International Journal of Medical Microbiology 302 (2012) 237–241

Contents lists available at SciVerse ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

Major clonal lineages in impetigo *Staphylococcus aureus* strains isolated in Czech and Slovak maternity hospitals

Vladislava Růžičková [a,*], Roman Pantůček [a], Petr Petráš [b], Ivana Machová [b], Karla Kostýlková [a], Jiří Doškař [a]

[a] *Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic*
[b] *National Reference Laboratory for Staphylococci, National Institute of Public Health, Prague, Czech Republic*

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form 19 March 2012
Accepted 2 April 2012

Keywords:
Staphylococcal skin infections
Exfoliative toxins
Multilocus sequence typing
DNA typing
Lysogeny

ABSTRACT

One hundred and twenty-seven exfoliative toxin-producing (ET-positive) strains of *Staphylococcus aureus* collected in 23 Czech and one Slovak maternity hospitals from 1998 to 2011 were genotypically characterized by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) profiling, *spa* gene polymorphism analysis, and ETA-converting prophage carriage, which resulted in the identification of 21 genotypes grouped into 4 clonal complexes (CC). Ninety-one isolates carried the *eta* gene alone whilst 12 isolates harboured only the *etb* gene. Two new, to date not defined, *spa* types (t6644 and t6645) and 2 novel sequence types (ST2194 and ST2195) were identified in the set of strains under study. The predominant CC121 occurred in 13 Czech hospitals. CC15, CC9, and ST88 (CC88) exclusively included *eta* gene-positive strains while the strains belonging to ST121 harboured the *eta* and/or *etb* genes. This study highlights not only significant genomic diversity among impetigo strains and the distribution of major genotypes disseminated in the Czech and Slovak maternity hospitals, but also reveals their impact in epidermolytic infections.

Introduction

Neonatal bullous impetigo and staphylococcal scalded skin syndrome (SSSS) are the most common human epidermolytic infections caused by *S. aureus* strains producing exfoliative toxins (ETs). SSSS predominantly affects neonates and infants, but immune system and renal impairment are known to be susceptibility factors in adults. Mortality among treated children is low and does not exceed 5% (Cribier et al., 1994; Gemmell, 1995). Blisters on the skin are caused mainly by exfoliative toxins A (ETA) and B (ETB) produced by some *S. aureus* strains. ETs recognize and cleave desmosomal cadherins in the superficial layers of the skin, which is directly responsible for the clinical manifestation of SSSS. The *eta* gene encoding ETA is carried by a prophage, located on the chromosome (O'Toole and Foster, 1987), whereas the *etb* gene encoding ETB is on a large plasmid (Warren, 1980; Yamaguchi et al., 2001). Recent studies have shown that the *eta* gene is carried by ETA-converting resident phages of the family *Siphoviridae* (Holochová et al., 2010a,b; Kahánková et al., 2010; Yamaguchi et al., 2000) classified into at least 6 groups (Holochová et al., 2010a).

Some studies have shown that in the USA, Europe, and Africa, the ETA production is expressed by more than 80% of the ET-producing *S. aureus* strains (Adesiyun et al., 1991; Cribier et al., 1994; de Azavedo and Arbuthnott, 1981); however in Japan, ETB strains prevail (Kondo et al., 1975; Yamasaki et al., 2005). Several papers dealing with the genotypic analysis of the impetigo isolates have been published (Dave et al., 1994; Mackenzie et al., 1995; Saiman et al., 1998). A recent study from Japan has reported the *eta* gene carriage among community-acquired methicillin-resistant *S. aureus* (CA-MRSA) strains (Ozaki et al., 2009; Shi et al., 2011). In Europe, a complex study of ET-positive *S. aureus* strains has not yet been reported. In a previous study, we described 2 outbreaks of nosocomial pemphigus neonatorum associated with *S. aureus* infection in 2 Czech hospitals in 1998 (Růžičková et al., 2003), but the MLST-based clonal affiliation was not determined.

In this paper, we report about detailed genotypic investigations of a collection of 127 ET-positive *S. aureus* strains most of which caused neonatal skin blistering disorders in 23 geographically distant maternity hospitals in the Czech Republic and in one Slovak hospital in 1998–2011. The aims of this study were to analyse the genetic profiles of these *S. aureus* strains, to determine their relatedness, and to elucidate whether there is any prevalent and widely distributed ET genotype lineage promoting epidermolysis

* Corresponding author at: Department of Experimental Biology, Laboratory of Molecular Diagnostics of Microorganisms, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic. Tel.: +420 549496827; fax: +420 549492570.
E-mail address: vladkar@sci.muni.cz (V. Růžičková).

1438-4221/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.ijmm.2012.04.001

Table 1
Genotypes of 127 ET-producing *S. aureus* strains.

Genotype	Number of strains	Genotype pattern[a]	Detection of ET genes[b]	Hospital no.	Year of isolation[b]	Clinical origin[b]
G-1	9	ST121:A:t916:L2:B1	*eta* (2)	23	1998 (5)	Child blister (4); mother breast (1)
			eta, *etb* (7)	1	2001 (1)	Child blister
					2008 (1)	Child SSSS
				13	2001 (1)	Child blister
				14	1999 (1)	Child SSSS
G-2	1	ST121:B:t6645:L2:B1	*eta*	23	1998 (1)	Child blister
G-3	1	ST121:C:t6644:L2:B1	*eta*, *etb*	10	2004 (1)	Child blister
G-4	16	ST121:D:t159:L6:B5	*eta* (6)	8	2004 (3)	Child blister (2); mother lochia (1)
			eta, *etb* (10)	9	2007 (9)	Child blister (8); nurse nose (1)
				12	2008 (3)	Child blister
				18	2008 (1)	Child blister
G-5	8	ST121:F:t159:L6:B5	*eta* (2)	6	2011 (2)	Child blister (1); child conjunctiva (1)
			eta, *etb* (6)	10	2001 (1)	Child blister
				13	2001 (1)	Child stool
				16	2006 (4)	Child blister (2); nurse nose (2)
G-6	1	ST121:E:t169:L7	*etb*	17	2003 (1)	Child arthritis
G-7	1	ST121:G:t159:L7	*etb*	21	2004 (1)	Child blister
G-8	10	ST121:G:t645:L7	*etb*	18	2008 (10)	Child blister (7); child nose (1)
						Mother nose (1); case unguent (1)
G-9	5	ST2195:I:t346:L4:B1	*eta*	5	1998 (5)	Child blister (4); table (1)
G-10	4	ST15:I:t084:L4:B3	*eta*	17	2010 (4)	Child blister (3); nurse nose (1)
G-11	11	ST15:J:t346:L3:B3	*eta*	6	2002 (4)	Child blister
				22	2003 (7)	Child blister (5); child stool (2)
G-12	9	ST582:H:t084:L3:B4	*eta*	19	2001 (5)	Child blister
				10	2008 (1)	Child blister
				20	2008 (3)	Child blister
G-13	10	ST582:I:t084:L4:B4	*eta*	3	2008 (1)	Child blister
				1	2010 (8)	Child blister (7); mother breast (1)
				2	2011 (1)	Child SSSS
G-14	3	ST9:K:t2700:L1:B3	*eta*	4	2002 (3)	Child blister (1); nurse nose (2)
G-15	1	ST9:K:t800:L1:B4	*eta*	4	2002 (1)	Nurse nose
G-16	2	ST9:M:t4794:L5:B4	*eta*	7	1998 (1)	Child nose
				23	1998 (1)	Mother breast
G-17	1	ST9:M:t4794:L4:B4	*eta*	9	2007 (1)	Mother nose
G-18	6	ST2194:L:t209:L5:B6	*eta*	4	2003 (5)	Child blister (3); nurse nose (1); table (1)
				4	2004 (1)	Child blister
G-19	11	ST109:L:t209:L5:B6	*eta*	15	2008 (2)	Child blister
				15	2009 (5)	Child blister (3); mother nose (2)
				15	2010 (2)	Child blister
				2	2009 (1)	Child blister
				2	2010 (1)	Nurse nose
G-20	7	ST88:N:t786:L6:B2	*eta*	11	2007 (5)	Child blister (3); nurse nose (2)
				6	2010 (2)	Child blister (1); table (1)
G-21	10	ST88:O:t186:L6:B2	*eta*	24	2003 (5)	Nurse hand (4); towel (1)
				24	2006 (3)	Child blister (2); nurse nose (1)
				24	2007 (2)	Child blister (2)

[a] Genotype patterns consist of MLST:PFGE type:*spa* type:Lysotype:ETA-B phage type. Lysotype (prophage carriage) determined as previously reported by Pantůček et al. (2004). Assignment to ETA-B phage type done according to Holochová et al. (2010a).
[b] Numbers of isolates are in parentheses.

in this region. Results of this study could importantly contribute to the present knowledge of the genotypic properties of yet clonally uncharacterized ET-positive strains having an impact in impetigo and SSSS infections.

Materials and methods

Bacterial strains

A collection of 127 ET-positive *S. aureus* strains isolated from 98 neonatal patients (90% had skin blisters), 24 carriers (mothers and/or nurses), and 5 items of hospital equipment in maternity hospitals where pemphigus neonatorum cases occurred, were reported from 1998 to 2011. Ninety-one strains were ETA-producers, 24 produced both ETA and ETB, and 12 of the strains synthesized only ETB (Table 1). For ET gene determination, the following *S. aureus* reference strains were used: ETA-producing strains CCM 2330 and CCM 7057, ETA- and ETB-producing strain CCM 7056, and ETA- and ETD-producing strain CCM 2331. The strains *S. aureus* NCTC 8325 and the prophageless *S. aureus* CCM 4890 were used as *eta* gene-negative controls. The quadruple lysogenic *S. aureus* CCM 7097 harbouring the prophages A, B, Fa, and Fb was used as a positive control (Pantůček et al., 2004). All the reference CCM strains were obtained from the Czech Collection of Microorganisms, Brno, Czech Republic (http://www.sci.muni.cz/ccm/). The positive control strains for multiplex PCR assays of ETA-converting prophages were reported previously (Holochová et al., 2010a).

Phenotypic characterization

ETA and ETB were detected using the Reverse Passive Latex Agglutination Kit (Denka Seiken for Unipath, Tokyo, Japan). For antibiotic susceptibility testing, the disc diffusion method was used, with the zone diameters measured at 24 h according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2011). The isolates were tested on Mueller Hinton agar using an oxacillin disc (1 μg) as well as cefoxitin disc (30 μg) (Oxoid, UK).

Genotypic characterization

For nucleic acid isolation, all the strains were subcultured in brain heart infusion broth (BHI, HiMedia, India) and incubated

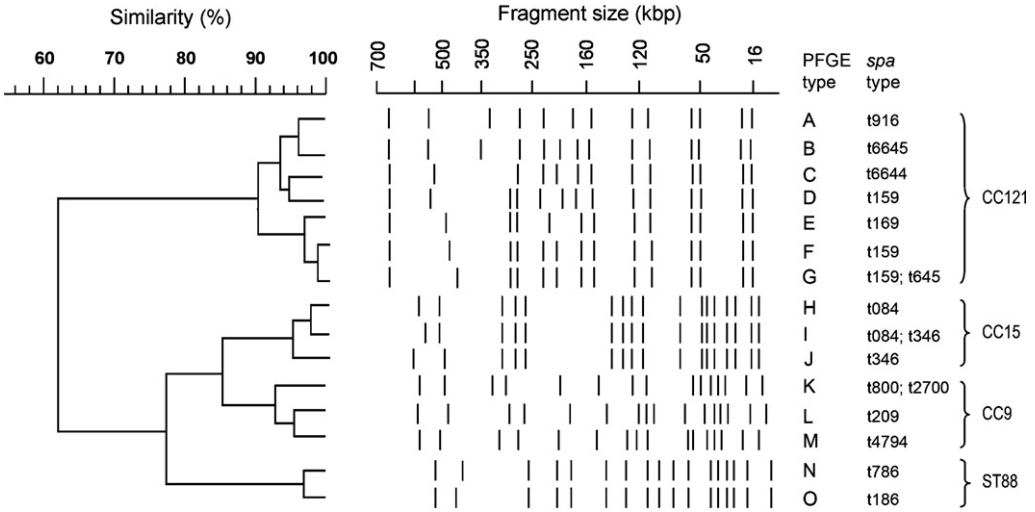

Fig. 1. Dendrogram demonstrating the degree of similarity of the SmaI-restriction patterns, constructed by the unweighted pair group method with arithmetic averages (UPGMA) and schematic representation of 15 PFGE types assigned to *spa* types and MLST CCs as identified in a set of 127 ET-positive *S. aureus* strains.

overnight with shaking at 37 °C. DNA for PCRs was obtained from lysostaphin (Dr. Petry Genmedics, Reutlingen, Germany)-treated cells and purified with the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions.

Genomic DNA for PFGE was prepared as described previously (Pantůček et al., 1996). SmaI macrorestriction fragments were separated with a CHEF Mapper (Bio-Rad Laboratories, Hercules, CA) under the following conditions: 1.2% agarose in 1× TAE electrophoresis buffer (0.04 M Tris/acetate, 0.001 M EDTA, pH 8.2) at 14 °C, 6 V cm^{-1}, pulse times of 1–55 s for 24 h. Digitized gel images were analysed by means of the Bionumerics version 6.0.1 software (Applied Maths, Kortrijk, Belgium).

Plasmid DNA was isolated from the *etb* gene-positive strains using the High Pure Plasmid Isolation Kit (Roche Diagnostics) according to the manufacturer's protocol with a minute modification based on bacterial lysostaphin lysis (30 μg/mL, 20 min/37 °C). DNA was digested with the restriction endonucleases EcoRI, XbaI, and PstI (Roche Diagnostics). Restriction endonuclease analysis of plasmids (REAP) was used for plasmid size determination.

PCR experiments and sequence typing

The *eta*, *etb*, and *etd* genes were detected by a multiplex PCR amplification as described previously (Růžičková et al., 2005). The reaction conditions for the multiplex PCR detecting 4 types of prophages (A, B, Fa, and Fb) and the multiplex PCR targeting 6 types of ETA-B prophages were as described previously (Holochová et al., 2010a; Pantůček et al., 2004).

Multilocus sequence typing (MLST) was performed according to Enright et al. (2000). The detection of polymorphisms in the X region of the staphylococcal protein A (*spa*) gene was performed as previously described (Shopsin et al., 1999) with the forward primer spa-1095F and the reverse primer spa-1517R. The PCR assays were conducted using a T-Gradient Thermal Cycler (Biometra, Göttingen, Germany). PCR products of the *spa* gene and 7 housekeeping genes for MLST were sequenced at the sequencing facility of Eurofins MWG Operon (Ebersberg, Germany) using the PCR primers.

Gene sequence analysis

The *spa* gene sequences were analysed with the Ridom StaphType version 2.2.1 software (Ridom, Münster, Germany). The nucleotide sequences of the novel *spa* types t6644 and t6645 obtained in this study have been deposited in the GenBank database under accession numbers JQ247760 and JQ247761, respectively. The electronic Based Upon Related Sequence Types (eBURST v3) algorithm available online at http://www.eburst.mlst.net was used to define clonal complexes (CCs) within the *S. aureus* collection. Two novel sequence types ST2194 (3-27-1-1-239-1-10) and ST2195 (13-13-1-1-20-11-13) were identified in this study. The sequence of the new *pta* allele 239 has been deposited in the GenBank database under accession no. JQ247762.

Results and discussion

From 1998 to 2011, the Czech National Reference Laboratory for Staphylococci investigated 6257 strains of *S. aureus* within an ongoing ETA- and/or ETB-positive *S. aureus* surveillance study in various regions of the Czech Republic and the Slovak Republic. Of these strains, 730 were ET-positive 78.6% of which were ETA producers, 4.4% ETB producers, and 17% both ETA and ETB producers. Five extensive nosocomial outbreaks of pemphigus neonatorum associated with *S. aureus* infection occurred in 5 maternity hospitals (hospital numbers 9, 11, 16, 18, and 24 as denoted in Table 1). The clinical symptoms of the infection varied from blisters anywhere on the body to multiple lesions complicated by conjunctivitis, and 3 cases were SSSS.

For the MLST and other genotype investigations, 127 ET-positive strains were selected. Ninety-eight strains were isolated from children, and 3 of them were identified as a source of acute SSSS. The remaining 29 isolates originated from human carriers and/or hospital equipment. All strains were susceptible to oxacillin and cefoxitin. The origin and genotypic characteristics of the impetigo *S. aureus* strains studied are summarized in Table 1.

The *eta* gene appeared alone in 91 strains recovered mostly from childrens' blisters, nose, stool, and conjunctiva, mother breast, mother and nurse noses, and hospital equipment. The concomitant presence of the *eta* and *etb* genes was observed in 24 isolates while the *etb* gene alone was identified in 12 strains.

For CC assignment of the strains, we performed the MLST which has been used primarily to study the molecular epidemiology and evolution of MRSA clones. Deurenberg and Stobberingh (2008) reported an overview of the major non-MRSA clones observed worldwide, such as CC7, CC9, CC12, CC15, CC25, CC51, and CC101, but CC diversity of impetigo-associated *S. aureus* have not yet been

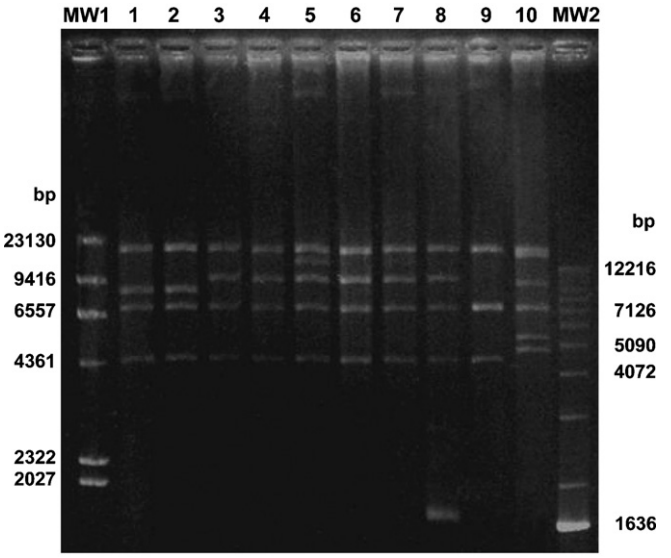

Fig. 2. Agarose gel electrophoresis of the EcoRI restriction fragments obtained from plasmid DNA of ET-producing strains. MW1, molecular weight marker Lambda-HindIII Digest (New England Biolabs); MW2, molecular weight marker MX (Roche Diagnostics, Germany). Lane 1, genotype G-7, plasmid profile pETB-1; lane 2, G-4, pETB-1; lane 3, G-8, pETB-2; lane 4, G-8, pETB-2; lanes 5–8, G-1, pETB-2; lane 9, G-6, pETB-3; lane 10, G-3, pETB-4. Genotypes are shown in Table 1.

completely known. Based on MLST analysis, the strains under study were grouped into 4 clonal complexes, i.e. CC121, CC15, CC9, and ST88 (CC88) as shown in Table 1 and Fig. 1.

A major clonal complex CC121, comprising exclusively ST121, included 47 strains isolated in 13 hospitals. The strains were classified into 8 genotypes. Based on analysis of SmaI macrorestriction fingerprints, 7 PFGE profiles could be distinguished in a set of CC121 strains. Sixteen strains collected in 4 hospitals were classified into the prevailing genotype G-4 which showed the unique PFGE profile D (Fig. 1). Clonal complex 121 as the only one included 12 strains which harboured the *etb* gene alone, located on the 38–41-kb plasmids showing 4 different REAP profiles (Fig. 2). The next prevailing G-8 (*spa* type t645) showed the same PFGE pattern as G-7 (*spa* type t159), and both harboured only the *etb* gene. The predominating *spa* type t159 was determined in 25 strains belonging to G-4, G-5, and G-7. Correlation between *spa* type t159 and CC121 affiliation was also determined in *S. aureus* strains investigated in Portugal recently (Conceição et al., 2011). Two ST121 strains, both isolated from a child's blisters were assigned to 2 not yet characterized *spa* types t6644 and t6645.

Thirty-nine CC15 strains were classified into sequence types ST15, ST582, and ST2195. The ST15 included 11 strains assigned to G-11 and 4 strains classified into G-10 which differed in the PFGE profile, lysotype, and *spa* type. ST582 comprised 19 strains classified into G-12 and G-13 differing in the PFGE fingerprints and the lysotype, but sharing the same *spa* type (t084). Five strains of the new ST2195 were identical in PFGE pattern and lysotype to those of G-10 and G-13. Surprisingly, 39 strains showing the PFGE fingerprints H, I, and J are highly similar to the SmaI pattern of ETA-producing *S. aureus* isolates reported by Mackenzie et al. (1995). The correspondence between the t084 *spa* type and affiliation to CC15 among non-MRSA strains has been previously shown (Argudín et al., 2009; Deurenberg and Stobberingh, 2008).

Among 24 CC9 strains tested, from which 9 were isolates from persons with nasal carriage, ST9 (7 isolates), ST109 (11 strains), and novel ST2194 (6 isolates) were determined. In 7 ST9 strains, the following genotypes were distinguished: G-14, G-15, G-16, and G-17. G-16 and G-17 (closely related to each other) are significantly different from G-18 and G-19. Strains assigned to the most frequently encountered genotype G-19 and those of G-18 (with novel ST2194) shared the PFGE pattern, lysotype, ETA-B6 prophage, and *spa* type.

Seventeen strains were assigned to ST88 (CC88). Seven isolates from 2 Czech hospitals belonged to genotype G-20. The remaining 10 strains collected in a Slovak hospital from 2003 to 2007 were classified into G-21. Their macrorestriction patterns were different from those of G-20 in 2 changes in the DNA fragments as shown in Fig. 1. Nevertheless, we consider the strains distributed into PFGE types N and O to be closely related members of ST88.

Most *S. aureus* strains are highly lysogenic (Goerke et al., 2009; Kahánková et al., 2010; Pantůček et al., 2004; Růžičková et al., 2003). Using the multiplex PCR (Pantůček et al., 2004), we distributed all strains into 7 lysogenic types (lysotypes) which harboured the following prophages: L1 (A, B, Fa), L2 (A, B, Fb), L3 (A, B), L4 (B), L5 (B, Fa), L6 (B, Fb), and L7 (Fb). The prevailing L6 covered strains belonging to ST121 and ST88. Types L1–6 harboured an *eta* gene-positive prophage B which is considered to be a major mobile element responsible for the dissemination of this gene among staphylococci.

Prophage carriage in the ETA-producing *S. aureus* strains has been well documented, and at least 6 different types of the ETA-converting phages have still been reported (Endo et al., 2003; Holochová et al., 2010a,b; Yamaguchi et al., 2000; Yoshizawa et al., 2000). Among the strains screened for the presence of *eta* gene-positive prophage B according to Holochová et al. (2010a), the most prevalent prophages ETA-B5 and ETA-B4 were detected (Table 1). The prophages ETA-B1, ETA-B2, ETA-B3, and ETA-B6 were evenly spread among the remaining strains.

Based on molecular analysis, ET-producing strains were grouped into 21 genotypes. Among the strains tested in this study, the most frequently found genotypes were the following: G-4/ST121 (in 4 hospitals), G-11/ST15 and G-19/ST109 (in 2 hospitals) followed by G-8/ST121 (in one hospital), G-13/ST582 (in 3 hospitals), and G-21/ST88 (in one hospital). Despite the adoption of a strong hygienic remedy, genotypes G-19 and G-21 continued to be detected in a hospital for 3 years. The geographically most widespread genotypes were G-1, G-4, and G-5, each being found in 4 hospitals. G-9 with novel ST2195 was only detected in 5 strains from hospital 5 and did not reemerge anywhere.

In this study, the genotyping methods applied to the characterization of 127 ET-positive strains allowed for their comparison at the molecular level. It has been shown previously that protein A (*spa*) gene typing in combination with another technique, in particular PFGE profiling, could be a highly discriminatory typing system for *S. aureus* (Růžičková et al., 2008; Shopsin et al., 1999). Our results proved *spa* typing to be an effective tool for increasing the accuracy of genotypic investigation of impetigo strains. As shown in Fig. 1, 4 PFGE-based clusters correspond to distinct CCs, each including related *spa* types. The differentiation of the closely related ETA-producing strains can be further refined by PCR detection of *eta* gene-positive prophages, as was confirmed previously (Holochová et al., 2010a).

We conclude that the causative ET strains are heterogeneous in their genotype patterns and did not have a recent common ancestor. The MLST approach was able to distinguish 4 clonal complexes among the strains studied, with the predominant CC121 occurring in 13 hospitals. This paper is the first report on the comprehensive genotypic characterization of the major lineages of ET-positive *S. aureus* strains in the Czech Republic contributing substantially to the knowledge of their genetic background and molecular epidemiology.

Acknowledgements

We would like to thank our colleagues in hospitals for providing us with *S. aureus* strains and epidemiological documentation.

We also thank E. Kodytkova for reviewing the manuscript. This work was supported by grants from the Czech Science Foundation (310/09/0459), Ministry of Education, Youth and Sports of the Czech Republic (MSM0021622415), and Internal Grant Agency of the Ministry of Health of the Czech Republic (NT12395-5/2011).

References

Adesiyun, A.A., Lenz, W., Schaal, K.P., 1991. Exfoliative toxin production by *Staphylococcus aureus* strains isolated from animals and human beings in Nigeria. Microbiologica 14, 357–362.

Argudín, M.A., Mendoza, M.C., Méndez, F.J., Martín, M.C., Guerra, B., Rodicio, M.R., 2009. Clonal complexes and diversity of exotoxin gene profiles in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from patients in a Spanish hospital. J. Clin. Microbiol. 47, 2097–2105.

CLSI, 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-first Informational Supplement. M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.

Conceição, T., Aires-de-Sousa, M., Pona, N., Brito, M.J., Barradas, C., Coelho, R., Sardinha, T., Sancho, L., de Sousa, G., Machado Mdo, C., de Lencastre, H., 2011. High prevalence of ST121 in community-associated methicillin-susceptible *Staphylococcus aureus* lineages responsible for skin and soft tissue infections in Portuguese children. Eur. J. Clin. Microbiol. Infect. Dis. 30, 293–297.

Cribier, B., Piemont, Y., Grosshans, E., 1994. Staphylococcal scalded skin syndrome in adults. A clinical review illustrated with a new case. J. Am. Acad. Dermatol. 30, 319–324.

Dave, J., Reith, S., Nash, J.Q., Marples, R.R., Dulake, C., 1994. A double outbreak of exfoliative toxin-producing strains of *Staphylococcus aureus* in a maternity unit. Epidemiol. Infect. 112, 103–114.

de Azavedo, J., Arbuthnott, J.P., 1981. Prevalence of epidermolytic toxin in clinical isolates of *Staphylococcus aureus*. J. Med. Microbiol. 14, 341–344.

Deurenberg, R.H., Stobberingh, E.E., 2008. The evolution of *Staphylococcus aureus*. Infect. Genet. Evol. 8, 747–763.

Endo, Y., Yamada, T., Matsunaga, K., Hayakawa, Y., Kaidoh, T., Takeuchi, S., 2003. Phage conversion of exfoliative toxin A in *Staphylococcus aureus* isolated from cows with mastitis. Vet. Microbiol. 96, 81–90.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J., Spratt, B.G., 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. 38, 1008–1015.

Gemmell, C.G., 1995. Staphylococcal scalded skin syndrome. J. Med. Microbiol. 43, 318–327.

Goerke, C., Pantůček, R., Holtfreter, S., Schulte, B., Zink, M., Grumann, D., Broker, B.M., Doskar, J., Wolz, C., 2009. Diversity of prophages in dominant *Staphylococcus aureus* clonal lineages. J. Bacteriol. 191, 3462–3468.

Holochová, P., Růžičková, V., Dostálová, L., Pantůček, R., Petráš, P., Doškař, J., 2010a. Rapid detection and differentiation of the exfoliative toxin A-producing *Staphylococcus aureus* strains based on phiETA prophage polymorphisms. Diagn. Microbiol. Infect. Dis. 66, 248–252.

Holochová, P., Růžičková, V., Pantůček, R., Petráš, P., Janisch, R., Doškař, J., 2010b. Genomic diversity of two lineages of exfoliative toxin A-converting phages predominating in *Staphylococcus aureus* strains in the Czech Republic. Res. Microbiol. 161, 260–267.

Kahánková, J., Pantůček, R., Goerke, C., Růžičková, V., Holochová, P., Doškař, J., 2010. Multilocus PCR typing strategy for differentiation of *Staphylococcus aureus* siphoviruses reflecting their modular genome structure. Environ. Microbiol. 12, 2527–2538.

Kondo, I., Sakurai, S., Sarai, Y., Futaki, S., 1975. Two serotypes of exfoliatin and their distribution in staphylococcal strains isolated from patients with scalded skin syndrome. J. Clin. Microbiol. 1, 397–400.

Mackenzie, A., Johnson, W., Heyes, B., Norrish, B., Jamieson, F., 1995. A prolonged outbreak of exfoliative toxin A-producing *Staphylococcus aureus* in a newborn nursery. Diagn. Microbiol. Infect. Dis. 21, 69–75.

O'Toole, P.W., Foster, T.J., 1987. Nucleotide sequence of the epidermolytic toxin A gene of *Staphylococcus aureus*. J. Bacteriol. 169, 3910–3915.

Ozaki, K., Takano, M., Higuchi, W., Takano, T., Yabe, S., Nitahara, Y., Nishiyama, A., Yamamoto, T., 2009. Genotypes, intrafamilial transmission, and virulence potential of nasal methicillin-resistant *Staphylococcus aureus* from children in the community. J. Infect. Chemother. 15, 84–91.

Pantůček, R., Doškař, J., Růžičková, V., Kašpárek, P., Oráčova, E., Kvardová, V., Rosypal, S., 2004. Identification of bacteriophage types and their carriage in *Staphylococcus aureus*. Arch. Virol. 149, 1689–1703.

Pantůček, R., Götz, F., Doškař, J., Rosypal, S., 1996. Genomic variability of *Staphylococcus aureus* and the other coagulase-positive *Staphylococcus* species estimated by macrorestriction analysis using pulsed-field gel electrophoresis. Int. J. Syst. Bacteriol. 46, 216–222.

Růžičková, V., Karpíšková, R., Pantůček, R., Pospíšilová, M., Černíková, P., Doškař, J., 2008. Genotype analysis of enterotoxin H-positive *Staphylococcus aureus* strains isolated from food samples in the Czech Republic. Int. J. Food Microbiol. 121, 60–65.

Růžičková, V., Pantůček, R., Petráš, P., Doškař, J., Sedláček, I., Rosypal, S., 2003. Molecular typing of exfoliative toxin-producing *Staphylococcus aureus* strains involved in epidermolytic infections. Int. J. Med. Microbiol. 292, 541–545.

Růžičková, V., Voller, J., Pantůček, R., Petráš, P., Doškař, J., 2005. Multiplex PCR for detection of three exfoliative toxin serotype genes in *Staphylococcus aureus*. Folia Microbiol. 50, 499–502.

Saiman, L., Jakob, K., Holmes, K.W., Whittier, S., Garzon, M.C., Rago, J.V., Schlievert, P.M., Della-Latta, P., 1998. Molecular epidemiology of staphylococcal scalded skin syndrome in premature infants. Pediatr. Infect. Dis. J. 17, 329–334.

Shi, D., Higuchi, W., Takano, T., Saito, K., Ozaki, K., Takano, M., Nitahara, Y., Yamamoto, T., 2011. Bullous impetigo in children infected with methicillin-resistant *Staphylococcus aureus* alone or in combination with methicillin-susceptible *S. aureus*: analysis of genetic characteristics, including assessment of exfoliative toxin gene carriage. J. Clin. Microbiol. 49, 1972–1974.

Shopsin, B., Gomez, M., Montgomery, S.O., Smith, D.H., Waddington, M., Dodge, D.E., Bost, D.A., Riehman, M., Naidich, S., Kreiswirth, B.N., 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J. Clin. Microbiol. 37, 3556–3563.

Warren, R.L., 1980. Exfoliative toxin plasmids of bacteriophage group 2 *Staphylococcus aureus*: sequence homology. Infect. Immun. 30, 601–606.

Yamaguchi, T., Hayashi, T., Takami, H., Nakasone, K., Ohnishi, M., Nakayama, K., Yamada, S., Komatsuzawa, H., Sugai, M., 2000. Phage conversion of exfoliative toxin A production in *Staphylococcus aureus*. Mol. Microbiol. 38, 694–705.

Yamaguchi, T., Hayashi, T., Takami, H., Ohnishi, M., Murata, T., Nakayama, K., Asakawa, K., Ohara, M., Komatsuzawa, H., Sugai, M., 2001. Complete nucleotide sequence of a *Staphylococcus aureus* exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. Infect. Immun. 69, 7760–7771.

Yamasaki, O., Yamaguchi, T., Sugai, M., Chapuis-Cellier, C., Arnaud, F., Vandenesch, F., Etienne, J., Lina, G., 2005. Clinical manifestations of staphylococcal scalded-skin syndrome depend on serotypes of exfoliative toxins. J. Clin. Microbiol. 43, 1890–1893.

Yoshizawa, Y., Sakurada, J., Sakurai, S., Machida, K., Kondo, I., Masuda, S., 2000. An exfoliative toxin A-converting phage isolated from *Staphylococcus aureus* strain ZM. Microbiol. Immunol. 44, 189–191.