Molecular analysis of community-acquired methicillin-susceptible and resistant *Staphylococcus aureus* isolates recovered from bacteraemic and osteomyelitis infections in children from Tunisia

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Abstract

Thirty-six children (27 boys, nine girls) that fulfilled CDC criteria for community-acquired infections were diagnosed with bacteraemia and/or osteomyelitis caused by *Staphylococcus aureus* during an 18-month period (2006–2008). Antibiotic susceptibility was determined by an agar dilution method. SCC*mec* type, carriage of *pvl* genes, *agr* type and *spa*-typing were determined using specific PCR protocols. Clonal relatedness was examined by pulsed field gel electrophoresis-*Sma*I and mutilocus sequence typing techniques. From the 36 isolates, eight (22%) corresponded to methicillin-resistant *Staphylococcus aureus* (MRSA) -*t*044/042-CC80/CC5-IVc-*pvl*$^+$-*agr*III/II. The highest genetic diversity was observed among the 28 community-acquired methicillin-susceptible *S. aureus* (CA-MSSA) isolates: 22 *spa*-variants that also grouped by multilocus sequence typing in CC1, CC5, CC6, CC8, CC30, CC80, CC97 and the singletons ST464, ST1467, ST1468 and ST1469. The *pvl* genes were detected in all eight CA-MRSA isolates and in eight CA-MSSA isolates (28%), being significantly more frequent among isolates causing osteoarticular infection (11 of 12, 92%) than in the bacteraemic isolates (six of 24, 25%). Based on patients' age, three groups were considered: newborns, infants and children. Bacteraemia was diagnosed in all newborns and infants, whereas in 42% of the children group osteomyelitis was the unique presentation. In most cases, the portal of entry was either the skin or unknown. In general, favourable outcome was observed, except in four cases—three of whom had severe complications and one died. In summary, we analysed the epidemiological and genetic background of community-acquired staphylococcal strains causing bacteraemic and/or osteomyelitis infections in children from Tunisia, describing three new sequence types and one novel *spa* type.

Keywords: Bacteraemia, clinical evolution, multilocus sequence typing, osteomyelitis, *pvl*, *spa*-typing
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Introduction

Infections caused by community-acquired *Staphylococcus aureus*, particularly methicillin-resistant, are increasing worldwide. An important characteristic of these isolates is their constant evolution to adapt virulence traits so continuous surveillance is needed [1]. Most of the community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infections usually present minor severity. However, serious manifestations with major clinical relevance, most of them linked to the presence of the *pvl* genes, including necrotizing fasciitis, pyomyositis, osteoarticular infections and pneumonia, have been described. CA-MRSA has replaced its methicillin-susceptible *S. aureus* (CA-MSSA) counterpart as the major cause of skin and soft tissue infections [2–4]. Additionally, the presence of the *pvl* genes among isolates causing acute osteomyelitis in children has been related to high inflammatory responses [5].

CA-MRSA usually has a particular epidemiology, the most successful lineage being USA300 [1]. Different studies have analysed the epidemiology of invasive infections caused by CA-MRSA, especially in developed countries, but few of

them focused on CA-MSSA despite this variant being possibly be more invasive [6,7]. A replacement in the CA-MSSA-*pvl*$^+$ lineages has been described [7].

Tunisia has one of the lowest rates of MRSA prevalence among Mediterranean countries, where the rate is generally high [8]. However, in our Children's Hospital of Tunis (Tunisia), *S. aureus* is the major pathogen (52%) causing bacteraemia and osteoarticular infections in previously healthy children (Hariga D, Smaoui H, Bouziri A, Hôpital d'Enfants de Tunis; Trifa M., Bouchoucha S., Smaoui H., Kechrid A., Microbiologic profile of acute hematogenous osteoarticular infections in children submitted for publication). The aim of this work was to establish the epidemiology of both CA-MRSA and CA-MSSA isolates causing bacteraemia or osteomyelitis in children from Tunisia, and to characterize the genetic background of these isolates.

Material and Methods

Bacterial strains

Thirty-six consecutive *S. aureus* isolates recovered from children with community-acquired invasive infection (September 2006–March 2008) attending the Children's Hospital of Tunis (Tunisia) were included (Table 1). This centre is a public, teaching hospital for paediatric patients, providing tertiary care for Tunis and surrounding areas. The hospital has 322 beds, admitting patients of all ages. In 2008 the paediatric population served by our hospital was 576 000, and during the period of the study the number of patients admitted to our institution was 30539. Clinical and epidemiological data were prospectively obtained. Infections were classified as community-acquired following the CDC criteria, i.e. detection

TABLE 1. Characteristics of newborns, infants and children, infection site, antibiotic treatment and staphylococcal strains included in this study

MLST (CC-ST)	*spa*-typing (CC-t)	PFGE	Strain	*mec*	*agr*	*pvl*	Resistance	Age	Sex	Portal of entry[a]	Infection	Treatment	Outcome[b]
Newborns													
CC80-ST728	CC359/224- t2883	7	9235	–	I	–		36 days	Male		B	Cf	
CC80-ST728	CC359/224-t189	7	664	–	I	–		52 days	Male		B	Cf+Gm	
CC80-ST728	CC359/224-t044	Ib	19 201	IVc	III	+	Km	58 days	Male	Skin	B	Ox+Gm	
ST464	CC359/224-t224	21	621	–	I	–	Km	10 days	Male		B	Ox+Gm	
CC5-ST8	CC062-t062	2	1287	IVc	II	+	Te, Tb	4 days	Male		B	Tc+Gm	
CC5-ST5	Singleton-t311	17	18 343	–	II	–	Te, Er	34 days	Male	Auricular	B	A/C	
Infants													
CC80- ST728	CC359/224-t044	Ia	10032	IVc	III	+	Te	8 months	Male		B	Vc+Gm	Surgery
CC80- ST728	CC359/224-t044	25	17489	–	III	+		1 year	Male		B	Ox+Gm	
CC80- ST728	CC359/224-t044	25	24422	–	III	+	Rf	2 years	Male	Skin	B	Ox+Gm	
CC1-ST1	Singleton-t127	13	15189	–	III	–	Er	5 months	Male		B	Ox+Gm	
CC1-ST199	t605[c]	15	7987	–	II	–	Er	2 years	Male		B	Ox+Gm	Dead
CC30-ST30	CC318-t318	3	18609	–	III	+		4 months	Male	Respiratory	B	Ox+Gm	
CC6-ST932	Cluster 4-t701	8	11632	–	I	–		5 months	Male	Skin	B	Ox+Gm	
ST1469[c]	Singleton-t903	11	15631	–	II	+		2 years	Male		B+O Hip	Ox+Gm	
ST1468[c]	Singleton-t2313	14	10282	–	I	–		2 years	Female		B+O Tibia	Fo+Cf	
Children													
CC80-ST728	CC359/224-t042	Ia	17366	IVc	III	+		8 years	Male		O Tibia	Tc+Gm	
CC80-ST728	CC359/224-t044	Ia	4931	IVc	III	+	Er, Km	13 years	Female		O Tibia-Femur	Tc+Py	Necrosis femur
CC80-ST728	CC359/224-t044	Ic	13452	IVc	III	+	Km	14 years	Male		B+O	Tc+Gm	
CC80-ST728	CC359/224-t044	Id	1109	IVc	III	+	Km	11 years	Male		O Ankle	Tc+Py	
CC80-ST728	CC359/224-t044	Ie	19913	IVc	III	+	Er, Km	8 years	Male		O	Tc+Gm	
CC80-ST728	Singleton t1951-	4	10198	–	III	+		11 years	Female		O Femur	Ox+Gm	
CC1-ST772	CC318-t3634	5	315	–	III	+		10 years	Male		O Femur	Ox+Gm	Sepsis
CC1-ST772	CC318-t113	5	18242	–	III	+	Te	10 years	Female		O Tibia	Ox+Gm	
CC1-ST772	CC318-t021	5	23522	–	III	+		7 years	Male		O Femur	Ox+Gm	Chronic O
CC1-ST1	Singleton-t127	10	23617	–	III	–		5 years	Male	Respiratory	B	Ox+Gm	
CC5-ST5	CC062-t5427	16	7141	–	II	–		8 years	Male		B	Ox+Gm	
CC5-ST5	CC062-t688	12	15459	–	II	–	Te, Cl	10 years	Male	Skin	B	A/C	
CC5-ST125	Singleton-t067	24	10015	–	III	–		13 years	Male		B	Ox+Gm	
CC6-ST932	Cluster 4-t701	23	3410	–	I	–	Er	3 years	Female	Skin	B	Ox+Gm	
CC6-ST932	Cluster 4-t701	22	6354	–	I	–		11 years	Female		B	Cf+Gm	Cardiopathy
CC8-ST8	Singleton-t1705	19	412	–	I	–		6 years	Female	Skin	B	Ox+Gm	
CC8-ST770	Singleton-t068	19	2140	–	I	–	Te	3 years	Female	Urinary	B	Cf+Fo	
CC30-ST30	CC318-t021	6	1718	–	III	–	Er	4 years	Male	Skin	O Hip	Ox+Gm	
CC30-ST30	CC318-t021	6	2140	–	III	–	Te, Er	11 years	Female	Respiratory	B	Ox+Gm	
CC97-ST97	CC359/224-t359	20	1016	–	I	–	Er	11 years	Male		B	Ox+Gm	
ST1467[c]	Cluster 4-t304	18	199	–	I	–	Er	5 years	Male	Skin abscess	B	Ox-Gm	

MLST, multilocus sequence typing; PFGE, pulsed field electrophoresis; CC, clonal complex; B, bacteraemia; O, osteomyelitis; Ox, oxacillin; Gm, gentamicin; Tc, teicoplanin; Cf, cefotaxime; Vc, vancomycin; A/C, amoxicillin/clavulanate; Fo, fosfomycin; Py, pristinamycin; Km, kanamycin; Te, tetracycline; Er, erythromycin; Tb, tobramycin; Cl, chloramphenicol.
[a]Only known portals are described.
[b]Only non-favourable outcome is reported.
[c]Newly described.