li M, Cheung GY, Hu J, Wang D, Joo HS, Deleo FR, et al. Comparative analysis of virulence and toxin expression of global community-associated methicillin-resistant *Staphylococcus aureus* strains. *J Infect Dis* 2010;**202**:1866—76.
23. Yamamoto T, Takano T, Yabe S, Higuchi W, Iwao Y, Isobe H, et al. Super-sticky familial infections caused by Panton—Valentine leukocidin-positive ST22 community-acquired methicillin-resistant *Staphylococcus aureus* in Japan. *J Infect Chemother* 2012;**18**:187—98.
24. Defres S, Marwick C, Nathwani D. MRSA as a cause of lung infection including airway infection, community-acquired pneumonia and hospital-acquired pneumonia. *Eur Respir J* 2009;**34**:1470—6.
25. Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med* 2007;**13**:1510—4.