Relapse of *Serratia marcescens* Sternal Osteitis 15 Years after the First Episode

Axelle Paquin,a Didier Lepelletier,a,b Céline Leprince,a Catherine Chamoux,a Michèle Treilhaud,c Philippe Despins,c Alain Reynaud,a,b and Stéphane Corvec,a,b

Service de Bactériologie-Hygiène Hospitalière, CHU de Nantes, 9 quai Moncousu, 44093 Nantes Cedex 01, France; Université de Nantes, EA 3826 Thérapeutiques Cliniques et Expérimentales des Infections, Faculté de Médecine, 44000 Nantes, France; and Institut du Thorax, CHU de Nantes, 44000 Nantes, France

Sternal osteitis, a potential consequence of cardiac surgery, remains rare. The bacteria involved belong mostly to the genus *Staphylococcus*. Sternal infections caused by *Serratia marcescens* are exceptional. We report an unusual recurrence of sternal infection with *S. marcescens*, 15 years after the initial episode. The identities of the isolates were determined by genomic analysis.

CASE REPORT

In March 1990, a 46-year-old man (body mass index, 37) was suffering from folliculitis. A bacteriological analysis was performed, yielding *Serratia marcescens* without any susceptibility testing. A local treatment of povidone-iodine was prescribed. In October 1990, the patient was admitted to the cardiac department for primitive dilated cardiomyopathy. The patient underwent an immediate cardiac transplantation for a major cardiovascular failure. Postoperative recovery was difficult due to a moderate dysfunction of the cardiac transplantation. Furthermore, 22 days after the transplantation, a pericardial effusion (0.8 liters) was drained in the surgical unit.

Gram staining of the pericardial effusion revealed numerous polymorphonuclear neutrophils without bacteria. Cultures performed on lactose agar plates (bioMérieux, Marcy l’Etoile, France) and 5% horse blood agar plates (bioMérieux) yielded numerous colonies of two different morphological types of Gram-negative bacilli (dry and mucous forms). Identification performed with an AP20E strip (bioMérieux) revealed two morphotypes of *S. marcescens* strains. Antibiotic susceptibility testing was performed by a disc diffusion method (Bio-Rad, Marnes-la-Coquette, France) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (4). Mucous and dry colonies were susceptible to β-lactams but resistant to different antibiotics, including aminoglycosides and cotrimoxazole. As he did not present with any systemic signs of infection, the patient was considered colonized and no antibiotic treatment was prescribed. The patient was discharged from the unit and regularly hospitalized in the rheumatology unit for thoracic and back pains treated with nonsteroid anti-inflammatory drugs. The patient developed metabolic syndrome: diabetes mellitus, hyperuricemia, which was associated with regular, but not quantifiable, consumption of a significant amount of alcohol. Although he suffered from essential arterial high blood pressure and from chronic renal failure, the cardiac transplantation dysfunction did not increase.

In 2005, 15 years after the cardiac transplantation, the patient was hospitalized for a fever (38.1°C) and an inflammatory prestenal pain in the lower third of the sternum with a discharge from a wound abscess. A thoracoabdominal computed tomography (CT) scan showed a prestenal infection with cutaneous infiltration surrounded by fluid, without mediastinitis. A surgery involving the removal of the sternal manubrium to drain the collection was discussed but not indicated because the surgery would be very damaging to the patient, with a high risk of mortality. Nevertheless, bone scintigraphy (Tc-99m and 67Ga) highlighted a sternal hyperactivity compatible with an evolutionary process in favor of septic sternitis.

Drainage of the collection under echography revealed a hemorrhagic fluid. An anatomopathological examination revealed an inflammatory reaction with numerous leukocytes and necrotic cells. Gram staining confirmed polymorphonuclear abundance without bacteria. Cultures performed on lactose agar plates and 5% horse blood agar plates were positive after 2 days, yielding two Gram-negative bacilli with the same cultural characteristics as the 1990 strains (dry and mucous forms). Identification with the GN ID card on the Vitek2 automatic system (bioMérieux) revealed two morphotypes of *S. marcescens* strains. Antibiotic susceptibility testing performed with the AST-N052 card on the Vitek2 automatic system (bioMérieux) revealed several differences in resistance patterns for the dry and mucous isolates compared with the first ones (Fig. 1).

Genotypic analysis performed by pulsed-field gel electrophoresis, after the extraction of genomic DNA and restriction by SpeI, revealed similar pulsortypes for all *S. marcescens* isolates recovered in 1990 and 2005, closely related according to Tenover criteria (two to three fragment differences in the compared profiles (15) (Fig. 1).

The patient received ceftriaxone (1 g per day alternatively to 2 g per day) and gentamicin (500 mg per day) to treat his prestenal infection. Disappearance of tumefaction and apyrexia occurred after 10 days of treatment.

Nine days after being discharged from the hospital, the patient was hospitalized again for pains, polypnea, and low blood pressure. After his admission in the intensive care unit, although the
blood cultures performed under antibiotics remained negative, the patient died from a multivisceral organ failure caused by metabolic disorders and acute renal failure.

Deep sternal wound infection remains rare but is involved in devastating complications associated with significant comorbidity, increased hospital mortality, and reduced long-term survival (6). Coagulase negative staphylococci (CNS) are isolated in 46% of cases with a microbiological etiology, Staphylococcus aureus in 26% of cases, and Gram-negative bacteria in 18% of cases (1).

Different postoperative mediastinitis can be distinguished as follows: (i) mediastinitis associated with obesity, chronic obstructive pulmonary disease, and sternal dehiscence, typically caused by CNS; (ii) mediastinitis following perioperative contamination of the mediastinal space, often caused by S. aureus; and (iii) mediastinitis mainly caused by the spreading of concomitant infections in other sites during the postoperative period, often caused by Gram-negative rods (7).

In this case report, two risk factor groups of sternal wound infection could be involved: patient related (diabetes mellitus, obesity, dilated cardiomyopathy) and procedure related (respiratory failure). Prior myocardial infarction, chronic obstructive pulmonary disease, and aortic calcification as well as operating time, reexploration for bleeding, were also reported previously (6).

Recurrent infective endocarditis due to the same microorganism remains rare and can be caused by relapse or reinfection, usually distinguished by the delay of recurrence of less or more than 6 months, respectively. In sternal wound infection, no data led us to distinguish reinfection to relapse. Clinical presentation caused by S. aureus and Gram-negative bacteria is more fulminant than CNS (7).

This is the first reported case of sternal S. marcescens osteitis, 15 years after the first episode. This resurgence suggests a possible persistence of S. marcescens over this period. Reinfection with the same strain seems to be improbable considering the genetic variability of this species but a persistent colonization of the skin that led to a new infection could not be excluded (5). Several hypotheses could explain bacterial persistence and resurgence after 15 years: (i) the limitation of antimicrobial agent penetration (2); (ii) the existence of dormant cells (11), and (iii) the phenotypic variations and the quorum-sensing system (16). On the other hand, other uncultivable microorganisms may have played a role in the infectious process during a potential polymicrobial infection with S. marcescens as part of the microbial community, but no other bacterium was recovered in the media incubated in different atmospheres during both episodes.

We could hypothesize that various strategies to resist against therapy have been developed by S. marcescens, such as biofilm production, withdrawal of variants into the bone intracellular environment, or the production of small-colony variants (SCV) (8, 11, 14).

In both episodes (1990 and 2005), we detected two morpho-logic phenotypes of S. marcescens. Phenotypic variations and SCV were previously described, especially in S. aureus recurrent and persistent infections many years after the initial infection but also in Gram-negative bacterial infections (11). These bacteria often reside inside human cells, avoiding host defenses and antimicrobial agents.

Indeed, bacteria could have decreased metabolic activity and virulence due to lower growth in biofilm and reduced cell wall synthesis, leading to partial tolerance to β-lactam antibiotics compared with their wild-type parents. The removal of biofilm releasing bacteria for a long period remains very difficult and needs long-term treatment. The deep sternal infection would have required surgery to reduce inoculum and facilitate antibiotic penetration (6), but the patient could not undergo another surgery because of a high risk of mortality.

Other virulence factors like lipopolysaccharide (LPS) O antigen and ShlA cytotoxicity could also play role in persistence. LPS O antigen plays an important role in both resistance to host defenses and adherence (9). ShlA cytotoxicity also results from a close contact of the pathogenic S. marcescens to the host cell, mediated by fimbrial adhesion. The cytotoxin production might kill immune cells during inflammation and help the bacteria to escape phagocytosis (3).

Finally, quorum sensing is also involved to control virulence factors, such as exoenzymes secretion (chitinase, lipase, chloroperoxidase) and biofilm formation (10). This complex system is the main regulatory pathway in the five stages of biofilm development: attachment, aggregation, SCV development, biofilm maturation, and detachment (8, 10, 14, 16). SCV could be consistently isolated at the time when mature variant colonies were observed, producing large amounts of capsular exopolysaccharide at late stages of biofilm development and coinciding with cell death and biofilm dispersal (8).

In conclusion, persistent and relapsing infection is an important clinical problem. The pathogenesis of chronicity and relapse is likely to be multifactorial and includes host factors, such as the presence of dead bone and tissue and shock or pressure on the bone, and bacterial factors, such as biofilm formation and reduced metabolism with SCV. The development of SCV represents one adaptive bacterial mechanism that may facilitate bacterial persistence with potential differences in resistance phenotypes (13). The vulnerability of the host (with different comorbidity factors) and a shock or an environmental stress could have induced a modification for the quiescent cryptic organisms, activating a more “toxic form” due to homeostatic disturbances. These concepts always
suggest the role of dormant, persistent, and difficult-to-culture bacteria in infectious diseases (11, 12).

ACKNOWLEDGMENT

We have no financial support or potential conflicts of interest to report for this paper.

REFERENCES