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A 12-year survey of methicillin-resistant *Staphylococcus aureus* infections in Greece: ST80-IV epidemic?

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of both healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) infections. Severe MRSA infections have been associated with the virulence factor Panton–Valentine leukocidin (PVL). The aim of this study was to investigate susceptibility patterns, the presence of toxin genes, including that encoding PVL, and clonality among MRSA isolates collected from patients in Greece over a 12-year period. MRSA isolates were collected from January 2001 to December 2012 from six different hospitals. Antibiotic susceptibility was determined with the disk diffusion method and the Etest. The presence of the toxic shock syndrome toxin-1 gene (*tst*), the enterotoxin gene cluster (*egc*) and the PVL gene was tested with PCR. The genotypic characteristics of the strains were analysed by SCC*mec* and *agr* typing, and clonality was determined with pulsed-field gel electrophoresis and multilocus sequence typing. An increasing rate of MRSA among *S. aureus* infections was detected up to 2008. The majority of PVL-positive MRSA isolates belonged to a single clone, sequence type (ST)80-IV, which was disseminated both in the community and in hospitals, especially during the warmest months of the year. Carriage of *tst* was associated with ST30-IV, whereas *egc* was distributed in different clones. CA-MRSA isolates were recovered mainly from skin and soft tissue infections, whereas HA-MRSA isolates were associated with surgical and wound infections. During the period 2001–2012, ST80-IV predominated in the community and infiltrated the hospital settings in Greece, successfully replacing other PVL-positive clones. The predominance of ST239-III in HA-MRSA infections was constant, whereas new clones have also emerged. Polyclonality was statistically significantly higher among CA-MRSA isolates and isolates from adult patients.

Keywords: Clones, epidemiology, Greece, methicillin-resistant *Staphylococcus aureus*, ST80-IV, toxins
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Introduction

Staphylococcus aureus is the main cause of purulent infections [1]. The classification of *S. aureus* as one of the most important human pathogens is largely based on its virulence potential and ubiquitous occurrence as a colonizer in humans, domestic animals, and livestock [2].

In the modern era, it is estimated that 25–35% of healthy human individuals carry *S. aureus* on the skin or mucous membranes [2]. This means that up to 2 billion individuals carry *S. aureus* worldwide, and conservative estimates based on Dutch and US prevalence data predict that *c.* 2–53 million people carry methicillin-resistant *S. aureus* (MRSA) [3]. MRSA is one of the most significant healthcare-associated pathogens, causing a wide range of infections. The first MRSA isolate was reported in 1960, and since then various clones have disseminated worldwide [2]. The prevalence of MRSA bacteraemia

varies from 1% in Norway to 67% in Japan [2,4]. Factors contributing to the occurrence of MRSA infections are cross-transmission via the hands of healthcare workers and high selective pressure exerted by broad-spectrum antibiotic therapy [5]. As a result of clonal spread, outbreaks of MRSA have been described in hospital settings worldwide, and MRSA has become endemic in many international healthcare settings (healthcare-associated MRSA (HA-MRSA)) [5]. Besides healthcare-associated infections, MRSA causes community-associated infections (community-associated MRSA (CA-MRSA)) among patients without predisposing risk factors, especially skin and soft tissue infections (SSTIs) and necrotizing pneumonia [2,3]. Genotyping of MRSA is important, in order to detect outbreaks, assess the dissemination of virulent strains, and understand its epidemiology. This is achieved by identifying the presence of important mobile genetic elements, which include the methicillin resistance gene *mecA* on the various SCC*mec* elements, the bacteriophage-encoded Panton–Valentine leukocidin (PVL) toxin, and many resistance determinants conveyed by plasmids or transposons [6–8]. PVL-positive MRSA isolates are strongly associated with SSTIs and severe community-onset pneumonia [9,10]. The first PVL-positive MRSA isolate in Greece was identified at the University General Hospital of Patras from an intravenous catheter culture of a premature baby in 1998, suggesting that it was acquired from a healthcare worker [11]. During the year 2000, another ten PVL-positive MRSA isolates were obtained from patients with underlying diseases, and it was obvious that there had been an increase in the incidence of CA-MRSA and HA-MRSA infections caused by PVL-positive strains [11]. A parallel increase was also observed in the overall percentage of total MRSA infections during the period of the above studies, probably caused by the spread of PVL-positive strains. It has been reported that CA-MRSA has a faster growth rate than HA-MRSA, resulting in successful colonization [12]. In another Greek study performed from 2001 to 2003, a significant increase in the number of PVL-positive *S. aureus* isolates, most of which were MRSA, was found [13]. Among CA-MRSA isolates, 72% carried the PVL genes, and 23% of HA-MRSA isolates were also PVL-positive [13]. The prevalence of CA-MRSA infections varies widely from one country to another [14]. For example, many differences exist between the epidemiology of CA-MRSA in the USA and that in Europe [2]. In the USA, CA-MRSA is one of the most common causes of SSTIs in patients from emergency departments, and one clone, USA300 (sequence type (ST)8-SCC*mec* IV), predominates [2]. In Europe, the prevalence of CA-MRSA is much lower, but it is increasing, especially in countries where the incidence of HA-MRSA is low, such as Denmark and The Netherlands [15]. Many different CA-MRSA clones have been identified, of which the ST80-SCC*mec* IV European clone is the most widely disseminated [15]. Recently, however, several European countries have been confronted with a rise in the prevalence of USA300 strains [14].

The aim of the present study was to estimate the prevalence and clonal distribution of PVL-positive MRSA among healthcare-associated and community patients with staphylococcal infections in a wide geographical area of Greece during a 12-year period.

Materials and Methods

Bacterial isolates and hospitals

Four thousand six hundred and fourteen MRSA isolates from six *S. aureus* collections were studied. These collections originated from six different Greek hospitals serving three-fifths of the total Greek population: University General Hospital of Patras (2259 isolates) and Karamandaneion Children's Hospital of Patras (291 isolates), both located in south-western Greece; 'P. & A. Kyriakou' Children's Hospital (139 isolates) and General Children's Hospital Pentelis (46 isolates), both located in Athens; University Hospital of Ioannina (80 isolates), located in north-western Greece; and University Hospital of Larissa (1799 isolates), located in central Greece. All isolates were recovered from different inpatients and outpatients with *S. aureus* infection (one isolate per patient) during a 12-year period from January 2001 to December 2012. Each isolate was sent to the National Reference Laboratory for Staphylococci, with a report describing demographics and clinical data: age and sex of the patients, clinical specimen and date of sampling, underlying disease, hospital ward, and whether the isolate was epidemiologically representative of a cluster. Children's hospitals admit patients up to the age of 14 years.

Isolates obtained within 48 h of hospital admission were defined as CA-MRSA, as were isolates from patients with infection diagnosed in an outpatient department, provided that there was no history in the past year of hospitalization, admission to a nursing home, skilled nursing facility, or hospice, or association with dialysis, surgery, permanent indwelling catheters, or medical devices. Isolates obtained >48 h following admission were defined as HA-MRSA [16].

S. aureus identification

Isolates were identified on the basis of colony morphology, Gram stain, catalase production, and the coagulase test (Slidex Staph Plus; bioMérieux, Marcy l'Etoile, France), and identification was verified with molecular methods (PCR for 16S rRNA and *nuc* genes) [17].