Prevalence of genes encoding extracellular virulence factors among meticillin-resistant *Staphylococcus aureus* isolates from the University Hospital, Olomouc, Czech Republic

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A rather fast and complicated progression of an infection caused by some strains of *Staphylococcus aureus* could be associated with the expression and co-action of virulence factor complexes in these strains. This study screened the antibiotic susceptibility and prevalence of virulence markers in isolates of meticillin-resistant *S. aureus* (MRSA) obtained from patients hospitalized at the University Hospital in Olomouc, Czech Republic. A total of 100 isolates was screened for 13 genes encoding extracellular virulence determinants (*tst*, *pvl*, *eta*, *etb*, *sea*, *seh*, *sec*, *sed*, *see*, *seg*, *seh*, *sei* and *sej*) and for their distribution in sample types. Eighty-nine isolates were positive for at least one of the genes. Genes for *etb*, *pvl*, *see* and *seh* were not detected in any of the MRSA isolates. No statistically significant differences in the occurrence of the determinants studied among sample types were found.

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INTRODUCTION

Staphylococcus aureus is one of the major nosocomial pathogens. Particular attention should be paid to meticillin-resistant *S. aureus* (MRSA). Resistance to meticillin is determined by the presence of the *mecA* gene encoding penicillin-binding protein with very low affinity to β -lactam antibiotics (Chambers, 1997). *S. aureus* produces a broad spectrum of extracellular and cell wall-associated virulence determinants (Foster, 2002). Among them, a wide variety of surface adhesins known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) has been described (Patti *et al.*, 1994).

The most common staphylococcal proteins anchored in the cell wall are proteins with affinity to fibrinogen (i.e. clumping factors A and B, encoded by the *clfA* and *clfB* genes, respectively), fibronectin (*fibA*), collagen (*cna*), sialoprotein (*bbp*), elastin (*ebpS*) and adhesins with unknown function (*sdrC* and *sdrE*) (Jonsson *et al.*, 1991; Josefsson *et al.*, 1998; McDevitt *et al.*, 1997; Ní Eidhin *et al.*, 1998; Park *et al.*, 1996; Speziale *et al.*, 1986; Tung *et al.*, 2000). The second group of virulence factors is represented by a family of bacterial proteins with superantigen activity:

enterotoxins A–E, G–R and U (encoded by the genes *sea*–*see*, *seg*–*ser* and *seu*), toxic shock syndrome toxin-1 (TSST-1, encoded by *tst*), exfoliative toxins A and B (*eta* and *etb*) and other toxins such as α -, β -, γ - and δ -toxin and the Panton–Valentine leukocidin (*pvl*) (Arbuthnott *et al.*, 1982; Bhakdi & Tranum-Jensen, 1991; Bohach *et al.*, 1990; Prevost *et al.*, 1995).

Apart from syndromes caused by toxin production, *S. aureus* pathogenesis results from synergistic interactions of a variety of the above-mentioned factors. Exfoliative toxin and pyrogenic toxin superantigen production enables *S. aureus* to cause staphylococcal scalded skin syndrome (SSSS), staphylococcal toxic shock syndrome and staphylococcal food poisoning. Experimental models indicate that the expression of receptors for fibrinogen and fibronectin is associated with endocarditis, whereas the presence of adhesins for sialoprotein, collagen and fibronectin is associated with arthritis and osteomyelitis (Cheung *et al.*, 1995; Johansson *et al.*, 2001; Tung *et al.*, 2000).

The aim of this study was to screen MRSA isolates at the University Hospital, Olomouc, for antimicrobial sensitivity and for the presence of different virulence factors, and to analyse the presence of such factors, alone or in combination, in relationship to the localization of the bacteria in the host organism in order to predict potentially dangerous isolates.

Abbreviations: MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*; SSSS, staphylococcal scalded skin syndrome.

METHODS

Bacterial isolates. *S. aureus* isolates were acquired from clinical samples obtained consecutively at the Department of Microbiology, Faculty of Medicine and Dentistry, Palacký University, and the University Hospital, Olomouc, during 2005–2006. The most important aspects were the time interval and total number ($n=100$) of MRSA isolates. In the case of multiple isolates acquired from the same patient, the first detected isolate of MRSA was included in the study. The group of patients comprised 43 % males and 57 %, females, with a mean age of 51 years (range 21–84 years). Identification of isolates was carried out by standard microbiological techniques using the commercially produced tests STAPHYtest16 (Pliva-Lachema Diagnostika) and Staphaurex Plus (Remel), which are capable of detecting coagulase (clumping factor), protein A and other specific antigens characteristic of *S. aureus*.

Resistance to meticillin was verified by the microdilution method and by detection of the *mecA* gene. Meticillin-resistant isolates ($n=100$) were examined for the presence of various virulence factors by PCR. Quality-control strains for PCR detection of virulence factors and as a control of antibiotic resistance determination were obtained from the Czech Collection of Microorganisms [*S. aureus* strains: CCM 5756 (enterotoxin A), CCM 5757 (enterotoxin B), CCM 5971 (enterotoxin C), CCM 5973 (enterotoxin D), CCM 5972 (enterotoxin E), CCM 7058 (exfoliatins A and B), and ATCC 29213 and ATCC 25923 for the antibiotic resistance protocol quality control] and from Dr P. Petráš at the National Reference Laboratory for Staphylococci, National Institute of Public Health of the Czech Republic, Praha, Czech Republic (strains for enterotoxins E, G, H, I and J, TSST-1 and Pantón–Valentine leukocidin).

Extraction of DNA. A simplified rapid method for extraction of crude staphylococcal DNA was applied. Briefly, isolates were grown on blood agar (Becton Dickinson) for 24 h, and a single colony was picked, resuspended in 100 µl sterile deionized water and heated at 99 °C for 15 min with mild shaking in a Thermomixer comfort (Eppendorf). The tubes were then centrifuged (1006 g, 5 min) to remove the sediment and the supernatant containing the crude extract of bacterial DNA was transferred into a new tube and frozen until used for PCR amplification.

PCR amplification. Several multiplex PCRs for the parallel detection of the presence of the following genes were performed: *tst*+*sea*+*pvl*, *seg*+*sei*, *eta*+*etb*, *seh*+*sej*, *seb*+*see* and *sec*+*sed*. In the case of unclear results, the presence of extracted bacterial DNA was confirmed by detection of the 16S rRNA gene with primers UNI_OL and UNI_OR (Sauer *et al.*, 2005). Identification of *S. aureus* was confirmed by detection of the species-specific *femB* gene, and meticillin resistance was verified by amplification of the *mecA* gene (Kobayashi *et al.*, 1994). The primers used are given in Table 1.

The reaction was performed in 50 µl containing: 5 µl 10× reaction buffer [100 mM Tris/HCl (pH 8.8), 500 mM KCl, 1 % Triton X-100, 15 mM MgCl₂], 0.4 µl dNTPs (10 mM each), 50 pmol each forward and reverse primers, 2.5 U *Taq* polymerase and 1 µl crude bacterial DNA (dNTPs were obtained from Promega, other components were from Top-Bio). The reaction mixture was overlaid with 50 µl mineral oil (Top-Bio) and amplified with a hot start in a MiniCycler (MJ Research) with initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 or 58 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The annealing temperature of 56 °C applied to multiplex mixtures used to amplify *tst*+*pvl*+*sea*, *seg*+*sei* and *seh*+*sej*, whereas 58 °C applied to *eta*+*etb*, *seb*+*see* and *sec*+*sed*. For the *sec*+*sed* multiplex mixture, the concentration of *sed* primers was doubled compared with the concentration of *sec* primers as this gave a more balanced amplification.

PCR products were analysed by gel electrophoresis in 2 % (w/v) agarose gel. The size of the amplified products was estimated by comparison with a DNA ladder (200–1500 bp; Top-Bio). To confirm the identity of the amplicons, restriction analysis with *Xba*I, *Sma*I and *Hae*III endonucleases was performed for randomly selected PCR products.

MIC determination. Antibiotic susceptibility was assessed by a standard microdilution method (CLSI, 2007). The breakpoint values were set as follows: 0.5 mg l⁻¹ for erythromycin and clindamycin, 1 mg l⁻¹ for ciprofloxacin, 2 mg l⁻¹ for tetracycline and vancomycin, 4 mg l⁻¹ for chloramphenicol and gentamicin, 8 mg l⁻¹ for teicoplanin and 32 mg l⁻¹ for cotrimoxazole. These are breakpoints defined by the National Reference Laboratory for Antibiotics, National Institute of Public Health, Praha, Czech Republic, and are commonly used in the Czech Republic. The MIC₅₀ and MIC₉₀ values were calculated as 50 and 90 % of the values of the MICs of the respective antibiotics.

Detection of penicillin-binding protein 2a (PBP2a) in all *S. aureus* strains was performed by latex agglutination (MRSA-Screen test; Denka Seiken), an adequate alternative to PCR detection of the *mecA* gene (Cavassini *et al.*, 1999; Felten *et al.*, 2002). Susceptibility to clindamycin, if simultaneous with resistance to erythromycin, was verified by a modified disc diffusion test (D-test) (Rich *et al.*, 2005). With respect to the importance of MRSA, the prevalence of virulence genes was analysed in relation to individual departments of the University Hospital, Olomouc (1433 beds, 157 intensive care beds), and in relation to clinical diagnoses.

PFGE analysis of macrorestriction patterns. The isolates were investigated by PFGE of fragments obtained by *Sma*I digestion (Sigma-Aldrich) using the CHEF-DR II pulsed-field gel electrophoresis system (Bio-Rad). The preparation of intact DNA was performed according to Pantůček *et al.* (1996). Restriction cleavage of blocks with embedded DNA was carried out in restriction endonuclease solution (5 µl *Sma*I restriction buffer, 5 µl 0.1 % BSA, 50 µl deionized water and 10 U *Sma*I). The blocks were incubated at 30 °C overnight. PFGE was performed in a 1.2 % agarose gel (Bio-Rad) in 1× TBE buffer and was carried out for 28 h at 14 °C, with pulse times of 1–75 s, at 5.0 V cm⁻¹ with λ DNA concatemers of 50–1000 kb (Sigma-Aldrich) as molecular mass markers. The gels were stained with ethidium bromide solution (1 µg ml⁻¹; Sigma-Aldrich).

RESULTS AND DISCUSSION

It was confirmed that a simplified and rapid extraction method for DNA was sufficient for the screening of virulence factors by PCR in staphylococcal isolates. All primers produced amplicons of predicted sizes, and restriction analysis of randomly selected amplicons also resulted in fragments of the predicted sizes, i.e. 421 and 138 bp for the *Hae*III-digested *tst* amplicon (559 bp), and 268 and 165 bp for the *Xba*I-digested *pvl* amplicon (433 bp). Further identification of *S. aureus* was carried out biochemically and by *femB* gene amplification. In the case of failed *femB* amplification, the presence of bacterial DNA was confirmed by PCR using the UNI_OL and UNI_OR primers, and biochemical identification of the isolate as *S. aureus* was reanalysed (eight cases). In all of these cases, other staphylococcal species were subsequently identified and the isolates were excluded from further study. Additional MRSA isolates were added to the study to substitute for these.

Table 1. Primers used for bacterial DNA and virulence factor complex detection

Primer	Sequence (5'→3')	Size of amplicon (bp)	Reference
UNI_OL	GTGTAGCGGTGAAATGCG	709	Sauer <i>et al.</i> (2005)
UNI_OR	ACGGGCGGTGTGTACAA		
<i>femB</i> -F	TTACAGAGTTAACTGTTACC	651	Kobayashi <i>et al.</i> (1994)
<i>femB</i> -R	ATACAAATCCAGCACGCTCT		
<i>mecA</i> -F	TCCAGATTACAACCTCACCAGG	162	Oliveira & De Lencastre (2002)
<i>mecA</i> -R	CCACTTCATATCTTGTAACG		
<i>tst</i> -F	GCTTGCGACAACCTGCTACAG	559	Monday & Bohach (1999)
<i>tst</i> -R	TGGATCCGTCATTGTTAT		
<i>pvl</i> -F	ATCATTAGGTAAAATGTCTGGACATGATCC	433	Lina <i>et al.</i> (1999)
<i>pvl</i> -R	GCATCAASTGTATTGGATAGCAAAAGC		
<i>sea</i> -F	GCAGGGAACAGCTTTAGGC	521	Monday & Bohach (1999)
<i>sea</i> -R	GTTCTGTAGAAGTATGAAACACG		
<i>seb</i> -F	ACATGTAATTTTGATATTCGCACTG	667	Løvseth <i>et al.</i> (2004)
<i>seb</i> -R	TGCAGGCATCATGTCATACCA		
<i>sec</i> -F	CTTGTATGTATGGAGGAATAACAA	284	Monday & Bohach (1999)
<i>sec</i> -R	TGCAGGCATCATATCATACCA		
<i>sed</i> -F	GTGGTGAATAGATAGGACTGC	385	Monday & Bohach (1999)
<i>sed</i> -R	ATATGAAGGTGCTCTGTGG		
<i>see</i> -F	TACCAATTAACCTGTGGATAGAC	171	Monday & Bohach (1999)
<i>see</i> -R	CTCTTTGCACCTTACCGC		
<i>seg</i> -F	CGTCTCCACCTGTTGAAGG	328	Monday & Bohach (1999)
<i>seg</i> -R	CCAAGTGATTGTCTATTGTCG		
<i>sei</i> -F	CAACTCGAATTTTCAACAGGTACC	466	Monday & Bohach (1999)
<i>sei</i> -R	CAGGCAGTCCATCTCCTG		
<i>seh</i> -F	CAACTGCTGATTTAGCTCAG	360	Monday & Bohach (1999)
<i>seh</i> -R	GTCGAATGAGTAATCTCTAGG		
<i>sej</i> -F	CATCAGAACTGTTGTTCCGCTAG	142	Monday & Bohach (1999)
<i>sej</i> -R	CTGAATTTTACCATCAAAGGTAC		
<i>eta</i> -F	GCAGGTGTTGATTAGCATT	93	Lee <i>et al.</i> (1987)
<i>eta</i> -R	AGATGTCCCTATTTTGTCTG		
<i>etb</i> -F	ACAAGCAAAAGAATACAGCG	226	Jackson & Iandolo (1986)
<i>etb</i> -R	GTTTTTGCTGCTTCTCTTG		

Antimicrobial determination

The resistance of *S. aureus* to meticillin was confirmed by detection of PBP2a and the *mecA* gene. The overall resistance to antibiotics is summarized in Table 2. The highest numbers of resistant isolates were found in relation to ciprofloxacin (85.0 %), erythromycin (62.0 %) and

clindamycin (60.0 %). No resistant isolates were recorded for vancomycin, teicoplanin and chloramphenicol.

Eleven isolates of *S. aureus* showed susceptibility to clindamycin simultaneously with resistance to erythromycin. However, as the D-test was positive, the susceptibility to clindamycin was apparently false in nine of these

Table 2. Susceptibility of MRSA isolates to antibiotics, including the MIC range, MIC₅₀ and MIC₉₀ values

Antibiotic	MIC range (mg l ⁻¹)	MIC ₅₀ (mg l ⁻¹)	MIC ₉₀ (mg l ⁻¹)	Resistant strains (%)
Erythromycin	0.03–8	1	2	62.0
Clindamycin	0.03–8	1	4	60.0
Chloramphenicol	≤1–4	2	4	0.0
Tetracycline	≤0.125–32	0.25	16	16.0
Gentamicin	0.125–64	0.25	32	24.0
Vancomycin	≤0.25–2	0.25	1	0.0
Teicoplanin	≤0.25–4	0.5	1	0.0
Ciprofloxacin	0.03–16	4	8	85.0
Cotrimoxazole	≤0.5–256	1	4	3.0

Table 3. Prevalence of genes encoding virulence determinants and frequency of these genes occurring together in the same isolate

Gene	Isolates (%) (n=100)	Gene combination	No. of MRSA-positive isolates (n=100)
<i>tst</i>	2.0	<i>sea</i>	6
<i>pvl</i>	0.0	<i>seb</i>	1
<i>eta</i>	10.0	<i>sea + eta</i>	3
<i>etb</i>	0.0	<i>seg + sei</i>	51
<i>sea</i>	12.0	<i>sej + sed</i>	1
<i>seb</i>	3.0	<i>eta + tst</i>	1
<i>sec</i>	2.0	<i>sea + seg + sei</i>	2
<i>sed</i>	17.0	<i>sec + seg + sei</i>	1
<i>see</i>	0.0	<i>eta + seg + sei</i>	4
<i>seg</i>	77.0	<i>sea + seg + sei + eta</i>	1
<i>seh</i>	0.0	<i>seb + tst + seg + sei</i>	1
<i>sei</i>	77.0	<i>seb + sec + seg + sei</i>	1
<i>sej</i>	17.0	<i>sej + seg + sei + sed</i>	15
		<i>eta + seg + sei + sej + sed</i>	1

(81.8 %). This type of MLS_B (macrolide, lincosamide and streptogramin B) resistance is caused by a change in the ribosome target site encoded by the *erm* gene (Rich *et al.*, 2005). Phenotypically, it is manifested by resistance to erythromycin together with susceptibility to clindamycin (the inducible type). In the case of the inducible type, resistance cannot be demonstrated by the common disc diffusion or microdilution methods, so the D-test has to be used. If its result is positive, the clindamycin result must be changed to resistant. The results of testing antibiotic resistance to other antimicrobial agents showed good

susceptibility to the agents with the exceptions of ciprofloxacin, erythromycin and clindamycin.

Frequency and distribution of virulence determinants

The prevalence of the detected genes among the studied isolates and the frequency of concurrent detection of various virulence factors are summarized in Table 3. Whereas no copy of the *pvl*, *etb*, *see* or *seh* gene was detected in any of the isolates, the *mecA* and *femB* genes occurred in all isolates, in accordance with the fact that only MRSA strains were included in the study.

A relatively high number of gene pattern combinations were observed. None of the 13 genes encoding virulence determinants was detected in 11 of the 100 *S. aureus* isolates, whereas 1 gene was detected in 7 isolates. In all remaining isolates (n=82), more than one gene was present in the same isolate. Fifty-six isolates harboured two genes simultaneously, seven isolates harboured three genes and in eighteen isolates four genes were detected. One isolate possessed five virulence genes.

The *seg* virulence determinant was always associated with the *sei* gene present in 77 of the tested isolates, and this pair was also the most common single combination of genes (51.0 %). These genes (together with *sem*, *sen* and *seo*) belong to the same enterotoxin gene cluster (*egc*) and this phenomenon of parallel incidence has been described in published reports (Jarraud *et al.*, 2001; Mempel *et al.*, 2003). Furthermore, the *sed + sej* genes occurred together exclusively (17.0 % of the tested isolates).

When looking at the site of origin of MRSA isolates, two major locations could be distinguished, skin and wounds

Table 4. Incidence of genes encoding virulence determinants by the type of clinical sample

Gene	No. (%) of isolates (n=100)						P value*	Odds ratio (95 % CI)†
	Skin and wound swabs (n=58)	Respiratory tract samples (n=25)	Blood cultures (n=5)	Catheters (n=5)	Urine (n=4)	Tissue (n=3)		
<i>tst</i>	1 (1.7)	—	1 (20.0)	—	—	—	1	—
<i>pvl</i>	—	—	—	—	—	—	—	—
<i>eta</i>	7 (12.1)	3 (12.0)	—	—	—	—	1	1.007 (0.256–3.896)
<i>etb</i>	—	—	—	—	—	—	—	—
<i>sea</i>	7 (12.1)	3 (12.0)	—	1 (20.0)	1 (25.0)	—	1	1.007 (0.256–3.896)
<i>seb</i>	—	2 (8.0)	1 (20.0)	—	—	—	0.161	0 (0–0.802)
<i>sec</i>	—	1 (0.4)	—	1 (20.0)	—	—	0.663	0 (0–1.649)
<i>sed</i>	9 (15.5)	7 (28.0)	—	1 (20.0)	—	—	0.308	0.475 (0.157–1.411)
<i>see</i>	—	—	—	—	—	—	—	—
<i>seg</i>	46 (79.3)	21 (84.0)	2 (40.0)	4 (80.0)	3 (75.0)	1 (33.3)	0.846	0.73 (0.223–2.431)
<i>seh</i>	—	—	—	—	—	—	—	—
<i>sei</i>	46 (79.3)	21 (84.0)	2 (40.0)	4 (80.0)	3 (75.0)	1 (33.3)	0.846	0.73 (0.223–2.431)
<i>sej</i>	9 (15.5)	7 (28.0)	—	1 (20.0)	—	—	0.308	0.475 (0.157–1.411)

*P value: χ^2 test with Yates' correction.

†CI, Confidence interval.

Table 5. Incidence of virulence genes found in this study compared with data in the literature

Gene	Incidence in this study (%)	Incidence in other studies (%)	Specification of <i>S. aureus</i> isolates	Reference
<i>tst</i>	2.0	18.3	Blood	Becker <i>et al.</i> (2003)
		22.4	Nasal	Becker <i>et al.</i> (2003)
<i>pvl</i>	0.0	4.9	Various	Holmes <i>et al.</i> (2005)
		15.3	MRSA of various origins	Wannet <i>et al.</i> (2005)
<i>eta</i>	10.0	1.2	Blood and nasal	Becker <i>et al.</i> (2003)
		6.0	Carriage	Peacock <i>et al.</i> (2002)
		22.0	Invasive	Peacock <i>et al.</i> (2002)
<i>etb</i>	0.0	0.0	Invasive	Peacock <i>et al.</i> (2002)
		3.0	Carriage	Peacock <i>et al.</i> (2002)
<i>sea</i>	12.0	15.9	Blood and nasal	Becker <i>et al.</i> (2003)
		19.6	Nasal swabs	Mehrotra <i>et al.</i> (2000)
		10.9	MSSA of various origins	Layer <i>et al.</i> (2006)
<i>seb</i>	3.0	7.0	Carriage	Peacock <i>et al.</i> (2002)
		9.0	Invasive	Peacock <i>et al.</i> (2002)
		5.6	Nasal swab	Mehrotra <i>et al.</i> (2000)
		5.9	Blood	Becker <i>et al.</i> (2003)
<i>sec</i>	2.0	8.7	Blood	Becker <i>et al.</i> (2003)
		13.8	Nasal	Becker <i>et al.</i> (2003)
		11.0	Carriage	Peacock <i>et al.</i> (2002)
		10.0	Invasive	Peacock <i>et al.</i> (2002)
<i>sed</i>	17.0	10.5	Blood	Becker <i>et al.</i> (2003)
		3.3	Nasal	Becker <i>et al.</i> (2003)
		15.8	MSSA of various origins	Layer <i>et al.</i> (2006)
<i>see</i>	0.0	0.0	Invasive	Peacock <i>et al.</i> (2002)
		0.5	Blood	Becker <i>et al.</i> (2003)
<i>seg</i>	77.0	55.0	Invasive	Peacock <i>et al.</i> (2002)
		55	Blood and nasal	Becker <i>et al.</i> (2003)
<i>seh</i>	0.0	15.0	Invasive	Peacock <i>et al.</i> (2002)
		10.0	Carriage	Peacock <i>et al.</i> (2002)
		5.4	Blood and nasal	Becker <i>et al.</i> (2003)
<i>sei</i>	77.0	52	Invasive	Peacock <i>et al.</i> (2002)
		55.0	Blood and nasal	Becker <i>et al.</i> (2003)
<i>sej</i>	17.0	10.5	Blood	Becker <i>et al.</i> (2003)
		3.3	Nasal	Becker <i>et al.</i> (2003)
		15.8	MSSA of various origins	Layer <i>et al.</i> (2006)

(58 isolates) and the respiratory tract (25 isolates). Of the 58 skin and wound swab isolates, 46 (79.3 %) were positive for the *seg* and *sei* genes, 9 (15.5 %) for the *sej* and *sed* genes, 7 (12.1 %) for the *eta* and *sea* genes, and 1 (1.7 %) for the *tst* gene. The *seg* and *sei* genes were found in 21 (84.0 %) of the 25 isolates from the respiratory tract, the *sej* and *sed* genes in 7 isolates (28.0 %), the *sea* and *eta* genes in 3 isolates (12.0 %), the *seb* gene in 2 isolates (8.0 %) and the *sec* gene in 1 isolate (0.4 %).

Differences in the occurrence of genes between skin and wounds versus the respiratory tract were not significant ($P > 0.05$), as demonstrated by the χ^2 test. The distribution of virulence genes in relation to diagnosis was not evaluated due to the very broad spectrum of underlying diagnoses.

The distribution of virulence determinants among isolates obtained from different types of clinical samples is summarized in Table 4. The results of the incidence of

these strains clearly showed that the highest incidence was observed in the department of surgery (36 %), the intensive care unit (22 %), the department of haemato-oncology (16 %) and the department of internal medicine (14 %). The incidence in other departments was 7 % (dermatology), 3 % (urology) and 2 % (neurology and orthopaedics). The diagnoses related to the incidence of MRSA were malignant diseases (26 %), traumas (24 %), diabetes mellitus (19 %), pneumonias (11 %), leg ulcers (9 %), bedsores (7 %) and others (4 %).

PFGE analysis of macrorestriction patterns

A comparison of 100 MRSA isolates revealed 46 different restriction patterns with 6 clonal types. Four isolates were repeatedly untypeable by PFGE. The majority of isolates (30) with the *seg* + *sei* genetic determinant were clustered in a single clonal type. Three isolates from this clonal type harboured an extra *eta* gene. The second most frequent

clonal type included 11 isolates with the *seg+sei* and *sed+sej* genes. The other clonal types contained four (three *sea+eta*, one with only *eta*), four (*seg+sei* and *sed+sej*), four (*seg+sei*) and three members (*seg+sei*).

The emergence of isolates possessing a certain spectrum of virulence genes is worrying, especially in relation to the increasing frequency of nosocomial MRSA strains. Some of the toxins may play important roles in specific pathogenic processes. The Pantone–Valentine leukocidin is produced by a large number of isolates from furuncles and carbuncles (Lina *et al.*, 1999), the TSST-1 often occurs together with septic shock and toxic shock syndromes (Uchiyama *et al.*, 1989), and exfoliative toxins are characteristic for isolates causing SSSS (Opal *et al.*, 1988).

However, relatively little is known about the occurrence of virulence factors among MRSA isolates. More information is available on the distribution of virulence factors among *S. aureus* isolates with no emphasis on antibiotic susceptibility, among methicillin-sensitive *S. aureus* (MSSA) strains or in mixed collections with MRSA; therefore, we will discuss our results further in the context of both MRSA-specific and non-specific data on virulence.

The incidence of virulence genes found in this study compared with data in the literature is summarized in Table 5. There is no higher association of the *sed* and *sej* genes with blood samples as found by Lehn *et al.* (1995).

A higher occurrence of exfoliative toxins is associated with SSSS diagnosis, where *eta* is present at higher rates (De Azavedo & Arbuthnott, 1981; Piemont & Monteil, 1983), but the prevalence shows geographical differences (in Japan, *etb* is more frequent) (Kondo *et al.*, 1975; Murono *et al.*, 1988). The higher incidence of *eta* in our isolates corresponds with data for geographical distribution together with *etb* for Germany (Elsner & Hartman, 1988) and France (Willard *et al.*, 1982). Surprisingly, the occurrence of the *eta* gene was almost the same for skin/wound isolates (12.1%), where it is associated with SSSS, as it was for isolates from the respiratory tract (12.0%).

More than half of the studied isolates (56) were classified into six clonal types. This result corresponds with the relatively small size of the University Hospital in Olomouc. The prevalence of different virulence genes in the same clonal type was surprising. The most frequent clonal type containing the *seg+sei* genes was represented by 30 isolates, including 3 isolates with an extra *eta* gene. In these three isolates, the PCR was repeated to detect the *eta* gene. The phenomenon of macrorestriction profiles with no detectable changes could be explained by changes located in bands of lower molecular mass where smeared bands were often detected, by different plasmid content not detectable by PFGE, or, conversely, by genetic changes associated with recombination events of different variable genetic elements resulting in similar sizes of a macrorestriction fragment that could be more difficult to detect due to a negligible shift of the high-molecular-mass bands.

Isolates with different virulence profiles but with the same macrorestriction profiles have been described in published reports (Issartel *et al.*, 2005; Loncarevic *et al.*, 2005).

This study contributes to the recognition of virulence gene prevalence among well-characterized MRSA clinical isolates in the Czech Republic. In conclusion, the prevalence of virulence genes in MRSA isolates is comparable with that reported for MSSA isolates in Germany and France. Different patterns of extracellular virulence factors were found in the studied group of isolates, and the fact that no highly virulent strains were detected among the MRSA isolates – suggesting an increased threat – from the University Hospital, Olomouc, between 2005 and 2006 can be viewed as the most positive outcome of the study.

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