Case Report

Fatal infections caused by methicillin-resistant *Staphylococcus aureus* of clonal complex 398: case presentations and molecular epidemiology

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 has emerged in livestock across Europe over the past 10 years.

Case presentation: Case 1 was a patient with a history of destructive chronic polyarthritis and immunosuppressive therapy who presented with dyspnoea and pain in the shoulders, back and hips. Microbiological analysis of tissue samples, punctures and blood cultures revealed MRSA. Echocardiography showed mitral valve endocarditis. The patient was treated with daptomycin and fosfomycin. Case 2 was a patient presenting with pneumonia after lung transplantation. Respiratory specimens and perianal swabs revealed MRSA. The patient was treated with teicoplanin and linezolid. The patients did not recover from their infections and died. The isolates belonged to *spa* types t2576 (case 1) and t011 (case 2), to sequence type 398 within CC398 as determined by multilocus sequence typing and to staphylococcal cassette chromosome *mec* type 5. An IdentiBAC Microarray revealed the absence of a bacteriophage integrating into the *hlyB* gene indicative of the livestock origin of the isolates. In 2013, 170 of 534 MRSA cases (31.8 %) among inpatients of the University Hospital Münster, Germany, were caused by closely related *spa* types clustering in one *spa*-CC indicative of CC398. Two of 12 MRSA isolates from blood cultures (16.7 %) were caused by isolates associated with MRSA CC398.

Conclusion: Livestock-associated MRSA CC398 is emerging as a cause of human infections. This observation is alarming and should inspire future efforts to control MRSA in livestock, forestall community spread and monitor changes of the occurrence of MRSA CC398 among cases of human infections.

Keywords: arthritis; endocarditis; livestock-associated; MRSA; pneumonia; *Staphylococcus aureus*.

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 as determined by multilocus sequence typing (MLST) has emerged in livestock across Europe over the past 10 years (European Food Safety

Authority, 2009). Whereas the risk of transmission to humans via contaminated food items is considered low, direct contact with livestock animals is a major risk factor for human colonization with MRSA CC398 (Köck *et al.*, 2009). This is corroborated by the detection of nasal carriage of MRSA CC398 in 77–100 % of pig farmers (Köck *et al.*, 2012). Many studies have also described cases of human infections due to MRSA CC398 including endocarditis, pneumonia and wound infections (Pan *et al.*, 2009; Schijffelen *et al.*, 2010).

Abbreviations: CC, clonal complex; ICU, intensive care unit; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; ST, sequence type; UKM, University Hospital of Münster.

We report two cases of fatal infections caused by MRSA CC398 among patients of the University Hospital of Münster (UKM), Germany. This tertiary-care hospital is located in an area characterized by a high density of pig farming, where MRSA CC398 has been found in 70 % of pig production units (Köck *et al.*, 2009). At the UKM, a general admission screening for MRSA has been implemented.

Case reports

Case 1

A 79-year-old female patient living on a pig-breeding farm with a history of destructive chronic polyarthritis and immunosuppressive treatment (methotrexate, leflunomide and etanercept) presented with dyspnoea and severe pain in her left shoulder, back and both hips. Intra-articular steroid injections were applied to both shoulders prior to admission. On examination, lymphoedema of the right arm, swelling of the left arm and mild elastic swelling of both shoulders were documented. The patient had subfebrile temperatures while being on antipyretics. Blood examinations revealed a white cell count of $1.6 \times 10^4 \mu\text{l}^{-1}$, platelet count of $1.64 \times 10^5 \mu\text{l}^{-1}$, C-reactive protein of 20.8 mg dl^{-1} (in healthy people $<0.5 \text{ mg/dl}$), procalcitonin of 2.18 ng ml^{-1} (in healthy people $<0.5 \text{ ng/dl}$) and a blood glucose level of 239 mg dl^{-1} . Radiography of both shoulders excluded fractures. A diagnostic puncture revealed purulent secretion. Transoesophageal echocardiography showed vegetations on the mitral valve, indicating endocarditis. Calculated antibiotic therapy was started with ampicillin/sulbactam (12 and 4 g day^{-1} , respectively) and gentamicin and clindamycin, both at standard doses. As nasal PCR-based MRSA admission screening (BD GeneOhm; Becton Dickinson) yielded a positive result, vancomycin was added to the regimen. Parallel nasal swabs and subsequent blood cultures, two specimens of shoulder tissue and punctures from the shoulder joints revealed MRSA isolates. Antibiotic therapy was changed to daptomycin on day 3 and fosfomycin was added on day 6 after admission. Nasal MRSA colonization was treated with mupirocin ointment. In spite of appropriate antibiotic therapy, echocardiography documented progressive mitral valve insufficiency after 1 week. As valve replacement was not an option for this patient, a best supportive care concept was confirmed. The patient succumbed to her infection 3 weeks after admission.

Case 2

A 49-year old female patient from a family of pig farmers was admitted for bilateral lung transplantation due to severe lung fibrosis caused by exogenous allergic alveolitis. After transplantation, the patient was treated in an intensive care unit (ICU) for 174 days due to multiple septic complications (not related to MRSA), weaning failure and acute kidney injury before being transferred to

a regular ward. Microbiological examinations during the first ICU stay excluded MRSA colonization by culture-based screening of the nares, pharynx and perianal region performed at admission and weekly thereafter. After 56 days on the regular ward, the patient was re-admitted to the ICU with septic shock, a temperature of 38.8°C , leucopenia of $1.72 \times 10^3 \mu\text{l}^{-1}$, C-reactive protein of 11.2 mg dl^{-1} and procalcitonin of 71 ng dl^{-1} . A broad anti-infective treatment was immediately initiated including meropenem, ciprofloxacin, teicoplanin and micafungin. Rapidly progressive multiorgan failure necessitated intubation and mechanical ventilation, high doses of catecholamines and continuous renal replacement therapy. Lung function deteriorated into severe acute respiratory distress syndrome and veno-venous extracorporeal membrane oxygenation was instituted within 24 h of ICU admission. Microbiological sampling during the second ICU stay revealed MRSA isolates in tracheal aspirates, bronchial lavages, and pharyngeal and perianal swabs. Teicoplanin was replaced by linezolid. Despite maximal treatment efforts, the patient did not recover and died.

Investigations

Culture of specimens

For microbiological analyses, all specimens were streaked directly on Columbia blood agar (Becton Dickinson). Swabs, tissue and liquid samples were placed in dextrose broth, incubated for 48 h and streaked again on Columbia blood agar in case of turbidity to increase sensitivity.

Characteristics of the case-related MRSA isolates

Using *spa* typing (Mellmann *et al.*, 2006), the isolates were found to belong to *spa* types t2576 (case 1) and t011 (case 2), which belong to sequence type (ST) 398 within CC398 as determined by MLST (Enright *et al.*, 2000). Isolates of both cases were additionally resistant to tetracycline, and the isolate of case 2 was also resistant to erythromycin, clindamycin and chloramphenicol.

Molecular analyses of the clinical MRSA isolates

Using the multiplex-PCR approach described by Kondo *et al.* (2007), the isolates were found to belong to staphylococcal cassette chromosome *mec* type 5, which has been shown to be a typical resistance cassette for livestock-associated MRSA (Lozano *et al.*, 2011). Molecular analyses of virulence and resistance genes of the patients' MRSA isolates by an IdentiBAC Microarray (Alere Technologies) (Monecke *et al.*, 2008), which determined the prevalence of 174 distinct genes, revealed that all three investigated isolates of patient 1 (blood culture, joint secretions and nasal) carried the same genes, while the isolate of patient 2 carried additional genes for macrolide/lincosamide (*ermC*) and chloramphenicol (*fexA*) resistance (Table 1). All MRSA

Table 1. Microarray data of MRSA isolates of individual patients (exemplified)

Gene	Patient 1			Patient 2	Protein
	Blood culture	Joint secretion	Nose	Respiratory tract	
Regulatory					
<i>agrI</i> (total)	+	+	+	+	Accessory gene regulator allele I
<i>agrII</i> (total)	—	—	—	—	Accessory gene regulator allele II
<i>agrIII</i> (total)	—	—	—	—	Accessory gene regulator allele III
<i>agrIV</i> (total)	—	—	—	—	Accessory gene regulator allele IV
Resistance					
<i>blaZ</i>	+	+	+	+	β -Lactamase
<i>ermC</i>	—	—	—	+	Macrolide/clindamycin resistance gene
<i>fexA</i>	—	—	—	+	Chloramphenicol/florfenicol resistance

isolates carried the *mecA* and *blaZ* genes confirming methicillin-resistance and the presence of a β -lactamase. Neither of the strains possessed genes encoding for *tst-1*, enterotoxins or the immune evasion cluster genes, which are carried on a bacteriophage integrated into the *hly* gene. Both strains carried important adhesin genes such as *fmbA* and *fmbB* (fibronectin-binding proteins), *clfA* and *clfB* (fibrinogen-binding proteins), *ebpS* (elastin-binding proteins) and *cna* (collagen-binding proteins), indicating the importance for the bacteria to adhere to host surfaces.

Epidemiology of MRSA CC398 at the UKM

In 2013, 534 individual cases of MRSA were detected (including cases of both colonization and infection) among inpatients of the UKM. Of these, 170 (31.8 %) were caused by closely related *spa* types (t034, *n*=79; t011, *n*=72; t898, *n*=4; t108, t1451 and t2011 each *n*=3; t2576, *n*=2; t1255, t2346, t4208 and t4652, each *n*=1) clustering in one *spa*-CC indicative of MLST CC398 (Köck *et al.*, 2013). In blood cultures derived from patients at the UKM, the proportion of MRSA from all *S. aureus* was 12/97 (12.4 %) in 2013 (duplicate isolates of the same patients were excluded). Two of the 12 MRSA isolates from blood cultures (16.7 %) were characterized by *spa* type (t034, *n*=1; t2576, *n*=1, patient case 1) associated with MRSA CC398.

Discussion

In this report, we have highlighted that MRSA CC398, which is widely disseminated as an asymptomatic colonizer among livestock animals, may cause fatal infections in humans, especially if this MRSA lineage affects patient populations prone to acquire severe, invasive infections in ICU settings. Fatal infections by MRSA CC398 have rarely been published. Only recently, Spanish authors reported the case of a patient who died from MRSA CC398 pleural empyema (Lozano *et al.*, 2011).

Epidemiologically, the origin of MRSA in case 1 was most likely direct animal contact. In case 2, the origin could be

persistent colonization of the skin, which may have remained undetected in the ICU, as only nasopharyngeal and perianal swabs were tested for MRSA in line with the routine screening procedure. Alternatively, the patient may have acquired MRSA ST398 during her stay on the regular ward (e.g. via contact with possibly ST398-colonized family member visitors or other persons). However, no other case of MRSA t011 colonization or infection was detected on the respective ward in the 12 months before the case patient.

The microarray results revealed an absence of the bacteriophage that integrates into the *hly* gene. This bacteriophage is detected in more than 80 % of clinical isolates and carries an immune evasion cluster, which protects the bacteria against the human immune system. Interestingly, this immune evasion cluster has been shown to be largely absent from those CC398 isolates belonging to the livestock clade, whereas it has been detected to be widespread among livestock-independent isolates of this CC (Price *et al.*, 2012).

The increase in CC398 MRSA confirms our recent observations that, among patients admitted to 39 hospitals in northwestern Germany, the proportion of MRSA CC398 among all MRSA cases detected from nasopharyngeal screening increased from 14 % in 2008 to 29 % in 2012 (Köck *et al.*, 2013). Moreover, MRSA CC398 accounted for 14 % of all MRSA from lower respiratory tract specimens and 8 % of all MRSA isolates from blood cultures in these hospitals in 2012 (Köck *et al.*, 2013).

In conclusion, livestock-associated MRSA CC398 is emerging as a cause of human infections. This observation is alarming and should inspire future efforts to control MRSA in livestock, forestall community spread and monitor changes in the occurrence of MRSA CC398 among cases of human infections.

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