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Mingling of human and veterinary strains of *Staphylococcus aureus*: An emerging issue in health-care systems

Sara Giordana Rimoldi[1‡], Annamaria Di Gregorio[1‡], Vittorio Sala[2], Eleonora De Faveri[2], Cristina Pagani[1], Pietro Olivieri[3], Claudio Savi[4], Anna Lisa Ridolfo[5], Antona Carlo[6] and Maria Rita Gismondo[7]

1. Laboratory of Microbiology, Virology and Bioemergency, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy; 2. Department of Veterinary Sciences and Public Health, Università di Milano, Italy; 3. Medical Management Staff, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy; 4. Cardiac Surgical Intensive Care Unit, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy; 5. Department of Infectious Diseases, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy; 6. Cardiac Surgery Unit, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy; 7. Laboratory of Microbiology, Virology and Bioemergency, ASST Fatebenefratelli Sacco-Polo Universitario, Via G.B. Grassi 74, 20157 Milan, Italy.

Corresponding author: Sara Giordana Rimoldi, e-mail: sara.rimoldi@asst-fbf-sacco.it
Co-authors: AD: annadigre@yahoo.it, VS: vittorio.sala@unimi.it, ED: defaverieleonora@gmail.com, CP: cristina.pagani@asst-fbf-sacco.it, PO: pietro.olivieri@asst-fbf-sacco.it, CS: claudio.savi@asst-fbf-sacco.it, ALR: annalisa.ridolfo@asst-fbf-sacco.it; AC: carlo.antona@unimi.it, MRG: mariarita.gismondo@unimi.it
‡Rimoldi and Di Gregorio equally contributed to the work.

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Abstract

Aim: Methicillin-resistant *Staphylococcus aureus* remains a leading cause of hospital and community infections. We report a retrospective molecular characterization of *S. aureus* strains from different settings: hospital workers and patients, and veterinarian surgeons and pets.

Materials and Methods: Eighty-nine *S. aureus* isolates obtained from nasal swabs of 10 patients, 17 health-care workers (HCWs), 9 pets, and 53 veterinarians were genotypically characterized by means of repetitive extragenic palindromic polymerase chain reaction (Rep PCR) and whole-genome sequencing.

Results: Thirteen different sequence types (STs) were detected: ST398, ST22, ST8, ST30, ST15, ST5, ST121, ST45, ST10, ST6, ST34, ST97, and ST1. Two new STs differing from ST22 and ST5 for a single multilocus sequence typing gene were also identified. Rep PCR documented a genetic relationship among isolates obtained from 5 veterinarians and 10 HCWs.

Conclusion: The large diversity of *S. aureus* strains detected may reflect a larger epidemiology within the hospital and community, in which companion animals likely act as a reservoir. We identified the circulation of ST5, ST8, ST15, ST22, ST30, ST45, and ST121 both in the hospital and veterinarian environment. Starting from the idea of a unique setting where our population lives, we consider the relationship between community- and hospital-acquired *S. aureus*.

Keywords: health-care workers, multilocus sequence typing, *S. aureus*, single-nucleotide polymorphisms, pets, veterinarians.

Introduction

Staphylococcus aureus is a major cause of health-care (HA)-associated infections worldwide [1,2]. It is one of the most prevalent causes of nosocomial bacteremia, hospital-acquired pneumonia, and surgical site infections, mainly in the intensive care settings [1,2]. In addition, methicillin-resistant *S. aureus* (MRSA), firstly reported in 1961, has quickly become one of the most important antibiotic-resistant nosocomial pathogens globally [3,4]. In one-third of the European countries, including Italy, MRSA continues to account for more than 25% of bloodstream infections despite efforts in infection control [5]. Nasal and extranasal carriage of MRSA often leads to invasive MRSA infection, as MRSA colonization is associated with an increased risk of acquiring MRSA infection during hospital stays [6]. Moreover, the pressure of colonization in health-care environment plays an important role in the subsequent dissemination of MRSA strains [7].

Although MRSA has long been considered an issue confined to health-care environment (HA-MRSA), its epidemiology has become more complex in the recent years. In the early 90s, MRSA strains genetically distinct from HA-MRSA have been recovered increasingly in the community from patients without previous HA contact (community-acquired [CA] - MRSA) [8,9]. Notably, CA-MRSA frequently carries genes for the cytotoxin Panton-Valentine leukocidin (PVL) that confers enhanced virulence [8,9].

MRSA which originated in the community and is associated with exposure to livestock ([LA]-MRSA) has also emerged in many countries [10,11].

LA-MRSA has shown the capacity to colonize humans and cause zoonotic infections in farm workers, abattoir workers, and veterinarians [10].

Furthermore, MRSA is increasingly reported in veterinary medicine, particularly, in small animals and equine practices [12-14]. In general, companion animal strains of MRSA differ from those in livestock and meat production animals. This is probably because in companion animals, MRSA infection is primarily a human disease, with the strains carried by human owners being passed to their animals [15]. Historically, MRSA infections in companion animals involved strains resembling human nosocomial strains, including epidemic MRSA [12]. When these epidemic HA-MRSA clones were observed in dogs [12-14], the assumption was that the direction of spread had been from humans to animals. However, this situation is changing rapidly, with strains of MRSA that is thought to have evolved in animals colonizing and infecting human attendants [16]. Like their companion animals, companion humans are more often colonized than infected, providing a reservoir for reinfection of their human and animal companions [17-19]. Human skin scales with MRSA are easily shed from leg ulcers, eczematous skin, and pressure areas during activities of daily living. Undetected colonized animals can provide a reservoir for continuing relapsing infection in humans [18-21].

Overall, therefore, MRSA can no longer be regarded as an exclusively HA problem, and it cannot be fought by hospital infection prevention and control measures alone. The expanding community reservoir of MRSA leads, in fact, to the inevitable infiltration and diffusion of CA-MRSA into hospitals through colonized/infected patients, visitors, and health-care workers (HCWs) [21-25]. Consequently, identifying reservoirs and tracking the source of MRSA diffusion within hospitals have become more difficult. Limitation in pathogens typing techniques available in routine clinical practice further hinders such investigations.

Molecular characterization methods are an essential tool for discriminating between isolates of epidemiologically important microorganisms. A variety of molecular typing methods [26,27] can be independently used to classify MRSA strains, including pulsed-field gel electrophoresis, multilocus sequence typing (MLST), or *Staphylococcus* protein A (*spa*) typing by sequencing the highly polymorphic *spa* gene. *Spa* type possesses high throughput and good interlaboratory reproducibility but can characterize *S. aureus* isolates within limited discrimination of a clonal complex. Greater discrimination such as provided with whole-genome sequencing (WGS) and single-nucleotide polymorphism (SNP) analysis could be useful to discern outbreak from non-outbreak strains in settings where similar strains are collected within a short timeframe [28,29].

This study was designed to investigate retrospectively whether *S. aureus* circulation in the cardiac-surgery area of ASST Fatebenefratelli–Sacco Hospital in Milan results from circulation/transmission of a heterogeneous mixing of *S. aureus* strains including those potentially originated from community/animal reservoirs. The causing of this rating was the occurrence during April 2015 of four cases of deep sternal wound infections (DSWIs) caused by *S. aureus*. The discovery of similar strains in some HCWs and veterinarians enrolled for a previous study [30] led us to extend our investigation on MRSA clones circulating among veterinarians and companion animals assisted by them.

Materials and Methods

Ethical approval

All examined isolates were cultured and preserved as part of the routine diagnostics (standard care) and local epidemiological surveillance regulations. Written informed consent for routine diagnostic and medical/epidemiological procedures was collected for each patient and healthcare operator. All data used in the study were previously anonymized, according to the requirements set by Italian Data Protection Code (leg. Decree 196/2003) and by the general authorizations issued by the Data Protection Authority. Approval by the Ethics Committee was therefore not required.

Study population

From January to December 2015, 365 nasal swabs collected from HCWs (98 physicians, nurses, and operating room and Intensive Care Unit (ICU) personnel), patients (10 subjects with DSWI), veterinarians (192), and pets (52 dogs and 13 cats) were investigated for the presence of *S. aureus* by the Laboratory of the Microbiology, Virology, and Bioemergency ASST Fatebenefratelli–Sacco Hospital, Milan, Italy. *S. aureus* isolates were characterized by means of antimicrobial susceptibility testing and genotyping techniques.

Microbiology

Nasal swabs were plated on Mannitol salt plates (BioMérieux, Marcy l'Etoile, France) that were incubated at 37°C overnight. Identification at the species level and antimicrobial susceptibility was determined using the Vitek2 automated system (BioMérieux) considering the criteria of the European Committee on Antimicrobial Susceptibility Testing breakpoints [31]. Isolates were stored at −80°C.

Real-time polymerase chain reaction (PCR)

Cultures were incubated on Columbia blood agar (BioMérieux). *S. aureus* DNA was extracted with the automatic extractor Easymag (BioMérieux) and analyzed with a real-time PCR (RealCycler SAMAPV, Progenie Molecular, Valencia, Spain) to detect the presence of the mecA gene, coding for methicillin resistance, and the PVL toxin gene.

Repetitive extragenic palindromic PCR (Rep-PCR)

Seventy-eight *S. aureus* isolates (7 methicillin-sensitive *S. aureus* [MSSA] and 71 MRSA) of HCWs, veterinaries, and pets were genotyped using an automated Rep-PCR (DiversiLab system; BioMérieux). Band patterns were compared by means of Pearson's correlation and modified Kullback–Leibler methods using the DiversiLab web-based software. The isolates whose band patterns were at least 95% similar were considered to be genetically related.

Next-generation sequencing (NGS)

Eighty-nine *S. aureus* isolates (9 MSSA and 80 MRSA) collected from HCWs, patients**,** veterinarians, and pets were fully sequenced with NGS on the Illumina MiSeq platform (kit v3, 600 cycles). Each of these isolates underwent molecular typing by an MLST, and 27 (17 HCWs and 10 patients who shared the same sequence types [STs]) of them underwent SNP analysis. WGS was performed at the Histology and Molecular Biology Section of the Army Medical Research Center in Rome.

MLST compared single nucleotide variants within 7 *S. aureus* reference genes (arcC, aroE, glpF, gmk, pta, tpiA, and yqiL) to a reference database [32]. WGS was used to obtain at the same time *in silico* data related to MLST and SNP analysis. We conducted *in silico* MLST analysis using the resources from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org).

Once all alleles were assigned a designation for the 7 MLST genes, an ST pattern was defined, comparing the whole-genome sequences against the MLST database.

SNP analysis was performed using the program kSNP v2.1.2 that defines SNP loci by an oligo of length k, identified by the Kchooser script, surrounding a central SNP allele [33]. A core SNP matrix including only SNPs detected at the loci that were present in all genomes was determined, and a maximum likelihood tree was visualized with the software Dendroscope v3.2.10 [34].

Results

Patient and HCW evaluation

From January to December 2015, 10 patients hospitalized in the cardiac-surgery area of our hospital had *S. aureus* isolated from surgical wound, including a cluster of 4 cases observed during April 2015. All the 10 patients were positive for *S. aureus* from nasal swabs. Two of 10 *S. aureus* strains were MSSA (*mecA* negative), whereas 8 were MRSA (*mecA* positive).

As part of an epidemiological investigation, 98 HCWs operating in the cardiac-surgery and ICU underwent nasal screening for *S. aureus,* and 17 (12%) resulted positive: 7 (41%) for MSSA and 10 (59%) for MRSA. All *S. aureus* isolates from patients and HCWs were negative for *PVL* gene.

WGS was performed to assess relationships among strains isolated from patients and HCWs. *In silico* extrapolation of MLST profiles of 27 whole genomes revealed 10 different STs: the most frequent was ST22 (12 strains), followed by ST121, ST30, ST8, and ST5 (2 strains each) and ST6, ST10, ST15, ST34, and ST45 (1 strain each). Two new STs differing from ST22 and ST5 for a single MLST gene were also identified (Table-1). In addition, SNP analysis allowed us to discriminate between samples with the same ST, such as in the case of the most predominant ST22 group (Figure-1).

WGS discounted patient-to-patient transmission as the only origin of the cluster of DWSI which occurred during April 2015. In fact, only 2 of 4 patients had the same ST (ST22) and similarities at SNP analysis, whereas the other 2 belonged to ST5 or ST30 clones. However, similarities of ST strains were detected among patients and HCWs: 3 patients and 3 HCWs shared the same ST22 strain, and 2 patient and HCWs pairs shared the same ST8 and ST30 strains, respectively.

Veterinarian and pet evaluation

Overall, 53 of 192 (27.6%) nasal swabs from veterinarians and 9 of 53 (16.9%) samples from pets were positive for MRSA. Among 62 whole genomes, *in silico* extrapolation of MLST profiles revealed 9 different STs: The most frequent was ST398 (45 strains),

Table-1: *S. aureus* (MSSA or MRSA) strains and MLST profiles detected in patients and operators in the cardiac-surgical area of L. Sacco Hospital, Milan, from January to December 2015.

ID analysis	Patient/operator ID	*S. aureus* strain	MLST
MRSA1	Operator 1	MRSA	ST22
MRSA2	Patient 1	MRSA	ST22
MSSA3	Patient 2	MSSA	ST22
MRSA4	Operator 2	MSSA	ST121
MSSA5	Patient 3	MSSA	New allele ST5
MRSA6	Operator 3	MRSA	ST121
MRSA7	Operator 4	MRSA	ST22
MRSA8	Operator 5	MRSA	ST22
MRSA9	Operator 6	MRSA	ST22
MRSA10	Patient 4	MRSA	ST5
MRSA11	Operator 7	MSSA	New allele ST22
MRSA12	Operator 8	MSSA	ST10
MRSA13	Operator 9	MSSA	ST15
MRSA14	Operator 10	MSSA	ST8
MRSA15	Operator 11	MSSA	ST30
MRSA16	Operator 12	MSSA	ST45
MRSA17	Patient 5	MRSA	ST8
MRSA18	Patient 6	MRSA	ST22
MRSA19	Patient 7	MRSA	ST30
MRSA20	Patient 8	MRSA	ST22
MRSA21	Patient 9	MRSA	ST22
MRSA22	Patient10	MRSA	ST5
MRSA23	Operator 13	MRSA	ST22
MRSA24	Operator 14	MRSA	ST22
MRSA25	Operator 15	MRSA	ST34
MRSA26	Operator 16	MRSA	ST6
MRSA27	Operator 17	MRSA	ST22

MSSA=Methicillin-sensitive *Staphylococcus aureus,* MRSA=Methicillin-resistant *Staphylococcus aureus*, *S. aureus=Staphylococcus aureus*, MLST: Multilocus sequence typing

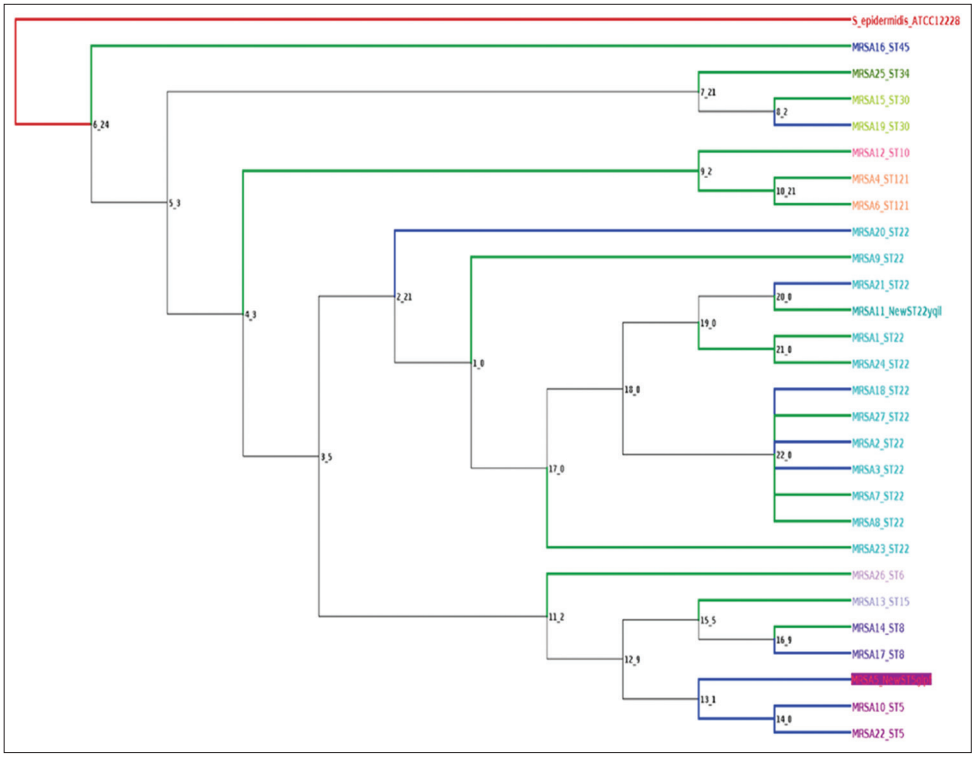

Figure-1: Dendrogram resulting from single-nucleotide polymorphisms analysis of *Staphylococcus aureus* strains.

followed by ST8, ST15, ST 45, and ST22 (2 strains each) and ST1, ST5, ST10, ST30, and ST97 (one strain each).

Even though 2 different predominant STs were found in the 2 settings of our study (ST398 for veterinarians and ST22 for HCWs and patients), the discovery of strains typical of animals (ST121 and ST45) in HCWs and the finding of some STs both in HCWs and veterinarians (ST5, ST8, ST10, ST15, ST22, and ST30) led us to investigate the clonal relationship between our hospital strains and those present in the veterinarian setting. It is interestingly to note that two HCWs carrying strains typical of animals lived with companion animals (a cat and a dog, respectively), but nasal swabs taken from their pets resulted negative.

Rep PCR evaluation

The clonal relationship among the 27 *S. aureus* strains obtained from HCWs and patients and the 62 obtained from veterinarians and pets was investigated by means of Rep-PCR. Using the Diversilab analysis software, we were able to obtain a hierarchical cluster representation of genomic similarities between this large number of samples. Overall, isolates presented a low similarity of sequence, confirming the results of MLST analysis.

With regard to the STs shared by veterinarians and HCWs, there was a correlation between human and animal ST121, and high similarity was found for ST45 shared by one HCW and one cat. Moreover, we observed the presence of indistinguishable strains (belonging to the same pattern) and related strains (belonging to different patterns, but assimilated to the same group) among HCWs and veterinarians.

A sharing pattern was reported in 3 situations: 1 case among 5 HCWs (ST22) and 1 veterinarian (ST unknown) and 2 cases among 1 HCW and 1 veterinarian (HCW ST10 and veterinary ST10). In the other 2 situations, the strains belonged to different patterns but were related in the same group: 1 case among 2 HCWs and 1 veterinarian and another among 1 HCW (ST15) and 1 veterinary (ST15).

Discussion

Increasing human and animal reservoirs outside the HA environment make more difficult to understand and control the diffusion of MRSA in hospitals. CA-MRSA is replacing classic hospital MRSA clones in many countries and have higher potential in transmission and virulence than HA-MRSA clones [8,9]. This poses new challenges for infection control, and physicians need to be aware of and understand the burden of this problem.

Molecular typing is essential for epidemiological studies and outbreak investigations. To this end, WGS has high discriminatory power and resolution and is well placed to become the gold standard in bacterial typing [27]. We used WGS to characterize bacterial isolates, which facilitated our understanding of the circulation of *S. aureus* in the cardiac-surgery area of our hospital and documented that patient-to-patient transmission was not the only pattern of diffusion. However, we were not able to establish whether transmission had taken place between HCWs and patients or *vice versa*.

WGS revealed ST121 and ST45 in two HCWs who lived with pets (cat and dog), but nasal swabs

from their animals resulted negative. However, the genomic identity between isolates obtained from dogs and cats and their owners has previously been documented in the literature, supporting the role of pets as reservoirs of CA-MRSA strains and their possible contribution to the maintenance and mixing of locally circulating strains [17-21].

Further investigation led us to seek clonality among the strains detected in our hospital and those found in veterinarians and pets. Rep-PCR revealed a correspondence between a cat and 1 of the 2 HCWs who lived with a cat. A high similarity (the same pattern or the same group) was also found among isolates from 5 veterinarians and 10 HCWs.

All these findings could reinforce the concept of an evolving epidemiology of *S. aureus* which involves both humans and animals [35]. The new epidemiology of MRSA, in particular, is closely related to the different environments of the animal species and humans with possible exchange of strains among the different epidemiological settings (community, hospital, and livestock).

Conclusion

Starting from the epidemiological circulation of infection in humans and animals and a unique setting where our population lived in close proximity (hospital and veterinarian workers, patients and pets), we considered the relationship between CA- and HA-*S. aureus*. In this context, the most effective strategy was the monitoring of circulating strains to track and locate them. For this reason, further investigations are needed to genomically characterize better the strains identified, especially the novel ones. For better epidemiological evaluation, it would be appropriate to expand the number of patients enrolled in the study. HA-MRSA, CA-MRSA, and LA-MRSA are an important novel problem that needs to be considered.

Authors' Contributions

SGR and AMD: Conception and design, laboratory work, data analysis, and manuscript write up. VS, ALR, and ED: Conception, design, and review of the manuscript. CA and MRG: Critical review of the manuscript. CS and PO: Patient's enrolment. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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