Case report

Genomic Characterization of an ST1153 PVL-producing Methicillin Resistant
Staphylococcus aureus Clinical Isolate in Italy

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SUMMARY

Methicillin-resistant Staphylococcus aureus (MRSA) clones are rapidly increasing beyond the hospital
into the community, livestock farming and environmental settings (Lepuschitz S et al. 2018). An Italian
man, a professional diver working in Egypt, was admitted to Infectious Diseases Clinic- ASST
Fatebenefratelli Sacco for ulcerative skin lesions. An MRSA strain was isolated from the lesions’
purulent exudate and the nasal colonization was also ascertained. The strain, characterized by whole
genome sequencing, resulted to be Panton–Valentine Leukocidin (PVL) positive, SCCmecl – spa-type
t504, and belonging to the sequence type 1153, sporadically described worldwide.

Keywords: MRSA, Staphylococcus aureus, ST1153, spa-type t504, Panton-Valentine Leukocidin, Italy

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INTRODUCTION
In the last two decades, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) emerged worldwide, representing a public health major concern (Zetola et al. 2005). Moreover, MRSA strains have been isolated from environmental sources in different countries (Levin-Edens et al. 2012; Tice et al. 2010; Goodwin et al. 2009; El-Shenawy et al. 2005). The majority of *S. aureus* isolated from skin and soft tissue infections harbors the Panton-Valentine Leukocidin (PVL) genes, encoding for a cytotoxin causing leukocyte destruction and tissue necrosis (Niemann et al. 2018). The molecular epidemiology and the disease burden of CA staphylococcal disease is highly variable in Europe and is clonally diverse (DeLeo, 2011). CA-MRSA clones harboring PVL genes have already been reported in Italy (Vignaroli et al. 2009; Bassetti et al. 2010).

CASE REPORT
In early April 2018, an Italian man in his 60s, working as professional diver in Egypt, was examined for multiple itching skin lesions, that evolved in a short time into cutaneous abscesses, by a dermatologist, who recommended a cycle of steroid therapy for one month. His medical history was notable for sporadic recurrence of cutaneous rashes in the previous 10 years. The patient also referred a hospitalization more than ten years before in Belgium, where he lived for years, due to an acute myocardial infarction. Because of the partial resolution of the clinical picture, two months later (June 2018) he requested a second opinion at the Infectious Diseases Clinic-ASST Fatebenefratelli Sacco. Here for the first time, a microbiological investigation was carried out and an MRSA strain was detected both in ulcerated skin lesions and in nasal swab. Due to the presence of ulcerated lesions the molecular analysis for the PVL genes detection was performed. The patient was discharged with a 10-day treatment of linezolid 600 mg twice a day. Due to poor tolerance of the drug, an additional hospitalization was needed and the therapy was changed with intravenous daptomycin (500 mg every 24 h) associated with amoxicillin-clavulanate (1 g 3 times a day). Finally, the patient was discharged in good condition without the appearance of new lesions. Given the peculiar clinical history of the patient we decided to analyze study the strain with the Whole Genome Sequencing (WGS) approach, investigating its possible origin and epidemiological links.

Both *S. aureus* strains collected from nasal (Sa_04-18_NC) and cutaneous (Sa_04-18_SI) swabs of the patient and from the other members of his family (wife and daughter) were characterized by phenotypic and molecular methods. The species identification and antimicrobial susceptibility profiles were accomplished by Vitek 2 system (BioMérieux) (EUCAST breakpoints, [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf)). Furthermore, the presence of *mecA* and PVL genes was investigated using the RealCycler SAMAPV real-time Polymerase Chain Reaction (Progenie Molecular, Valencia, Spain). Both Sa_04-18_SI and Sa_04-18_NC strains resulted susceptible to all antibiotics tested except for oxacillin (>2 mg/L), fusidic acid (>16 mg/L) and benzilpenicillin (>0.25 mg/L); the cefoxitin test was
positive. Molecular typing revealed that Sa_04-18_SI and Sa_04-18_NC strains belonged to spa-type t504 and harbored the SCCmec type I genetic element, typical of hospital-acquired MRSA. The nasal swab from the patient’s daughter was positive for a different strain, methicillin susceptible SA PVL-negative; while no colonization was found for his wife.

The Sa_04-18_SI strain was subjected to WGS typing. After DNA extraction with the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA), the genomic libraries were sequenced on a Pgm apparatus (Ion Torrent) with a 2 by 250 paired-end run. The genome assembly was obtained using SPAdes (Bankevich et al. 2012) and in silico characterized.

All the results of SCCmecFinder (Kaya et al. 2018), spaTyper 1.0 (Bartels et al. 2014) and PVL (lukS and lukF) genes BLAST (Altschul et al. 1990) searches confirmed the molecular data. Furthermore, the in silico MLST determination, accomplished by MLST 2.0 tool (Larsen et al. 2012), assigned the strain to the Sequence Type (ST) 1153, sporadically isolated in the Mediterranean area.

Finally, a phylogenetic analysis was performed on a dataset including the Sa_04-18_SI genome, the S. aureus reference strain NCTC_8325 and 100 genomes from the PATRIC database (Wattam et al. 2017) selected to be the most genetically similar to Sa_04-18_SI, on the basis of the Mash distance (Ondov et al. 2016). Selected genomes were subjected to SNP calling analysis (Gaiarsa et al. 2015) using the NCTC_8325 strain as reference. Furthermore, an SNPs-based phylogenetic reconstruction was performed by RAxML software (Stamatakis 2014) with 100 pseudo bootstrap replicates, setting the best evolutionary model selected by ModelTest-NG (Darriba et al. 2017). SNP calling produced an alignment of 43,122 bases; the best phylogenetic model found was TVM and the obtained phylogenetic tree (Figure 1) clustered the Sa_04-18_SI strain to the only two ST1153 strains present in PATRIC database.

**DISCUSSION**

In this work we describe the first ST1153 PVL-producing MRSA strain, isolated from recurrent human skin infections, eventually resolved after an appropriate PVL diagnosis and the consequent antibiotic therapy.

Unfortunately, the patient’s long (10-year) clinical history and the lack of previous microbiological investigations made it impossible to date the initial acquisition of the ST1153 MRSA strain. The main risk factors for the onset of the infection might be the hospitalization in Belgium and the patient’s profession as a diver.

The ST1153 PVL-producing MRSA has already been sporadically described in Asia (Aung et al. 2016) and Africa (Figure 2), from both human samples and camel’s milk in Egypt (Ali et al. 2017), but never in Europe. We had no evidences for the intrafamilial transmission of the strain, possibly due to the fact that all the members of the family were adults (Cocchi et al. 2013). All these data led us to hypothesize that the ST1153 MRSA strain might be acquired from the environment, possibly from the seawater in
Egypt, considering the patient’s job. Indeed, PVL-producing MRSA strains isolated from seawater have been already reported in the literature (Plano et al. 2013).

To obtain a more detailed epidemiological reconstruction, the Sa_04-18_SI strain was included in a global S. aureus phylogeny. The tree clustered Sa_04-18_SI only with two strains belonging to the ST1153, harboring PVL genes and sampled in Thailand. With such a limited ST1153 dataset, this result cannot be considered as strong evidence for an epidemiological link among the three strains. The difficulties that we met in this epidemiological reconstruction reveal that a greater effort on WGS surveillance programs is necessary, particularly for non-predominant clones.

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References


**Figure 1. Phylogenetic reconstruction**

Phylogenetic reconstruction of the relationships between the studied isolate (Sa_04-18_SI) and a dataset of 100 genomes selected to be the most similar in PATRIC database. MLST profiles of the isolates are mapped on the tree using the color code reported in the legend.
Figure 2. Geographical distribution of ST1153 *Staphylococcus aureus*

The map represents the sampling sites and the collection years of the ST1153 *S. aureus* isolates reported in literature and in this study. In details, the human icon refers to the studied isolate (Sa_04-18_SI), sampled in Egypt in 2017. The remaining isolates are represented with violet *S. aureus* shaped icons. When the exact sampling year was not available in literature, the sampling period was inferred from the paper and reported in brackets. All the strains derived from human samples, with the exception of one isolated from camel’s milk.