Emerging ST121/agr4 community-associated methicillin-resistant Staphylococcus aureus (MRSA) with strong adhesin and cytolytic activities: trigger for MRSA pneumonia and fatal aspiration pneumonia in an influenza-infected elderly

T.-W. Wan1,2, Y. Tomita3, N. Saita4, K. Konno4, Y. Iwao1, W.-C. Hung5, L.-J. Teng2 and T. Yamamoto1
1) Department of Epidemiology, Genomics, and Evolution, International Medical Education and Research Centre, Niigata, Japan, 2) Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan, 3) Developmental Therapeutics Branch, Centre for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, 4) Kanno Hospital, Fukushima, Japan and 5) Department of Microbiology and Immunology, Kaohsiung Medical University, Kaohsiung, Taiwan

Abstract

The pathogenesis of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) pneumonia in influenza-infected elderly individuals has not yet been elucidated in detail. In the present study, a 92-year-old male infected with influenza developed CA-MRSA pneumonia. His CA-MRSA was an emerging type, with the subsequent development of fatal aspiration pneumonia. Resistance to erythromycin/ clindamycin (inducible-type) and gentamicin was detected. Pneumonia improved with the administration of levofloxacin, but with the subsequent development of fatal aspiration pneumonia. Hence, characteristic CA-MRSA with strong adhesin and cytolytic activities triggered influenza-related sequential complications.

Keywords: Community-associated methicillin-resistant Staphylococcus aureus, elderly community-acquired pneumonia, fatal aspiration pneumonia, influenza, ST121/agr4 lineage

Introduction

Staphylococcus aureus is a major human pathogen that causes skin and soft-tissue infections, life-threatening infections such as pneumonia and sepsis, and toxicoses including toxic shock syndrome. Methicillin-susceptible S. aureus (MSSA) has evolved as methicillin-resistant S. aureus (MRSA) through the acquisition of staphylococcal cassette chromosome mec (SCCmec) [1–3].

Two types of MRSA have been identified [1]: traditional MRSA, which emerged in hospitals in 1961 and is now classified as healthcare-associated MRSA (HA-MRSA) with global examples of ST239/SCCmecII and ST5/SCCmecII [4,5], and community-associated MRSA (CA-MRSA), which emerged in the community in 1997–1999 with global examples of ST8/ SCCmecIV (USA300), ST30/SCCmecIV, ST80/SCCmecIV and ST59/SCCmecV [3,5,6,7]. Hence, globally disseminated ST30/ agr3 (but not ST121/agr4) MSSA actually evolved as global CA-MRSA [8].

CA-MRSA possesses distinct virulence factors from those of HA-MRSA, for example, it more strongly expresses cytolytic peptides, such as phenol-soluble modulins (PSMs) [6,9,10], and frequently produces Panton–Valentine leucocidin (PVL) [6,7,10,11]. Successful CA-MRSA also has unique virulence factors, as typically shown with USA300 [3,6,10].

The elderly are susceptible to S. aureus community-acquired pneumonia (CAP) that requires hospitalization [12], and this may be due to a functionally dysregulated host immune system [13]. Moreover, influenza-infected elderly individuals are at risk of bacterial pneumonia; influenza impairs host immunological mechanisms [14] and promotes co-infections or sequential infections with S. aureus or CA-MRSA [14–16]. Although PVL is not necessary [16], the pathogenesis of influenza-related CA-MRSA CAP remains unclear.

We herein isolated ST121/agr4 CA-MRSA from influenza-related MRSA CAP for the first time. We then attempted to elucidate the molecular features of CA-MRSA and gain novel
insights into the pathogenesis of influenza-related, CA-MRSA-triggered sequential complications.

Case

A 92-year-old man was diagnosed with influenza A using an influenza rapid diagnostic (antigen detection) test on 25 January 2013, and was treated with oseltamivir phosphate (150 mg per day); however, no improvements were noted in his symptoms. He was admitted to a hospital on 27 January 2013 (day 1) with fever and progressive dyspnoea. He had a previous history of cerebral infarction, but did not need regular home visits by healthcare workers and did not have a urinary catheter. He did not have established risk factors for HA-MRSA infections such as a history of hospitalization, surgery, haemodialysis, the presence of a permanent indwelling catheter or percutaneous medical device, or residence in a long-term care facility in the past year [1]. He also had no previous MRSA infections or history of recent antibiotic use. On admission, his white blood cell count and C-reactive protein level were 20,200/μL and 33.3 mg/dL, respectively. Chest radiography revealed bilateral pulmonary infiltrates (Fig. 1): an infiltrative shadow in the right lung (arrow 1) and consolidation with air bronchograms on the lateral side of the left middle lung field (arrow 2). The pattern of the chest radiography findings, a peripherally distributed parenchymal abnormality (arrow 2), is more frequent with MRSA pneumonia than with MSSA pneumonia [17]. Respiratory failure (SpO2, 76%) was noted. Chemotherapy was initiated with a drip infusion of piperacillin (2 g every 12 h). On day 2, sputum was examined, MRSA was detected in cultures (at 10⁷ CFU/mL), and the presence of intracellular MRSA in polymorphs was confirmed microscopically; no other pathogens were detected. On day 5, oral levofloxacin (500 mg every 24 h) was initiated based on the results of drug-susceptibility testing for MRSA (see Supplementary material, Table S1). His respiratory status rapidly improved, and his white blood cell count and C-reactive protein level also improved (4,500/μL and 1.7 mg/dL, respectively) on day 14 (7 February 2013). Hospitalization was continued due to severe anorexia; however, he developed aspiration pneumonia on 11 April and died on 19 April. This case of MRSA, epidemiologically classified as CA-MRSA [1], was named KT1.

Characterization of microbes

The molecular typing of MRSA, such as sequence type (ST), clonal complex (CC), spa, agr, SCCmec [2], and Coagulase (Coa), was performed as described previously [18]. Forty-nine virulence genes were analysed by PCR [18]: three leucocidin genes ( lukSF, lukE-lukD and lukM), five haemolysin genes (hla, hib, hlg, hlg-v and hld), the peptide cytolysin, PSMα (psmα), 19 staphylococcal superantigen genes, named enterotoxin (SE) or enterotoxin-like (SEI) (tst, sea-e, seg-j, selk-r and selu), staphylococcal exotoxin (set) genes, a staphylococcal superantigen-like gene cluster (ssl), 3 exfoliative toxin (ET) genes (eta/b and etf), the epidermal cell differentiation inhibitor gene (edin), 14 adhesin genes (icaA/D, eno, fib, fnbA/B, ebpS, clfA/B, sdrC-E, cna and bbp), and the arginine catabolic mobile element-arcA gene. MRSA plasmids were analysed as described previously [18]. Bacterial susceptibility testing was performed using the agar dilution method with Mueller–Hinton agar [18]. The mRNA expression level of the PSMα gene (psmα) was examined using an RT-PCR assay [18]. Data were analysed statistically using the Student’s t-test. The level of significance was defined as p < 0.05.

KT1 exhibited ST121/agr4 and was positive for the enterotoxin gene cluster (egc) with seg, sei, seln, selo and selu [19] (Fig. 2a), similar to global ST121/agr4 MSSA [8]. However, KT1 was negative for PVL, and its spa type (spoligo1493(t5110)) was divergent from global ST121/agr4 MSSA [8,20].

KT1 carried SCCmecV, a characteristic SCCmec of CA-MRSA [2,3,5], and oxacillin and imipenem resistance levels were low, which is consistent with CA-MRSA [5,18]. KT1 carried the characteristic combination of the adhesin genes, cna (for collagen binding) and bbp (for bone sialoglycoprotein binding). Moreover, the

FIG. 1. Chest X-ray of the patient on admission day 1. A chest radiograph shows bilateral pulmonary infiltrates. Arrow 1 indicates an infiltrative shadow overlapping the second arch in the middle to lower field of the right lung, while arrow 2 indicates consolidation with air bronchograms on the lateral side of the left middle lung field.
**PSMA gene expression level of KT1 was significantly higher than that of HA-MRSA (p <0.01), similar to CA-MRSA USA300 (Fig. 2b). Possible ancestral ST121/agr4 MSSA strains also exhibited the same virulence characteristics (see Supplementary material, Fig. S1); those ST121/agr4 MSSA strains (n=14) were PVL+, Coa V, cna+, bbp+, and egc+, and harbored stronger PSMα activities than other MSSA strains [21].

KT1 carried an ermC plasmid (pWK1), specifying for inducible clindamycin resistance, and the aacA-aphD gene encoding for gentamicin resistance (Fig. 2a; see Supplementary material, Table S1). KT1 was susceptible to generally recommended anti-MRSA agents (see Supplementary material, Table S1).

**Discussion**

ST121/agr4 MRSA (KT1) met the CDC criteria for CA-MRSA [1]. Moreover, KT1 was bacteriologically consistent with CA-MRSA, based on the SCCmecV carriage [2,3,5], SCCmec (mecA) -mediated low level of resistance to oxacillin and imipenem [2,5,18], weaker multidrug resistance [5], and strong cytolytic activity [6,9,10]. Although elderly MRSA pneumonia in Japan is mostly from ST5/SCCmecII HA-MRSA and, hence, resistant to fluoroquinone [5], MRSA CAP in the present study was a rare fluoroquinone-susceptible case. KT1 exhibited gentamicin and inducible-clindamycin resistance, similar to CA-MRSA in Japan. We herein described the first case of MRSA CAP from ST121/agr4 CA-MRSA (KT1), originated from PVL+ ST121/agr4 MSSA (including spa type t159), which was globally disseminated in association with skin and soft-tissue infections and necrotizing pneumonia [8,20]. A unique feature of KT1 over other ST121/agr4 MSSA was PVL–, spa t5110 and SCCmecV+.

Influenza impairs host immunological mechanisms [14], promoting the development of CA-MRSA pneumonia [14,16]. The present case also had influenza-related CA-MRSA CAP, with chest radiography findings showing MRSA pneumonia [17], and was successfully treated with levofloxacin.
We strongly speculate that CA-MRSA associated with CAP harbours unique adhesin and toxic activities that act against the influenza-impaired respiratory tract mucosa. The present case was PVL-independent, which is consistent with previous findings [16]. KT1 (the ST121/agr4 lineage, irrespective of PVL+ or PVL– and MRSA or MSSA) harboured strong adhesin and toxic activities, as characterized by collagen and bacteio-protein adhesins, six egc-encoded superantigens, and the strong expression of PSMa. Collagen adhesin is considered to be associated with CAP, possibly through MRSA adherence to damaged tissues, for example, due to viral infection [22].

Globally disseminated ST30/agr3/SCCmecIV CA-MRSA, which has been associated with severe MRSA CAP, also harboured the same adhesin and toxic activities [23,24].

The present CA-MRSA CAP case, possibly in combination with his previous history of cerebral infarction, developed a decline in the activities of daily living. Pneumonia events have been associated with the loss of physical functioning [25]. In addition, in our patient, CA-MRSA CAP may also have damaged deglutition functions, possibly due to the strong adhesin and cytolytic activities of ST121/agr4 CA-MRSA, resulting in aspiration pneumonia.

ST121 CA-MRSA has been isolated from skin and soft-tissue infections, such as impetigo; however, its genetic characteristics remain unknown [26,27]; KT1 was negative for impetigo-associated ETs. The ST121/agr4 lineage may be evolving, in two ways, as PVL+, ET+ impetigo-associated CA-MRSA and as PVL– (or PVL+) CAP-associated CA-MRSA.

In conclusion, we isolated ST121/agr4 CA-MRSA (KT1) from influenza-related CA-MRSA CAP for the first time. KT1 originated from globally disseminated PVL– ST121/agr4 MSSA (with strong adhesin and cytolytic activities), with the unique KT1’ feature of PVL–/spasf5110/SCCmecV+. Pneumonia improved with levofloxacin administration, but with the subsequent development of fatal aspiration pneumonia. Hence, professional ST121/agr4 CA-MRSA, with high adhesin and cytolytic activities, triggered influenza-related sequential complications (CAP and aspiration pneumonia).

Conflict of interest

None declared.

Acknowledgements

We thank L. K. McDougal and L. L. McDonald for the USA300-type strain, K. Hiramatsu for the ST5/SCCmecII reference strains, W. Higuchi for technical information and for manuscript revision and submission, and A. Nishiyama for technical information.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2016.05.011.

References

[1] 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.
[2] International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. Antimicrob Agents Chemother 2009;53:4961–7.
[3] David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010;23:616–87.
[4] Harris SR, Feil EJ, Holden MT, QuaI MA, Nickerson EK, Charvatrza N, et al. Evolution of MRSA during hospital transmission and intercontinental spread. Science 2010;327:469–74.
[5] Yamamoto T, Hung WC, Takano T, Nishiyama A. Genetic nature and virulence of community-associated methicillin-resistant Staphylococcus aureus. BioMedicine 2013;3:2–18.
[6] Diep BA, Otto M. The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol 2008;16:361–9.
[7] DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant Staphylococcus aureus. Lancet 2010;375:1557–68.
[8] Rasigade JP, Laurent F, Lina G, Meugnier H, Bes M, Vandenesch F, et al. Global distribution and evolution of Panton–Valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus, 1981–2007. J Infect Dis 2010;201:1589–97.
[9] 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.
[10] Thurlow LR, Joshi GS, Richardson AR. Virulence strategies of the dominant USA300 lineage of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). FEMS Immunol Med Microbiol 2012;65:5–22.
[11] Zhang K, McClure JA, Elsaed S, Tan J, Conly JM. Coexistence of Panton–Valentine leukocidin-positive and -negative community-associated methicillin-resistant Staphylococcus aureus USA400 sibling strains in a large Canadian health-care region. J Infect Dis 2008;197:195–204.
[12] Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Branley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med 2015;373:415–27.
[13] Furpol T, Le Page A, Forcin C, Wiskowski JM, Dupuis G, Larbi A. Cellular signaling in the aging immune system. Curr Opin Immunol 2014;29:105–11.
[14] Rynda-Apple A, Robinson KM, Alcorn JF. Influenza and bacterial superinfection: illuminating the immunologic mechanisms of disease. Infect Immun 2015;83:7364–70.
[15] Hagenan JC, Uyeki TM, Francis JS, Jernigan DB, Wheeler JG, Bridges CB, et al. Severe community-acquired pneumonia due to
Staphylococcus aureus, 2003–04 influenza season. Emerg Infect Dis 2006;12:894–9.

[16] Murray RJ, Robinson JO, White JN, Hughes F, Coombs GW, Pearson JC, et al. Community-acquired pneumonia due to pandemic A(H1N1)2009 influenza virus and methicillin resistant Staphylococcus aureus co-infection. PLoS One 2010;5:e8705.

[17] Morikawa K, Okada F, Ando Y, Ishii R, Matsushita S, Ono A, et al. Methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus pneumonia: comparison of clinical and thin-section CT findings. Br J Radiol 2012;85:e168–75.

[18] Khokhlova OE, Hung WC, Wan TW, Iwao Y, Takano T, Higuchi W, et al. Healthcare- and community-associated methicillin-resistant Staphylococcus aureus (MRSA) and fatal pneumonia with pediatric deaths in Krasnoyarsk, Siberian Russia: unique MRSA’s multiple virulence factors, genome, and stepwise evolution. PLoS One 2015;10:e0128017.

[19] Collery MM, Smyth DS, Tumilty JJ, Twohig JM, Smyth CJ. Associations between enterotoxin gene cluster types egc1, egc2 and egc3, agr types, enterotoxin and enterotoxin-like gene profiles, and molecular typing characteristics of human nasal carriage and animal isolates of Staphylococcus aureus. J Med Microbiol 2009;58:13–25.

[20] Rao Q, Shang W, Hu X, Rao X. Staphylococcus aureus ST121: a globally disseminated hypervirulent clone. J Med Microbiol 2015;64:1462–73.

[21] Sawanobori E, Hung WC, Takano T, Hachuda K, Horiuchi T, Higuchi W, et al. Emergence of Panton–Valentine leukocidin-positive ST59 methicillin-susceptible Staphylococcus aureus with high cytolytic peptide expression in association with community-acquired pediatric osteomyelitis complicated by pulmonary embolism. J Microbiol Immunol Infect 2015;48:565–73.

[22] de Bentzmann S, Tristan A, Etienne J, Brousse N, Vandenesch F, Lina G. Staphylococcus aureus isolates associated with necrotizing pneumonia bind to basement membrane type I and IV collagens and laminin. J Infect Dis 2004;190:1506–15.

[23] Ito T, Iljima M, Fukushima T, Nonoyama M, Ishii M, Baranovich T, et al. Pediatric pneumonia death caused by community-acquired methicillin-resistant Staphylococcus aureus. Japan. Emerg Infect Dis 2008;14:1312–4.

[24] Isobe H, Takano T, Nishiyama A, Hung WC, Kuniyuki S, Shibuya Y, et al. Evolution and virulence of Panton–Valentine leukocidin-positive ST30 methicillin-resistant Staphylococcus aureus in the past 30 years in Japan. Biomed Res 2012;33:97–109.

[25] Hoogendijk EO, Del Campo N, Rolland Y, Demougeot L, Gérard S, Vellas B. Adverse effects of pneumonia on physical functioning in nursing home residents: results from the INCUR study. Arch Gerontol Geriatr 2016;65:116–21.

[26] Chheng K, Tarquinio S, Wuthiekanun V, Sin L, Thaipadungpanit J, Amornchaisri P, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus associated with pediatric infection in Cambodia. PLoS One 2009;4:e6630.

[27] Kikuta H, Shibata M, Nakata S, Yamanaka T, Sakata H, Akizawa K, et al. Predominant dissemination of PVL-negative CC89 MRSA with SCCmec type II in children with impetigo in Japan. Int J Pediatr 2011;2011:143872.