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The molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Czech Republic

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Objectives: To gain data on the current molecular epidemiology and resistance of MRSA in the Czech Republic.

Methods: Between September 2017 and January 2018, a total of 441 single-patient MRSA isolates were collected from 11 Czech hospitals and analysed by *spa* typing, SCC*mec* typing, antibiotic susceptibility testing, detection of the PVL toxin and the *arcA* gene.

Results: Of all MRSA isolates, 81.41% ($n = 359$) belonged to the CC5-MRSA clone represented by the *spa* types t003 ($n = 136$), t586 ($n = 92$), t014 ($n = 81$), t002 ($n = 20$) and other *spa* types ($n = 30$); a majority of the CC5 isolates ($n = 348$, 96.94%) carried SCC*mec* type II. The occurrence of CC5-MRSA was more likely in older inpatients and associated with a healthcare origin ($P < 0.001$). The CC5-MRSA isolates were resistant to more antimicrobial drugs compared with the other MRSAs ($P < 0.001$). Interestingly, t586 was detected in blood samples more often than the other *spa* types and, contrary to other *spa* types belonging to CC5-MRSA, t586 was not associated with patients of advanced age. Other frequently found lineages were CC8 ($n = 17$), CC398 ($n = 11$) and CC59 ($n = 10$). The presence of the PVL was detected in 8.62% ($n = 38$) of the MRSA isolates.

Conclusions: The healthcare-associated CC5-MRSA-II lineage (t003, t586, t014) was found to be predominant in the Czech Republic. t586 is a newly emerging *spa* type in the Czech Republic, yet reported rarely in other countries. Our observations stress the need for MRSA surveillance in the Czech Republic in order to monitor changes in MRSA epidemiology.

Introduction

Staphylococcus aureus is one of the leading causes of healthcare-associated infections in Europe[1] and MRSA has become a substantial burden in terms of healthcare costs, morbidity and mortality.[2,3]

Since 2000, the Czech Republic has been actively participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), which collects data on the prevalence of MRSA isolates cultured from blood and CSF. The EARS-Net data showed an overall increase in the prevalence of MRSA among invasive isolates from 4% in 2000 to 13% in 2005 in the Czech Republic (European Antimicrobial Resistance Surveillance System, EARSS Annual Report 2005, https://www.rivm.nl/bibliotheek/rapporten/210624001.pdf). Since then, the prevalence of MRSA in the Czech Republic has remained at the same level, which is slightly lower than the EU population-weighted mean of 16.9%.[4]

The characterization of MRSA isolates is not included in the EARS-net reporting so data on the molecular epidemiology of MRSA are available only from a few time-limited studies. Between 1996 and 1997, the ST239-MRSA-III (Brazilian/Hungarian) clone was found to predominate (80%), followed by the ST247-MRSA-I (Iberian) clone (12%) in 59 isolates investigated.[5] In a follow-up study conducted in 21 Czech hospitals between 2001 and 2002, from 100 characterized MRSA isolates, ST239-MRSA-III still predominated (74%).[6]

In 2006, the European multicentre study observed a decline in ST239-MRSA-III and CC5/ST225-MRSA-II (t003) was found to be the new dominant clone in the Czech Republic, accounting for 34 (66.67%) of 51 invasive MRSA isolates followed by 7 (13.7%) isolates of t032 (CC22-MRSA-IV, EMRSA-15).[7] In a subsequent study in 2011, among the 49 submitted MRSA isolates, t003 (61.2%) was still found to be dominant, followed by t014 (14.3%), and both belonged to the same CC5/ST225-MRSA-II clone.[8]

To map the current epidemiology of MRSA in the Czech Republic, MRSA isolates from 11 hospitals were collected. They were characterized by *spa* typing, SCC*mec* typing, assessed for Panton–Valentine leucocidin (PVL) genes and subject to antimicrobial susceptibility testing.

Materials and methods

Ethics

Ethics approval and informed consent were not required for this study since it was laboratory based. MRSA isolates were obtained as part of routine diagnostic testing and the data were anonymized. The results of the study had no impact on the patients' care.

Study design

Between September 2017 and January 2018, 11 microbiological departments, of six tertiary and five secondary care hospitals, collected non-duplicated (single-patient) MRSA isolates. Both groups of MRSA isolates that were cultured either from the site of infection or from nasal, throat or skin screening swabs (asymptomatic colonization) of inpatients and outpatients were included. The MRSA screening of patients with risk factors for developing an MRSA infection (history of MRSA infection/colonization, ICU admission, surgery, hospitalization abroad or in hospital with high MRSA prevalence) was based on the national standard operating procedure.[9]

The following anonymized data were collected: age, gender, source of isolation and diagnosis associated with MRSA isolation. The origin of the MRSA isolate was defined as hospital associated (HA-MRSA) when the symptoms of an infection and the collection of an MRSA-positive sample happened more than 48 h after admission, or, if in the year before MRSA isolation, the patient was hospitalized; any infections that did not meet these criteria were classified as community associated (CA-MRSA).[7,10]

Identification of isolates

The identification of *S. aureus* isolates was confirmed using a MALDI-TOF mass spectrometer Biotyper v 3.1 (Bruker Daltonics). The presence of the *mecA* gene was determined by quantitative PCR (qPCR) in order to confirm the genetic base of methicillin resistance. Moreover, the genes *lukSF-PV*, encoding PVL, and the gene *arcA*, specific for the arginine catabolic mobile element (ACME), were also detected.[11,12]

Antimicrobial susceptibility testing

Antimicrobial susceptibility to oxacillin, cefoxitin, erythromycin, clindamycin, gentamicin, tetracycline, rifampicin, ofloxacin, vancomycin, teicoplanin, trimethoprim/sulfamethoxazole, linezolid, tigecycline and ceftaroline was determined by the disc diffusion method and/or the broth microdilution method, depending on the routine practice of the participating microbiological laboratories. Resistance to teicoplanin and linezolid, which was detected by disc diffusion, was confirmed by MIC determination using the gradient diffusion (Oxoid) or broth microdilution method (Erba Lachema). The current EUCAST breakpoints were applied.[13] Inducible clindamycin resistance was tested by D-test.[13]

Clonal analysis

spa typing was performed according to the protocol published on the website of the European Network of Laboratories for sequence-based typing of microbial pathogens (http://www.seqnet.org).[14] To increase the discriminatory power of *spa* typing, PCR-based SCC*mec* typing was performed as described previously.[15,16] The *spa* types were assigned using the Ridom StaphType software (Ridom, Germany). To assess the relatedness of detected *spa* types, the BURP (Based Upon Repeat Pattern) algorithm was used. The MLST STs were inferred from the *spa* type using information available in the SpaServer database (http://spaserver.ridom.de) or from previously published studies.[17] Information about STs and the relatedness of the *spa* types was used to assign isolates to MRSA clonal complexes (CCs). For 12 selected isolates, MLST was performed as described previously.[18]

Statistical analysis

Differences between groups were evaluated using the χ^2 test for categorical variables and the Wilcoxon test for continuous variables. The Spearman correlation coefficient was used to determine the correlation between the age of the patient and the level of resistance of the MRSA isolates (i.e. the number of antimicrobial drugs to which the isolate was resistant). The *P* values were adjusted for multiple comparisons using the Holm–Bonferroni method and a *P* value of ≤ 0.05 was considered statistically significant. Analyses were conducted using the R statistical software version 3.5.1.

Results

Patient data

During a 5 month study, 441 non-duplicated MRSA isolates from 11 hospitals were collected. The geographical location of the participating hospitals and the numbers of MRSA isolates submitted from each hospital are shown in Figure 1 and Table 1.

The median age of patients was 67.06 years (range 1 day to 107 years), 284 (64.40%) of patients were male, 303 (68.71%) were inpatients and 136 (30.84%) were outpatients. For two isolates, the inpatient/outpatient origin was unknown. Inpatients were older than outpatients (median 69 versus 62 years; $P = 0.006$).

MRSA isolates were derived most frequently from skin and soft tissue infections (SSTIs; $n = 222$), asymptomatic colonization (MRSA carriage; $n = 112$), respiratory tract infections (RTIs; $n = 53$), bloodstream infections (BSIs; $n = 20$), urinary tract infections ($n = 20$) and bone and joint infections ($n = 14$). Patients with MRSA asymptomatic colonization were significantly younger compared with patients with MRSA infection (median age 60.5 versus 69 years; $P < 0.001$); there was no significant difference in the origin ($P = 0.329$), inpatient/outpatient setting ($P = 0.063$), level of resistance ($P = 0.749$) or patient's gender ($P = 0.886$). The detailed epidemiological characteristics of the MRSA isolates cultured from

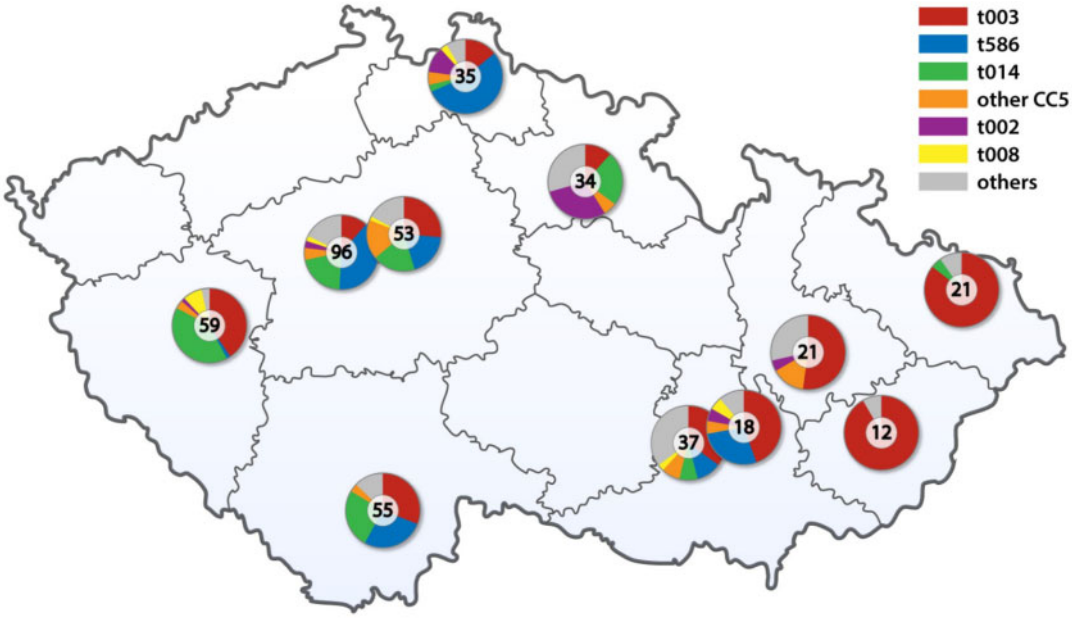

Figure 1. The distribution of participating hospitals in the study. The pie charts show the most common *spa* types identified per hospital. The numbers in the centre represent the number of MRSA isolates sent for molecular characterization. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 1. Distribution of MRSA *spa* types and CCs in 11 participating hospitals in the Czech Republic between September 2017 and January 2018

	CC5					CC8									
Hospital	t002	t003	t014	t586	other CC5	t008	other CC8	CC398	CC59	CC30	CC1	CC22	CC20	CC97	Other CCs
A ($n=96$)	3	11	20	38	5	2	3	2	0	2	1	1	2	1	5
B ($n=53$)	0	14	10	10	9	1	0	0	3	1	1	0	1	0	3
C ($n=18$)	1	8	0	5	1	1	0	0	0	0	1	0	0	0	1
D ($n=37$)	0	13	3	4	3	1	1	2	2	2	1	1	0	0	4
E ($n=55$)	0	17	14	15	2	0	1	2	1	0	0	0	0	0	3
F ($n=34$)	10	4	8	0	3	0	1	4	0	1	0	2	0	1	0
G ($n=35$)	4	5	1	19	2	1	0	0	2	0	1	0	0	0	0
H ($n=21$)	1	11	0	0	3	0	0	1	2	1	1	1	0	0	0
I ($n=59$)	1	24	24	1	2	5	0	0	0	0	0	0	1	0	1
J ($n=12$)	0	11	0	0	0	0	0	0	0	0	0	0	0	1	0
K ($n=21$)	0	18	1	0	0	0	0	0	0	0	0	0	1	1	0
Total ($n=441$)	20	136	81	92	30	11	6	11	10	7	6	5	5	4	17

infections by CC are shown in Table S1 (available as Supplementary data at *JAC* Online).

By origin, 70.52% of isolates were HA-MRSA ($n=311$) and 16.78% were CA-MRSA ($n=74$); in 12.70% ($n=56$) the origin of the isolates was unknown. Patients with HA-MRSA were older compared with patients with CA-MRSA ($P<0.001$; median 69 versus 33.5 years) (Table 2).

Antimicrobial susceptibility testing

Except for oxacillin and cefoxitin, the MRSA isolates were found to be frequently resistant to erythromycin (87.98%), clindamycin (84.81%) and ofloxacin (82.77%). The resistance to clindamycin was inducible in 51 isolates. The resistance of the MRSA isolates to other antimicrobials did not reach 10% except for gentamicin (14.51%) and tetracycline (10.43%). Linezolid resistance, detected by disc diffusion ($n=1$), was confirmed by the gradient diffusion method (MIC = 6 mg/L). For the isolate resistant to teicoplanin ($n=1$), an MIC determination by broth microdilution (MIC = 0.5 mg/L) did not confirm a resistant phenotype. On average, the isolates were resistant to three of all the tested antimicrobials (excluding cefoxitin and oxacillin) and a highly MDR phenotype was observed in 13 (2.95%) isolates; these isolates were resistant to five or more different antibiotics, including combinations of

Table 2. Statistical analysis of the correlation between MRSA type and patients' characteristics (Czech Republic between September 2017 and January 2018) ($n = 441$)

	CC5 ($n = 359$)		*spa* type t003 ($n = 136$)		*spa* type t014 ($n = 81$)		*spa* type t586 ($n = 92$)		PVL ($n = 38$)	
		P value		*P* value		*P* value		*P* value		*P* value
Age, years (median)	70	<0.001	71.5	<0.001	72	0.002	67	0.666	31.5	<0.001
infection	71	<0.001	72	0.002	74	0.003	68.5	1.000	31.5	<0.001
colonization	66	0.023	67.5	0.030	69	0.039	12.5	0.009	32	0.354
No. of males (%)	226 (62.95)	0.669	85 (62.50)	1.000	49 (60.49)	0.494	59 (64.13)	1.000	25 (65.79)	0.992
infection	168 (62.67)	0.137	66 (61.11)	0.801	29 (58.00)	0.356	44 (64.71)	1.000	23 (67.65)	0.853
colonization	58 (63.74)	1.000	19 (67.86)	1.000	20 (64.52)	1.000	15 (62.50)	1.000	2 (50.00)	0.623
% of dominant MRSA origin[a]	91.53 (HA)	<0.001	89.42 (HA)	0.054	97.33 (HA)	<0.001	93.83 (HA)	0.008	83.33 (CA)	<0.001
infection	90.63 (HA)	<0.001	88.46 (HA)	0.060	97.83 (HA)	0.003	93.44 (HA)	0.013	84.38 (CA)	<0.001
colonization	93.98 (HA)	<0.001	92.31 (HA)	1.000	96.55 (HA)	0.317	95.00 (HA)	0.591	75.00 (CA)	0.037
% of inpatients	75.49	<0.001	66.91	1.000	90.12	<0.001	82.22	0.018	21.05	<0.001
infection	73.31	<0.001	63.89	0.801	90.00	0.001	78.79	0.074	20.59	<0.001
colonization	83.52	0.031	78.57	1.000	90.32	0.317	91.67	0.471	25.00	0.091
Resistance (median)[b]	3	<0.001	3	0.007	3	<0.001	3	0.111	2	<0.001
infection	3	<0.001	3	0.012	3	<0.001	3	0.315	2	<0.001
colonization	3	<0.001	3	1.000	3	0.098	3	0.471	1	0.037
MRSA colonization isolates (%)	91 (25.35)	0.753	28 (20.59)	0.458	31 (38.27)	0.010	24 (26.09)	1.000	4 (10.53)	0.089

Groups of isolates, determined by molecular characteristics (top row), were compared separately against all remaining isolates for selected demographical characteristics (first column). Differences between groups were evaluated using the χ^2 test for categorical variables and the Wilcoxon test for continuous variables.
$P \leq 0.05$ was considered statistically significant.
Isolates of unknown origin ($n = 56$), age ($n = 1$) and inpatient/outpatient setting ($n = 2$) were excluded from calculation.
[a]Dominant origin in parentheses.
[b]Median number of antimicrobials to which the isolate was resistant.

erythromycin, clindamycin, gentamicin, ofloxacin, tetracycline, rifampicin, trimethoprim/sulfamethoxazole and linezolid (Table 3). The mean number of antibiotics to which the isolates were resistant differed significantly between HA-MRSA and CA-MRSA (two versus three, $P < 0.001$). The mean number of antimicrobial drugs to which the isolate was resistant also correlated with the patients' advanced age (Spearman's correlation coefficient $\rho = 0.237$; $P < 0.001$). No difference was observed ($P = 0.749$) in antibiotic resistance between colonizing and infecting MRSA isolates (Table 2).

Clonal analysis

For 343 MRSA isolates, the MLST CC was inferred from the *spa* type using the SpaServer database or taken from previously published data. The designation of the MLST CC could not be determined in 92 isolates of *spa* type t586, due to an insufficient number of repeats for BURP analysis in this *spa* type. Thus, we performed MLST in four randomly chosen isolates representing t586 and the results confirmed that t586 belongs to ST225, which is a single-locus variant of ST5 and belongs to CC5. MLST was also performed for six singleton *spa* types: t073 (CC45/ST45), t457 (CC5/ST225), t665 (CC30/ST1472), t7093 (CC8/ST5712), t16230 (CC88/ST88) and t17929 (CC30/ST1472).

The following SCC*mec* types were detected through SCC*mec* typing: I ($n = 2$), II ($n = 350$), IVa ($n = 23$), IVb ($n = 4$), IVc ($n = 16$), IVd ($n = 1$), V ($n = 22$), V_T ($n = 8$) and VI ($n = 1$). Four isolates possessed an unspecified type IV and 10 isolates were non-typeable (Table 4 and Table S2).

CC5-MRSA lineage

From 441 MRSA isolates, 58 different *spa* types belonging to 18 MLST CCs were identified (Table 4 and Table S2). The clonal structure of the MRSA isolates in our study was dominated by *spa* types belonging to CC5-MRSA, which constituted 81.41% ($n = 359$) of isolates; CC5-MRSA was represented mainly by the ST225 *spa* types t003 ($n = 136$; 30.84%; all hospitals), t586 ($n = 92$; 20.86%; 7/11 hospitals), t014 ($n = 81$; 18.37%; 8/11 hospitals) and ST5 *spa* type t002 ($n = 20$; 4.54%; 6/11 hospitals) (Figure 1). An SCC*mec* type II was detected in a majority of the CC5-MRSA isolates ($n = 348$), although two isolates were non-typeable. The age of the patients infected or colonized by CC5-MRSA strains was significantly higher than that of patients with MRSA belonging to other CCs ($P < 0.001$; median age 70 versus 33 years). The same pattern was observed when comparing the age of the patients infected or colonized with t003 or t014 only with the age of patients infected or colonized by all other *spa* types (71.5 versus 64.5 years;

Table 3. Antimicrobial resistance profiles of MRSA isolates according to isolate origin (Czech Republic between September 2017 and January 2018)

Additional resistance to oxacillin and cefoxitin[a]	HA-MRSA		CA-MRSA		Unknown origin		
	no. (%)	*spa* types	no. (%)	*spa* types	no. (%)	*spa* types	Total (%)
ML, OFX	223 (78.25)	t002, t003, t014, t045, t264, t586, t626, t893, t1623, t2379, t3195	19 (6.67)	t003, **t008**, t457, t586	43 (15.09)	t003, t014, t586, t1282	285 (64.63)
ML, OFX, GEN	37 (86.05)	t002, t003, t014, t586, t3195	4 (9.30)	t002, **t005**, t014	2 (4.65)	t003, t014	43 (9.75)
No additional resistance	7 (26.92)	**t019**, t026, t073, t091, t2461	18 (69.23)	t015, **t019**, **t044**, t091, **t105**, **t197**, t267, **t311**, t359, t437, t586, **t701**, t1576, **t4179**, **t17930**	1 (3.85)	t148	26 (5.90)
ML, TET	10 (45.45)	t011, t024, t034, **t437**, t898, t4087	11 (50.00)	**t034**, t127, **t437**, **t665**, **t13245**, **t17929**	1 (4.55)	t127	22 (4.99)
ML	6 (40.00)	t008, t664, t1954, t7093	8 (53.33)	**t008**, t010, t330, **t437**, **t441**, **t622**	1 (6.67)	**t002**	15 (3.40)
OFX	4 (44.44)	t003, t032, t586, t1282	3 (33.33)	**t008**, t311, t747	2 (22.22)	t008, t1282	9 (2.04)
TET, GEN	2 (33.33)	t011, t127	3 (50.00)	t011, t084	1 (16.67)	t085	6 (1.36)
ML, OFX, TET	4 (66.67)	t002, t003, t586	0	–	2 (33.33)	t003	6 (1.36)
ML, OFX, RIF	3 (60.00)	t003	0	–	2 (40.00)	t003	5 (1.13)
TET	2 (50.00)	t011, t786	2 (50.00)	t688, t16230	0	–	4 (0.91)
ML, OFX, GEN, RIF, SXT	4 (100.00)	t586	0	–	0	–	4 (0.91)
ML, OFX, TET, GEN	2 (50.00)	t003, t586	2 (50.00)	t127, t014	0	–	4 (0.91)
ML, OFX, SXT	3 (100.00)	t003, t014, t045	0	–	0	–	3 (0.68)
ML, OFX, GEN, RIF	1 (50.00)	t014	0	–	1 (50.00)	t586	2 (0.45)
ML, GEN, RIF	1 (100.00)	**t355**	0	–	0	–	1 (0.23)
Other combinations[b]	2 (33.33)	t003, t045	4 (66.67)	t005, t037, t127, t267	0	–	6 (1.36)
Total[c] (%)	311 (70.52)		74 (16.78)		56 (12.70)		441 (100.00)

ML, macrolides (erythromycin) and lincosamides (clindamycin); GEN, gentamicin; TET, tetracycline; RIF, rifampicin; OFX, ofloxacin; SXT, trimethoprim/sulfamethoxazole; LZD, linezolid.
[a]Of the 393 ML-resistant isolates, 369 were resistant to both erythromycin and clindamycin, and of these 51 showed inducible resistance to clindamycin; 19 isolates were resistant to erythromycin and 5 to clindamycin only.
[b]GEN ($n = 1$; t267; CA-MRSA); ML, OFX, TET, LZD ($n = 1$; t003, HA-MRSA); ML, OFX, TET, SXT ($n = 1$; t045; HA-MRSA); ML, TET, GEN ($n = 1$; t127; CA-MRSA; PVL+); OFX, GEN ($n = 1$; t005; CA-MRSA, PVL+); OFX, TET, GEN, RIF ($n = 1$; t037, CA-MRSA).
[c]*spa* types of corresponding origin of isolates carrying the genes for PVL toxin are in bold.

Table 4. Clonal structure of MRSA isolates (Czech Republic between September 2017 and January 2018)

CC	No. (%)	Origin[a] (%)	SCC*mec* types (no.)	Most frequent *spa* types			Other (no.)
				1st (no.)	2nd (no.)	3rd (no.)	
CC5	359 (81.41)	HA-MRSA (78.27)	II (348); I (2); IV (1); IVa (1), IVc (3); V (1); VI (1); NT (2)	t003 (136)	t586 (92)	t014 (81)	t002 (20); t010 (1); t045 (7); t105 (2); t264 (2); t311 (2); t457(1); t626 (2); t688 (1); t893 (1); t1282 (3); t1623 (5); t1954 (1); t3195 (2)
CC8	17 (3.85)	CA-MRSA (64.71)	IVa (7); IVc (4); IVd (1), NT (5)	t008 (11)	–	–	t024; t197; t622; t1576; t7093; t17930
CC398	11 (2.49)	HA-MRSA (63.64)	IVa (2); V (9)	t034 (6)	t011 (4)	t898 (1)	–
CC59	10 (2.27)	CA-MRSA (70.00)	IV (1); V (1); V_T (8)	t437 (8)	t441 (1)	t13245 (1)	–
CC30	7 (1.59)	CA-MRSA (71.43)	IVa (2); IVb (1); IVc (4)	t019 (4)	–	–	t665; t4179; t17929
CC1	6 (1.36)	CA-MRSA (66.67)	IVa (3); V (3)	t127 (6)	–	–	–
CC22	5 (1.13)	CA-MRSA (80.00)	IVa (3); IV (2)	t005 (3)	t032 (1)	t747 (1)	–
CC20	5 (1.13)	HA-MRSA (80.00)	IVc (4); V (1)	t2461 (2)	–	–	t148; t664; t4087
CC97	4 (0.91)	CA-MRSA (100.00)	IVa (2); IVb (1); V (1)	t267 (2)	t359 (2)	–	–
Other CC[b]	17 (3.85)	–	II (2); IVa (3); IVb (2); IVc (1); V (6); NT (3)	–	–	–	–

NT, non-typeable.
For 343 MRSA isolates the MLST CC was inferred from the *spa* type using the SpaServer database or taken from previously published data. The designation of the MLST CC could not be determined in 92 isolates of *spa* type t586, due to an insufficient number of repeats for BURP analysis in this *spa* type. Thus, we performed the MLST in four randomly chosen isolates representing t586 and the results confirmed that t586 belongs to ST225, which is a single-locus variant of ST5 and belongs to CC5. The MLST was also performed for six singleton spa types: t073 (CC45/ST45), t457 (CC5/ST225), t665 (CC30/ST1472), t7093 (CC8/ST5712), t16230 (CC88/ST88) and t17929 (CC30/ST1472).
[a]Prevailing origin of isolates from corresponding CC; percentage of isolates with predominant origin stated in parentheses.
[b]Other CCs included (no. of isolates; *spa* types): CC6 (1; t701), CC7 (2; t091), CC15 (3; t084, t085), CC45 (4; t015, t026, t073, t330), CC80 (1; t044), CC88 (2; t786, t16230), CC133 (2; t2379), CC152 (1; t355), ST239 (1; t037). For details see Table S2.

$P < 0.001$; 72 versus 66 years; $P = 0.002$, respectively). No significant difference was found between the age of patients with t586 MRSA and the age of patients infected or colonized by other *spa* types (67 versus 67.5 years; $P = 0.666$) (Table 2). Of note, a collection of t586 isolates ($n = 92$) included 14 isolates (11 isolates from colonization, 2 isolates from RTI and 1 isolate from SSTI) from the neonatology department of a single hospital.

In the CC5-MRSA isolates, an HA origin was more frequent than a CA origin ($P < 0.001$; 281/311 of HA-MRSA isolates) and the CC5-MRSA isolates were more often derived from inpatients ($P < 0.001$); CC5-MRSA also formed a significant part of the CA-MRSA cases (35.14%; 26/74). Compared with other *spa* types, the CC5-MRSA *spa* type t014 was associated with asymptomatic colonization ($P = 0.010$; 38.27%; 31/81 isolates versus 22.50%; 81/360 isolates). In addition, no correlations were found for t003 or t586 *spa* types ($P = 0.458$ and 1.000, respectively) (Table 2).

In 268 (81.46%) cases, CC5-MRSA isolates were isolated from an infection; *spa* types t003, t014 and t586 were the cause of 108 (32.83%), 50 (15.20%) and 68 (20.67%) infections, respectively. The distribution of these *spa* types in different kinds of infections corresponded to their relative abundance, except for a notable increase in the frequency of t586 (40%; 8 of 20 BSI isolates) and the absence of t014 among BSI isolates (Table S2).

Other MRSA lineages

The remaining 41 *spa* types (82 isolates) belonged to 17 different CCs. Of the non-CC5 lineages, the most frequently identified was CC8-MRSA (t008, t024, t197, t622, t1576, t7093, t17930), represented by 17 isolates; the CC8-MRSA included 10 PVL-positive isolates; six (t008, t622) of them were also ACME positive and SCC*mec* type IVa, one t008-MRSA-IVa (PVL+) isolate lacked ACME; three PVL-positive isolates (t008, t197, t17930) carried SCC*mec* type IVc. Other lineages were represented by 10 isolates (t437, t441, t13245) belonging to CC59-MRSA (SCC*mec* types IV, V and V_T) and seven isolates belonged to CC30 (t019, t665, t4179, t17929). The ST80-MRSA-IVc was represented by one isolate (t044) and lineages CC398-MRSA-IVa/V ($n = 11$) and CC1-MRSA-IVa/V ($n = 6$) were also identified; other lineages were represented by five or fewer isolates (Table 4).

Collectively, non-CC5 isolates were identified in 48 cases as CA-MRSA (58.54%) and in 30 cases as HA-MRSA (36.59%); in 4 of the isolates (4.88%) the origin was unknown. The CC398-MRSA (2.25%; 7/311) was the second most frequent cause of HA-MRSA cases after the CC5-MRSA isolates. In CA-MRSA, the most frequent non-CC5 lineages were CC8-MRSA (14.86%; 11/74) and CC59-MRSA (9.46%; 7/74). In addition, 12 lineages (CC1, CC6, CC7, CC15,

CC22, CC30, CC45, CC80, CC88, CC97, ST239 and CC398) caused five or fewer CA-MRSA infections each, but together represented a significant portion of the CA-MRSA cases (40.54%; 30/74). In the non-CC5 lineages, the outpatients (60.98%, 50 isolates) were found to be more affected than inpatients ($P < 0.001$).

PVL

The presence of PVL was detected in 38 isolates. Of these, 31 were from SSTIs, 4 were detected through screening, 2 originated from blood cultures and 1 originated from an RTI (pneumonia). The age of the patients from whom the PVL-positive isolates were recovered differed significantly from the age of patients infected by PVL-negative isolates (median 31.5 versus 69 years; $P < 0.001$). PVL-positive isolates were more frequently CA-MRSA ($P < 0.001$; 30/38 isolates) and demonstrated resistance to fewer antimicrobial drugs ($P < 0.001$; two antibiotics on average) (Table 2). There was no significant difference in the gender distribution between PVL-positive and -negative isolates ($P = 0.992$). In PVL-positive MRSA, the most frequently identified clones were t008-CC8-MRSA-IVa/IVc ($n = 7$) and t437-CC59-MRSA-V/V_T ($n = 7$) and these were detected in 6 and 5 of 11 participating hospitals, respectively. SCC*mec* types detected among PVL-positive isolates included IV ($n = 1$; t311), IVa ($n = 12$; t005, t008, t622, t665, t17929), IVb ($n = 2$; t019, t701), IVc ($n = 11$; t002, t008, t019, t044, t105, t197, t4179, t17930), V ($n = 4$; t034, t127, t355, t437) and V_T ($n = 8$; t437, t441, t13245). The *spa* types of all PVL-positive isolates are summarized in Table 3.

Discussion

Our study aimed to gain data on the clonal structure of MRSA circulating in the Czech Republic. This study presents a large collection of 441 MRSA isolates collected by 11 hospitals covering a substantial area of the country during a 5 month period.

Our results confirmed the continuing dominance of CC5/ST225-MRSA-II (mainly t003, t586 and t014) in Czech hospitals in 2017 and patterns of MRSA epidemiology similar to other Central European countries. In our study, the prevalence of t003 (up to 90% of isolates) was found to be the highest in the Moravian/Silesian regions (East, North East) and, given that t003 was found recently to be the most common *spa* type among MRSA isolates in southern Poland,[19] it suggests that there may be some cross-border transmission. The CC5-MRSA-II clone (t002, t003, t014, t626, t10303), which makes up 37.2% of all MRSA isolates, was dominant in south-east Austria in 2012.[20] In a study investigating ICU patients from eight European countries, the *spa* types t003 and t002 were also found frequently among isolates from Slovenia, Luxemburg and Greece[21] as well as in patients in the Czech Republic suffering from cystic fibrosis.[22] A recent study of 62 invasive MRSA isolates, collected between 2010 and 2016 in one Czech tertiary hospital, confirmed the continuing presence of the clone in Czech healthcare facilities since all but one isolate belonged to CC5/ST225-MRSA-II (t002, t003, t014 and related).[23]

When the data on the epidemiology of MRSA were compared with those of countries neighbouring the Czech Republic, the CC22-MRSA (t032) clone was found to be predominant in a tertiary hospital in Saxony, Germany, where it constituted almost 50% of MRSA isolates in 2015 and 2016,[24] and in Austria, where a significant increase of this clone was detected in 2012;[20] in our study only one isolate of CC22 MRSA (t032) was found.

In our study, we identified an emergence of the *spa* type t586 that belongs to the CC5/ST225-MRSA clone; it is an interesting observation since until now isolates of t586 were reported rarely in MRSA isolates from studies conducted in other countries, such as Germany, Belgium, Sweden, New Zealand, the USA and China.[25–30] In 2012, a single t586 was identified among 38 isolates during an investigation into an outbreak of MRSA cases in a Czech secondary hospital.[31] In our current study, the occurrence of t586 was highly variable among hospitals. Even though t586 was not detected in eastern and western parts of the country, it was frequently found in central Bohemia and in neighbouring southern and northern regions of the country. We suggest that the t586 is probably a descendant of t003, t014 or a genetically related *spa* type, because they belong to the same ST225 and possess the same SCC*mec* type II; however, the independent introduction of t586 from abroad and its subsequent spread cannot be ruled out without the use of highly discriminatory methods like WGS. Similar to t003 and t014, t586 was associated with an HA origin or inpatients, but it is worth noting that its association with patients of a higher age was not confirmed. This could be explained, in part, by an endemic presence of t586 in the neonatology department in one of the participating hospitals. Moreover, t586 was also the most frequent cause of BSIs (8 from 20 cases), underlining its possible higher virulence; however, the total number of BSI isolates was low and further epidemiological monitoring is needed.

Eleven isolates belonged to the CC398 (t011, t034). CC398 was reported previously in more than 10% of MRSA cases in European countries with a high livestock density, such as Belgium, Denmark, Spain, the Netherlands and Slovenia,[32] although data from the Czech Republic were not included. However, the later Czech study identified CC398 as dominant (150 of 159 isolates) in MRSA collected from livestock and food of animal origin.[33] CC398 isolates are frequently resistant to tetracycline, a drug used frequently in the veterinary sector,[34] and this was also found in all CC398 isolates in this study, but CC398 isolates are increasingly being detected in people who have had no contact with livestock.[35]

In our analysis of CA-MRSA, the isolates belonged more frequently to the CC8-MRSA lineage. A variant of this lineage, known as the USA300 clone, often carries PVL genes, SCC*mec* type IV and ACME and, after its emergence in the 1990s, completely changed MRSA epidemiology in the USA. An epidemic of USA300 led to an increase in MRSA infections of community origin in the USA[36] and within a few years USA300 replaced older HA-MRSA clones and, in different US medical centres, became the leading cause of SSTIs and invasive infections caused by MRSA.[37] In our study, CC8-MRSA was the most frequent non-CC5 lineage found in 7 of 11 participating hospitals. In the 10 PVL-positive CC8-MRSA isolates identified in our study, four different resistance profiles were detected and 6 isolates (originating from five hospitals) had the characteristics of USA300 (PVL+, SCC*mec* type IVa, ACME+). Observed variability suggests independent introduction rather than epidemic spread of CC8-MRSA. The repeated introduction of the USA300 clone into Europe from North America, where its prevalence is high, was also suggested by a study analysing the genetic relatedness of USA300 isolates collected across Europe.[38] From 2002 to 2009, 21 CA-MRSA isolates were collected in the Czech Republic and CC8-MRSA ($n = 13$) was found to be the most frequent clone. The same study

also reported that ST80-MRSA (i.e. the European CA-MRSA) was the second most frequent, with a total of five isolates; however, in our study ST80-MRSA-IVc was only found in one isolate (t044).[39]

CC59-MRSA was the second most frequent CA-MRSA lineage, with 10 isolates that were collected in five different hospitals; in 7 isolates, CA origin was recorded. CC59-MRSA is represented by the Asian CA-MRSA (t437 and related, PVL+, SCC*mec* type V or V_T), a high-risk epidemic clone with a high prevalence of CA-MRSA in China,[40] but it has also been detected globally.[17] The occurrence of CC59 across Europe was confirmed by a study of t437 ($n = 147$) isolates from 11 European countries collected during a 10 year period. The high genetic similarity between t437 isolates from Europe and China, where this strain is endemic, pointed to the recent introduction of CC59 from Asian countries to Europe, and its rapid spread.[41]

The CA-MRSA cases in our study were, in general, associated with patients of a lower age, the outpatient setting, a lower number of tested antibiotics to which the isolate was resistant, and the presence of PVL. Similar findings were reported previously by David and Daum.[37]

It is worth noting that a significant portion of the CA-MRSA cases in our study were caused by HA-MRSA lineage CC5-MRSA, which could be explained partly, in some cases, by an unreported link to healthcare exposure. However, the blurring of the lines between classical HA-MRSA and CA-MRSA lineages has already been reported to be the result of an increasing exposure of the general population to healthcare.[42] On the other hand, detection of CC8-MRSA, CC59-MRSA and CC398-MRSA clones in our study could indicate the successful establishment of these epidemic clones in in both a community and a healthcare environment in the Czech Republic. The replacement of HA-MRSA clones by clones of CA origin in hospitals was observed in multiple studies and was followed by changes in MRSA epidemiology that affect the severity and types of infections, age groups, antibiotic treatment, etc.[36,43,44] This trend is supported theoretically by mathematical modelling and takes into account the growing reservoir of CA-MRSA in the community and the constant influx of CA-MRSA clones into hospitals.[45] Further, it stresses the need for the regular surveillance of the clonal structure of MRSA on a national and international level in order to track and analyse epidemiological changes that could have a significant impact on infection prevention and treatment strategies.

Limitations of the study

This study has a number of potential limitations. The susceptibility of isolates to glycopeptide antibiotics (vancomycin and teicoplanin) was tested using the disc diffusion method and only isolates showing a resistant phenotype were retested by the gradient diffusion method. Although glycopeptide resistance is rare in *S. aureus*, the use of the disc diffusion method is no longer recommended since the method cannot reliably detect glycopeptide resistance.[13] Isolates were assigned to *S. aureus* CCs based on the detected *spa* type; however, in cases of rare *spa* types and STs, or in lineages where large genome recombination has occurred, such as in ST239, ST2249, ST34 and ST42,[46,47] the CC assignment based on *spa* type may be less reliable. In addition, SCC*mec* typing was performed in order to increase the discriminatory power of *spa* typing. The results of our study could have been influenced by the indiscriminate inclusion of isolates, including those originating from possible outbreaks, which led to an overrepresentation of specific strains in our data. However, based on the available data, an accurate exclusion of outbreak isolates was not possible.

Conclusions

The healthcare-associated CC5-MRSA lineage (t003, t586, t014) was found to be predominant in the Czech Republic. Interestingly, t586 is a newly emerging *spa* type that so far has been reported only rarely in the Czech Republic and other countries. Our observations stress the need for MRSA surveillance in the Czech Republic in order to monitor changes in MRSA epidemiology.

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Transparency declarations

All authors: none to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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