OPTICAL MAPPING REVEALS A GENETIC INSERTION IN COLONIZING *STAPHYLOCOCCUS AUREUS* ISOLATED FROM DIABETIC FOOT ULCERS

A. SOTTO[1], G. LINA[2], JL. RICHARD[3], G. SKORSKI[4], CW. DYKES[5], J. ETIENNE[2], J.P. LAVIGNE[1,6]

[1]INSERM Espri 26, Université Montpellier 1, Nîmes, France, [2]CNR Staphylocoques, INSERM, Lyon, France, [3]Service des Maladies Métaboliques et Endocriniennes, [6]Laboratoire de Bactériologie, CHU Caremeau, Nîmes, France, [4]Phylogene SA, Bernis, France, [5]Opgen Inc, Gaithersburg, MD, USA

INTRODUCTION

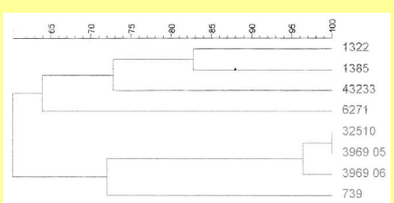
Recently we highlighted the coexistence of two *Staphylococcus aureus* populations on diabetic foot ulcers (DFU): one colonizing with low virulence potential and one infecting with high virulence potential (Sotto et al, Diabetes Care, 2008. 31:2318).

The purpose of this study was to assess the epidemiological links and genomic rearrangements between these 2 *S. aureus* populations in the aim to develop a tool to discriminate non-infected from infected DFU.

RESULTS

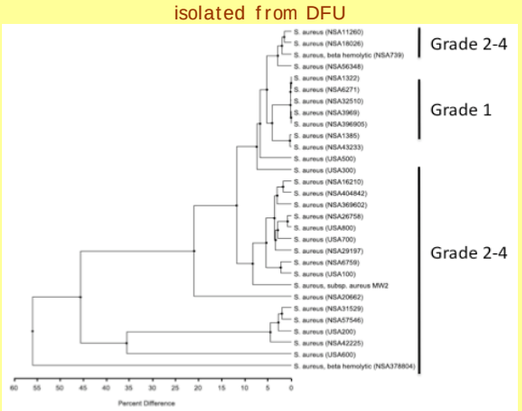
Using PFGE (Fig. 2), no clonal link was detected between the different strains notably those isolated in Grade 1 ulcers (~70% homology). Using optical maps, we identified that colonizing *S. aureus* (Grade 1) belonged to a clonal group (C_I) near to USA300 and infecting *S. aureus* (Grade 2-4) to three clonal groups (C_I, C_{II} near to USA800, C_{III} near to USA200) (Fig. 3). Four infecting strains belonged to C_I clone like the colonizing strains with more than 90% of homology between these stains. Optical maps allowed the identification, approximate location, and characterization of a genetic insertion of a DNA element between the colonizing and infecting strains (Fig. 4). This insertion was exclusively present in all Grade 1 strains and allowed the distinction between the 2 populations (Fig. 5).

Fig. 2. UPMGA tree of PFGE from *S. aureus* isolated from Grade 1 DFU



Comparison between strains isolated from Grade 1 DFU (NSA1322, 1385, 43233, 6271, 32510, 396905, 396906) and with one strain isolated from Grade 2-4 DFU (739) demonstrated that those isolated from different ulcers had no clonal link.

Fig. 3. UPMGA tree of optical maps of *S. aureus* isolated from DFU



A clonal link was shown between all strains isolated from Grade 1 ulcers and with some strains isolated from Grade 2-4 DFU (notably NSA739).

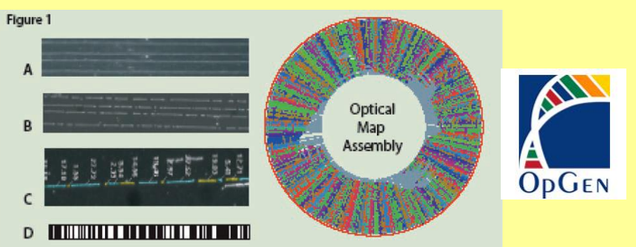
MATERIALS AND METHODS

Population. Diabetic patients hospitalized in a diabetic foot department in Nîmes University Hospital (France) with a foot ulcer were prospectively enrolled if they had been free of antibiotic treatment over the previous 6 months. At admission, ulcers were classified as infected (Grade 2-4) or noninfected (Grade 1) on the basis of clinical examination, according to the International Working Group on the Diabetic Foot system. Only patients carrying *S. aureus* as the sole pathogen were included. Twenty-two patients harboring Diabetic Foot Ulcers were enrolled.

Studied strains. Eight strains isolated from Grade 1 ulcers, 4 strains from Grade 2, 7 from Grade 3 and 3 from Grade 4 were enrolled. 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.

PFGE. Macrorestriction analysis of *Sma*I-digested chromosomal DNA was performed by Pulsed Field Gel Electrophoresis (PFGE) with the CHEF DRII system (BioRad) (11). The PFGE patterns were analyzed by Gel compar software (Applied Math, Kortrijk, Belgium) and compared by the algorithmic clustering method known as the unweighted-pair group method using arithmetic averages with the Dice coefficient of similarity.

Optical Mapping was used for comparative genome analysis. This technique utilizes whole genome restriction mapping and is depicted in Fig. 1.



Optical Maps were constructed at OpGen (Madison, WI). (A) High molecular weight DNA from each isolate was immobilized as individual molecules onto an Optical Chip, (B) digested with restriction enzyme Xba I and fluorescently stained with YOYO-1 (Invitrogen), (C) the stained restriction fragments were analyzed by automated fluorescent microscopy and the size and order of restriction fragments for each molecule were captured with OpGen software. Collections of ordered restriction maps - Optical Maps. (D) Optical Maps are represented as "barcodes," where the vertical lines indicate the locations of cut sites and the space between lines represents the size of the restriction fragments.

Fig. 4. Optical map alignements within *S. aureus* isolated from Grade 1 and Grade 2-4 DFU

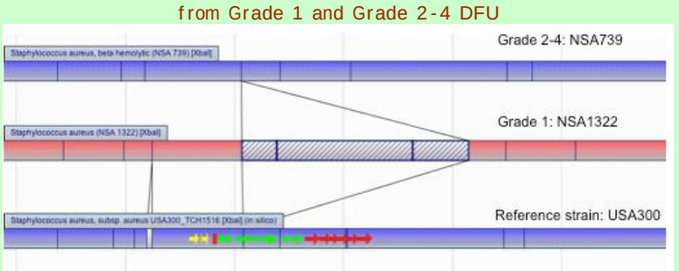
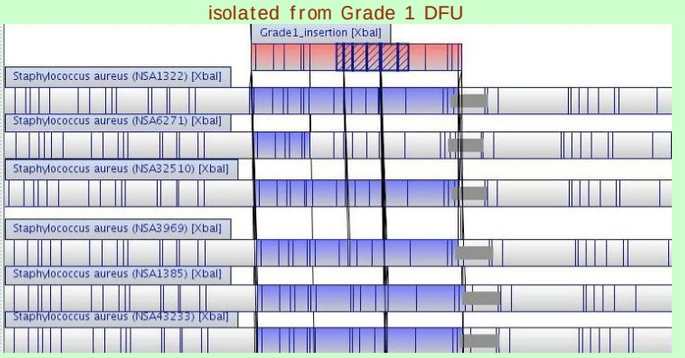


Fig. 5. Optical map alignements of genetic insertion in S. aureus isolated from Grade 1 DFU



CONCLUSION

Comparative whole genome analysis by Optical Mapping may be a useful alternative to PFGE due to its capability to easily generate ordered whole genome restriction fragment maps, identify areas of relatedness or differences between isolates of the same or different species and to determine genome size. Optical map was interesting to discriminate colonizing and infecting *S. aureus* in DFU and could contribute to more appropriate use of antibiotics.