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SHORT COMMUNICATION

Epidemiology of necrotizing infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes* at an Iowa hospital

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Summary The present study was performed to characterize the epidemiology of necrotizing soft tissue infection caused by *Streptococcus pyogenes* ($n = 14$) and *Staphylococcus aureus* ($n = 14$) isolates collected at the University of Iowa Hospitals and Clinics. An additional 9 *S. pyogenes* isolates were collected from patients being treated for mild respiratory infections and served as a comparison sample in the analysis. Patient data corresponding to the isolates ($n = 37$) were also collected in order to identify risk factors or comorbid conditions possibly correlated with necrotizing fasciitis (NF). The prevalence of methicillin-resistant *S. aureus* among the study isolates was 35.7% (5/14), and the prevalence of the Panton—Valentine leukocidin (PVL) gene was 57% (8/14). The *S. pyogenes* NF (wound) isolates ($n = 14$) belonged to 10 different *emm* types, none of which appeared to be associated with more severe disease when compared to the milder infection (throat) samples ($n = 9$). Comorbid conditions such as diabetes and cardiovascular disease were significantly associated with NF. The results indicate that there may be a high prevalence of the PVL virulence factor in NF infections and that *spa* type t008 may be responsible for the increasing incidence of *S. aureus* NF infections in Iowa.

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Introduction

In the United States, the incidence of necrotizing infections is approximately 0.04 cases per 1000 persons-years [1]; nevertheless, the disease is often characterized by its rapid progression and high mortality rate, which is estimated to range from 25% to 35% [1,2], though some studies have reported a mortality rate as high as 50% [3]. Most necrotizing infection cases are caused by *Streptococcus pyogenes*; however, the incidence of necrotizing fasciitis (NF) with *Staphylococcus aureus* identified as the primary pathogen is on the rise [4,5].

To date, little is known about the pathogenicity of necrotizing infections, primarily regarding molecular characterization and virulence factors, as well as host factors that may be correlated with severe necrotizing infections. Three notable virulence factors associated with *S. pyogenes* infection include *emm* type, mutations in the *covR*/*covS* system, and the presence of superantigens. Previous research has indicated that certain *emm* types may be responsible for necrotizing *S. pyogenes* infections, particularly *emm* types 1, 3, and 12 [6—8].

A key virulence factor of interest for *S. aureus* infections is the presence of the Panton—Valentine leukocidin (PVL) gene. Of particular interest, two previous studies performed molecular typing on subsets of methicillin-resistant *S. aureus* (MRSA) isolates causing NF and reported the presence of the PVL gene in 100% of the samples (5/5) [4,9].

Along with molecular characterization, numerous studies have investigated the association between certain host factors and an increased risk of NF. Studies examining patient medical histories have identified a multitude of factors, including prior trauma, surgery, nonsteroidal anti-inflammatory drug use, burns, chronic alcohol consumption, immunosuppressive drug use, cancer, diabetes, obesity, and renal disease, among others, that may be correlated with NF [10—12]. However, studies also have reported the occurrence of necrotizing infections in patients with no known risk factors or comorbid conditions [4,11].

The previously described findings demonstrate the need for further research regarding the etiology of NF. The goals of our study were to determine the molecular characterization of NF caused by *S. pyogenes* and *S. aureus*, including *emm* type (*S. pyogenes*), *spa* type, and the presence of the PVL and *mecA* genes (*S. aureus*), and to identify whether specific host factors are correlated with severe necrotizing infections among patients at the University of Iowa Hospitals and Clinics.

Materials and methods

Study isolates ($n = 38$) were collected and stored at the University of Iowa Hospitals and Clinics Pathology Department between January 2011 and September 2012. Fourteen *S. aureus* and 14 *S. pyogenes* samples were collected from cases of necrotizing infection; the remaining 10 *S. pyogenes* isolates were obtained from throat cultures collected from patients being treated for mild respiratory infections and served as a comparison in the analysis. Isolates were attained from the Pathology Department in October 2012 following IRB approval, and were analyzed at the Center for Emerging Infectious Diseases with molecular typing.

Genomic DNA extraction was performed using the Wizard Genomic DNA preparation kit (Promega, WI). Polymerase chain reaction (PCR) was performed to detect *mecA* and PVL genes (*lukS*, *lukF*) present in the *S. aureus* isolates [13,14]. The staphylococcus protein A (*spa*) gene was amplified using SpaF (5′-GAACAA-CGTAACGGCTTCATCC-3′) and 1514R (5′-CAGCAGTAGTGCCGTTTGCCT-3′), as previously described [15,16]; *emm* typing was carried out for all *S. pyogenes* isolates [17], and 16s rRNA PCR was performed with all isolates to confirm the species [18]. Upon completion of 16s rRNA PCR, 1 isolate among the *S. pyogenes* throat samples was found to belong to the *Streptococcus parasanguinis* species, and was subsequently excluded from further analysis, leaving 9 throat infection isolates. Multilocus sequence typing was completed on all but 1 study isolate [19].

All *S. aureus* and 23 *S. pyogenes* isolates were tested for antibiotic susceptibility by using the VITEK 2 System (bioMérieux). We used the AST-GP71 and AST-ST01 cards of the VITEK 2 System for the antibiotic susceptibility testing of *S. aureus* and *S. pyogenes*, respectively. *S. aureus* isolates were tested for susceptibility to benzylpenicillin, oxacillin, tetracycline, erythromycin, ciprofloxacin, moxifloxacin, minocycline, clindamycin, trimethoprim—sulfamethoxazole, quinupristin/dalfopristin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin, rifampicin, minocycline, tigecycline, and nitrofurantoin. *S. pyogenes* isolates were tested for susceptibility to benzylpenicillin, ampicillin, cefotaxime, ceftriaxone, tetracycline, erythromycin, clindamycin, trimethoprim—sulfamethoxazole, levofloxacin, linezolid, and vancomycin. Isolates showing intermediate levels of susceptibility were classified as resistant. *S. aureus* isolates that were resistant to 3 or more classes of antimicrobials or that were