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# Epidemiology of *Staphylococcus aureus* in Italy: First nationwide survey, 2012

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ABSTRACT

A 3-month epidemiological study to determine the prevalence and antibiotic resistance of *Staphylococcus aureus* nosocomial infections was performed in 52 centres throughout Italy in 2012. A total of 21,873 pathogens were analysed. The prevalence of *S. aureus* among all nosocomial pathogens isolated in that period was 11.6% ($n = 2541$), whilst the prevalence of methicillin-resistant *S. aureus* (MRSA) among the *S. aureus* was 35.8% ($n = 910$). All tested antimicrobials demonstrated ≥92.2% susceptibility against methicillin-susceptible *S. aureus*, with the exception of clindamycin (89.7%) and erythromycin (84.2%). Among MRSA, percentages of resistance ranged from 12.6% to >39% for tetracycline, rifampicin, clindamycin and gentamicin; higher percentages were found for erythromycin (65.4%) and fluoroquinolones (72.3–85.8%). Overall, the glycopeptide minimum inhibitory concentration (MIC) distribution showed that 58.3% of strains possessed MICs of 1–2 mg/L and few strains were linezolid- or daptomycin-resistant. Molecular characterisation was performed on 102 MRSA selected from Northern, Central and Southern regions. Five major clones were found: Italian/ST228-I (t001-t023-t041-t1686-t3217), 33.3%; USA500/ST8-IV (t008), 17.6%; E-MRSA15/ST22-IVh (t020-t025-t032-t223), 16.7%; USA100/ST5-II (t002-t653-t1349-t2164-t3217-t388), 14.7%; and Brazilian/ST239/241-III (t030-t037), 3.9%. Five PVL-positive CA-MRSA isolates, belonging to USA300 and minor clones, were also identified. In conclusion, this first nationwide surveillance study showed that in Italy, *S. aureus* infections accounted for 11.6% of all nosocomial infections; MRSA accounted for approximately one-third of the *S. aureus* isolates and these were multidrug-resistant organisms. Five major MRSA epidemic clones were observed and were inter-regionally distributed, with ST228-SCC*mec*I becoming predominant.


## 1. Introduction

Staphylococcal infections represent a serious challenge for clinicians, particularly for serious infections such as bacteraemia, severe pneumonia and skin infections. Indeed, staphylococci represent the second most frequent cause of hospital-acquired infections (HAIs) in Europe (12.3%) [1].

Among *Staphylococcus aureus* isolates, methicillin-resistant *S. aureus* (MRSA) represents the major cause of antibiotic-resistant HAIs, accounting for 16.7% of isolates in Europe [2,3]. The worldwide diffusion of MRSA increases morbidity and mortality rates in HAIs, however they do not replace methicillin-susceptible *S. aureus* (MSSA) strains as causative agents of infections but add a further burden to *S. aureus* diseases [4,5]. European surveillance data, supported by the European Centre for Disease Prevention and Control (ECDC), show remarkable annual variations in the incidence of MRSA bloodstream infections (BSIs) among European Union member states, ranging from 1% in Norway, Sweden and Denmark to >50% in Romania and Portugal. In Italy, the incidence of MRSA BSIs in 2012 was >35% [6]. In a previous study conducted in four Italian hospitals between 2007 and 2009, we also

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documented a frequency of MRSA in BSIs and infective endocarditis of 21% and 28%, respectively [7]. In various European countries, above all England, the incidence of hospital-associated MRSA (HA-MRSA) infections has shown a significant decrease during the last decade, emphasising the role of control and prevention measures as the correct strategy for the treatment of nosocomial infections [4,6,8,9].

*S. aureus* molecular epidemiological analysis revealed that only a few adapted genetic backgrounds acquired and maintained antibiotic resistance [above all, staphylococcal cassette chromosome *mec* (SCC*mec*)] and virulence traits [10]. Despite worldwide diffusion of MRSA, only a few major clones are able to survive and become established in hospital settings, making their eradication difficult [5,11,12]. We previously documented the alternation of multiresistant MRSA clones in 1990–2007, with the establishment of ST228-SCC*mec*I over the period 2000–2007 [13], confirming that MRSA epidemiology undergoes changing clonal waves over time and in diverse geographic areas, with a shift into new reservoirs, leading to the spread of MRSA from and to healthcare institutions (HA-MRSA), the community [community-associated MRSA (CA-MRSA) since 2000] and livestock [livestock-associated MRSA (LA-MRSA)] [14,15].

The aim of this study was to assess the recent epidemiological situation of *S. aureus* isolates in Italy in 2012, providing a 3-month picture of the prevalence and antimicrobial features of clinical isolates and supplying an accurate analysis and distribution of MRSA clones in Italy.

## 2. Materials and methods

### 2.1. Study design and strain collection

The AMCLI (Associazione Microbiologi Clinici Italiani; the Italian Society of Clinical Microbiologists) proposed a project named CoSA (Comitato di Studio per gli Antimicrobici; Antimicrobial Study Group) on the evaluation of the prevalence and antimicrobial resistance in *S. aureus*. Institutions recruited were geographically and demographically representative of Italy. The participating institutions (PIs) were asked to compile a questionnaire and to identify and test *S. aureus* strains for antimicrobial agents using routine procedures. We report here just some parts, while all of the data were only used internally to double-check information.

Consecutive non-replicate clinical isolates were collected by the PIs between 15 June and 15 July 2012 and 15 September to 15 November 2012 from documented BSIs, lower respiratory tract infections (LRTIs) and skin and soft-tissue infections (SSTIs) and were dispatched to the reference laboratory [Laboratory of Molecular Microbiology and Antibiotic Resistance (LMMAR); http://www.labmicrobiologia.unict.it/] of the University of Catania (Catania, Italy), generally accompanied by additional information including sample number, date of isolation, origin of clinical specimen, epidemiological context (hospital-acquired when symptoms developed >48 h after admission, or community-onset).

A total of 2541 *S. aureus* strains were collected from 52 centres in 15 Italian regions. Three macroregions were defined as follows: Northern, Trentino-Alto Adige, Piedmont, Friuli-Venezia Giulia, Veneto and Lombardy; Central, Emilia-Romagna, Liguria, Tuscany, Marche and Lazio; and Southern, Campania, Apulia, Basilicata, Calabria and Sicily.

Of the original 2541 *S. aureus* strains, only 1684 were included in this study. The remaining 857 were excluded because of incomplete data (antibiotic susceptibilities and/or source of infection) or the lack of compliance with the selection criteria (other source of infection than BSI, LRTI and SSTI).

### 2.2. Identification and susceptibility testing

Initially, *S. aureus* identification and resistance testing was done locally in the laboratories of the PIs with automatic systems [VITEK®2 (bioMérieux, Marcy-l'Étoile, France), MicroScan® (Siemens HealthCare, Milan, Italy) and Phoenix™ (BD Diagnostic Systems, Sparks, MD)] following the manufacturer's instructions. The antimicrobial panels included oxacillin/cefoxitin for the identification of MRSA and MSSA as well as the principal antimicrobial agents teicoplanin, vancomycin, daptomycin, linezolid, clindamycin, erythromycin, mupirocin, gentamicin, moxifloxacin, levofloxacin, tetracycline, trimethoprim/sulfamethoxazole (SXT), rifampicin and tigecycline. Minimum inhibitory concentrations (MICs) were reported to the reference laboratory and were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretative criteria [16].

In the reference laboratory, species confirmation was carried out by colony amplification of the *Staphylococcus*-specific *tuf* gene [17]. Methicillin resistance was re-evaluated as suggested by EUCAST and Clinical and Laboratory Standards Institute (CLSI) guidelines [16,18].

MICs for seven antibiotics [teicoplanin (Aventis, West Malling, UK), vancomycin, rifampicin, levofloxacin (Sigma Chemical, St. Louis, MO), tigecycline, linezolid (Pfizer Inc., New York, NY) and daptomycin (Novartis, Basel, Switzerland)] were re-tested by the broth microdilution (BMD) method and were interpreted using EUCAST and CLSI guidelines [16,18].

The antimicrobial susceptibility profiles of the MRSA isolates were tested by the disk diffusion method against gentamicin, erythromycin, clindamycin [considering also the double-disk diffusion method (D-zone test)], tetracycline, ciprofloxacin, SXT and rifampicin (Oxoid Ltd., Thermo Scientific, Milan, Italy).

Control strains Mu50-NRS1 [vancomycin-intermediate *S. aureus* (VISA)], Mu3-NRS2 [heterogeneous VISA (hVISA)], ATCC 29213 (vancomycin-susceptible *S. aureus*), USA100-NRS382, USA300-NRS384, USA400-NRS123, USA500-NRS385, COL-NRS100, ATCC® BAA-44™ (ATCC Bacteriology Collection, LGC Standard; BEI Resources, formerly NARSA Collection, Manassas, VA), E-MRSA15-gentamicin-susceptible, HU25-Brazilian and 004/210-Italian clones were included in the study for phenotypic and genotypic assays [13,19].

### 2.3. Molecular characterisation

Molecular analysis was performed on 102 MRSA strains selected because they were representative of different antimicrobial susceptibility profiles (Northern region, 40/294; Central region, 42/223; and Southern region, 20/123). MRSA strains were analysed by SCC*mec* typing and subtyping, detection of the Panton–Valentine leucocidin (PVL)-coding genes *lukS–lukF* and the arginine catabolic mobile element (ACME) locus, multilocus sequence typing (MLST) (http://saureus.mlst.net/) and *spa* typing. Pulsed-field gel electrophoresis (PFGE) was used to define initial possible relationships among the MRSA isolates and their clonal definition. Clones were typed using the following nomenclature: ST-MRSA-SCC*mec*. All analyses were performed as previously described [13,15].

The *spa* types were clustered into a *spa* clonal complex if a single genetic event could account for the observed sequence divergence, according to the *Based Upon Repeat Pattern Algorithm* with the Ridom StaphType v.1.5 software package (Ridom Bioinformatics, Ridom GmbH, Munster, Germany). The default setting recommended by the manufacturer was used (http://www.spaserver.ridom.de/).