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 hlb 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*.

**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, meticillin-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 × 10^9 μl^-1, platelet count of 1.64 × 10^11 μl^-1, C-reactive protein of 20.8 mg dl^-1 (in healthy people <0.5 mg/dl), procalcitonin of 2.18 ng ml^-1 (in healthy people <0.5 mg/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 × 10^9 μl^-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 extracorporal 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 was additionally resistant to tetracycline, and the isolate of case 2 was also resistant to erythromycin, clindamycin and chloramphenicol.
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 hlb gene. Both strains carried important adhesin genes such as fnbA and fnbB (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 hlb 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.

References


