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

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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

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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