Staphylococcus aureus nosocomial infections: the role of a rapid and low-cost characterization for the establishment of a surveillance system

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SUMMARY

Continuous surveillance on resistance patterns and characterization of *Staphylococcus aureus* represent simple and low-cost techniques to understand and evaluate the effectiveness of infection control and antimicrobial prescribing measures. In this study we analyzed the antibiotic susceptibility and trends for *S. aureus* strains collected from bacteraemia cases in a five year period. Between 2004 and 2008 we noted a progressive decrease in the number of *S. aureus* isolates compared to all pathogens from clinical specimens and *S. aureus* bloodstream infections (BSI) reflected a similar trend. In particular we analyzed 185 isolates from blood cultures: 89 isolates were MSSA and 96 isolates were MRSA. Molecular SCC*mec* typing of these strains showed an absolute prevalence of types I and II, whereas five *spa* types from 96 isolates were obtained. Resistance pattern analysis allowed us to place MRSA strains into 12 antibiotypes and the major antibiotype was resistant to penicillin, gentamicin, erythromycin, clindamycin and ciprofloxacin. The predominant antibiotype among the MSSA isolates was resistant only to penicillin. In addition, 19.1% of MSSA are susceptible to all antibiotics tested. We also found a close association between antibiotyping 1 and genotyping t002/SCC*mec*I of MRSA strains, suggesting a nosocomial scenario dominated by a few particular clones.

KEY WORDS: Staphylococcus aureus, MRSA, antibiotic susceptibility, Antibiotype, SCC*mec* and *spa* typing, *pvl*

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INTRODUCTION

Most of the world literature shows that *Staphylococcus aureus* is one of the main pathogens responsible for a number of infections in hospital settings, with considerable morbidity and mortality (Deurenberg *et al.,* 2008; Cosgrove *et al.,* 2003; Engemann *et al.,* 2003; Ho *et al.,* 2009). By studying the evolution steps and the virulence features of this microorganism during the last 50 years, two events can be seen: the development, in 1961, of resistance to methicillin (due to the acquisition of the *mecA* gene (Kuhl *et al.,* 1978; Hiramatsu *et al.,* 1996), which caused widespread outbreaks of healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) (Brumfitt *et al.,* 1989; Voss *et al.,* 1995), and the emergence, at the end of 1990s, of virulent community-associated MRSA (CA-MRSA) (Shapiro *et al.,* 2009; Hidron *et al.,* 2009; Kanerva *et al.,* 2009) clones producing Panton-Valentine leukocidin (PVL) (Vandenesch *et al*., 2003; Yu *et al.,* 2008; Daskalaki *et al.,* 2009). First isolated in outpatient clinics and emergency departments and causing skin and soft-tissue infections, CA-MRSA recently became widely disseminated in healthcare facilities as well (Boyce, 2008; Popovich *et al.,* 2008) and was isolated from different clinical materials including blood (Naber *et al.,* 2009; Naber, 2009; Pan *et al.,* 2009; Park *et al.,* 2009; Thompson *et al.,* 2008).

The *mec*A gene is carried by "staphylococcal cas-

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sette chromosome *mec*" (SCC*mec*) and encodes for the production of a low-affinity penicillin-binding protein (PBP-2a). There is no *mec*A homolog in methicillin-susceptible strains of *S. aureus* (MSSA strains), nevertheless recently similar genes have been reported in *S. sciurii* or in other coagulase negative staphylococci (Pantosti *et al.,* 2007). The molecular mechanism of this transfer is still unknown.

Transposition is the most plausible method and the genes required for the excision and integration of SCC*mec* into the staphylococcal chromosome have been identified and, currently, eight differently organized SCC*mec* elements have been characterized (McClure *et al.,* 2010). The genes for PVL are found in high prevalence in CA-MRSA strains, but have been recently described in MSSA strains as well (Berglund *et al.,* 2008; Tinelli *et al*., 2009).

The MRSA infection control in healthcare communities requires a specific strategy based on combined interventions (Siegel *et al*., 2007; Struelens, 2009). The main target is the prevention of bacteraemia, that is associated with higher mortality and hospital charges (Blot *et al.,* 2002; Cosgrove *et al.,* 2003). The clinical microbiology laboratory contributes by providing more information about the molecular characterization of *S. aureus* strains and their antimicrobial resistance pattern; antibiotyping and molecular typing are key functions for epidemiological investigation of hospital-onset *S. aureus* infection (van Berkum *et al.,* 2007; Cornaglia *et al.,* 2004*;* Marcel *et al.,* 2008).

Diagnosis and hospital management of *S. aureus* infection is a major problem in our Institution as well. The Policlinico San Donato is a research hospital and treatment centre (IRCCS or "Istituto di Ricovero e Cura a Carattere Scientifico") with a department of Cardiac Surgery which provides the highest number of cardiosurgical interventions in Italy (about 2.000 per annum); in the post-surgery Intensive Care Unit (45 beds) all patients are exposed to multiple risk factors for nosocomial infection: mechanical ventilation, indwelling catheters and a great variety of other medical devices.

The aims of the current study were: to evaluate the rate of *S. aureus* from several specimens, compared to all the pathogens isolated in the last five-year period; to describe the antimicrobial susceptibility of methicillin-susceptible and methicillin-resistant clinical isolates of *S. aureus* from blood cultures; to investigate the possible appearance of CA-MRSA as the cause of bacteraemia; to check the possibility of carrying out a periodic surveillance of MRSA, based on SCC*mec* and *spa* typing together with screening for *pvl* and phenotyping based on resistance patterns, as previously described (Ganga *et al.,* 2009; Ender *et al.,* 2009).

MATERIALS AND METHODS

Bacterial isolates

A group of 2247 consecutive, non-duplicate *S. aureus* strains were included in this study during a period of 5 years (2004-2008), isolated from patients in the Hospital of San Donato Milanese (a suburban area near Milan, in northern Italy). All strains were identified by the Clinical Microbiology Laboratory and recovered from several sources including surgical site (wound swabs, pus), respiratory material (sputum, bronchoalveolar lavage, bronchoaspirate), blood cultures and other materials (various corporeal fluids, tissues, prosthesis materials). The molecular characterization was only performed for MRSA strains from blood culture by the Clinical Research Laboratory.

Traditional microbiological analysis

Isolation and identification

All strains were cultured and identified using internal protocols, approved for and routinely applied in general bacteriology procedures in the clinical microbiology laboratory. Blood culture was conducted by Bact Alert and selective media (bioMérieux, Marcy l'Etoile, France); identification of *S. aureus* was performed with ID 32 Staph panel in the automated ATB Expression System (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

Antibiotic susceptibility tests were performed with ATB Staph panel in the automated breakpoint ATB Expression System; in vitro susceptibility was confirmed on Muller Hinton agar plates using the disc diffusion method with commercial discs of Oxacillin, Cefoxitin (to detect susceptibility to methicillin), Erythromicyn and

Clindamycin (to detect inducible resistance to Clindamycin) (bioMérieux, Marcy l'Etoile, France) and Oxacillin MIC by E-test (AB Biodisk, Solna, Sweden). The tests were performed following the Clinical and Laboratory Standard Institute (CLSI) standards (Clinical and Laboratory Standards Institute, 2008). Antimicrobial susceptibility tests and disk diffusion method has been performed on both MRSA and MSSA. All isolates of blood culture were stored in agar collection for further investigation by the Clinical Microbiology Laboratory.

Molecular analysis

DNA extraction for amplification

For rapid DNA extraction, approximately ten colonies were suspended in 50 µl of sterile distilled water and heated at 99°C for 10 minutes. The samples were then centrifuged for 2 minutes at 2000 X *g*. After centrifugation DNA concentration was assessed spectrophotometrically and samples were stored at -20°C.

Molecular detection of the nuc and mecA genes by Real-Time PCR

Isolates of blood cultures resistant to oxacillin according to the automated breakpoint method and the disk diffusion technique were confirmed as *S. aureus* methicillin-resistant by Real-Time PCR detection of the *nuc* and *mec*A genes respectively. Detection of these genes was performed with the commercial kit "Real-Time PCR BioDect *S. aureus* MRSA assay" (Biodiversity, Brescia, Italy) in a BioRad IQ5 (BioRad Laboratories, USA) apparatus according to the manufacturer's recommendations (Brakstad *et al., 1992;* Chambers, 1997). For each run, an MSSA strain (ATCC 29213) and a MRSA strain (ATCC 43300) were used respectively as negative and positive controls.

Molecular typing

SCC*mec* typing was performed by PCR as described by Zhang *et al.* (Zhang *et al.,* 2005) of all MRSA isolates. The MRSA control strains included type I (NCTC10442), type II (N315), type III (85/2082), type IVa (CLS-2207), type IVb (CLS-5827), type IVc (CLS-1040), type IVd (JCSC4469) and type V (WIS [WBG8318]-JCSC3624).

spa gene was amplified by PCR as described by Shopsin *et al.* (Shopsin *et al.,* 1999). Sequence types were determined with the database accessible via http://ridom.de/spaserver.

Detection of pvl

Pvl gene was detected by PCR as described by Jarraud *et al.* (Jarraud *et al.,* 2002).

Statistical analysis

We used the ANOVA test for statistical analysis. *P* value of <0.05 was considered to indicate statistical significance.

RESULTS

Traditional microbiological analysis identified in our 2247 *S. aureus* series, 1063 (47.3%) strains resistant to methicillin (MRSA) and 1184 (52.7%) strains sensitive to methicillin (MSSA). Real-Time PCR analysis confirmed the traditional microbiological results in all bloodstream infections considered. The different clinical sources are listed in Table 1; for each material the number of pathogens, *S. aureus* and MRSA isolates per year with the respective annual percentages (MRSA compared to all pathogens and to *S. aureus*) are listed. The cumulative isolation rates of *S. aureus* in the five years in surgical sites, respiratory materials, other materials and blood cultures was 32.4%, 17.7%, 9.7% and 8.4% respectively. Although a progressive reduction in the incidence of *S. aureus* compared to all pathogens was found (from 26.2% to 19.1%), our data do not show statistically significant differences. The percentage of MRSA (compared to *S. aureus*) fluctuated during the five-year period, ranging from 49.6% to 64.6% ($p>0.05$).

Otherwise, the incidence of *S. aureus* in the bloodstream infections decreased by almost 50% between 2004 and 2008. We screened 185 *S. aureus* strains from blood cultures: 96 (52%) were MRSA and 89 (48%) MSSA; their susceptibility profiles are described in Table 2. All the strains from blood cultures were susceptible to vancomycin, nitrofurantoin and minocycline; penicillin was, in absolute, the least efficient antibacterial agent, with about 90% strains resistant to it. More then 80% of MRSA were resistant to gentamicin, erythromycin, clindamycin and ciprofloxacin, while a total of 17 MSSA (19% of MSSA and 9% of all *S. aureus*) were susceptible

TABLE 1 - *Total number of all Pathogens, S. aureus and MRSA strains for each material per year.*

	Surgical site	*Respirat. material*	*Other material*	*Blood cultures*	*Total*
2004					
Tot. Pat	1121	548	320	321	2310
Tot. S. aureus	408	132	26	40	606
Tot. MRSA	161	109	14	21	305
% MRSA/Pat	14,4	19,9	4,4	6,5	13,2
% MRSA/S.aureus	39,5	82,6	53,8	52,5	57,1
2005					
Tot. Pat	1038	364	300	390	2092
Tot. S. aureus	318	71	30	31	450
Tot. MRSA	111	62	14	13	200
% MRSA/Pat	107	17,0	4,7	3,3	9,6
% MRSA/S.aureus	34,9	87,3	46,7	41,9	52,7
2006					
Tot. Pat	928	189	336	428	1881
Tot. S. aureus	288	16	23	43	370
Tot. MRSA	136	14	14	27	191
% MRSA/Pat	14,7	7,4	4,2	6,3	10,2
% MRSA/S.aureus	47,2	87,5	60,9	62,8	64,6
2007					
Tot. Pat	1098	226	227	534	2085
Tot. S. aureus	356	33	32	38	459
Tot. MRSA	143	23	14	17	197
% MRSA/Pat	13,0	10,2	6,2	3,2	9,4
% MRSA/S.aureus	40,2	69,7	43,8	44,7	49,6
2008					
Tot. Pat	836	242	283	536	1897
Tot. S. aureus	255	42	32	33	362
Tot. MRSA	102	33	17	18	170
% MRSA/Pat	12,2	13,6	6,0	3,4	9,0
% MRSA/S.aureus	40,0	78,6	53,1	54,5	56,6

TABLE 2 - *Antimicrobial susceptibility of S. aureus (MSSA and MRSA) from blood cultures, 2004 to 2008.*

	MSSA n= 89			*MRSA n= 96*		
	S	*R*	*Resistance rate (%)*	*S*	*R*	*Resistance rate (%)*
Penicillin	20	69	77,5	0	96	100
Gentamicin	80	9	10,1	19	77	80,2
Tetracycline	84	5	5,6	91	5	5,2
Minocycline	89	0	-	96	0	-
Erythromycin	78	11	12,4	6	90	93,8
Clindamycin	84	5	5,6	18	78	81,3
Ciprofloxacin	89	0	-	1	95	99,0
Nitrofurantoin	89	0	-	96	0	-
Fusidic acid	89	0	-	94	2	2,1
Trimethoprim sulfamethoxazole	89	0	-	82	14	14,6
Rifampin	86	3	3,4	86	10	10,4
Vancomycin	89	0	-	96	0	-
Oxacillin	89	0	-	0	96	100

to all the antimicrobials tested. Antibiogram-based phenotyping was primarily performed for an immediate clinical purpose to guide chemotherapy.

Retrospectively, the various antimicrobial patterns (both of MSSA and MRSA) were grouped in the present study and (only for MRSA) combined with SCC*mec* and *spa* typing.

The predominant antibiotype among MSSA strains was characterized by resistance to penicillin only, which was observed in 53 isolates (59.6%) (Table 3A). The remaining MSSA strains were distributed in eleven antibiotypes, ten of which had various resistances, from a minimum of one up to a maximum of four antibiotics. MRSA strains were divided into twelve antibiotypes, illustrated in Table 3B.

The predominant antibiotype among MRSA (58.3%) was resistant to penicillin, gentamicin, erythromycin, clindamycin and ciprofloxacin.

TABLE 3 - *Antibiotyping of S. aureus (MSSA and MRSA) from blood cultures, 2004 to 2008.*

A

MSSA n= 89													*n*	*(%)***
*Antibiotype**	*Resistance phenotypes*													
	PEN	*GEN*	*TET*	*MIN*	*ERY*	*CLI*	*CIP*	*NIT*	*FA*	*SXT*	*RIF*	*VAN*		
1	+	-	-	-	-	-	-	-	-	-	-	-	53	(59,6)
2	+	-	-	-	+	+	-	-	-	-	-	-	4	(4,5)
3	+	+	-	-	-	-	-	-	-	-	-	-	3	(3,4)
4	+	+	-	-	-	-	-	-	-	-	+	-	3	(3,4)
5	-	-	-	-	+	-	-	-	-	-	-	-	2	(2,3)
6	+	-	+	-	-	-	-	-	-	-	-	-	2	(2,3)
7	+	+	-	-	+	-	-	-	-	-	-	-	1	(1,1)
8	-	-	+	-	+	-	-	-	-	-	-	-	1	(1,1)
9	+	+	-	-	+	+	-	-	-	-	-	-	1	(1,1)
10	+	-	+	-	+	-	-	-	-	-	-	-	1	(1,1)
11	+	+	+	-	+	-	-	-	-	-	-	-	1	(1,1)
12	SUSCEPTIBLE TO ALL ANTIBIOTICS												17	(19,1)

B

MRSA n= 96													*n*	*(%)***
*Antibiotype**	*Resistance phenotypes*													
	PEN	*GEN*	*TET*	*MIN*	*ERY*	*CLI*	*CIP*	*NIT*	*FA*	*SXT*	*RIF*	*VAN*		
1	+	+	-	-	+	+	+	-	-	-	-	-	56	(58.3)
2	+	-	-	-	+	-	+	-	-	-	-	-	12	(12.5)
3	+	+	-	-	+	+	+	-	-	+	-	-	7	(7.3)
4	+	+	-	-	+	+	+	-	-	-	+	-	5	(5.2)
5	+	-	-	-	-	-	+	-	-	-	-	-	4	(4.2)
6	+	+	+	-	+	+	+	-	-	+	+	-	3	(3.1)
7	+	-	-	-	+	+	+	-	-	-	-	-	3	(3.1)
8	+	+	-	-	-	-	+	-	-	+	-	-	2	(2.1)
9	+	+	+	-	+	+	+	-	-	-	+	-	1	(1.0)
10	+	+	-	-	+	+	+	-	+	+	+	-	1	(1.0)
11	+	+	+	-	+	+	-	-	-	-	-	-	1	(1.0)
12	+	+	-	-	+	+	+	-	+	+	-	-	1	(1.0)

PEN, penicillin; GEN, gentamicin; TET tetracycline, MIN, minocycline; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; NIT, nitrofurantoin; FA, fusidic acid; SXT, trimethoprim sulfamethoxazole; RIF, rifampin; VAN, vancomycin. *Number of antibiotypes in MSSA and MRSA isolates from blood cultures. **Percentages in parentheses are based on the total number of isolates in each group (MSSA=89; MRSA=96).

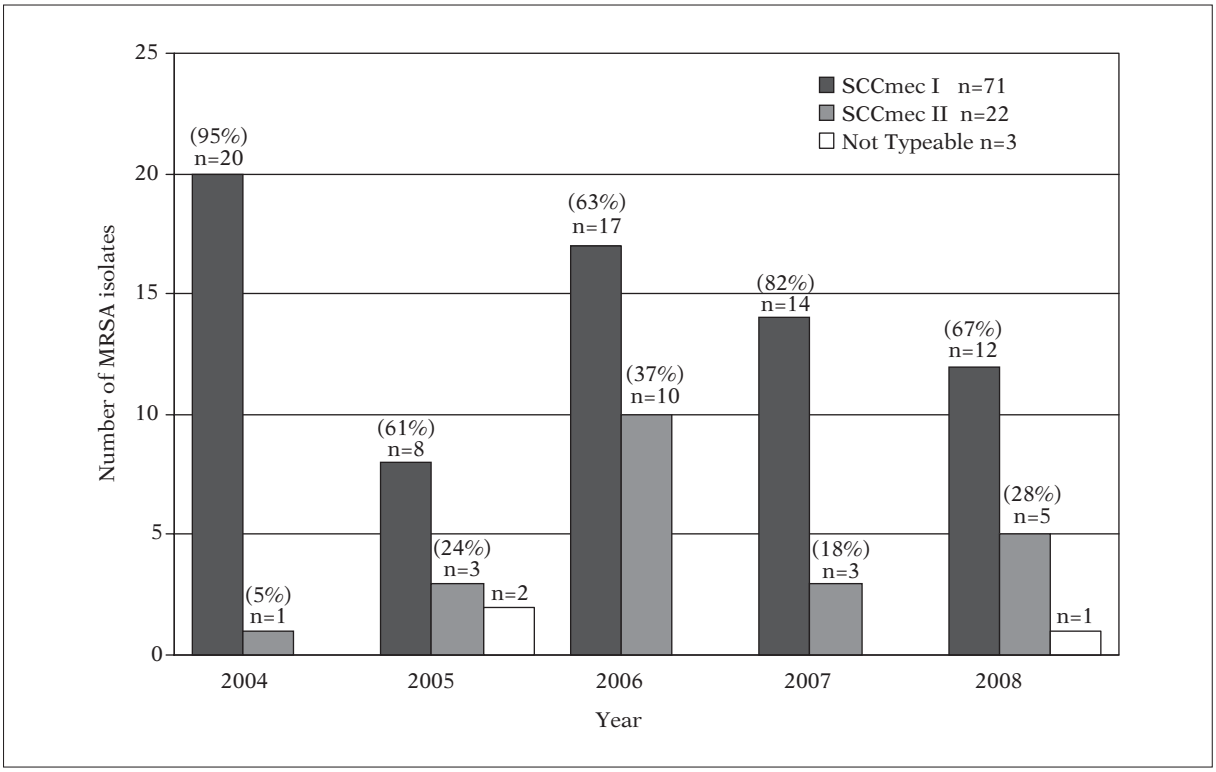

FIGURE 1 - *SCCmec types distribution of MRSA from blood cultures per year.*

The antibiotypes of MRSA are characterized by a higher grade of antimicrobial resistance compared to MSSA, with an average of about five and a maximum of eight resistances over methicillin-resistance. Only four strains (4.2%) were resistant to only two classes of antibiotics.

All 96 MRSA strains were grouped according to their SCC*mec* elements profile. Overall, two SCC*mec* types, namely I and II, were found. The change in distribution of SCC*mec* types between 2004 and 2008 is summarized in Figure 1. The most common SCC*mec* type was SCC*mec* type I, which was found in 71 isolates (71/96, 74%). SCC*mec* type II was the second predominant type, which accounted for 22 isolates (22/96, 23%) and only three of these (3%) were nontypeable with Zhang method. *spa* typing of blood culture isolates yielded 5 *spa* types (Table 4). The most predominant *spa* type was t002, constituting 58.3% (56/96) of all isolates. The proportions of t008, t041, t057 and t1569 were 20.8% (20/96), 13.5% (13/96), 5.2% (5/96) and 2% (2/96), respectively. Combination of these two typing molecular methods has provided eight different genotypes. The PVL genes were not present in any of the strains considered (data not shown). A strong association between antibiotyping and genotyping of MRSA strains from blood cultures was noted: 80% (n=45) of strains in the predominant antibiotype 1 was t002/SCC*mec* I (Table 5). Furthermore 50% of MRSA antibiotype 2 were in the t041/SCC*mec* I group and 5 strains in the t002/SCC*mec* II group, finally all the antibiotype 3 strains were t041/SCC*mec* I.

TABLE 4 - *Correlation between spa and SCCmec typing of MRSA from blood culture, 2004 to 2008*

Spa type	*SCCmec I*	*SCCmec II*	*SCCmec NT*
t002	48	5	3
t008	3	17	-
t041	13	-	-
t057	5	-	-
t1569	2	-	-

TABLE 5 - *Correlation between genotyping and antibiotyping of MRSA from blood culture, 2004 to 2008.*

Genotype (spa type/ SCCmec type)	*t002/I*	*t008/I*	*t041/I*	*t057/I*	*t1569/I*	*t002/II*	*t008/II*	*t002/NT*
Antibiotype								
1	45	2					9	
2			6			5		1
3			7					
4	3						2	
5				3			1	
6					2		1	
7							2	1
8						1		1
9				1				
10						1		
11		1						
12				1				

DISCUSSION

S. aureus represents one of the most serious gram-positive bacterial infections in nosocomial and community settings.

The severity of *S. aureus* infections is linked to the different potential infected tissues (ranging from skin and soft tissues to lower respiratory tracts and bloodstream) and is further aggravated by the bacterial potential to develop multiple antimicrobial resistances.

Hospitalized patients show a high frequency of *S. aureus* infections due to their weak immune system and frequent injections and catheterizations. Moreover, in these kind of patients, *S. aureus* can lead to life-threatening infections such as endocarditis and osteomyelitis.

Through these major characteristics, and for the recent emergence of *S. aureus* strains resistant to glycopeptide antibiotics, the survey of *S. aureus* infections, performed even in small hospital settings, could be a useful strategy to evaluate regional epidemiology, to clarify the origin of the infection distinguishing Hospital Acquired (HA) and Community Acquired (CA) strains, and finally to assess the potential efficacy of prevention plans.

In this study we summarized the results arose from a five consecutive years *S. aureus* surveillance system composed of both a phenotype and a genotype characterization adopted in the hospital of San Donato Milanese (Milan, Italy).

S. aureus strains were recovered from different materials and analyzed with traditional microbiological assays to determine their antimicrobial susceptibility. Genetic analysis was finally performed on the MRSA strain isolated from blood cultures.

Preliminary overall strains identification (n=2247) revealed an equal presence of methicillin-sensitive strains (MSSA n=1184; 52.7%) and methicillin-resistant strains (MRSA n=1063; 47.3%). A similar strain distribution was observed even in the overall *S. aureus* bloodstream infections: 185 blood cultures analysis disclosed 96 (52%) and 89 (48%) MRSA and MSSA series respectively.

Antimicrobial susceptibility testing performed with traditional microbiological analysis over blood culture isolated strains showed a low variability in antimicrobial resistant pattern and identified a predominant and recurrent phenotype among both MSSA and MRSA strains surrounded by a small set of low frequency alternate phenotypes. In particular, the common phenotype revealed in 56 strains (58.3%) of the MRSA series was characterized by resistance to penicillin, gentamicin, erythromycin, clindamycin and ciprofloxacin, whereas the predominant MSSA resistance pattern (59.6% of the overall MSSA

blood cultures isolates) was characterized by resistance to penicillin only.
Genetic SCC*mec* analysis performed on the blood cultures MRSA strains, as described by Zhang *et al.,* 2005, was found to be consistent with the phenotype results. In fact, SCC*mec* characterization noted the presence of only two chromosomal cassettes types, the SCC*mec* type I and the SCC*mec* type II both associated to healthcare associated methicillin-resistant S. aureus (HA-MRSA), with a high prevalence (74%) of the SCC*mec* type I only. Finally, the *spa* typing, applied to clarify the genotype of MRSA strains, gave further evidence to antibiotype and SCC*mec* typing results, and identified a confined number of genotypes with a net prevalence (58.3%) of the t002 type.
The first major observation derived from the antibiotype-genotype correlation calculated in the bloodstream MRSA showed a small heterogeneity of strains causing bloodstream infections and the total absence of CA-MRSA, thus suggesting a nosocomial scenario dominated by a few particularly well adapted HA-MRSA strains, first of which the t002/SCC*mec* I strain, and sporadic different clones; similar conditions have already been described in literature (Hallin *et al.,* 2008). Further investigation of SCC*mec* "not typable" strains showed that they did not correlate, either in time (isolation date) or in space (hospital department).
A second important observation of this survey is the overall progressive decrease in the number of total *S. aureus* isolates both in all clinical sites and in blood cultures during our five year observation period. Different hypotheses can be formulated regarding the various clinical sources. For example, the remarkable reduction noticed in the isolates from respiratory materials in 2006 is probably caused by the introduction, in the microbiology laboratory, of different criteria to assess the quality of specimens (number of squamous epithelial cells per field) and the consequent rejection of inadequate materials. Concerning bloodstream infections, we noted a decreasing trend similar to that observed for respiratory materials, with a substantial decline from 2004 (12,5%) to 2008 (6,2%). In addition, SCC*mec* typing shows a change in type I recovery from blood, from 95% in 2004 through 67% in 2008. We suppose that the observed decrease of this kind of infection was caused by different factors (not technical), according to the results of some authors who recently reported a change in the burden of MRSA diseases (Burton *et al.,* 2009; Eveillard *et al.,* 2009).
The combination of these two major observation, the antibiotype-genotype correlation and the amount of MRSA infections, have encouraged us to improve specific prevention measures into the hospital setting, principally focused on interrupting cross-contamination between healthcare workers and patients.
In recent years, in our hospital, various strategies to prevent and control MRSA serious infections have been implemented, namely an intense communication and education campaign about hand hygiene and contact precaution. We hope that the observed MRSA decrease could represent an initial result of such strategy.
Finally, we think that SCC*mec* with spa typing, as adopted in this survey study, could represent an important molecular tool available to understand the genetic background of MRSA strains and how they are related, in order to investigate the major trends and events such as, for example, the emergence of CA-MRSA.
The association of this molecular characterization with antibiotyping and screening for *pvl* genes can represent a simple, achievable and cost-effective technique for a continuous surveillance of *S. aureus* infections, even in hospital contexts, where time and budget greatly influence the whole process.
We would recommend applying this combination of methods in case of epidemic events in order to make an initial characterization of closely or possibly related strains and to distinguish isolates that are certainly not part of the outbreak. In endemic situations, these methods can be used in addition to the criteria for referral of a few representative isolates to a national or international typing laboratory, as recommended by the International Union of Microbiology Societes' European Staphylococcal Typing Network (Cookson *et al.,* 2008).

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