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EPIDEMIOLOGY OF Staphylococcus aureus STRAINS METHICILLIN-RESISTANT AND SENSITIVE ISOLATED IN CARDIOSURGICAL AREA OF THE ITALIAN L. SACCO HOSPITAL

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INTRODUCTION/PURPOSE

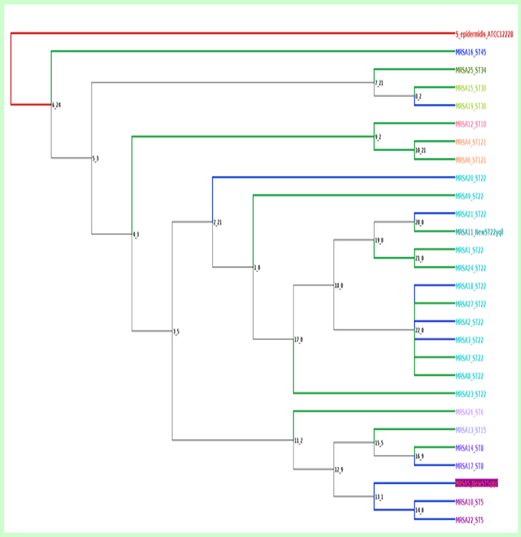
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common and important cause of infection in the intensive care unit (ICU) setting. It may be acquired from colonized or infected patients and healthcare workers (HCWs). Moreover community reservoirs have been implicated in MRSA introduction by increasing colonization prevalence among patients, visitors and workers.
Preceding MRSA colonization is a risk factor for subsequent MRSA infections[1]. The colonizing bacterial strains may serve as endogenous reservoirs for overt clinical infections or may spread to other patients[2].
Molecular genetics studies revealed that most MRSA isolates were healthcare-associated clones and that nasal and clinical isolates exhibited up to 75% shared identity[3].
As a consequence, it is important to confirm the linkage between nasal carriage and clinical MRSA isolates to develop the best strategy to avoid systemic infections by decolonization methods.
However, identifying reservoirs and tracking the source of implicated strains has proven difficult. Limitation in genotyping techniques available in clinical practice may hinder the investigation of MRSA outbreaks in healthcare settings.
During April 2015 4 patients hospitalized in Cardiosurgical Area at the "Luigi Sacco" Hospital in Milan due to a deep sternal wound infection (DSWI) resulted positive for S. aureus.
Our study was designed to retrospectively evaluate the epidemiology of Staphylococcus aureus strains collected in Cardiosurgical Area at the "Luigi Sacco" Hospital, Milan (Italy).

1 Natural history of community-acquired methicillin-resistant Staphylococcus aureus colonization and infection in soldiers Ellis MW, Hospenthal DR, Dooley DP, et al. Clin Infect Dis. 2004
2 Methicillin-resistant Staphylococcus aureus (MRSA) nares colonization at hospital admission and its effect on subsequent MRSA infection. Davis KA, Stewart JJ, Crouch HK, et al. Clin Infect Dis. 2004;
3. Risk Factors of Methicillin-Resistant Staphylococcus aureus Infection and Correlation With Nasal Colonization Based on Molecular Genotyping in Medical Intensive Care Unit: A Prospective Observational Study
Kuo-Chin Kao, MD, Chun-Bing Chen, MD, Han-Chung Hu, MD et al.; Medicine. 2015

METHODS

From January to Dicember 2015 dreinage by a negative pressure wound therapy (NPWT) device was collected from 15 patients and nasal swabs were collected from 98 HCWs of Cardiosurgical Area at the "Luigi Sacco" Hospital in Milan.
Microorganisms identification and susceptibility test were performed using Vitek2 (Biomerieux, Marcy l'Etoille, France).
***S.aureus* isolates were 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 Panton-Valentine leucocidine toxin (PVL) gene.**
The whole-genome sequencing (WGS) approach was used to obtain at the same time in silico data related multi locus sequence type (MLST) and SNP analysis of the S. aureus isolates collected from 10 patients and 17 HCWs.

Fig 1: Dendogram resulting from the SNPs analysis of *S. aureus* strains detected



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 ST5glpf
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 ST22yqii
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

Tab 1: kind of *S. aureus* (MSSA or MRSA) and relative MLST profile detected in patients and operators of Cardisurgical Area of L. Sacco Hospital, Milan from January to Dicember 2015

RESULTS

2 out of 10 (20%) *S.aureus* strains collected from patients were MSSA (Methicillin-sensitive Staphylococcus aureus) (mecA negative), while 8 out of 10 (80%) were MRSA (mecA positive).
17 out of 98 HCWs (17%) were positive to *S. aureus*: 7 out of 17 (41%) were MSSA and 10 out of 17 (59%) were MRSA.
No PVL toxin gene was detected.
In silico extrapolation of MLST profiles revealed 10 different sequence types (STs): the most represented was ST22 (12 strains), followed by ST5, ST8, ST30 and ST121 (2 strains) and ST6, ST10, ST15, ST34 and ST45 (owned each by a single strain). Two new STs , differing from ST5 and ST22 for a single MLST gene, were also identified. (Tab. 1) Finally, the SNPs analysis allowed to discriminate between samples with the same ST such as in the case of the most predominant ST22 group (Fig. 1)

CONCLUSIONS

The diversity of isolates detected in our epidemiological evaluation in Cardiosurgical Area may reflect a larger epidemiology within the hospital or community, which would require further molecular investigations.
ST5, ST8, ST15, ST22 and ST30 are reported in other italian hospitals indicating a wide distribution of these STs in our Country. Moreover the finding of STs typical of animals like ST45 and ST121, should lead to better consider the relationship between HCWs and their pets. (Fig.2)
The data obtained demonstrate that genotyping is an indispensable tool of epidemiological investigation and that WGS is emerging as a gold standard in bacterial typing

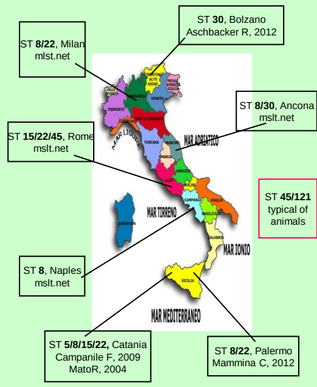


Fig 2: Geographycal distribution of different sequence types of *S. auereus* strains in Italy