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Changes in the rates and population structure of methicillin-resistant *Staphylococcus aureus* (MRSA) from bloodstream infections: A single-centre experience (2000–2015)

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ABSTRACT

Objectives: The aim of this study was to assess the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infections (BSIs) and the population structure of MRSA isolates recovered between 2000–2015 in a tertiary-care hospital in Athens, Greece.
Methods: Non-duplicate MRSA blood isolates recovered during the study period were examined. Antimicrobial susceptibility testing was performed by Kirby–Bauer and gradient strip methods. Carriage of PVL and *mecA* genes was examined by PCR. Genetic relatedness of the isolates was studied by SCC*mec*, *spa* and multilocus sequence typing.
Results: A total of 398 MRSA BSI cases were identified. A decreasing trend in incidence from 1.69/10 000 patient-days in 2000 to 1.39/10 000 patient-days in 2015 ($P = 0.038$) and in prevalence from 64.7% to 36.4% ($P = 0.008$), respectively, was observed, whereas the incidence of methicillin-susceptible *S. aureus* BSI increased. MRSA isolates exhibiting resistance to common antistaphylococcal agents (excluding glycopeptides and the newer antistaphylococcals) decreased from 84.8% in 2000 to 0% in 2011 and were progressively 'replaced' by more susceptible phenotypes. A strong association between antimicrobial resistance phenotype and molecular type was observed. The pandemic HA-MRSA clone ST239-III progressively declined in parallel with increasing isolation frequency of two clonal complexes (CCs): HA-MRSA CC5, with the majority of isolates belonging to ST5-II; and CA-MRSA CC80, represented mainly by ST80-IV-t044, PVL+.
Conclusion: The decline in MRSA BSI rates observed in our institution was associated with changes in population structure of the organism. This decline may be related to biological properties of the prevailing MRSA clones.

1. Introduction

The epidemiology of *Staphylococcus aureus* infections has been changing over the last years [1]. Several multicentre reports have shown that methicillin-resistant *S. aureus* (MRSA) infections are declining in certain geographic areas, including Europe [2–4]. According to the latest data (2013–2016) from the European Antimicrobial Resistance Surveillance Network (EARS-Net), more than one-third of both low and high MRSA prevalence countries reported significantly decreasing trends, and the population-weighted mean MRSA percentage has dropped from 18.1% in 2013 to 13.7% in 2016 [5]. The reasons for this MRSA-specific decline are not fully understood. Despite this positive development, MRSA remains a public-health priority in Europe, as 10 of 30 countries reported MRSA prevalence rates >25%, including Greece [6].

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In addition to changes in MRSA infection rates, the molecular epidemiology of these organisms undergoes alterations over time; new MRSA clones arise, expand and then decline, being replaced by others [7–9]. Lately, the emergence of community-acquired MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA) has also contributed to profound changes in the molecular epidemiology of MRSA infections [10–13]. Moreover, MRSA clones of community origin have entered healthcare facilities replacing the traditional hospital-associated MRSA (HA-MRSA) clones [8,11,14,15].

In this study, time trends in the rate of MRSA bloodstream infections (BSIs) as well as the temporal dynamics of MRSA clones circulating over a 16-year period (2000–2015) in a single institution in Greece were examined.

2. Materials and methods

2.1. Setting and bacterial isolates

This study was conducted in 'Laikon' General Hospital, a 550-bed tertiary-care hospital located in Athens (Greece), during a 16-year period (1 January 2000 to 31 December 2015). Isolates in the study originated from blood cultures from patients with suspected bacteraemia and incubated in a BacT/ALERT® 3D automated system (bioMérieux, Marcy-l'Étoile, France). All blood MRSA isolates had been collected and preserved at −80 °C. The first MRSA isolate per patient was re-cultured for further testing. Species identification was performed by standard methodology supplemented with the API® Staph identification system (bioMérieux) and/or MicroScan ID/AST panels (Siemens SA, Athens, Greece). Methicillin resistance was also determined using a 30 μg cefoxitin disk.

2.2. Antimicrobial susceptibility testing

Susceptibility testing to penicillin, gentamicin (Gm), trimethoprim/sulfamethoxazole (Sxt), tetracycline (Tet), rifampicin (Ra), fusidic acid (Fa), erythromycin (Ery), clindamycin (Cl), ciprofloxacin (Cip), vancomycin, teicoplanin, linezolid and quinupristin/dalfopristin was performed by the Kirby–Bauer disk diffusion method and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [16], except for fusidic acid for which European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used [17]. Minimum inhibitory concentrations (MICs) were determined using MicroScan ID/AST panels. MICs for vancomycin, teicoplanin, linezolid and daptomycin were also determined using the gradient strip method (Etest strips; bioMérieux).

2.3. Molecular typing

Genomic DNA for molecular typing was prepared using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). All isolates were examined for the presence of the *mecA* gene by PCR. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing/subtyping and detection of Panton–Valentine leukocidin (PVL)-encoding genes (*lukS*/*lukF*-PV) were performed on all strains isolated during the years 2000–2001, 2004–2005, 2009–2010, 2012–2013 and 2014–2015 according to previously published protocols [18–20]. Multilocus sequence typing (MLST) and staphylococcal protein A (*spa*) typing were performed on representative isolates from each SCC*mec* type, evenly distributed across the study period, using previously published protocols [21,22] and the guidelines on the respective websites (http://saureus.mlst.net; http://spa.ridom.de/index.shtml). PCR products were purified using a NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany). Sequencing was performed using a BigDye® Terminator kit v.3.1 (Applied Biosystems, Foster City, CA) and an ABI 3730 DNA Analyser (Applied Biosystems). Allele numbers and sequence types (STs) were assigned using the software of the MLST database (http://saureus.mlst.net/sql/multiplelocus.asp). *spa* types were assigned using the BioNumerics Demo Web Server (http://bnas.applied-maths.com/spaupload.aspx).

2.4. Statistical analysis

Trends in the annual incidence and prevalence of MRSA as well as the incidence of methicillin-susceptible *S. aureus* (MSSA) were studied by linear regression analysis using GraphPad Prism v.6.01 (GraphPad Software Inc., La Jolla, CA). The χ^2 test was used for categorical variables and the Student's *t*-test or Mann–Whitney *U*-test were used for continuous variables. Statistical significance was set at 0.05. The study was approved by the Institution Review Board of 'Laikon' General Hospital.

3. Results

3.1. MRSA infection rates

A total of 398 cases of MRSA BSI were identified during 2000–2015. As shown in Fig. 1, the annual incidence of MRSA BSIs fluctuated considerably, with a decreasing trend from 1.69/10 000 patient-days (PD) in 2000 to 1.39/10 000 PD in 2015 [annual decrease, -0.04 ± 0.02 per 10 000 PD, 95% confidence interval (CI) −0.003 to −0.08; $R^2 = 0.27$; $P = 0.038$]. Moreover, the prevalence of MRSA decreased significantly from 64.7% in 2000 to 36.4% in 2015 (annual decrease, -1.9 ± 0.6%, 95% CI −0.6 to −3.2; $R^2 = 0.4$; $P = 0.008$). In contrast, there was an increasing trend in the incidence (annual increase 0.065 ± 0.024 per 10 000 PD, 95% CI 0.014 to 0.11; $R^2 = 0.35$; $P = 0.02$) and prevalence (annual increase, 2.3 ± 0.6 per 10 000 PD, 95% CI 1.07 to 3.6; $R^2 = 0.45$; $P = 0.02$) of MSSA BSIs.

3.2. Antimicrobial susceptibility profile

The resistance rates to commonly used antibiotics are shown in Table 1. None of the isolates exhibited resistance to vancomycin, teicoplanin, linezolid, daptomycin or quinupristin/dalfopristin. A substantial decrease in resistance rates to rifampicin, Sxt, gentamicin, tetracycline and fusidic acid was observed from >90% in 2000 to 4.2%, 4.2%, 8.3%, 37.5% and 50.0%, respectively, in 2015. The five most common resistance phenotypes, comprising 77.1% of isolates, are shown in Fig. 2. The remaining 22.9% of isolates displayed 32 different phenotypes, including 1 to 12 isolates each. Of note, the multidrug-resistant phenotype CipEryClTetFaRaGmSxt decreased from 84.8% in 2000 to 0% in 2011 and thereafter, while the frequency of less extensive multiresistance phenotypes such as CipEryClTetFa, CipEryCl and TetFa increased overtime.

3.3. Molecular typing

All isolates ($n = 237$) recovered during the periods 2000–2001 ($n = 63$), 2004–2005 ($n = 58$), 2009–2010 ($n = 41$), 2012–2013 ($n = 38$) and 2014–2015 ($n = 37$) were subjected to SCC*mec* typing. SCC*mec* types II, III, IV (a–d,g,h) and IV (e,f) were detected in 75, 104, 33 and 17 isolates, respectively; 8 isolates were non-typeable. As shown in Fig. 3, isolates harbouring the SCC*mec* III element decreased from 93.7% in 2000–2001 to 14.6% in 2009–2010 and disappeared thereafter, whereas those containing the SCC*mec* II and IV elements increased from 1.6% and 4.8% in 2000–2001 to 64.9% and 27% in 2014–2015, respectively. Of note, SCC*mec* IV (e,f) prevailed over SCC*mec* IV (a–d,g,h) during the period 2000–2005, whereas the reverse was observed during 2012–2015 when the

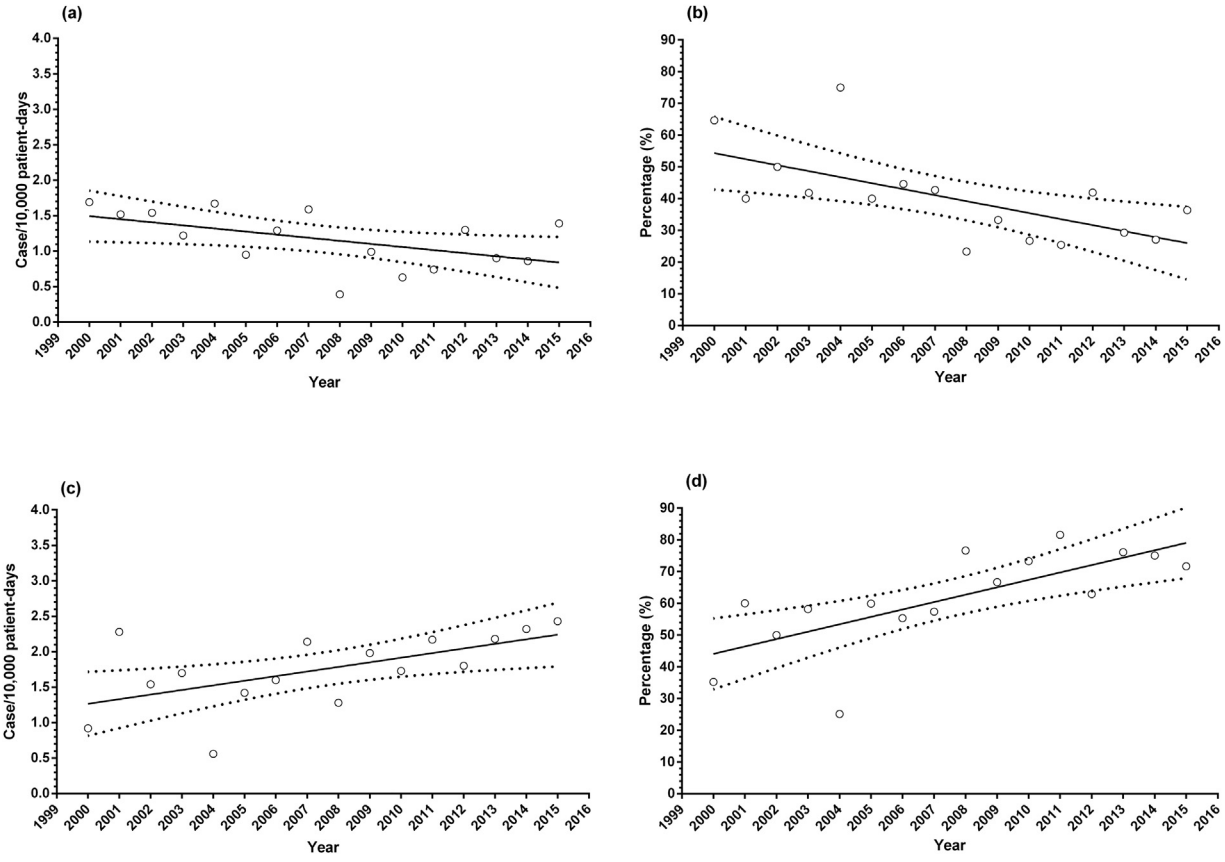

Fig. 1. (a,b) Incidence (a) and prevalence (b) of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infections (BSIs), and (c,d) incidence (c) and prevalence (d) of methicillin-susceptible *S. aureus* (MSSA) BSIs between 2000 and 2015. Open circles indicate observed values, the continuous line indicates the trend over time, and interrupted lines indicate the 95% confidence intervals.

subtypes (a–d,g,h) prevailed over subtypes (e,f). The percentage of PVL-positive isolates increased from 0% in 2000–2001 to 22% during 2009–2010, reaching 36.8% in 2012–2013 and 21.6% in 2014–2015.

A strong association between resistance phenotype and SCC*mec* type was observed, as 84 of the 87 isolates with the resistance phenotype CipEryClTetFaRaGmSxt harboured SCC*mec* III, 56 of the 65 isolates with the resistance phenotype CipEryCl or CipEryCl-TetFa harboured SCC*mec* II, and 9 of the 10 isolates with the resistance phenotype TetFa harboured SCC*mec* IV (a–d,g,h). Also, 11 of the 14 isolates resistant only to β-lactams harboured the SCC*mec* IV (e,f) element ($P < 0.001$).

A total of 83 isolates were subjected to MLST and *spa* typing, including 29 from SCC*mec* II, 14 from SCC*mec* III, 25 from SCC*mec* IV

Table 1
Rates of antimicrobial resistance among 398 methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection isolates (2000–2015).

Year of isolation	No. of isolates	Percentage resistant[a]							
		Gm	Sxt	Tet	Ra	Fa	Ery	Cl	Cip
2000	33	97.0	97.0	97.0	90.9	97.0	93.9	90.9	97.0
2001	30	83.3	90.0	86.7	86.7	83.3	76.7	76.7	93.3
2002	32	90.6	87.5	90.6	90.6	90.6	87.5	87.5	93.8
2003	28	82.1	85.7	82.1	82.1	85.7	89.3	89.3	92.9
2004	36	77.8	77.8	77.8	72.2	80.6	86.1	86.1	91.7
2005	22	63.6	63.6	68.2	63.6	68.2	81.8	77.3	86.4
2006	29	72.4	72.4	82.8	72.4	82.8	89.7	89.7	96.6
2007	38	68.4	68.4	81.6	63.2	81.6	84.2	84.2	89.5
2008	10	60.0	60.0	60.0	40.0	70.0	70.0	70.0	90.0
2009	25	32.0	12.0	48.0	24.0	44.0	60.0	60.0	64.0
2010	16	12.5	12.5	43.8	12.5	37.5	56.3	56.3	56.3
2011	16	12.5	6.3	56.3	6.3	50.0	75.0	56.3	56.3
2012	26	3.8	0.0	38.5	3.8	46.2	69.2	61.5	65.4
2013	17	0.0	0.0	41.2	0.0	47.1	88.2	88.2	82.4
2014	16	6.3	0.0	43.8	0.0	62.5	81.3	81.3	68.8
2015	24	8.3	4.2	37.5	4.2	50	87.5	87.5	83.3
Total	398	56.9	54.3	69.1	52.4	80.7	81.4	79.7	84.2

Gm, gentamicin; Sxt, trimethoprim/sulfamethoxazole; Tet, tetracycline; Ra, rifampicin; fa, fusidic acid; Ery, erythromycin; Cl, clindamycin; Cip, ciprofloxacin.
[a] All isolates were susceptible to vancomycin, teicoplanin, linezolid, daptomycin and quinupristin/dalfopristin.

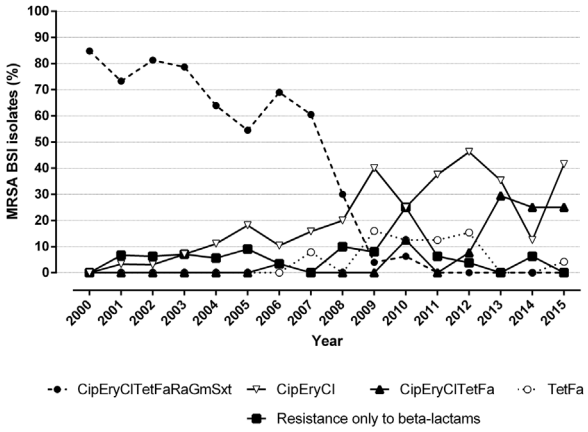

Fig. 2. Distribution of resistance phenotypes of 398 methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection (BSI) isolates (2000–2015). Cip, ciprofloxacin; Ery, erythromycin; Cl, clindamycin; Tet, tetracycline; Fa, fusidic acid; Ra, rifampicin; Gm, gentamicin; Sxt, trimethoprim/sulfamethoxazole.

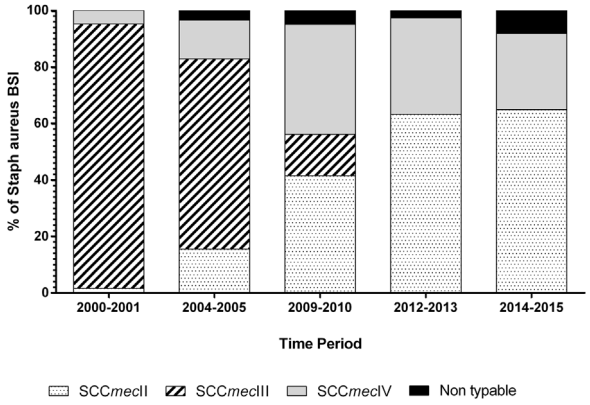

Fig. 3. Distribution of staphylococcal cassette chromosome *mec* (SCC*mec*) types of 237 methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection (BSI) isolates, by time period.

(a–d,g,h) and 15 from SCC*mec* IV (e,f) (Table 2). All SCC*mec* III isolates belonged to ST239 [clonal complex (CC) 8], *spa* t037, whereas the SCC*mec* II isolates belonged to six different STs [ST36 ($n = 5$), ST5 ($n = 13$), ST105 ($n = 3$), ST225 ($n = 4$), ST2092 ($n = 3$) and ST2599 ($n = 1$)]. Of note, among the SCC*mec* II types isolated during the period 2000–2010, the predominant STs were ST36 (CC30), *spa* t018 and ST5 (CC5), whereas among those isolated during the period 2011–2015 the predominant STs were ST5, ST225 and ST105 (CC5) exhibiting a variety of *spa* types. Finally, among 25 SCC*mec* IV (a–d,g,h) isolates, 19 belonged to ST80 [*spa* types t044 ($n = 16$), t003 ($n = 1$), t042 ($n = 1$) and t131 ($n = 1$)], all of which were PVL-positive, 2 belonged to ST217 and one each belonged to ST728, ST3032, ST22 and ST6; whereas among the 15 SCC*mec* IV (e,f) isolates, 11 belonged to ST30 [*spa* types t018 ($n = 10$) and t1642 ($n = 1$)], two to ST1 (USA400) and one each to ST22 and ST8 (USA300).

4. Discussion

A significant decrease both in the incidence and prevalence rates of MRSA BSIs as well as a concomitant increase in the incidence of MSSA BSIs were observed in our institution over a 16-year period.

The decline in MRSA infections was associated with considerable changes in the population structure of this organism. The pandemic HA-MRSA clone ST239-III (CC8), which has prevailed in many geographic areas for half a century, followed the international declining trend in our hospital and, after 2010, was replaced mainly by two CCs, namely HA-MRSA CC5, with the majority of isolates belonging to ST5-II, and CA-MRSA CC80, represented mainly by ST80-IV-t044, PVL+. A third population, displaying resistance only to β-lactams, CC30-ST30-IV-t018, PVL–, emerged in 2001, expanded up to 2010 but then disappeared. Notably, ST22, the most rapidly expanding clone in Europe [23], was almost absent in our institution and only sporadic cases of the USA300 and USA400 clones were observed.

Factors that have contributed to these profound changes in our setting are unclear. The simultaneous increase of MSSA BSIs suggests that the phenomenon is not related to infection control interventions. Similar observations have been made by other investigators who have shown that the decline of MRSA infections preceded the intense infection control programme implemented in the UK in the previous decade [24,25]. This decline was attributed to differential spread of two major UK MRSA clones (ST22 and

Table 2
Staphylococcal cassette chromosome *mec* (SCC*mec*) types, sequence types (ST) and prevailing resistance phenotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection isolates by time period.

SCC*mec* type	2000–2010		2011–2015	
	Subjected to MLST and *spa* typing ($n = 49$)	Prevailing resistance phenotypes	Subjected to MLST and *spa* typing ($n = 34$)	Prevailing resistance phenotypes
III ($n = 104$) II ($n = 75$)	ST239[a] ($n = 14$) ST36[b] ($n = 5$) ST5 ($n = 4$) ST105 ($n = 2$) ST2092 ($n = 1$)	Cip,Ery,Cl,Tet, Fa,Ra,Gm,Sxt ($n = 10$) Cip,Ery,Cl ($n = 12$)	$n = 0$ ST5 ($n = 9$) ST105 ($n = 1$) ST225 ($n = 4$) ST2092 ($n = 2$) ST2599 ($n = 1$)	$n = 0$ Cip,Ery,Cl ($n = 6$) Cip,Ery,Cl,Tet,Fa ($n = 6$) Cip,Ery,Cl,Fa ($n = 5$)
IV (a–d,g,h) ($n = 33$)	ST80[c] ($n = 8$) ST217 ($n = 2$) ST728 ($n = 1$)	Te,Fa ($n = 6$)	ST80[c] ($n = 11$) ST3032 ($n = 1$) ST6 ($n = 1$) ST22 ($n = 1$)	Tet,Fa ($n = 5$) Ery,Cl,Tet,Fa ($n = 3$)
IV (e,f) ($n = 17$)	ST30 ($n = 11$) ST22 ($n = 1$)	Resistance only to β-lactams ($n = 9$)	ST1 ($n = 2$) ST8 ($n = 1$)	Ery,Cl,Tet ($n = 1$) Ery,Cl ($n = 1$) Cip,Ery,Cl ($n = 1$)

MLST, multilocus sequence typing; *spa*, staphylococcal protein A; Cip, ciprofloxacin; Ery, erythromycin; Cl, clindamycin; Tet, tetracycline; Fa, fusidic acid; Ra, rifampicin; Gm, gentamicin; Sxt, trimethoprim/sulfamethoxazole.

[a] All isolates belonged to *spa* t037.

[b] All isolates belonged to *spa* t018.

[c] All isolates were Panton–Valentine leukocidin (PVL)-positive and 16/19 belonged to *spa* t044.

ST36); ST36 declined markedly during 2006–2010 and ST22 became the dominant MRSA clone thereafter. Similar events, i.e. emergence, expansion and decline of an MRSA clone with replacement by another clone, have occurred repeatedly in the MRSA evolutionary process [7,8]. Occurrence of the community-acquired European MRSA clone ST80-IV as a cause of BSI in this study is noteworthy. In a recent survey from Greece focused on skin and soft-tissue infections, ST80-IV was responsible for 11.2% of HA-MRSA infections [26]. Taken together, these findings suggest the establishment of this clone in our hospital setting. Several reports since 2003 suggest that the MRSA clones of community origin have begun to replace or overtake traditional HA-MRSA clones in several countries [11,14,26–28]. Using a mathematical model, D'Agata et al. predicted that CA-MRSA would become the dominant MRSA clones in hospitals, with competitive exclusion or near exclusion of the traditional HA-MRSA owing to an expanding reservoir of MRSA in the community and their continuous influx into the hospital [29].

It is worth noting that during the last period of the present study (2014–2015), 27% of MRSA BSIs were caused by CA-MRSA clones carrying the SCC*mec* IV element, the majority of which belonged to the community clone ST80. These findings, as pointed out by others, indicate that clones of community origin when inside the hospital behave more like HA-MRSA and can cause not only skin and soft-tissue infections but also serious invasive infections with similar disease profiles [30,31]. More worrisome is the potential of CA-MRSA clones to become multidrug-resistant in healthcare settings [32–34]. Indeed, in the current study one ST80-IV isolate exhibited resistance to seven different classes of non-β-lactam antibiotics, namely quinolones, macrolides, clindamycin, tetracyclines, fusidic acid, rifampicin and gentamicin.

The current findings should not be interpreted without considering several limitations. First, the analysis was retrospective and thus it was not possible to distinguish patients with HA from those with CA infections. This distinction, however, is of limited importance since both the CA-MRSA and HA-MRSA clones shuttle between community and hospitals as mentioned previously. A second limitation is that all of the patients were from a single institution and may partly reflect local trends. Nevertheless, the declining trend in MRSA BSI rates observed in our hospital is in accordance with national trends (see http://www.mednet.gr/whonet).

5. Conclusions

A decline in MRSA BSI rates was associated with changes in the population structure of this organism; the pandemic HA-MRSA clone ST239-III (CC8) was replaced mainly by HA ST5-II (CC5) and CA ST80-IV-t044, PVL+ European clones. A plausible hypothesis is that the observed changes in our setting are not anthropogenic but rather associated with the biological properties of the currently prevailing clones and reflect the evolutionary process of the organism.

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Competing interests

None declared.

Ethical approval

This study was approved by the Institution Review Board of 'Laikon' General Hospital (Athens, Greece).

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