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Isolation and Molecular Characterization of Methicillin – Resistant Staphylococcus Aureus (MRSA) In Hospital Patients

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ABSTRACT

Staphylococcus spp. it is one of the genera most frequently implicated in the etiology of hospital infections, especially in nosocomial wards. *Staphylococcus aureus* is one of the most frequently isolated pathogenic microorganisms in hospital infections, capable of causing septicemia and infections of the skin, respiratory system, and soft tissues. Furthermore, the spread of infections caused by the methicillin-resistant *Staphylococcus aureus* (MRSA) species is constantly increasing and is reaching worrying levels in various countries of the world, including Italy, in continuous and rapid expansion, even outside hospitals. In this study, strains of *Staphylococcus* with methicillin resistance in hospitalized patients were identified and characterized through a phenotypic and genotypic approach. In all the methicillin resistant strains analyzed, a high resistance to other classes of antibiotics tested was found, in accordance with the findings of the European Center for Disease Prevention and Control (CDC) and numerous studies at national and world level. On some isolated MRSA strains, a molecular epidemiological study was conducted to understand the origin and spread of circulating clones. All these have been identified by molecular approach aimed at genetic research and identification, by means of Multi Locus Sequence Typing (MLST), typing of the Staphylococcal Cassette Chromosome mec (SCCmec complex) and spa typing, typing of the repeated variable region of protein A.

Using the MLST profile, 5 different clones of *S. aureus* were identified in several hospital departments, 4 of which already circulating in Italy and worldwide, while one is not yet reported in Italy. The application and deepening of these techniques have provided an overview of the spread and development of MRSA strains in the hospital setting.

Abbreviations: CA-MRSA: Community-Acquired Methicillin-Resistant *S. aureus*; CDC: Centers for Disease Control and Prevention; CoNS: Coagulase-negative staphylococc; HA-MRSA: Hospital-Acquired Methicillin-Resistant *S. aureus*; MDR: Multi Drug Resistance; MIC: Minimal Inhibitory Concentration; MLST: Multi-Locus Sequence Typing; MRSA: Methicillin-Resistant *S. aureus*; PBP: Penicillin Binding Protein; PCR: Polymerase Chain Reaction; PST: Penicillin Streptomycin and Tetracycline – Resistant; PSTE: Penicillin streptomycin tetracycline and erythromycin – Resistant; PVL: Panton-Valentine Leukocidin; SCCmec: Staphylococcal Cassette Chromosome mec; ST: Sequence Type; TSST: Toxic Shock Syndrome Toxin; VRSA: Vancomycin-Resistant *Staphylococcus Aureus*

Introduction

Staphylococci are gram positive bacteria belonging to the Staphylococcaceae family. They are catalase positive, spherical in shape arranged in clusters or tetrads, non-spore-forming, and immobile. Many staphylococci can grow under various conditions, in the presence and absence of oxygen, with another market concentration (10% NaCl) and a temperature between 18 °C and 40 °C. Staphylococci are found mainly on the skin and mucous membranes of mammals, some species have a preferential host such as *Staphylococcus hominis* in humans, while others such as *Staphylococcus* aureus, find it in more hosts. *S. aureus* is present on the skin and mucous membranes in 20-30% of healthy people. Adolescents and adults often carry short-term or persistent *S. aureus*, approximately 15% of healthy adults are persistent carriers. The adult is colonized by *S. aureus* for a 30-50%, 20% of the population in a persistent way. There are also conditions such as diabetes, drug addiction, immunodeficiency that support colonization and proliferation and transmission [1-3]. *S. aureus* is one of the most common and important human pathogens, both in the community and in the hospital. The most common *S. aureus* infections, defined as staphylococcal, are of the supportive type, affect various organs and systems with a high and variable degree of virulence. Infections affect the skin, cutaneous glands, and subcutaneous soft tissues. There may be localizations in the site of abscesses in various organs, therefore infections in surgical wounds and systemic forms.

Other infections are represented by Ritter's disease or burned skin syndrome, due to the epidermolysin staphylococcus produced. It is a toxin capable of detaching the superficial layers of the skin and by the toxic shock syndrome, TSST-1, also deriving from action of a toxin that involves symptoms such as: fever, hypotension, desquamative erythroderma and organ symptoms [1,4,5]. The main factors that increase susceptibility to infections are the prolonged or inefficient antibiotic or corticosteroid therapies, the use of invasive procedures (vascular and bladder catheterization, tracheal intubation, etc.), prolonged hospitalization and surgical interventions [6,7]. *S. aureus* is also responsible for food poisoning, due to the multiplication in foods of strains of *S. aureus* producing toxins resistant to cooking temperatures and the action of digestive proteolytic enzymes [8,9]. *S. aureus* is provided with a polysaccharide capsule, with phagocytic power, neutralized by specific antibodies. On the cell surface there are proteins that are able to cooperate with those of the host, such as fibronectin and fibrinogen, playing the role of adhesions. Among these, the clumping factor is a protein which, interacting with fibrinogen, forms aggregates that can be highlighted on the slide. Another important surface protein of *S. aureus* is protein A.

This is involved in complement activation, inhibits the phagocytosis of the bacterium by polymorphonuclear leukocytes, invokes hypersensitization and stimulation of lymphocyte production, contributing significantly to increase the virulence of *S. aureus* [3,10]. Furthermore, *S. aureus* has always been an absolute protagonist of acquired antibiotic resistance. Of particular importance and interest was the evolution of the resistance of *S. aureus* to β-lactam antibiotics, characterized by two distinct periods of hospital infections. A first hospital infection, which developed early (around the early fifties of the last century) and rapidly spread all over the world, was sustained by penicillin-resistant strains, which became such having acquired the ability to produce penicillinase [11]. The end after 10 years thanks to the advent of new antibiotics (such as penicillinase-resistant penicillin and the first cephalosporin's), even if the phenotypic and genotypic characteristic of β-lactamase production remained definitively acquired by most of both hospital community. A second hospital infection, still ongoing today, is that sustained by methicillin-resistant strains (internationally known with the acronym MRSA, methicillin-resistant *S. aureus*), that is, competent of resisting methicillin, the progenitor of penicillinase-resistant penicillins [4]. Methicillin is characterized by an acyl group in 6 'which sterically prevents attachment to the β-lactam ring, thus preserving its activity even in the presence of β-lactamase [12,13].

Furthermore, MRSA are resistant not only to penicillinase-resistant penicillins but to all β-lactams, and in addition they are characterized by a demonstrated multi-resistance [9,14]. The onset of MRSA has occurred over time in at least three different areas that have seen changes in those involved in infections: hospitalized people, therefore nosocomial infections, people outside the hospital community and animals. The presence of MRSA was reported for the first time as a nosocomial infection (hospital - acquired MRSA, HA -MRSA), affecting hospitalized patients, so much so that up to the 1970s strains of MRSA represented the major cause of hospital infections. The beginning and spread of HA-MRSA has been associated with typical risk factors related to the hospital environment and isolates from patients who were MRSA negative at hospital admission or MRSA isolates are still defined as HA-MRSA. Between 1970 and 1990 several HA-MRSA epidemics occurred in the USA and Japan; pandemics followed by some cases in Europe [15-17]. Since the 1990s, invasive MRSA infections of the skin have occurred in patients who are not hospitalized and who did not possess characteristics to be attributable to HA-MRSA strains [18-20]. The *S. aureus* that affects such infections are called community-acquired MRSA (CA-MRSA). Described for the first time in the United States, they are potentially dangerous even for the

"healthy" population, and are, unfortunately, responsible for most of the children's deaths. It was possible to discriminate between HA-MRSA and CA-MRSA strains thanks to not only phenotypic but above all genotypic characteristics.

Most infections caused by CA-MRSA involve skin and soft tissue, and some also produce the toxin PVL [21-24]. *S. aureus* owes its resistance to methicillin to the presence in the SCCmec cassette of the gene encoding a variant of the penicillin binding protein (PBP) referred to as PBP2a. Beta-lactam antibiotics work by binding PBPs to the wall, inhibiting the synthesis of peptidoglycan, the main component of the bacterial wall, thus causing cell death. The PBP2 variant is unable to bind β-lactams, so the synthesis activity can continue, making the action of these ineffective. It is a form of resistance that develops with the production of a protein like the drug's target, but not susceptible to it. The mecA gene is regulated by the Mecl repressor and the β-lactam sensitive transmembrane signal transducer, MecRI. In the absence of β-lactam antibiotics, MecI represses the transcription of all the genes of the mec complex, therefore not only mecA, but also MecRI and mecI. MecRI with an autocatalytic cut activates the cytoplasmic metallo-protease domain, which splits the link between Mecl and the operator region of the mecA gene, allowing the transcription and production of PBP2a, in the presence of β-lactam. Therefore, the staphylococcal chromosomal cassette mec (SCCmec) is the main genetic determinant able to discriminate between the two groups of HA and CA-MRSA [11,21,25,26]. SCCmec is a mobile genomic island that encodes various resistance determinants. Currently 8 different types of SCCmec have been described. Types I, II, III and VIII are associated with HA-MRSA.

While type IV, V, VI and VII are associated with CA-MRSA, virulent mainly, which mainly affected previously healthy young subjects. Therefore, according to the single clone theory, the cassette would have been introduced only once in *S. aureus* with horizontal transfer from a species of *Staphylococcus*, therefore MRSA would have a single precursor, unlike the multiple clone theory which predicts that there have been different events and factors involving different strains of *S. aureus* [27,28]. Multi-Locus Sequence Typing (MLST) demonstrated that the 5 pandemic clones of MRSA evolved from only two genetically distinct ancestral backgrounds: one dating back to the earliest European MRSA strains and to MSSA strains circulating in Denmark towards the end of the 1950s, and the other, a completely different background, attributable to MRSA strains originally isolated in the USA, Japan and in pediatric patients from different parts of the world [29,30].

The first European MRSA isolates were characterized by belonging to the same phage group, resistance to penicillin, streptomycin, tetracycline (PST) and occasionally to erythromycin (PSTE), by a low MIC (minimum inhibitory concentration) of methicillin (6-25 μg/ml), and a heterogeneous expression of resistance [31,32]. These strains have evolved to the current clone called Iberic, which has acquired additional resistance determinants (some resident on mobile elements, such as plasmid pUB110 and transposon Tn554) and is often resistant to the most common antibiotics except co-trimoxazole. And glycopeptides.

The Brazilian and Hungarian clones would also have derived from the first background. The New York / Japan and Pediatric clones would have derived from the second background. The Iberic, Hungarian and New York / Japan clones is sensitive only to co-trimoxazole and glycopeptides. The Brazilian clone is sensitive only to spectinomycin and glycopeptides. The pediatric clone is resistant only to oxacillin, penicillin, gentamicin, and occasionally erythromycin [13,31]. Epidemiologically, the various reports relating to the isolation of Community MRSA strains outline a European reality characterized by a polyclonal character. In Italy, several clones have been described such as ST88, ST30, ST8, ST72 and ST813. On the contrary in the United States, there is the diffusion of a clone called USA300, belonging to the ST8 and USA400 [16,33,34]. The main HA-MRSA clones circulating in the world belong to the clonal complexes CC5, which includes ST5 SCCmec type II (New York / Japan); ST5-IV pediatric, ST228-I (southern German); The CC8 with ST250-I (Archaic clone), ST8-IV (EMRSA-2, -6), ST8-II (Irish), ST239-III (Brazilian / Portuguese), ST247-I (Iberian); The CC22 with ST22-IV (EMRSA-15); CC30 with ST36-II (EMRSA-16); The CC45 with ST45-IV (Berlin) [35,36]. The aim of this work was to characterize the presence of methicillin resistance in *Staphylococcus* spp. by phenotypic and genotypic methods isolated from hospitalized patients.

In addition, an epidemiological-molecular study was performed on some MRSA isolates from various departments, applying MLST, to understand the origin and spread of circulating clones.

Materials and Methods

Bacterial Isolates

Eighty-one *Staphylococcus* spp. strains were isolated and identified. methicillin resistant from patients at the University Hospital of Sassari, Sardinia, Italy. The strains were isolated respectively from 14 blood cultures, 41 samples from the respiratory tract (bronchus aspirate, sputum, nasal, and pharyngeal swabs); 14 from swabs and wound fluids and 12 from other anatomical sites (skin swabs, urine, other). Biochemical identification and antibiogram were performed on all isolates, using the VITEK 2 automated system (Advance Expert System 4.01 software, Biomerieux, Rome, Italy) before being subjected to molecular investigation.

DNA Extraction

Two methods were used for DNA extraction: simple boiling or boiling prep and the use of the DNeasy Blood & Tissue Kit - (QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden). Boiling prep. Some colonies (4 or 5 colonies) were collected and resuspended in 150µl of sterile double-distilled water and boiled at 100°C for 10 min, to lysate the bacterial wall and obtain the escape of the DNA. Next it was centrifuged at 10000 rpm for 3 min, allowing the separation between the pellet (the bacterial lysate) and the supernatant containing the DNA. One µl of supernatant was used in the PCR reactions. The DNA thus extracted are stored at - 20 °C. The instructions of the DNA producers were followed extraction DNeasy Blood & Tissue Kit (QD). Bacterial strains were grown in liquid Luria Broth medium under stirring at 37 °C overnight. Pellet was obtained from 1.5 ml of bacterial culture by centrifugation at 7500 rpm for 10 min. The bacterial pellet was resuspended in 180µl of enzymatic lysis buffer (20 mM Tris HCl at pH 8.0, 2 mM sodium EDTA, 1.2% Triton X-100, lysozyme, 20mg/ml) and incubated for 30 min at 37 °C. Then Buffer AL is added with 25µl of Proteinase K (100mg/ml) and incubated at 56 °C for 30 min for further lysis. The lysate thus obtained was added with 200µl of ethanol is transferred to the columns provided by the kit and centrifuged at 8000 rpm for 1 min. This is followed by 2 washes with 500µl of washing Buffer (AW2).

The DNA was then eluted from the column by adding 100µl of double distilled water and centrifuging at 8000 rpm for 1 min. The DNA thus extracted is stored at -20 °C until use.

Detention of *S. aureus* using PCR Amplification

Validation of *S. aureus* species identification was performed by PCR using the species-specific primers [37]. Primers were as follows: Fw, SAU1 5'AGGGTTTGAAGGCGAATGGG 3'; and RV, SAU2 (reverse) 5'CAATTTGTCGGTCGAGTTTGCTG3'. The reaction was carried out in a final volume of 25µl which included 22µl of Platinum® PCR Supermix (Hot start recombinant Taq DNA polymerase, buffer 22 mM Tris-HCl at pH8.4, 55 mM KCl, 1.65 mM $MgCl_2$, 220µM dNTPs, Invitrogen), 1µl of DNA sample and 1µl of each primer (final 0.5µM concentration). The amplification program consisted of an initial denaturation step at 95 °C for 10 min, 35 cycles of denaturing at 95 °C for 30 sec, annealing at 61 °C for 30 sec and extension at 72°C for 2 min; and a final extension at 72°C for 10 min. PCR products were analysed by electrophoresis on a 1% agarose gel, previously stained with GelRed® Nucleic Acid Gel Stain, 10,000X (Biotium, Inc. Landing Parkway. Fremont, CA), and run at 5 V/cm for 40 min. The molecular marker used was a 100 bp ladder (Invitrogen, Waltham, Massachusetts, USA). The sizes of the PCR products sequenced after PCR were 296 bp amplicon.

Detection of the mecA, mecC (mecALGA251), spa e pvl genes using Multiplex PCR in *S. aureus* Sample

Was designed a Multiplex PCR for 13 samples identified as *S. aureus* and 14 invasive CoNS strains, isolated from all blood culture samples, from several departments (intensive care unit, surgery, hematology, pneumology, medical pathology, ENT, nephrology, and dialysis departments) (23,52) to detect the mecA regulatory genes, MecC, spa and pvl genes. Primers: mecA P4, 5´TCCAGATTACAACTTCACCAGG 3´; mecA P7, 5´CCACTTCATATCTTGTAACG 3´; spa-1113F, 5´ TAAAGACGATCCTTCGGTGAGC 3´; spa-1514R, 5´ CAGCAGTAGTGCCGTTTGCTT 3´, to amplify mecC, mecALGA251 MultiFP, 5´ GAAAAAAAGGCTTAGAACGCCTC 3´; mecALGA251 MultiRP, 5´ GAAGATCTTTTCCGTTTTCAGC 3´; pvl-F, 5´ GCTGGACAAAACTTCTTGGAATAT 3´; pvl-R, 5´ GATAGGACACCAATAAATTCTGGATTG 3´. A 50µl PCR reaction contained final concentration 1 U of Platinum Taq DNA Polymerase (Invitrogen); 0.25 mmol/L of each dNTP (GeneAmp, Applied Biosystems, Warrington, UK); 4 mmol/L of MgCl2; 0.4 µmol/L of each of forward and reverse primers (spa; mecA; mecALGA251; pvl) and 2 µl of DNA template. The amplification program consisted of an initial denaturation step at 94 °C for 5 min, 30 cycles of denaturing at 94 °C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 1 min: and a final extension at 72°C for 10 min.

The sizes of the expected PCR products were 162 bp for mecA, 138 bp for mecC, 85 bp for the gene encoding Panton Valentine Leukocidin (pvl) 180-600 bp for spa fragment (the absence of fragment spa indicates that the isolate is not a *S. aureus*) [37,38].

Multilocus Sequence Typing

MLST with standard primers introduced by the MLST database was performed on 7 MRSA isolates based on seven housekeeping genes (arcC, aroE, glpF, gmK, pta, tpiA and yqiL) as described by Enright et al. (2000). The following seven housekeeping genes were used in the final MLST scheme, and the fragments were amplified by using the primers shown in (Table 1). PCRs were carried out with 25 µl reaction volumes containing 1 µL of chromosomal DNA (approximately 0.5 mg), 1.25 µL of each primer, 21,5 µl di Platinum® PCR Supermix (Hot start recombinant Taq DNA polymerase, buffer 22 mmol/L Tris-HCl a pH8.4, 55 mmol/L KCl, 1.65 mmol/L $MgCl_2$, 220 µM dNTP, Invitrogen). The PCR was performed in a PTC-200 DNA engine (MJ Research, Boston, Mass.) with an initial 3 min denaturation at 94°C, followed by 30 cycles of denaturing at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72°C for 30 sec; and a final extension at 72°C for 5 min. The amplification products were purified with a MinElute 96 UF PCR purification kit (QIAGEN, Venlo, and The Netherlands) and the samples were

sent to the sequencing service, Sequencing Service LMU Munich, Germany (http://www.gi.bio.lmu.de/sequencing). Allele numbers and sequence types (STs) were assigned according to the *S. aureus* MLST website (http://saureus. mlst.net). Trace files of putative novel alleles and the allelic profiles of novel STs were sent to the database for allele or ST number assignment and admission into the database.

Table 1: Sequences of primers used in the Multiplex PCR.

Gene	Primer	Sequences (5'-3')	Gene Size
Carbamate kinase (arcC)	arcC-Up arcC-Dn	TTGATTCACCAGCGCGTATTGTC AGGTATCTGCTTCAATCAGCG	456 pb
Shikimate dehydrogenase (aroE)	aroE-Up aroE-Dn	ATCGGAAATCCTATTTCACATTC GGTGTTGTATTAATAACGATATC	456 pb
Glycerol kinase (glpF)	glpF-Up glpF-Dn	CTAGGAACTGCAATCTTAATCC TGGTAAAATCGCATGTCCAATTC	465 pb
Guanylate kinase (gmk)	gmk-Up Gmk-Dn	ATCGTTTTATCGGGACCATC TCATTAACTACAACGTAATCGTA	429 pb
Phosphate acetyltransferase (pta)	pta-Up pta-Dn	GTTAAAATCGTATTACCTGAAGG GACCCTTTTGTTGAAAAGCTTAA	474 pb
Triosephosphate isomerase (tpi)	tpi-Up Tpi-Dn	TCGTTCATTCTGAACGTCGTGAA TTTGCACCTTCTAACAATTGTAC	402 pb
Acetyl coenzyme A acetyltransferase (yqiL)	yqiL-Up yqiL-Dn	CAGCATACAGGACACCTATTGGC CGTTGAGGAATCGATACTGGAAC	516 pb

Statistical Analysis

Statistical analysis was performed using Statgraphics Centurion® XV for Windows.

Results

In this study, 81 strains of *Staphylococcus* spp. were recovered from infected blood samples (17%), respiratory tract samples (51%), wounds (17%) and samples of various kinds (15%). Of the 81 strains, the majority came from inpatients in intensive care (84%). Strains identified included the following Staphylococcus species: 84% Coagulase negative staphylococci (CoNS) of which *S. epidermidis, S. haemolyticus, S. hominis, S. warnerii*, and *S. aureus* (16 % n=13) (Figure 1).

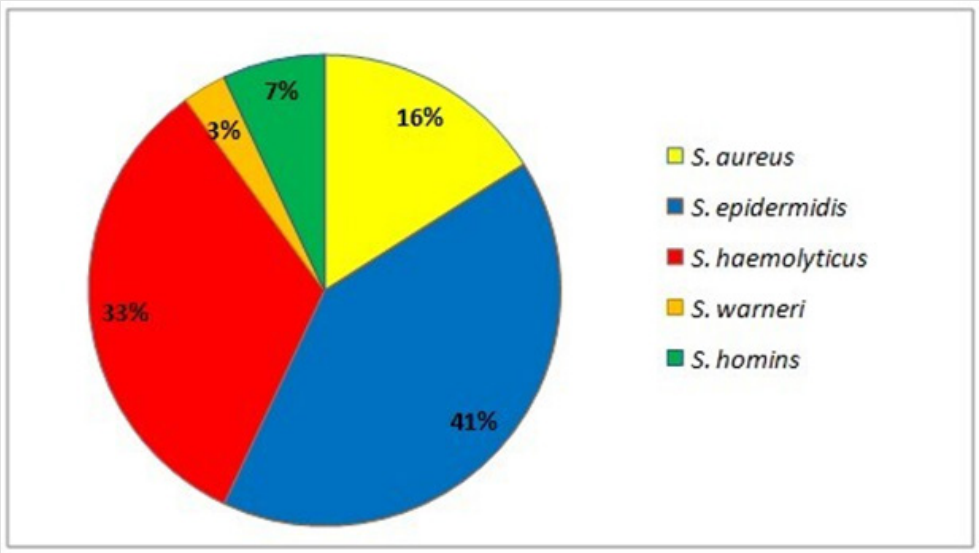

Figure 1: *Staphylococcus* spp. identified by the Vitek2 biochemical system.

Antimicrobial Susceptibility

The following resistance patterns were observed among *Staphylococcus* spp. isolates: cefoxitin (95%), oxacillin (81%), benzyl penicillin (97%), gentamicin (77%), levofloxacin (85%), erythromycin (86%), clindamycin (48%), and trimethoprim sulfamethoxazole (43%). All isolates were susceptible to vancomycin, teicoplanin, linezolid and tigecycline. On the contrary, all Staphylococcus spp. isolates were sensitive to vancomycin, teicoplanin, linezolid and tigecycline. Of 13 *Staphylococcus aureus* isolates, 11 (85%) were MRSA and MDR. The predominant resistance profile among MDR isolates included a resistance profile to 7 antibiotics (53.9%) followed by 6 antibiotics (7.7%), 5 antibiotics (15.3%), 3 antibiotic (7.7%) and 2 antibiotics (15.3%) simultaneously.

Distribution of mecA, mecC ($mecA_{LGA251}$), spa and pvl

Multiplex-PCR analysis for detection of different mecA, mecC (mecALGA251), spa and pvl revealed the mecA gene for methicillin resistance in all 14 CoNS (100%) and 11 of 13 of the MRSA (84.6%). The mecC gene was found in 9 MRSA isolates (69.2%). All MRSA samples have showed the presence of spa and the absence of pvl. On the other hand, the previous genes (spa and pvl) were not found in 14 CoNS strains.

MLST

According to the MLST method, isolates were assigned to five different sequence types (STs) (ST5 in 1 strain, ST8 in 1 strain, ST10 in 1 strain, ST22 in 2 strains, and ST228 in 2 strains). Furthermore, the 3 MRSA of care unit were belonged to ST8 (n = 1) and ST228 (n = 2), the strain isolated from the Surgical Clinic showed ST5, from hematology the ST10, while the isolates of Infectious Diseases (n = 1) and of Pneumology (n = 1) were ST22.

Discussion

S. aureus is one of the species most frequently implicated in the etiology of hospital infections in different parts of the world, especially in the intensive care, pneumology, hematology, and surgery departments [39,40]. Although with lower percentages, CoNS are also emerging as important opportunistic pathogens, and are often involved in hospital epidemics [41,42]. This study, in agreement with these studies, highlighted beyond the isolation of *S. aureus*, a high percentage of CoNS from clinical samples from acutely patients, confirming the growing involvement of these problems in nosocomial infections. The MRSA spread infections is increasing and is achieving worrying levels in several countries, including Italy. Since *Staphylococcus* spp., in particular MRSA is transmitted through infected people, or vehicles, the first strategy to contain this spread may therefore concern the implementation of prevention, as suggested by the guidelines [43,44]. In this work, all methicillin resistant strains were found to have high resistance to other classes of tested, in accordance with what was reported by the European Center for Disease Prevention and Control (CDC) [45]. The mecA gene was considered the "golden standard" for detecting methicillin resistance in MRSA, however, recently methicillin-resistant mecA negative strains have been found, in which the presence is associated with the mecC analogue (mecALGA251).

In this work 97% of methicillin-resistant staphylococci had showed the presence of the mecA gene. Instead, in two isolates, despite being resistant to methicillin from the analysis with Vitek2, they did not possess the mecA and cC genes, highlighting, as reported by other authors, the limits of the phenotypic systems [46,47]. The data confirmed that HA-MRSA showed the virulence gene of Protein A (spa) but not the Leukocidin Panton - Valentine (pvl) gene, usually associated with CA-MRSA a community circulation [48]. Through the MLST profile have been identified 5 different clones of *S. aureus*, 4 of which ST5, ST8, ST22 and ST228 already circulating in Italy and worldwide, while the ST10 was not yet reported in Italy, was present only at community and veterinary level, confirming the trend of diffusion and exchange between CA-MRSA and HA-MRSA [49]. The ST5 profile strain from surgical clinic, linked to the type of sequence of a HA-MRSA widespread throughout the world and responsible for nosocomial, tract, mucosal and wound complications. Strains of ST8 and ST228 were identified in the intensive care unit isolates, detecting the circulation of at least two different clones in this unit. The presence of strains with characteristics such as to be included in ST8 and ST228, found to be circulating in both hospital and community settings, has been reported throughout the world [3,31,43].

Furthermore, MRSA with ST22 type sequence had been isolated from different types of samples from infectious disease and pneumology department, clone was found mainly in hospital and outpatient clinics, but also in communities and in animals in close contact with humans (dogs and cats) [3,46]. Finally, in this work, a type of ST10 sequence never reported in Italy was found coming from a nasal swab of the hematology department.

Conclusion

In conclusion, this study demonstrated the importance of constant supervision of the clones circulating in the several hospital departments, colonization, and the probable, but already possible, diffusion and exchange of strains found in the hospital and then in the community. This study was conducted on clinical samples that were chosen to represent the reality nosocomial situation. Although conducted on a restricted number of samples, it provides a database for the design of targeted screening and preventive molecular diagnostics.

Conflict of Interest

Declare that I have no economic interest or conflict of interest.

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