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Prevalence of *cna*, *fnbA* and *fnbB* adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant

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Abstract

Here are reported data on virulence determinants of *Staphylococcus aureus* from orthopedic surgical infections, emphasizing on the genes encoding fibronectin (*fnbA*, *fnbB*) and collagen (*cna*) adhesins. 191 *S. aureus* strains from orthopedic infections (53 from internal fixation devices, 29 external fixation devices, 15 knee arthroprostheses, 30 hip arthroprostheses, 45 surgical reconstruction and 19 non-associated to medical devices) were investigated for the presence of the genes of the collagen-binding protein Cna and of the two fibronectin-binding proteins, FnbA and FnbB. 87 (46%) strains were found to be *cna*+ without significant variations across the different surgical categories considered. Conversely, the *fnbA* and the *fnbB* genes were almost always present in all surgical categories. The finding that, among the investigated adhesins, fibronectin-adhesins are present in the majority of the implant associated *S. aureus* clinical isolates encourages the development of strategies to specifically block the interaction of bacteria with matrix fibronectin by antagonist ligands.

Keywords: Collagen adhesion gene (*cna*); Fibronectin-binding protein; Bacterial adhesion; Biomaterial-associated infections; *Staphylococcus aureus*

1. Introduction

For *Staphylococcus aureus*, a very common pathogen involved in implant-associated infections, a series of adhesins have been recently identified that, during the early phases of cell adhesion, can potentially favor the active anchoring of bacteria to specific extracellular matrix proteins adsorbed on biomaterials surfaces. These adhesins, also named MSCRAMMs (the acronym for "microbial surface components recognizing adhesive matrix molecules") [1], are protein components of the microbial surface that are able to interact with and bind to a variety of relevant mammalian extracellular proteins. Among adhesins, two fibronectin-binding proteins, (FnbA and FnbB), three fibrinogen-binding proteins (ClfA, ClfB and Efb), a collagen-binding protein (Cna), the elastin-binding protein (EbpS [2]) and the bone sialoprotein-binding protein (Bbp [3]) have been well characterized. Cna, FnbA and FnbB have been proved significantly to contribute to tissue

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colonization in various pathological conditions such as eye keratitis [4–6], osteomyelitis and septic arthritis [7–9], and indwelling medical devices [10].

In *S. aureus*, different MSCRAMM proteins often coexist with other crucial surface components such as the polysaccharide intercellular adhesin (PIA) and the biofilm-associated protein (Bap) [11]. Adhesins expression can be temporally modulated as a function of the cell cycle and the phase of infection [2,12,13].

Diverse studies have shown that FnbA, CNA and PIA are significantly more common in invasive isolates and that they contribute independently to virulence and their effect appears to be cumulative [14]. A key to interpret such cumulative contribution is that, although co-existent, they explicate their specific action in distinct phases of the infection process.

This molecular epidemiological study aimed at investigating the prevalence of selected adhesion mechanisms in a collection of 191 *S. aureus* strains isolated from orthopedic infections, 127 of which from infections clearly associated to prosthetic implant materials. PCR-based techniques were utilized to detect the gene encoding for the collagen-binding adhesin (*cna*) and the genes of the two fibronectin-binding proteins, respectively, *fnbA* and *fnbB*. In order to ascertain possible differences related to the typology of the implant, the isolates associated to prosthetic materials were further grouped in 4 different categories.

2. Materials and methods

2.1. Bacterial isolates

The 191 staphylococcal strains used in this study were clinical isolates derived from orthopedic infections sequentially observed in patients of the Rizzoli Orthopedic Institute over a 20 months period. Clinical infections were identified based on criteria and definitions previously described in Arciola et al. [15], opportunely adapted to surgical and prosthesis associated infections: stage 1, colonization at the surgical wound or implant surface; stage 2, localized infection, revealed by local inflammatory signs at biopsy; stage 3, systemic infection, documented by bacteraemia and stage 4, sepsis. Strains were isolated from tissue biopsies. Depending on the type of orthopedic implant infected, 127 of these strains were classified into 4 different categories: internal fixation devices, external fixation devices, hip arthroprostheses, and knee arthroprostheses. The category of internal fixation devices included a variety of prosthetic implants such as plates, pins, screws and so on. Conversely, 19 strains were isolated from infections that were not associated to medical devices such as those post-trauma. The remaining 45 isolates classified in the category of reconstructive surgery included infections developed following interventions of reconstructive surgery in oncology, pelvis surgery, tendon and ligaments reconstructions. In these 45 cases, the device could not be correctly classified in one of the 4 categories, the complete patient history was not promptly available, the infection could potentially have started from a suture used in a surgical procedure, but there was no sound proved association with biomaterials. All isolates were characterized by means of classic microbiological methods. In particular, the staphylococcal species was identified by Api-Staph test (Biomérieux, France), an identification kit, and coagulase test. Bacterial strains coming from the same surgical unit, or even when coming from different units but isolated within the same week, were subjected to automated EcoRI ribotyping by RiboPrinter® (DuPont) in order to check if they were originating from monoclonal outbreaks. A clonal relationship among these strains was excluded, because they, compared with the 303 ribotypes of the Dupont Release (2003 updating), appeared as individual strains and therefore all the collected isolates were included in the study. For the storage, a single colony of each isolate was seeded in 8 ml of trypticase soy broth (TSB) and incubated for 24 h at 37 °C. The broth culture, supplemented with 15% glycerol, was finally fractionated in 1-ml aliquots and stored at −80 °C.

2.2. Detection of cna sequence

The sequence of *cna* gene (Accession No. M81736) was checked by the GenBank Sequence Database of the National Centre for Biotechnology Information. A couple of primers specific for *cna* gene were picked by the program Primer3 as previously described [16,17]. The respective sequences were: 5′-AAAGCGTTGCCTAGTGGAGA (forward primer) and 5′-AGTGCCTTCCCAAACCTTTT (reverse primer), including a region of 192 bp (corresponding to nucleotides 1291–1482). Both primers were synthesized by M-Medical Genenco (Firenze, Italy). PCRs were performed in a DNA thermal cycler model Gene Amp PCR System 9600, Perkin Elmer. PCR conditions were those described in [16]. Electrophoretic patterns in agarose gels were analyzed by a ''Scanner Agfa Arcus II'' image analyzer equipped with the software GelPro Analyzer 3.0.

2.3. Binding of bacteria to immobilized collagen

The phenotypic ability of all *S. aureus* strains to bind to collagen type II and IV (all from Sigma) was assessed following an adaptation of the procedure already described by Gatermann and Meyer [18], as mentioned in [16]. Briefly collagen (50 μg/ml in PBS) was dispensed in 96-wells microtiter plates (5 μg/well) for 16 h at 4 °C. Wells were then washed with PBS. Fresh cultures of bacteria in TBS were pelleted, washed with PBS and