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SHORT REPORT
Carriage of virulence factors and molecular characteristics of *Staphylococcus aureus* isolates associated with bloodstream, and skin and soft tissue infections in children

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SUMMARY

We investigated the virulence gene carriage and molecular type characteristics of *Staphylococcus aureus* isolates from bloodstream infections (BSIs) and skin and soft tissue infections (SSTIs) in children. A total of 71 isolates, 16 of which were methicillin-resistant *S. aureus* (MRSA), were investigated by PCR for virulence-associated gene profiles, sequence type and *spa* type. This revealed that 76·7% and 53·7% of the SSTI and BSI isolates, respectively, exhibited simultaneous carriage of $\geqslant$10 virulence genes. Compared to BSI isolates, carriage rates for *hla*, *hlb*, *cna*, *clfA*, *seb*, *sec* and *pvl* genes were significantly higher in SSTI isolates. By contrast, carriage of *eta*, *etb* and *sea* was significantly higher for BSI isolates. Thirty-four sequence types (STs) and 36 *spa* types were identified in the 71 isolates and included 14 novel STs and four novel *spa* types. ST59-MRSA-IV/V-t437 was the most common clone in the MRSA isolates. We concluded that virulence determinants are widely distributed in isolates of *S. aureus* strains from children with BSIs and SSTIs, with an unexpectedly high rate in SSTI isolates. Future profiling of *S. aureus* virulence determinants may allow the prediction of severity and outcome for children with these infections.

Key words: Bloodstream infection, molecular typing, skin and soft tissue infection, *Staphylococcus aureus*, virulence genes.

Staphylococcus aureus is responsible for a wide range of severe infections, including skin and soft tissue infection (SSTI), device-associated infection, pneumonia, septic arthritis, endocarditis, osteomyelitis, sepsis, and bloodstream infection (BSI). The incidence of severe *S. aureus* infections in children in the UK has increased during the past three decades, with the incidence of methicillin-resistant *S. aureus* (MRSA) bacteraemia in infants rising from 1% to

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15% by the turn of the century [1]. This experience is mirrored worldwide and the resulting higher mortality and longer hospital stays associated with such infections has stirred global concern.

Superantigens which mediate colonization of the host, invasion of damaged skin and mucosa, dissemination through the body, and evasion of host defence mechanisms are important virulence factors for *S. aureus* [2]. These include exfoliative toxins, toxic shock syndrome toxin-1, and the staphylococcal enterotoxins (SEs) [3]. In addition to the five classic SEs (Sea Seb, Sec, Sed, See), several other microbial surface components have been described including fibrinogen-binding proteins (ClfA, ClfB), Sdr protein family, and collagen-binding proteins which are responsible for initial contact with host cells and adhesion of staphylococcal cells to the extracellular matrix of these cells [4].

Molecular methods for strain typing [e.g. pulsed-field gel electrophoresis, *spa* typing, multilocus sequence typing (MLST)] have provided better insight into the epidemiology of *S. aureus* and monitoring the evolution of pandemic MRSA clones [5]. In addition, PCR-based techniques have allowed the identification of a large number of virulence-associated genes and methicillin resistance gene cassettes. We used a selection of these techniques to determine the rates of carriage of virulence genes and strain genetic diversity in *S. aureus* isolates from SSTIs and BSIs in children attending two provincial hospital centres in China.

A total of 71 *S. aureus* isolates was recovered from children aged 0–7 years with BSIs and SSTIs from January 2010 to December 2010 in either Jiangxi Provincial Children's Hospital, Nanchang, central China (69 isolates) or the First Affiliated Hospital of Wenzhou Medical College, Wenzhou, eastern China (two isolates). Forty-one isolates were from BSIs, and 30 from SSTIs. SSTI was identified by the presence of erythema and swelling, localized pain, and/or fever. In order to limit the possibility that *S. aureus* isolates represented skin colonization or culture contamination, only the isolates from patients with increased white blood cell count and fever were included. Impetigo was the most common presentation for SSTI (50%), followed by cellulitis (20%), abscesses (16·7%) and wound infections (13·3%). SSTI was located predominantly on the trunk (73·3%), and three patients with SSTI developed bacteraemia.

S. aureus was identified by Gram stain, positive catalase and coagulase test results, and fully-automated Vitek microbiology analysis (bioMérieux, France). *S. aureus* ATCC25923 was used as a control strain. All isolates were digested with lysostaphin (1 mg/ml) (Sangon, China) at 37 °C for 1 h and DNA was extracted using the Genomic DNA Extraction kit (Sangon) and stored at −20 °C. The *mecA*, and *pvl* genes were detected using a PCR protocol described in our previous study [5]. Isolates positive for *mecA* gene were confirmed as MRSA using MRSA N315 as control strain. A MRSA isolate harbouring *pvl* identified in our previous study was used as a positive control for detection of *pvl* carriage [5]. Twenty-one virulence genes, including superantigen genes (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *tst*, *eta*, *etb*), adhesion genes (*clfA*, *cna*, *sdrC*, *sdrD*, *sdrE*), *icaA, arcA, hla* and *hlb* were detected using PCR assays with primers and conditions as described previously [5]. *S. aureus* isolates of known virulence gene profile [5] were used as positive control strains. SCC*mec* typing was performed using a multiplex PCR as described previously [6], and MRSA isolates with unexpected fragment patterns were defined as non-typable (NT). MRSA isolates with suspected SCC*mec*III or SCC*mec*V elements were re-characterized using the multiplex PCRs (M-PCR1 and M-PCR2) described by Kondo *et al*. [7]. Strains NCTC 10442 (SCC*mec*I), N315 (SCC*mec*II), 85/2082 (SCC*mec*III), JCSC 4744 (SCC*mec*IV) and WZ153 (SCC*mec*V) were used as reference strains for SCC*mec* typing. The *spa* variable repeat region from each *S. aureus* isolate was amplified as described previously [8] and *spa* types were assigned using the database website (http://www.ridom.de/spaserver). MLST was performed as described previously [9] and DNA sequences were compared with known alleles from the MLST database (http://saureus.mlst.net). STs were identified according to allelic patterns and novel types were deposited in the MLST database. Statistical differences between groups were determined by χ^2 test using SPSS v. 11.0 (SPSS Inc., USA), and $P<0{\cdot}05$ was considered statistically significant.

Sixteen (22·5%, 16/71) isolates positive for *mecA*, including 10 BSI isolates (24·4%, 10/41) and six SSTI isolates (20·0%, 6/30) were identified as MRSA. SCC*mec* types II, III, IV and V accounted for 1, 2, 5, and 8 of MRSA isolates, respectively.

The invasive potential of *S. aureus* is defined on a molecular basis by carriage of a battery of virulence determinants associated with adhesion, acquisition of nutrients, and evasion of host immunological responses [10]. The majority (63·4%) of all *S. aureus* isolates, comprising 76·7% and 53·7% of SSTI and

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Table 1. *Frequency (%) of virulence genes in* S. aureus *isolates from bloodstream infections (BSI) and skin and soft tissue infections (SSTI) in children*

Virulence gene	*S. aureus*	BSI	SSTI	*P* value[a]
icaA	97·2	95·1	100	>0·05
sdrC	98·6	100	96·7	>0·05
sdrD	47·9	56·1	36·7	>0·05
sdrE	63·4	61·0	66·7	>0·05
hla	88·7	80·5	100	<0·05
hlb	83·1	73·2	96·7	<0·05
cna	36·6	19·5	60·0	<0·05
clfA	81·7	70·7	96·7	<0·05
arcA	0	0	0	
eta	19·7	31·7	3·3	<0·05
etb	11·3	19·5	0	<0·05
sea	22·5	39·0	0	<0·05
seb	76·1	63·4	93·3	<0·05
sec	25·4	14·6	40·0	<0·05
sed	18·3	24·4	10·0	>0·05
see	0	0	0	
seg	74·6	82·9	63·3	>0·05
seh	1·4	1	0	
sei	80·3	78·0	83·3	>0·05
sej	12·7	19·5	3·3	>0·05
tst	0	0	0	
pvl	66·2	51·2	86·7	<0·05

BSI isolates, respectively, harboured ⩾10 virulence genes and this difference was statistically significant ($P<0·05$). SSTI isolates were also characterized by more diverse virulence gene profiles. The percentage frequencies for virulence genes in all isolates are listed in Table 1. All isolates harboured more than five tested virulence genes. At least one enterotoxin gene was carried by all but two (97·2%) of the tested isolates. All tested virulence genes except *arcA, tst* and *see* were detected. Eight genes (*icaA*, *sdrC*, *hla*, *hlb*, *clfA*, *seb*, *seg*, *sei*) were identified in about 75% of isolates and seven genes (*eta*, *etb*, *sea*, *sec*, *sed*, *seh*, *sej*) were found in ⩽25% isolates. Compared with BSI isolates, the carriage rates for *hla*, *hlb*, *cna*, *clfA*, *seb*, *sec* and *pvl* genes in SSTI isolates were significantly higher, while those of *eta*, *etb* and *sea* were significantly lower. SSTI isolates consistently lacked *etb*, *sea* and *seh*, and these genes were found only in BSI isolates. The frequency of *pvl* gene was significantly higher in SSTI isolates (86·7%) than in BSI isolates (51·2%, $P<0·05$).

The carriage of *pvl* by *S. aureus* is strongly associated with SSTIs [11], and the rates found here markedly exceeded its frequency of 23% in both SSTI and BSI isolates from adults reported in our earlier studies [5, 12]. This suggests that PVL toxin may be a particularly important virulence factor for *S. aureus* infections arising in children. The enterotoxin Sea triggers overexpression of inflammatory mediators associated with shock, and *sea* carriage correlates with severity of infection by *S. aureus* [13]. It is interesting that in the present study *sea* was only detected in BSI isolates in contrast to the high frequency (93·4%) of the *seb* gene in SSTI isolates. This may indicate that enterotoxins contributing to invasive *S. aureus* infections in children may differ in function from those contributing to adult infections. The Sdr proteins encoded by the tandemly arrayed *sdrC*, *sdrD*, and *sdrE* genes have different roles in *S. aureus* pathogenicity [14]. The majority of isolates from both groups of infections in children possessed more than two *sdr* loci, with *sdrC* (98·4%) being the most prevalent.

Molecular characterization of *S. aureus* isolates is necessary for understanding the evolutionary mechanisms and geographical spread of the organism. MRSA isolates from different geographical areas have demonstrated considerable genetic diversity. In this study 34 different STs were identified, of which ST120 (11·3%, 8/71) and ST25 (11·3%, 8/71) were the most prevalent, followed by ST121 (9·9%, 7/71), ST88 (8·5%, 6/71), ST59 (8·5%, 6/71) and ST6 (7·0%, 5/71). ST15 and ST188 also accounted for three and two isolates, respectively. All novel STs and 12 other STs were represented by single isolates only. Thirteen novel STs including ST2201, ST2202, ST2203, ST2204, ST2206, ST2207, ST2208, ST2209, ST2210, ST2211, ST2212, ST2213 and ST2214, were identified in BSI isolates but only a single novel type (ST2424) was found in the SSTI isolates. BSI isolates were markedly more genetically diverse than those from SSTI isolates (30 STs *vs.* nine STs); ST6 (five isolates) and ST121 (seven isolates), respectively were exclusively associated with BSI and SSTI isolates.

The genetic diversity of the isolates was confirmed by the finding of 36 *spa* types in the 71 tested, four of which were novel (t9518, t10738, t10739, t10740). t437 was the most prevalent type and accounted for 14·1%, followed by t159 (12·7%), t189 (7·0%) and t349 (5·6%); other *spa* types accounted for 1–3 isolates. Types t437 and t189 were the most common in BSI isolates while t159 was the most prevalent in SSTI isolates. All t189 isolates were from BSIs and 8/9 t159 isolates were from SSTIs. Five, six, five and four *spa* types were found in eight ST120, eight ST25, six ST88 and five ST6 isolates, respectively. *spa* types t159,

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t170 and t2019 accounted for five, one and one ST121 isolates while type t437 was identified in six ST59 isolates. In addition to ST59 one isolate each of four STs were identified in 10 t437 isolates. ST120 (three isolates), ST121 (five isolates) and ST2213 (one isolate), were all *spa* typed as t159. With regard to clones of the 16 MRSA isolates, five were MRSA-ST59- SCC*mec*VI/V-t437-*pvl*(+), comprising four SSTI isolates and one BSI isolate. Of the remaining 55 MSSA isolates, ST25 (14·5%) was the most prevalent, followed by ST120 and ST121, accounting for 12·7% each.

In summary, the molecular characteristics of *S. aureus* isolates from children with BSIs and SSTIs showed considerable genetic heterogeneity. Furthermore, several STs identified in children such as ST6, ST121 and ST120 are comparatively rare in China as the most predominant clone of CA-MRSA previously identified in SSTIs of children in Beijing was ST59-MRSA-SCC*mec*IVa-t437 which accounted for 6/14 infections [15]. We identified the latter clone in 5/16 MRSA isolates and although the small numbers of isolates in both this and the Beijing studies preclude firm conclusions being made, nevertheless the data underline the association of diverse genetic types of *S. aureus* with different infections.

In conclusion, we found a high carriage rate for multiple virulence genes in *S. aureus* BSI and SSTI isolates from children, particularly SSTI isolates which harboured more and had greater heterogeneity of virulence determinants than BSI isolates. We conclude that virulence gene carriage and molecular type characteristics differ for *S. aureus* isolates associated with BSIs and SSTIs in children, and that unique virulence factors may play a role in invasive *S. aureus* infections in children compared to adults.

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DECLARATION OF INTEREST

None.

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