Development of antimicrobial resistance in *Staphylococcus lugdunensis* during treatment—report of a case of bacterial arthritis, vertebral osteomyelitis and infective endocarditis

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Staphylococcus lugdunensis is a recently described coagulase-negative staphylococcus (CNS) that has been associated with a wide range of human infections [1,2].

The most frequent type of infection reported is infective endocarditis which may involve prosthetic as well as native valves [3]. In this paper we present a patient with bacterial arthritis in both knee joints (one with and one without an arthroplasty), vertebral osteomyelitis and infective endocarditis of native mitral and aortic valves.

The patient was a 79-year-old male, non-smoking, former lumberjack with a history of psoriasis and hypertension. He had seropositive rheumatoid arthritis with general joint affection. His rheumatoid arthritis was treated with corticosteroids, methotrexate and non-steroidal anti-inflammatory drugs. He underwent total replacement of the left knee in November 1992 because of severe osteoarthritis. The right knee did not show any signs of osteoarthritis until the end of 1994.

He was admitted in April 1995 due to cellulitis of the right elbow and an infected ulcer on the left foot. Blood cultures showed growth of CNS, later verified to be *S. lugdunensis*. After initial intravenous treatment with benzylpenicillin and dicloxacillin for 2 days, the patient was treated with oral clindamycin for 2 weeks and the clinical course was uneventful. On follow-up at the beginning of May, the patient was well and the C-reactive protein (CRP) serum concentration was 21 mg/L.

Nine days later he was readmitted due to bacterial arthritis of the right knee. Cultures were positive for CNS (later shown to be *S. lugdunensis*) in both blood and synovial fluid. This time, treatment comprised intravenous cloxacillin for 6 days, then oral flucloxacillin 1 g three times a day for 6 weeks, and then flucloxacillin 0.5 g three times a day for 7 months, in accordance with the susceptibility test.

A systolic heart murmur, not previously recognized, led to a transthoracic echocardiography (TTE) being performed in August 1995. The TTE showed mild-to-moderate aortic and mitral valve regurgitation. No vegetations were seen. The patient was followed with repeated determinations of CRP and ESR values (CRP 10–20 mg/L and ESR 20–30 mm/1 h) for several months, pending total replacement of the right knee because of osteonecrosis. This was performed in November 1995. Antibiotic prophylaxis was given as intravenous cloxacillin 2 g three times a day for 7 days, and the patient was discharged with oral flucloxacillin 0.5 g three times a day, which was continued until January 1996.

In June 1996 the patient was admitted once more but now presented symptoms of swelling, pain, effusion and warmth of the left prosthetic knee. Arthrocenthesis was performed and the patient was given oral flucloxacillin. The symptoms recurred over the following year. The CRP levels were 40–80 mg/L and the ESR was 50–100 mm/1 h when the symptoms were present. X-ray investigation of the left knee from this period showed signs of osteitis. Altogether, six cultures of synovial fluid were taken from the left knee during this period, and they all showed growth of CNS, which in February 1997 was typed as *S. lugdunensis*. Initially, the patient was treated with oral flucloxacillin, but repeated susceptibility tests showed that the isolate had developed resistance to flucloxacillin in December 1996 and the treatment was changed to ciprofloxacin (Table 1). Later, the isolated strain also showed a significantly increased MIC for ciprofloxacin, and the antibiotic treatment was changed to clindamycin.

In June 1997 the patient presented with a history of lower back pain and arthralgia of the hips, and at this time the CRP was 175 mg/L. The antibiotic regimen was changed to oral rifampicin plus ciprofloxacin. The patient improved, the CRP level decreased, and the patient remained well until the end of August 1997, when he was readmitted due to fever, back and hip pains. A magnetic resonance imaging (MRI) scan showed signs of vertebral osteomyelitis, and TTE showed calcification of the aortic and the mitral valves but no signs of endocarditic vegetations. Mild-to-moderate mitral and aortic valve regurgitation was present, but no signs of cardiac decompensation. Blood cultures showed growth of *S. lugdunensis*, *and* the strain had now developed resistance to rifampicin. Corticosteroids

Table 1 Change in antibiotic susceptibility using the E test (MIC values, mg/L) over time for the *Staphylococcus lugdunensis* strain isolated from blood and synovial fluid

Date	β-Lactamase	Oxacillin	Gentamicin	Ciprofloxacin	Clindamycin	Fusidic acid	Rifampicin	Vancomycin
1995								
10 April, blood	Negative	0.38	0.064	0.19	0.032	0.064	0.004	1.0
13 May, blood	Negative	0.38	0.064	0.19	0.032	0.064	0.004	1.0
13 May, synovial fluid	Negative	0.38	0.094	0.19	0.032	0.064	0.004	1.0
16 May, synovial fluid	Negative	0.38	0.094	0.19	0.032	0.064	0.003	1.0
1996								
17 December, synovial fluid	Negative	4.0	0.064	0.125	0.016	0.016	0.002	0.75
1997								
24 February, synovial fluid	Negative	1.5	0.094	1.5	0.023	0.064	0.003	1.0
6 May, synovial fluid	Negative	1.5	0.094	4.0	0.047	0.064	0.003	1.0
28 June, synovial fluid	Negative	1.5	0.094	3.0	0.125	0.094	0.004	1.0
22 August, blood	Negative	1.5	0.094	4.0	0.094	0.094	> 32	1.0
7 September, blood	Negative	1.5	0.19	6.0	0.094	0.094	> 32	1.0

and methotrexate were discontinued and the patient was treated with intravenous clindamycin. However, the blood cultures were still positive after 1 week of intravenous treatment. A renewed TEE now showed a vegetation on the mitral valve. Treatment was changed to intravenous vancomycin, and the patient improved gradually, the fever subsided and the CRP decreased. Treatment with intravenous vancomycin was continued for 55 days.

However, at the end of October the patient deteriorated rapidly and developed shortness of breath due to pneumonia and heart failure. TTE at this time showed vegetations on both the aortic and mitral valves and severe mitral and aortic valve regurgitation. Ciprofloxacin was added to intravenous vancomycin, and had a temporary effect on temperature and CRP levels. The patient died in acute respiratory and circulatory failure at the beginning of November 1997.

Autopsy showed pneumonia, vertebral osteomyelitis and infective endocarditis with vegetations of the mitral and aortic valves, including perforation of both valves.

The collection of stored frozen samples at the Department of Clinical Microbiology was reviewed and 10 isolates were found (Table 1). These strains were thawed and typed to species level using STAPH-ZYM (Rosco Diagnostica, Taastrup, Denmark) and ornithine decarboxylase (ODC) (KEY Scientific Products, Round Rock, TX, USA). All 10 isolates were typed as *S. lugdunensis* biotype 3360–3 ODC+. All were positive for clumping factor (Slidex Staph-Kit, bioMerieux, Marcy l'Etoile, France) and all were coagulase negative. MICs were determined using the E-test (AB Biodisk, Solna, Sweden). Pulsed-field gel electrophoresis (PFGE) (Gene Path system, Bio-Rad Laboratories, Hercules, CA, USA) was performed, and all 10 isolates showed an indistinguishable pattern (Figure 1).

Three isolates (from 10 April 1995, 17 December 1996 and 22 August 1997) were tested for the *mecA* and *nuc* genes using PCR [4,5] (Swedish Institute for Infectious Disease Control, Solna, Sweden), and all these isolates were negative for both *mec*A and *nuc* genes.

S. lugdunensis emerges as an unusually virulent coagulase-negative staphylococcus causing various types of infections, ranging from superficial skin infections to life-threatening endocarditis [1,3].

In the present case, the patient had a chronic persistent infection caused by *S. lugdunensis* with manifestations from four different locations, bacterial arthritis of both knee joints (with and without prosthesis), vertebral osteomyelitis and infective endocarditis.

Bacterial arthritis caused by *S. lugdunensis* has been reported only once before, and in that case as a complication following arthroscopy [6].

S. lugdunensis may be considered as part of the resident flora of the normal skin and may be found over the entire surface of human skin [1]. The most common types of infection due to *S. lugdunensis* have been found to be skin and soft tissue infections. In addition, during surgical procedures the microorganism may be introduced into the wound at the time of operation, and in this way infection may be a result of contamination.

Specific virulence factors similar to those in *S. aureus* have not yet been identified, but toxins such as SLUSH [7,8],

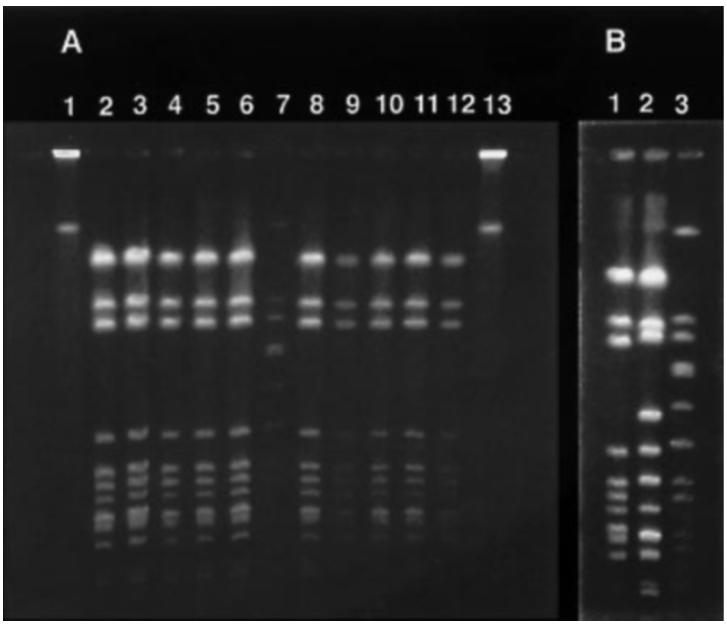

Figure 1 Pulsed-field gel electrophoresis of 10 *S. lugdunensis* isolates from a patient with chronic bacterial arthritis, vertebral osteomyelitis and infective endocarditis over 29 months. (A) Lanes 1 and 13: lambda ladder. Lanes 2, 3, 11 and 12: isolates from blood. Lanes 4, 5, 6, 8, 9 and 10: isolates from synovial fluid. Lane 7: *S. aureus* ATCC 35556 control strain. (B) Lane 1: same as lane 8 of panel A. Lane 2: *S. lugdunensis* ATCC 43809 control strain. Lane 3: *S. aureus* ATCC 35556 control strain.

enzymes such as proteases, lipases and esterases, and glycocalyx have been reported [9].

The first case of vertebral osteomyelitis due to *S. lugdunensis* was reported in 1996 [10]. In that report, the patient was also being treated with corticosteroids, due to polymyalgia rheumatica, at the onset of symptoms. The patient was, however, successfully treated with isoxazolyl–penicilllin.

A number of case reports and reviews of infective endocarditis due to *S. lugdunensis* are accumulating in the literature [3]. *S. lugdunensis* appears to be a potent valvular pathogen causing endocarditis of both native and prosthetic valves. The portal of entry is not usually identified, and a predilection for valvular abnormalities and immunocompromised patients has been found [3]. In the present case, the site of entry was probably the infected knee, and valvular disease might have been present since the echocardiography in 1995 revealed mild-to-moderate regurgitation of both the aortic and the mitral valve. The patient was also receiving treatment with corticosteroids and methotrexate.

A long delay between a probable infection due to *S. lugdunensis* and the manifestation of endocarditis has been reported previously [11].

A most striking finding is the high case-fatality rate despite adequate antimicrobial treatment. In a report of 11 cases with a mortality rate of 8/11 (73%), none of the fatalities was attributed to inadequate antibiotic therapy [3]. However, the taking of consecutive cultures during antibiotic treatment, allowing evaluation of subsequent development of antibiotic resistance, has not been reported previously.

Surgery with valve replacement is often required, since the endocarditis is aggressive and destructive. In addition, patients who undergo valve replacement appear to have a more favorable outcome than those who do not [3,12]. In the present case, when surgery was considered, the patient was not clinically in a condition suitable for major surgery and he died as a result of circulatory and respiratory failure.

Although the patient was treated with antibiotics to which the isolated strain was, in the initial stages, fully susceptible, the patient still presented with recurrent episodes of bacteremia. Repeated cultures from the synovial fluid of the knee prosthesis also showed growth of *S. lugdunensis* despite adequate antibiotic therapy in accordance with in vitro susceptibility tests.

During the various courses of antibiotic treatment, the *S. lugdunensis* strain developed a significant increase in MIC values at different times for different antibiotics; isoxazolyl–penicillin, ciprofloxacin and rifampicin, respectively. These changes correlated with the antibiotic regimen given. In addition, during treatment with clindamycin the patient developed vertebral osteomyelitis, and during treatment with ciprofloxacin and rifampicin infective endocarditis was established.

Most of the strains of *S. lugdunensis* previously analyzed were sensitive to the majority of tested antibiotics, although resistance has rarely been found to aminoglycosides, macrolides and clindamycin. Strains producing β-lactamase have been reported [2,3,13–15].

In the present case, the 10 isolates from blood and joint fluid cultures of the patient were indistinguishable when analyzed with PFGE. The presence of plasmids has been described [14], but this aspect was not investigated. The strain did not possess the *mecA* gene determined by the PCR method and it did not produce β-lactamase. The resistance mechanism against β-lactam antibiotics may be due to specific changes in the penicillin-binding protein affinity or other modifications of the composition of the cell wall. Resistance to rifampicin and ciprofloxacin may be due to chromosomal mutations resulting in changes in RNA polymerase and DNA gyrase or topoisomerase, respectively.

The present case illustrates the propensity of *S. lugdunensis* to cause endocarditis and the difficulties of controlling the infection even when antibiotics were given according to the findings from continual susceptibility testing. Over time, the infecting strain developed increases in MIC values for the antimicrobial agents used for treatment. We hypothesize that the presence of antimicrobial agents may select for clones with increased MIC values and finally development of antibiotic resistance in *S. lugdunensis*. Based on the present case report, endocarditis caused by *S. lugdunensis* must be treated aggressively with antibiotics, and early referral for valve replacement is mandatory for optimal treatment.

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