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SHORT REPORT
Clonal structure of *Staphylococcus aureus* colonizing children with sickle cell anaemia and healthy controls

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SUMMARY

Children with sickle cell anaemia (SCA) might carry hospital-associated bacterial lineages due to frequent hospital stays and antibiotic treatments. In this study we compared *Staphylococcus aureus* from SCA patients ($n=73$) and healthy children ($n=143$) in a cross-sectional study in Gabon. *S. aureus* carriage did not differ between children with SCA ($n=34$, 46·6%) and controls matched for age, residence and sex ($n=67$, 46·9%). Both groups shared similar *S. aureus* genotypes. This finding points towards a transmission of *S. aureus* between both groups in the community. We conclude that resistance rates from population-based studies with healthy participants could therefore also be used to guide treatment and prophylaxis of endogenous infections in children with SCA despite a different selection pressure.

Key words: Bacterial typing, *Staphylococcus aureus*, surveillance.

Sickle cell anaemia (SCA) is a hereditary disorder of the beta-globulin which affects about 1% of all newborns in Africa [1]. SCA patients suffer from hyposplenism due to hypoperfusion of the spleen [2]. This predisposes to infections with encapsulated bacteria such as *Streptococcus* (*Str.*) *pneumoniae*, *Haemophilus influenzae* or *Staphylococcus* (*S.*) *aureus* [3]. Antibiotic prophylaxis is therefore recommended to reduce mortality and morbidity [4]. Despite this selection pressure, we recently reported that resistance rates and capsular types of *Str. pneumoniae*, *H. influenzae* and *S. aureus* did not differ between children with SCA and healthy controls [3]. This might be due to a similar genetic background of these isolates. To test this hypothesis we compared genotypes of *S. aureus* isolated from this cohort and associated certain lineages with Panton–Valentine leukocidin (PVL) which is highly prevalent in Gabon [5].

Ethical approval was received from the regional ethics committee, Lambaréné (CERIL). Legal guardians of the children provided a signed documented informed consent prior to enrolment.

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Children with SCA and controls were recruited in a 1:2 ratio. SCA patients ($n=73$) who attended the regular consultation for SCA at the Albert Schweitzer Hospital, Lambaréné, Gabon were enrolled. Healthy controls ($n=143$) were subsequently screened for the matching criteria of age (± 2 years), residence and sex. If we failed to find children meeting all matching criteria we gave priority to age over residence and residence over sex. For two SCA patients we did not find any matched controls and for one SCA patient only one control was enrolled. Exclusion criteria were (i) signs of any type of infection and (ii) blood transfusion for children with unknown sickle cell state in the past 3 months. The two study groups (SCA patients *vs.* healthy controls) have been described recently and are comparable in terms of mean age (7·43 *vs.* 7·44 years), proportion of females (42·5% *vs.* 47·6%) and residence (urban 4·1 *vs.* 3·5%, semi-urban 82·2% *vs.* 81·1%, rural 13·7 *vs.* 15·4%) [3].

A standardized questionnaire on a patient's history and risk factors for *S. aureus* carriage [age; frequency of hand washing; passive smoking; crowding and sanitation; parents' education, categorized as 'primary school' (école élémentaire), 'junior high' (collège) and 'high school/college' (lycée/université)] was applied (see Supplementary online material).

Nasal and pharyngeal swabs were cultured onto blood agar plates with an aztreonam disc (Oxoid, UK) for 18–24 h. Colonies were tested for positive catalase reaction and latex agglutination test (Pastorex Staph-Plus; Bio-Rad Laboratories, France). PCR was applied to confirm *S. aureus* species by *nuc* detection and to detect the PVL encoding genes (*luk S-PV*/*luk F-PV*) [5].

S. aureus protein A typing (*spa* typing) was performed for each isolate [6]. If a participant was colonized at different sites with *S. aureus* isolates belonging to the same *spa* type, we only included one isolate in the analysis to exclude the bias of multiple samples. Multilocus sequence typing (MLST) was performed exemplarily for one randomly selected isolate of each *spa* type [7]; sequence types (ST) and clonal complexes (CC) were assigned in accordance to the *S. aureus* MLST database (http://saureus.mlst.net).

We compared categorical data using the χ^2 test or Fisher's exact test and calculated the odds ratio (OR) with the 95% confidence interval (CI). To analyse confounders of risk factors for *S. aureus* carriage, all categorical variables with a P value $<0·2$ were used for logistic regression analysis with a stepwise backward elimination. The level of statistical significance was set at $P<0·05$. Mean values of continuous variables were compared using Student's t test. All analyses were performed with 'R' (http://cran.r-project.org) and the package 'epicalc'.

The proportion of participants that suffered from various infectious diseases during the 12 months prior to enrolment, according to the personal health record file, was significantly higher in SCA patients than in healthy controls with respect to pneumonia (50·7% *vs.* 2·2%, OR 46·6, 95% CI 13·3–243·3, $P<0·005$), urinary tract infection (6·9% *vs.* 0·7%, OR 10·2, 95% CI 1·1– 482·1, $P<0·02$), and skin and soft tissue infection (12·3% *vs.* 4·9%, OR 2·7, 95% CI 0·9–9·0, $P=0·48$). This was reflected by a higher mean number of days of hospitalization for children with SCA compared to controls (6·2 *vs.* 0·5 days, $P=0·0005$).

The *S. aureus* carriage rates were similar in SCA patients ($n=34$, 46·6%) and controls ($n=67$, 46·9%). We merged the two groups to assess risk factors for *S. aureus* carriage in a multivariate analysis. The age group of children aged <5 years and higher education of a parent (i.e. high school or college degree) were independent risk factors for non-carriage (OR 0·22, 95% CI 0·11–0·42, $P=0·00001$ and OR 0·49, 95% CI 0·27–0·90, $P=0·02$, respectively). To the best of our knowledge the impact of the parents' educational level on *S. aureus* colonization in children has not yet been investigated. However, our finding is in line with reports from the USA where lower social status is associated with higher risk for the emergence of methicillin-resistant *S. aureus* (MRSA) [8]. In our study population, MRSA was only found in one SCA patient (t939, ST45) and one healthy control (t3202, ST88) [3].

Frequent hand washing (>3 times per day) was a strong independent risk factor for *S. aureus* carriage (OR 14·26, 95% CI 3·94–70·40, $P=0·0002$). This is surprising as hand washing is seen as one tool to reduce transmission of *S. aureus*. Re-acquisition might occur when sharing towels with others immediately after hand washing.

In 26 (35·6%) matched case-controls triplets, both the SCA patient and at least one control were colonized with *S. aureus*. In nine (12·3%) groups, the same *S. aureus spa* type was found in the SCA patient and at least one healthy control indicating transmission in the community setting. The *spa* types which were found in SCA patients and their respective control were t084 ($n=5$), t062, t148, t3662 and t6694 ($n=1$ each). The distribution of *S. aureus* genotypes according to *spa* typing and MLST was balanced

Table 1. *Distribution of genotypes in* Staphylococcus aureus *isolates from sickle cell anaemia* (*SCA*) *patients and healthy children*

CC	ST	No. of isolates (%)	*spa* type (no. of isolates)		OR (95% CI)	*P*
			SCA patients ($n=46$)	Healthy controls ($n=72$)		
CC1	ST1	4 (3·39)	**t127** (1), **t1931** (2)	**t1931** (1)	2·44 (0·27–30·09)	0·38
	ST2337	1 (0·85)	—	t4776 (1)		
CC5	ST5	17 (14·41)	t002 (1), **t062** (1), t071 (1), t311 (2), t319 (1), t2121 (1)	t002 (2), t062 (1), t232 (1), **t311** (3), t319 (2), t2121 (1)	1·34 (0·51–3·48)	0·5
	ST6	2 (1·70)	—	t1131 (1), t6618 (1)		
	ST8	3 (2·54)	**t1476** (3)	—		
	ST72	5 (4·24)	t148 (2)	t148 (3)		
CC15	ST15	34 (28·81)	**t084** (11), **t279** (1), t346 (1), **t6636** (1)	**t084** (16), **t230** (1), **t279** (1), **t346** (1), t774 (1)	1·17 (0·5–2·72)	0·69
	ST25	4 (3·39)	**t3662** (3)	**t3662** (1)		
	ST97	1 (0·85)	—	t267 (1)		
	ST188	1 (0·85)	—	t189 (1)		
	ST573	1 (0·85)	—	t6637 (1)		
CC30	ST30	1 (0·85)	**t1055** (1)	—	∞ (0·04–∞)	0·39
CC45	ST45	7 (5·93)	t939 (1), t6619 (1)	t939 (2), t4976 (1), t6552 (1), t6619 (1)	0·39 (0·07–1·6)	0·24
	ST508	6 (5·09)	t1510 (1)	t635 (1), t2784 (1), t5575 (1), t6550 (1), t6551 (1)		
	ST1745	1 (0·85)	—	t6242 (1)		
CC88	ST88	4 (3·39)	t2723 (2)	t2723 (1), t3202 (1)	1·59 (0·11–22·6)	0·64
CC101	ST101	6 (5·09)	t056 (1), t4679 (1), t6708 (1)	t4679 (3)	1·6 (0·2–12·48)	0·68
CC121	ST120	1 (0·85)	—	**t645** (1)	0·38 (0·01–4·01)	0·65
	ST121	2 (1·70)	t317 (1)	**t159** (1)		
	ST1746	2 (1·70)	—	**t314** (1), **t940** (1)		
CC152	ST152	10 (8·48)	**t355** (3)	**t355** (6), **t1096** (1)	0·65 (0·1–3·04)	0·74
CC707	ST707	2 (1·70)	—	**t1458** (2)	0 (0–8·34)	0·52
singleton	ST2338	2 (1·70)	**t6694** (1)	**t6694** (1)	0·78 (0·01–15·38)	1
	ST2349	1 (0·85)	—	t6553 (1)		

CC, Clonal complex; ST, sequence type; OR, odds ratio; CI, confidence interval.
PVL-positive isolates are in bold.

in both groups (Table 1). The most frequent ST was ST15 (28·81%, $n=34$), followed by ST5 (14·41%, $n=17$) and ST152 (8·48%, $n=10$). In general, it is assumed that all carrier isolates have the potential to become invasive, whatever the clonal lineage the isolate belongs to [9]. However, some STs might be more virulent than others [5]. The distribution of STs in our study is similar to the population structure of *S. aureus* from asymptomatic Malian carriers [10]. However, isolates belonging to ST15 and related STs were only the second most frequent ST in a German study, while other STs were only sporadically detected (ST5) or were not found (ST152) [11].

The proportion of PVL was 46·6% ($n=55$) and did not differ between groups. The PVL prevalence in Gabon is higher compared to asymptomatic carriers from Europe (0·65%) [11]. PVL can be associated with skin and soft tissue infection or necrotizing pneumonia and could therefore be a risk for life-threatening infections. PVL-positive isolates ($n=55$) were clonal and associated with ST15, ST152, ST5 and ST1 (Table 1).

In conclusion, *S. aureus* isolates from children with SCA and healthy controls share the same genetic background despite frequent hospital stays and antibiotic treatments in SCA patients. This suggests that

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results from community-based colonization studies with healthy participants (i.e. resistance studies) could be used to guide treatment and prophylaxis of endogenous infections in children with SCA, despite different selection pressure.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268812002270.

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DECLARATION OF INTEREST

None.

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