

# Virulence Gene Profile of a Methicillin-Sensitive *Staphylococcus aureus* Associated with Severe Necrotizing Fasciitis

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## ABSTRACT

**Background.** A 72-year-old diabetic man developed a benign appearing papular eruption with cellulitis on his left leg. Blood cultures and tissue specimens were positive for a methicillin-sensitive strain of *Staphylococcus aureus*. Over the next 3 weeks this initially localized infection developed into a rapidly-spreading necrotizing fasciitis despite antimicrobial therapy and multiple surgical debridements. An amputation was required to control the infection.

**Methods.** Phenotypic and genetic characterizations of the *S. aureus* strain implicated in the infection were performed in order to determine antimicrobial resistance patterns and the presence or absence of virulence factors.

**Results.** The strain was sensitive to all classes of antimicrobial agents tested, including penicillin. It lacked Panton-Valentine leukocidin genes and several superantigen genes commonly associated with methicillin-resistant *S. aureus*. An unusual feature was the presence of the enterotoxin gene cluster *seg*, *sei*, *sem*, *sen*, and *seo*.

**Conclusions.** Necrotizing fasciitis due to methicillin-sensitive strains of *S. aureus* is uncommon. We postulate that the poor outcome associated with this infection was due in part to the presence of an enterotoxin gene cluster harbored by the infecting strain and diabetes of the patient.

## INTRODUCTION

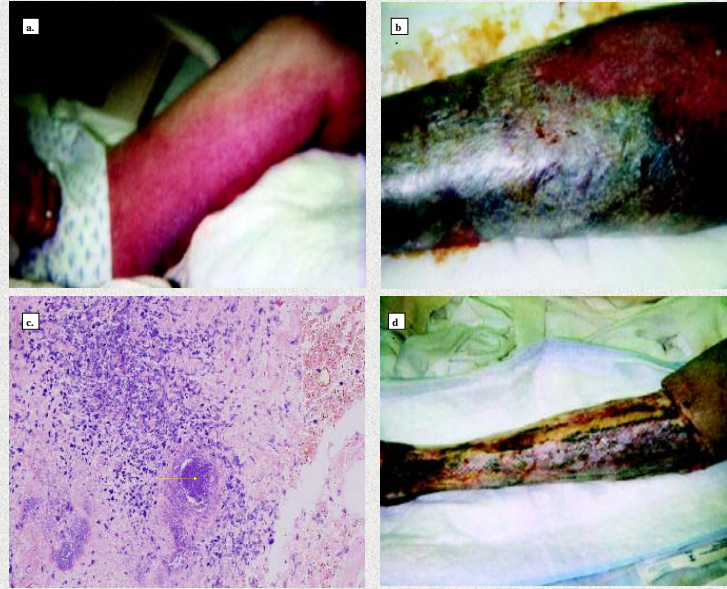
Necrotizing fasciitis is a rare soft tissue infection that primarily involves superficial fascia and results in the extensive undermining and necrosis of the surrounding tissue. *S. aureus* is thought to be an uncommon agent of this disease. However, rare cases of rapidly progressing, necrotizing fasciitis have been described in diabetic patients and patients with chronic use of steroid containing medications. Miller et al. recently described cases of necrotizing fasciitis due to Panton-Valentine leukocidin (PVL) producing community-associated methicillin-resistant *S. aureus* (CA-MRSA) in Los Angeles. In the other few cases reported so far, virulence profiles and genotype of the *S. aureus* strains has not been described in detail.

With the global epidemic of CA-MRSA, a better understanding of how virulence factors of *S. aureus* relate to specific clinical syndromes would be useful. Here we describe a severe case of necrotizing fasciitis in a diabetic patient due to a highly virulent methicillin sensitive *S. aureus* (MSSA) strain that originated in the community.

## CASE HISTORY

A 72-year-old man presented to his local physician with a 1-day history of swelling and erythematous papular eruption on his left ankle. Despite doing yard work when he noticed the rash, he did not remember trauma to the area. At the initial visit he was febrile and was diagnosed with cellulitis. The patient was treated with intravenous ciprofloxacin and clindamycin for 5 days with no improvement. Two blood cultures drawn at the time of presentation yielded MSSA. The MSSA strain was susceptible to all classes of antimicrobial agents tested, including penicillin.

The patient was transferred to the Marshfield Clinic with continued worsening erythema and edema on his left leg (Figure 1a). The leg was purpuric and there was necrosis along the medial aspect of the left leg and thigh (Figure 1b). Edema was present outside the area of erythema and purpura. His admission temperature was 101.4°F. He had a leucocytosis of 14,400/mm<sup>3</sup>. His past medical history was significant for type 2 diabetes mellitus, coronary artery disease, hyperlipidemia, hypertension, and thrombocytopenia. The patient was allergic to penicillin. After vancomycin was added to his intravenous antibiotic regimen, the decision was made to emergently take the patient to the operating room for aggressive debridement of the skin and soft tissue of his left thigh and leg with the presumptive diagnosis of necrotizing fasciitis. Intraoperative histological tissue examination indicated focal areas of necrosis with acute inflammation in the skin and subcutaneous tissue (Figure 1c). Fascial biopsy showed mild acute inflammation. The underlying skeletal muscle was involved with what appeared to be reactive inflammatory changes. Microbiological culture of the tissue sample taken from the wound grew a clonally related MSSA. The ciprofloxacin was discontinued and rifampin was started. The patient subsequently underwent two more operative debridements (Figure 1d). On hospital day 7 his wound cultures were negative and on day 10, his wounds were grafted with porcine skin. He was transferred to a hospital closer to his home on hospitalization day 16. He continued to receive intravenous antibiotics, but unfortunately his clinical picture worsened and he underwent an above the knee amputation. He recovered from his surgery and was later discharged to home.



**Figure 1.** **1a**, Appearance of the left leg on admission to the hospital. There is extensive erythema and purpura involving the entire left leg. Edema is present outside the area of erythema and necrosis is developing along the medial aspect of the thigh. **1b**, Extensive necrosis of the leg. The infection rapidly progressed to frank necrosis of the skin and subcutaneous tissues necessitating surgical debridement. **1c**, Histological appearance of deep subcutaneous tissue from surgical debridement. There is a dense inflammatory infiltrate of neutrophils, lymphocytes, and macrophages. A bacterial colony is present within the tissue (arrow). **1d**, Appearance of the left leg after two surgical debridement procedures. After extensive debridement the wounds were grafted with porcine skin. Despite continued antibiotic therapy his condition worsened and the patient required an above the knee amputation.

## MATERIALS AND METHODS

- Bacterial identification was done by routine microbiological methods and 16S rDNA PCR and sequencing.
- Genotyping by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing.
- The etiologic agent was screened by PCR for the *mecA* gene, staphylococcal enterotoxin genes, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, and *seo*, exfoliative toxin genes, *eta* and *etb*, toxic shock syndrome toxin, *stx*, leukocidin genes *lukSF-PV*, *lukE*, *lukD*, and  $\alpha$ ,  $\beta$ ,  $\delta$ , and  $\gamma$ -toxin genes, *hla*, *hfb*, *hld*, and *hlg*.
- Screened by PCR for seven additional virulence genes, *bsa*, *ear*, *seg2*, *sel2*, *sec4*, *set16*, and *lpl10*.
- Also screened for fibronectin binding protein genes, *fnbA* and *fnbB*, collagen binding protein, *cna*, intracellular adhesion, *icaA*, clumping factors, *clfA* and *clfB*, adhesion factors, *sdrD* and *sdrE*.

## RESULTS

*S. aureus* was confirmed by 16S rDNA PCR and sequencing. A 734 base pair sequence of the 16S rRNA gene of this isolate, WI-MSSA184, had 100% sequence identity with *S. aureus* strains, including a known virulent MSSA strain, MSSA476.

The isolate showed susceptibility to: cefazolin, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, tetracycline, trimethoprim sulfamethoxazole, levofloxacin, linezolid, and amoxicillin/sulbactam. *SmaI* macrorestricted PFGE pattern of the isolate was closely related to the type strain of USA600 MRSA clone, except for the absence of DNA fragment containing the *mecA* element. The MLST allelic profile (10-14-8-6-10-3-2) identified it as sequence type 45, which was consistent with the sequence type determined for the type strain USA600 clonal group.

Typing by the surface protein A gene showed it to be a *spa* type 917. As expected, the isolate was negative for the *mecA* gene and other genetic elements of staphylococcal cassette chromosome SCC<sub>mec</sub>. The strain was also negative for leukocidin genes (*lukSF-PV*, *lukE*, and *lukD*), exfoliative toxin genes (*eta* and *etb*), as well as enterotoxin genes (*sea*, *seb*, *sec*, *sed*, *see*, *seh*, *sei*, *sek*, and *sel*) and *fnbB*. However, the strain harbored  $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\gamma$ -toxin genes and was positive for the enterotoxin gene cluster (*seg*, *sei*, *sem*, *sen*, and *seo*) and the newer toxin genes, *bsa*, *sel16* and *lpl10*. The strain was positive for clumping factors, *clfA* and *clfB*, fibronectin binding protein, *fnbA*, collagen binding adhesion, *cna*, and intracellular adhesion, *icaA*.

The deep subcutaneous tissue obtained during surgical debridement showed a dense inflammatory infiltrate of neutrophils, lymphocytes, and macrophages, as well as bacterial colonies within the tissue (Figure 1c).

## DISCUSSION

Necrotizing fasciitis is a syndrome caused predominantly due to polymicrobial infection and includes pathogens such as *Streptococcus pyogenes* and species of *Bacteroides*, *Clostridium*, *Peptostreptococcus*, and members of *Enterobacteriaceae*. There have been rare reports of monobacterial necrotizing fasciitis caused by *S. aureus*. In all these reports, the clinical course was indolent. In this report, the course of infection was slow during the first few days, but progressed rapidly thereafter to frank fascial necrosis. Despite prompt antibiotic coverage, the patient required an above the knee amputation to prevent further necrosis.

This WI-MSSA184 strain belonged to ST45, a clone not previously reported to be hypervirulent. A search of the MLST database showed that ST45 has been found among nasal carriers and associated with nosocomial infections. Both MRSA and MSSA ST45 phenotypes have been reported from several European countries and at least one case from New York ([www.s aureus.mlst.net](http://www.s aureus.mlst.net)). Even though the strain in this case was a ST45 type, it should be considered uncommon due to its susceptibility to penicillin. Given the severity of the necrotizing fasciitis, it was surprising to see that it lacked leukocidin genes, *lukD* and *lukE*, tissue necrotizing factor, PVL, and genes for several superantigens (SEA, SEB, SEC, SED, SEE, SEH, SEI, SEK, and SEL). The MSSA strain was positive for staphylococcal adhesin genes, *clfA* and *clfB*, that are capable of contributing to the initial attachment of the fascia followed by FnbA acting in the necrosis of fascia.

This strain's virulence likely came from a cluster of genes that belonged to the superantigen family, the family of proteins capable of triggering a massive toxic shock response. We speculate that products of superantigen genes, *seg*, *sei*, *sem*, *sen*, and *seo*, played a role in the severity of the syndrome. Our understanding of the staphylococcal *egc* is far from complete given the limited literature available on their role in pathogens. Toxins made by *egc* appear to be less in quantity than the classical enterotoxins, but EGC toxins are capable of evading immune response due to lack of neutralization by the human sera. In one study *egc* was found to be less commonly present than *sea* in *S. aureus* strains that caused sepsis with or without septic shock. However, in another study, *seg* and *sei*, two genes of the *egc* were implicated in causing staphylococcal toxic shock syndrome and staphylococcal scarlet fever. In this context it seems plausible that EGC toxins could add to the severity of infections in certain individuals. We speculate that the diabetic patient's immune response to the EGC toxins may have been inadequate.

Toxin producing strains of *S. aureus* have recently been described in association with purpura fulminans and Waterhouse-Friderichsen syndrome. Despite the number of these cases being relatively small, these diseases along with necrotizing fasciitis could be viewed as examples of *S. aureus*-associated emerging illnesses.

This case report serves as a sobering reminder to clinicians that initially benign appearing soft tissue infections caused by a MSSA harboring the appropriate virulence arsenal can progress to more severe disease, especially in compromised individuals. It also underscores the need for a better understanding how virulence factors are associated with specific clinical syndromes.

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