Cluster of Infections Caused by Methicillin-Resistant *Staphylococcus pseudintermedius* in Humans in a Tertiary Hospital

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The dog-associated *Staphylococcus pseudintermedius* is a rare pathogen in humans. Here we describe a cluster of infections caused by the methicillin-resistant *S. pseudintermedius* clone ST71-J-t02-II–III. It involved four elderly patients at a tertiary hospital. Three patients had wound infections, and the strain had a tendency to cause bullous skin lesions.

## CASE REPORTS

**Case 1.** On 15 March 2011, a 61-year-old male with diabetes mellitus, severe sepsis, and constant penicillin V treatment (1 g once a day) due to recurrent erysipelas was admitted to the Department of Infectious Diseases, Uppsala University Hospital, Uppsala, Sweden. Two days before admission, he had finished a 2-week ciprofloxacin treatment for a urinary tract infection. On physical examination, he had erythematous elbows and lower extremities, minor chronic leg ulcers, and a body temperature of 37.6°C. Laboratory analysis showed a C-reactive protein (CRP) level of 115 mg/liter (reference range, <5 mg/liter) and a leukocyte particle concentration (LPC) of 5.4 × 10^9/liter (reference range, 3.5 × 10^9 to 9.0 × 10^9/liter). Treatment with ceftriaxone at 2 g once a day and clindamycin at 300 mg three times a day was initiated. Three days later, the patient’s clinical status had improved, and the cultures from the leg ulcers yielded growth of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). The ceftriaxone treatment was terminated, but clindamycin was kept for a total of 13 days. Before the patient’s discharge on 22 March, the prophylactic penicillin V dose was doubled. The patient was readmitted 2 weeks later with pneumonia caused by respiratory syncytial virus. Before the causative agent was identified, he received a 2-day course of piperacillin-tazobactam and vancomycin. On 12 April, the patient visited the Wound Centre at the Department of Dermatology due to deteriorated leg ulcers. A bullous lesion (diameter, ~2 cm) was noticed on his right foot. A culture from the lesion showed growth of MRSP. Treatment with linezolid at 600 mg twice a day lasted for 13 days. Nearly 3 years and 26 months later, the last case appeared, a 75-year-old male with diabetes mellitus and a liver transplant was admitted to the intensive care unit due to sepsis and liver failure. He was treated with piperacillin-tazobactam at 4 g three times a day, and the immunosuppressive treatment was adjusted. He was transferred to the Department of Infectious Diseases on 3 June, when his clinical status and laboratory parameters had improved. X-rays of his lungs showed pleural effusion on the right side, and blood cultures yielded growth of *Klebsiella pneumoniae* and *Citrobacter* spp. Piperacillin-tazobactam was exchanged for meropenem at 0.5 g three times daily. Vancomycin at 1 g three times a day was added on 12 June, when there was growth of *Enterococcus faecium* in the blood and MRSP (≥10^6 CFU/ml) in sputum. Thoracentesis was performed 5 days later, and the therapy was altered to trimethoprim-sulfamethoxazole at 800/160 mg twice a day and linezolid at 600 mg twice a day. The antibiotic treatment was finished when the patient was discharged on 27 June.

**Case 2.** On 21 March 2011, an 82-year-old male with a chronic diabetic foot ulcer was admitted to the Department of Infectious Diseases. On arrival, he had a body temperature of 39.3°C and his right calf was swollen and sore. Laboratory analysis revealed a CRP level of 95 mg/liter and an LPC of 11.7 × 10^9/liter. He was treated with penicillin G at 3 g three times a day and with a single dose of gentamicin (520 mg). The ulcer culture yielded growth of group G streptococcus and skin flora. The blood cultures were negative. Four days later, the CRP levels had dropped to 45 mg/liter, but bullous lesions had appeared on his right leg. On 5 April, penicillin G was replaced with penicillin V at 1 g three times a day, and the treatment was terminated 8 days later when he was discharged.

About 1 month later, the leg was once again swollen and had multiple bullous lesions. Flucloxacinil was prescribed by his general practitioner, and the culture revealed growth of MRSP and group G streptococci. On 3 June, the ulcer had improved. No further clinical information is available. The cultures remained positive for MRSP until 1 November.

**Case 3.** On 5 June 2011, a 64-year-old male with diabetes mellitus and a liver transplant was admitted to the intensive care unit due to sepsis and liver failure. He was treated with piperacillin-tazobactam at 4 g three times a day, and the immunosuppressive treatment was adjusted. He was transferred to the Department of Infectious Diseases a day later, when his clinical status and laboratory parameters had improved. X-rays of his lungs showed pleural effusion on the right side, and blood cultures yielded growth of *Klebsiella pneumoniae* and *Citrobacter* spp. Piperacillin-tazobactam was exchanged for meropenem at 0.5 g three times daily. Vancomycin at 1 g three times a day was added on 12 June, when there was growth of *Enterococcus faecium* in the blood and MRSP (≥10^6 CFU/ml) in sputum. Thoracentesis was performed 5 days later, and the therapy was altered to trimethoprim-sulfamethoxazole at 800/160 mg twice a day and linezolid at 600 mg twice a day. The antibiotic treatment was finished when the patient was discharged on 27 June.

**Data Availability:** Further clinical information is available. The cultures remained positive for MRSP until 1 November.

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patient had received the same therapy as described above. Her ulcers healed slowly without additional treatment, and on 9 September, there was no longer growth of MRSP.

The samples collected for culture were inoculated onto blood, chocolate (Becton, Dickinson and Company, Sparks, MD, USA), and S. aureus ID agar (SAID; bioMérieux, La Balme Les Grottes, France). After 48 h of incubation, relatively large yellowish colonies grew on blood or chocolate agar and large green colonies grew on SAID. The bacteria were both coagulase and DNase positive, but the outcome of the StaphaurexPlus test (Remel, Oxoid AB, Sollentuna, Sweden) varied. Antibiotic susceptibility to oxacillin, cefoxitin, clindamycin, erythromycin, fusidic acid, gentamicin, tobramycin, rifampin, trimethoprim-sulfamethoxazole, moxifloxacin, and linezolid was determined by disk diffusion according to the Swedish Reference Group for Antibiotics (SRGA) (www.srga.org). The inhibition zones for oxacillin were in the range of 6 to 10 mm, whereas those for cefoxitin classified the isolates as susceptible (range, 23 to 31 mm; mean, 29.5 mm) when breakpoints for S. aureus were used. The isolates were otherwise resistant to all tested antibiotics except linezolid, rifampin, and fusidic acid. A PCR (1) revealed the mechanism of the oxacillin resistance: all isolates carried the meca gene. However, none of them were nuc gene positive; hence, 16S rRNA gene sequencing was required to identify the isolates as S. pseudintermedius.

The MRSP isolates were sent to the National Veterinary Institute for further epidemiological analyses, including pulsed-field gel electrophoresis for comparison with animal isolates (2), spa typing (3), and SCCmec typing (4). In addition to previously mentioned antimicrobial substances, susceptibility against tetracycline was tested using VetMIC (National Veterinary Institute, Uppsala, Sweden), an MIC-based system for antimicrobial susceptibility testing. The results showed that the human isolates belonged to spa type t02, harbored SCCmecII and -III, and were susceptible to tetracycline. None of the human isolates exhibited a pulsed-field gel electrophoresis (PFGE) pattern identical to that of any of the earlier analyzed dog or cat isolates, but isolates from patients 1 and 4 had the same PFGE pattern.

To obtain the genetic background of the bullous skin lesions, the presence of four exfoliative toxin genes was investigated (5–7). The isolates were PCR positive only for the Staphylococcus intermedius exfoliative toxin (SIET) gene. LukF-S-PV genes encoding Panton-Valentine leukotoxin (PVL) could not be detected by the routine method used for S. aureus (8). Due to the severity of two of the infections, and the fact that the SIET gene has been questioned as a true exfoliative toxin gene (7), the whole genome of the isolate from the first patient was sequenced on the IonTorrent system according to the manufacturer’s instructions (LifeTechnologies, Carlsbad, CA, USA). The reads with a length of 400 bp were assembled into a draft genome using MIRA v3.9.9 (9, 10) with the recommended settings from LifeTechnologies. Using BLAST, the online database http://www.pubmlst.org/spseudintermedius/ (9, 11), guidelines available at http://www.sccmec.org/Pages/SCCClassificationEN.html, and a method described by Moodley et al. (3), the isolate was confirmed to belong to the ST71-1-t02-II–III clone. The toxin genes identified were the SIET gene and lukF/ S-PV of S. intermedius (GenBank accession number X79188).

Infection control professionals were contacted after the first case, and contact tracing was initiated. This patient (case 1) had no cats or dogs and denied any recent animal contact. However, almost all staff members who had nursed the patient in the infectious diseases ward or at the Wound Center were dog owners. Culture samples from noses and present wounds were collected from their dogs, but the contact tracing yielded no additional MRSP isolates. Patient 2 was the only animal owner among the patients. He had several cats on his farm, but none of them suffered from any skin disorder. Although patients 1 and 2 stayed at the same ward for a brief period, they were never in contact with each other or nursed by the same staff members. Furthermore, when patient 2 was admitted to the same ward as patient 1, patient 1 had been treated for his MRSP-induced infection for almost a week and the rooms for these patients were not close to each other. The only connection that could be established in time and space was between patients 1 and 4, the two patients with MRSP isolates with identical PFGE patterns. They had visited the Wound Center repeatedly prior to their infections, and on some occasions they had been there at the same time.

This is a rare report of a cluster of MRSP infections among human patients in a tertiary hospital. It was caused by the epidemic clone ST71-J-t02-II–III. Although the animal source(s) could not be identified, a direct or an indirect patient-to-patient transmission of this bacterium very likely occurred and not only carriage but infection followed. None of these types of events have, to our knowledge, been documented earlier.

S. pseudintermedius belongs to the S. intermedius group, a group of bacteria which commonly colonize dogs and, to a lesser extent, cats. It is a major cause of canine pyoderma, and pet owners and veterinary staff run the risk of zoonotic transmission of MRSP, especially if the dogs suffer from pyoderma (12). MRSP colonization among owners of infected dogs is, however, transient and uncommon (13). Human infections with S. pseudintermedius are rare and usually local and associated with bite wounds (14), but there are isolated reports of bacteremia, brain abscess, endocarditis, sinusitis, otitis, infected leg ulcers, and pneumonia (15, 16).

The patients involved in this clustering all had local infections, but the infections were not associated with any animal bites and the colonization period was not transient. It is possible that the age of the patients and their underlying conditions, including diabetes mellitus, chronic skin problems, and immunosuppression, facilitated both the colonization and the infectious process. The bullous skin lesions found on two of the patients indicated, however, that the bacterium was not just a relatively harmless opportunist. It made use of its virulence potential by producing an exfoliative toxin. Whether there was any PVL production was not quite as clear.

Staphylococcal species may transfer staphylococcal cassette chromosome (SCC) between species (17), and MRSP harbors a new combination of two SCCmec elements (II and III) (18). The inhibition zone around the cefoxitin disc is usually too large to raise any suspicions of methicillin resistance for someone used to the much smaller zones caused by methicillin-resistant S. aureus (MRSA), and thus it is easy to overlook S. pseudintermedius carrying the meca gene. In veterinary microbiology laboratories, oxacillin is therefore used instead of cefoxitin. Since 2014, EUCAST (www.eucast.org) has made new breakpoints for cefoxitin available for S. pseudintermedius (resistance breakpoint, <35 mm). Whether this will solve the problem remains to be seen.

The meca gene in the SCCmec element removes the most important class of antibiotics for treatment, the beta-lactams. Like many
MRSA strains, this MRSP clone is, in addition, resistant to several other classes of antibiotics. It is therefore difficult to treat, and its multiresistant profile was advantageous during selective pressure: before isolation of the bacterium, at least three of the four patients had been exposed to antibiotics which did not cover the bacterium.

In recent years, isolates of MRSP have been reported worldwide (15). In Sweden, the incidence of MRSP from dogs increased from 13 cases in 2006 to a peak of 121 cases in 2009 (19). Thirty-nine cases were reported 3 years later. The ST71–J–02–II–III clone has been most successful in Europe (20), and it seems to be persistent in the Uppsala region. Since 2011, there have been 1 to 3 human cases each year in this region, and interestingly, they have all occurred during the summer months.

New zoonotic pathogens and their virulence factors are difficult to identify due to the high degree of specialization in human microbiology laboratories. MRSA and S. aureus toxins are no problem, but veterinary Staphylococcus spp., including MRSP, and their specific toxins are. Conventional phenotypic and PCR methods geared for human pathogens do not suffice, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) will stop at the S. intermedius level without current taxonomy updates. With whole-genome sequencing, it was possible to correctly identify all virulence factors and type the isolate, but the cost was relatively high and continues to be.

To summarize, this is a rare documented cluster of MRSP infections in humans in a hospital. No animal source was identified, but a probable direct or indirect patient-to-patient transmission was reported. This may be the first sign of MRSP as an emerging zoonotic pathogen, and attention should be paid to multiresistant staphylococci which resemble S. aureus but do not quite fulfill the identification criteria for this bacterium.

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