Distribution of *edin* in *Staphylococcus aureus* isolated from diabetic foot ulcers

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Abstract

Staphylococcus aureus is both a common colonizer of human skin and the most frequently isolated pathogen in diabetes foot infections (DFIs). The spread of DFI to soft tissue and bony structures is a major causal factor for lower-limb amputation. It is therefore of great importance to differentiate colonizing from infecting strains of *S. aureus*. Epidermal cell differentiation inhibitors known as EDIN and EDIN-like factors, a group of toxins targeting RhoA master regulator of the actin cytoskeleton, may confer virulence properties on *S. aureus*. In this study, for the first time, analysis of *S. aureus* strains, recovered in DFIs at an initial stage and during the follow-up, showed that 71.4% of *edin*-positive strains were associated with moderate-to-severe infections (grades 3 and 4 of the IDSA/IWGDF classification) compared with 28.6% of *edin*-positive strains associated with low-grade infections. Most of these strains were *edin-B* positive (86.7%) and belonged to CC25/28-MSSA (n = 10). One *edin-B*-positive ST152-MSSA strain was negative for the two highly prevalent predictive markers of infecting strains (*lukDE* and *hlgv*). Collectively, this points towards the *edin-B* encoding gene as a *bonafide* subsidiary predictive risk marker of DFI.

Keywords: Colonization, diabetic foot ulcer, EDIN, oligonucleotide array, severe infection, *Staphylococcus aureus*
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Introduction

Foot ulcers are common in diabetic patients, with a prevalence rate as high as 25% [1]. These ulcers frequently become infected, and spread of infection to soft tissue and bony structures is a major causal factor for lower-limb amputation [2]. *Staphylococcus aureus* is the most frequently isolated pathogen in diabetic foot infections (DFIs). Both the occurrence and progression of staphylococcal infection result from the action of a variety of virulence factors combined with the immunological status of the host [3, 4]. Bacterial factors are extremely heterogeneous in structure and mode of action, giving the bacteria specific metabolic and adhesive properties, as well as offering them a protection against the large body of innate immune effectors [3]. Defining the contributing role of virulence factors by combining cellular microbiological techniques and epidemiological approaches would be of interest in predicting *S. aureus*-associated risk of pejorative infections [5].

The epidermal differentiation factor (EDIN) and EDIN-like factors of *S. aureus* belong to such factors of well-determined mode of action, although their implication in staphylococcal infection remains ill defined. EDIN belongs to a family of bacterial exotoxins targeting the small host protein RhoA, which they inhibit [6, 7]. This small GTPase is a major

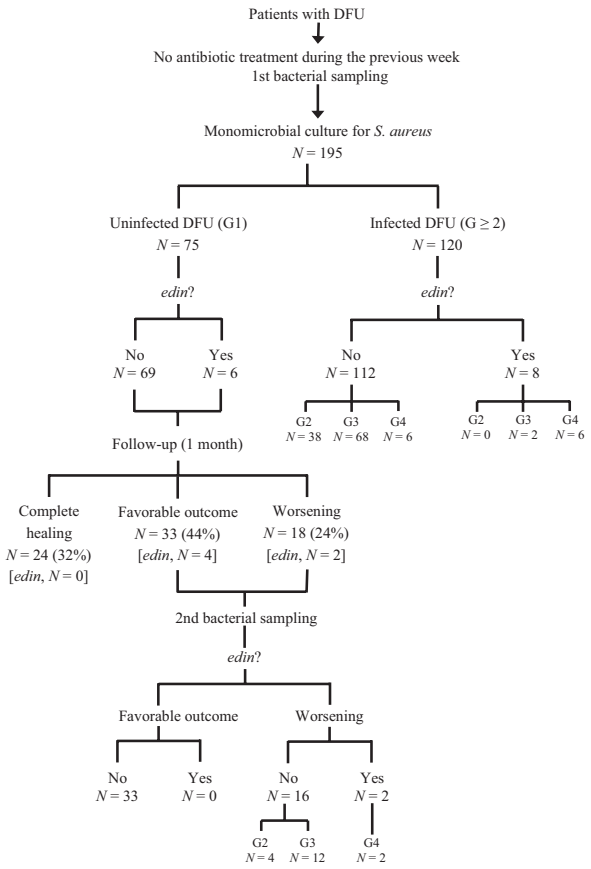
regulator of the host cell actin cytoskeleton [8]. Several cell biology studies have revealed that inhibition of RhoA has a detrimental impact on the cohesion of epithelium and endothelium barriers, which is likely to favour bacterial dissemination [9, 10]. Inhibition of RhoA also impairs complement-mediated phagocytosis [11]. As a whole, a large number of studies aimed at investigating the impact of RhoA inhibition, point towards a contributing role of EDIN factors in bacterial colonization and host tissue invasion [5, 12]. Thus defining the distribution of *edin*-encoding genes in clinical isolates of *S. aureus* might help in predicting the risk factors for severe and spreading infection.

A previous multicentre study conducted on clinical isolates of *S. aureus* had suggested that two clonal complexes (CC) and an array of virulence factors can be used to discriminate uninfected from infected diabetic foot ulcers (DFUs) [13]. Indeed, we determined that colonizing *S. aureus* strains (grade 1) with a favourable outcome are linked to the clonal complexes CC5/CC8. In parallel, we defined a group of virulence factors associated with worse outcomes of DFU, notably *sea, sei, lukDE* and *cap8* [14]. Refined analysis of virulence factors associated with infecting strains, using DNA array technology, identified two highly prevalent factors, *lukDE* and *hlgv*, as suitable genetic markers to predict risk of infection by *S. aureus* ($p < 0.005$). Nevertheless, it would be interesting to determine additional markers, which might be used to discriminate a higher number of colonizing from infecting strains of *S. aureus* in DFU. We therefore analysed the distribution of *edin* genes in clinical isolates of *S. aureus* responsible for diabetic foot infection (DFI) in a French national collection of 195 isolates.

Patients and Methods

Study design

We prospectively enrolled a sample of outpatients attending one of 12 participating French foot clinics between 1 April 2008 and 30 June 2010 for any type of DFU, after informed consent was obtained (Fig. 1). This study was approved by the local ethics committee (South Mediterranean III) and carried out in accordance with the Declaration of Helsinki as revised in 2000. Patients were included if they had not received any antibiotic agents in the previous week. Every patient was examined by trained physicians to grade infection severity. According to the IDSA-IWGDF criteria [15, 16], wounds were considered either uninfected (grade 1) or infected (grade ≥ 2). After wound debridement, samples for bacterial culture were obtained by swabbing the wound base, needle aspiration or tissue biopsy and immediately sent to the bacteriology



G1, G2, G3 and G4 refer to Grade 1, 2, 3, and 4 of infection according to the IDSA/IWGDF classification system

FIG. 1. Flowchart of the study.

department. Only patients with monomicrobial culture for *S. aureus* were included in the study. Antibiotic treatment was not prescribed in patients with uninfected ulcers. Thereafter, patients were closely monitored for 30 days to definitively assess the wound status (infected/uninfected). If the wound condition was worsening before the follow-up visit, patients were instructed to return to the outpatient department for early review and a further sample was taken for bacterial culture. At the follow-up visit, if the wound was healing but not completely re-epithelialized, a sample for bacterial culture was obtained, and the outcome was considered as favourable. For completely re-epithelialized wounds, no microbiological specimens were sampled and the wound was considered healed.

Microbiological study

Genus, species and antibiotic susceptibilities were determined using the Vitek 2 card (BioMérieux, Marcy-l'Etoile, France) and interpreted according to the recommendations of the French Society for Microbiology [17]. Susceptibility to methicillin was screened by agar diffusion using cefoxitin disks (BioRad, Marnes-La-Coquette, France) [17].