Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton–Valentine leukocidin genes

S. Breurec[1], C. Fall[1], R. Pouillot[2], P. Boisier[3], S. Brisse[4], F. Diene-Sarr[1], S. Djibo[3], J. Etienne[5], M. C. Fonkoua[2], J. D. Perrier-Gros-Claude[6], C. E. Ramarokoto[7], F. Randrianirina[7], J. M. Thiberge[4], S. B. Zriouil[8,9], the Working Group on *Staphylococcus aureus* infections*, B. Garin[1,†] and F. Laurent[5,†]

1) *Institut Pasteur, Dakar, Senegal,* 2) *Centre Pasteur, Yaounde, Cameroon,* 3) *CERMES, Niamey, Niger,* 4) *Institut Pasteur, Paris,* 5) *University of Lyon, National Reference Centre for Staphylococci, Lyon, France,* 6) *Institut Pasteur, Casablanca, Morocco,* 7) *Institut Pasteur, Antananarivo, Madagascar,* 8) *Ibn Rochd University Hospital and* 9) *Hassan II University, Casablanca, Morocco*

Abstract

The epidemiology of methicillin-susceptible *Staphylococcus aureus* (MSSA) in Africa is poorly documented. From January 2007 to March 2008, 555 *S. aureus* isolates were collected from five African towns in Cameroon, Madagascar, Morocco, Niger, and Senegal; among these, 456 unique isolates were susceptible to methicillin. Approximately 50% of the MSSA isolates from each different participating centre were randomly selected for further molecular analysis. Of the 228 isolates investigated, 132 (58%) belonged to five major multilocus sequence typing (MLST) clonal complexes (CCs) (CC1, CC15, CC30, CC121 and CC152) that were not related to any successful methicillin-resistant *S. aureus* (MRSA) clones previously identified in the same study population. The *luk*-PV genes encoding Panton–Valentine leukocidin (PVL), present in 130 isolates overall (57%), were highly prevalent in isolates from Cameroon, Niger, and Senegal (West and Central Africa). This finding is of major concern, with regard to both a source of severe infections and a potential reservoir for PVL genes. This overrepresentation of PVL in MSSA could lead to the emergence and spread of successful, highly virulent PVL-positive MRSA clones, a phenomenon that has already started in Africa.

Keywords: Africa, clones, community-acquired infections, hospital infections, methicillin-susceptible *Staphylococcus aureus*, Panton–Valentine leukocidin
Original Submission: 7 April 2010; **Revised Submission:** 17 June 2010; **Accepted:** 20 June 2010
Editor: J.-L. Mainardi
Article published online: 29 July 2010
Clin Microbiol Infect 2011; **17:** 633–639
10.1111/j.1469-0691.2010.03320.x

Corresponding author: S. Breurec, Unité de Biologie Médicale et Environnementale, Institut Pasteur, 36 avenue Pasteur, BP 220, Dakar, Senegal
E-mail: sbreurec@pasteur.sn

*The Working Group on *Staphylococcus aureus* infections includes: A. Maudry, Institut Pasteur, Dakar, Senegal; A. Seck, Institut Pasteur, Dakar, Senegal; A. Tall, Institut Pasteur, Dakar, Senegal; M. Cisse, Le Dantec University Hospital, Dakar, Senegal; A. Gaye-Diallo, Le Dantec University Hospital, Dakar; P. I. Ndiaye, Le Dantec University Hospital, Dakar, Senegal; S. Guyomard, Principal Hospital, Dakar, Senegal; S. Ka, Principal Hospital, Dakar, Senegal; A. Sow, Principal Hospital, Dakar, Senegal; K. A. Wade, Principal Hospital, Dakar, Senegal; M. Sembene, Cheikh Anta Diop University, Dakar, Senegal; M. Bekkali, Hassan II University, Casablanca, Morocco; N. El Mdaghri, Ibn Rochd University Hospital, Casablanca, Morocco; M. Timinouni, Institut Pasteur, Casablanca, Morocco; K. Zerouali, Ibn Rochd University Hospital, Casablanca, Morocco; J. F. Carod, Institut Pasteur, Antananarivo, Madagascar; J. B. Chretien, Institut Pasteur, Antananarivo, Madagascar; E. Ratsima, Institut Pasteur, Antananarivo, Madagascar; V. Richard, Institut Pasteur, Antananarivo, Madagascar; L. Vaillant, Institut Pasteur, Antananarivo, Madagascar; A. Ngandjio, Centre Pasteur, Yaounde, Cameroon; M. Tejiokem, Centre Pasteur, Yaounde, Cameroon; and A. Zakou, CERMES, Niamey, Niger.
†These authors contributed equally to this study.

Introduction

Staphylococcus aureus is a major human pathogen and the leading cause of hospital-associated infections. First reported in 1961 [1], methicillin-resistant *S. aureus* (MRSA) has become endemic in hospitals worldwide. In the 1990s, outbreaks of community-associated MRSA (CA-MRSA) infection became increasingly frequent [2]. Methicillin-susceptible *S. aureus* (MSSA) lineages have been the subject of fewer molecular studies than MRSA [3], but knowledge of MSSA

genetic backgrounds and associated virulence factors is important for understanding the dynamics of the emergence of hospital-acquired MRSA and CA-MRSA.

It is now established that the population structure of MSSA is genetically more diverse than that of MRSA, and that MRSA originated from a limited number of epidemic MSSA lineages through transfer of the staphylococcal cassette chromosome *mec* (SCC*mec*) [3]. This suggests that some MSSA genetic backgrounds may not provide a stable genetic environment for SCC*mec* integration [3]. Few data on the population structure of MSSA lineages, their relationship to MRSA lineages and associated virulence factors are available in Africa as compared with industrialized countries [4–10], but ongoing surveillance of MSSA lineages is important to anticipate the emergence of virulent MRSA.

Here, we report the molecular characterization of MSSA isolates in five major African towns, namely Antananarivo (Madagascar), Casablanca (Morocco, Maghreb), Dakar (Senegal, West Africa), Niamey (Niger, West Africa), and Yaounde (Cameroon, Central Africa), by means of accessory gene regulator (*agr*) typing, multilocus sequence typing (MLST), staphylococcal protein A (*spa*) typing, and toxin profiling. Antimicrobial susceptibility was also evaluated.

Materials and Methods

Study population

All patients with suspected staphylococcal infection were pre-included in seven major tertiary-care centres located in five major African towns, namely Antananarivo (Madagascar), Casablanca (Morocco), Niamey (Niger), Dakar (Senegal), and Yaounde (Cameroon), between January 2007 and March 2008 (January 2007 to June 2007 in Casablanca). Patients were subsequently included in the study if *S. aureus* infection was confirmed. A standardized specific medical questionnaire was completed during hospitalization, to collect demographic data, the medical history over the previous 12 months, the reason for admission, and the time between admission and sample collection for culture. If more than one *S. aureus* isolate with the same *spa* type was recovered from the same patient, only the first was included. Isolates were considered to be community-acquired if recovered by culture from a sample obtained within 48 h after admission in a patient with no risk factors for nosocomial acquisition in the previous year, namely hospitalization or surgery, use of an indwelling catheter or a percutaneous device, or frequent exposure to healthcare facilities for an underlying chronic disorder. All other isolates were considered to be hospital-acquired. Owing to the large number of strains collected, about half of the MSSA isolates from each different participating centre were randomly selected for further molecular analysis. The study protocol was approved by local ethics committees.

Microbiological analysis, DNA extraction, *mecA* detection, *agr* typing, and toxin gene profiling

S. aureus identification and genomic DNA extraction were performed as previously described [3]. Isolates were screened for genes encoding staphylococcal enterotoxins A, C, D, H, M, P, and R (*sea*, *sec*, *sed*, *seh*, *sem*, *sep*, and *ser*), toxic shock syndrome toxin 1 (*tst*), exfoliative toxins A, B, and D (*eta* and *etb*), Panton–Valentine leukocidin (PVL) (*luk*-PV), class F *Luk*M leukocidin (*lukM*), and β-haemolysin (*hlb*), as previously described [11].

Spa typing

Spa sequence typing was performed on *S. aureus* isolates with the Ridom Staph Type standard protocol (http://www.ridom.com/doc/Ridom_spa_sequencing.pdf) and the Ridom *Spa*Server, which automatically analyses *spa* repeats and assigns *spa* types (http://spa.server.ridom.de). By application of the BURP algorithm implemented in Ridom StaphType software version 1.4 (Ridom GmbH, Würzburg, Germany), *spa* types with more than four repeats were clustered into different *spa* clonal complexes (CCs), with the calculated cost between members of a group being 4 or less.

MLST

The associated MLST CCs were allocated with the Ridom *Spa*Server [12], if possible. To confirm the concordance between the MLST and *spa* CCs, MLST was performed as described elsewhere [13] on a set of representative strains from each *spa* type found within the five major *spa* CCs, each *spa* CC including more than 20 strains, and also within the *spa* CCs corresponding to three major MRSA CCs (associated MLST CC5, CC8, and CC88) described elsewhere [3,4].

Antimicrobial susceptibility

Susceptibility to penicillin, oxacillin, cefoxitin, gentamicin, kanamycin, tobramycin, tetracycline, erythromycin, lincomycin, pristinamycin, fosfomycin, fusidic acid, rifampicin, pefloxacin, co-trimoxazole and vancomycin was determined by each participating laboratory, following the guidelines of the French Society for Microbiology. *S. aureus* strain ATCC 25923 was used for quality control. External quality control was ensured by the French National Antibiotic Reference Centre (Institut Pasteur, Paris).

Statistical analysis

Pearson's chi-square test and Fisher's exact test were used as appropriate to test associations between qualitative variables. Significance was assumed at $p < 0.05$.

Results and Discussion

In total, 542 *S. aureus*-infected patients were included during the study period. The relatively small size of the sample reflects the clinical situation in Africa, including a lack of routine bacteriological samples and frequent initiation of antibiotic therapy before biological sampling. Six hundred and nine biological samples were taken, and 555 *S. aureus* isolates were collected; among these, 456 unique isolates were susceptible to methicillin. Sixty-one per cent of isolates were recovered from male patients. The patients' mean age was 29.8 years (range 1–84 years; median 26 years, 25th percentile 13 years, 75th percentile 44 years). About half of the MSSA isolates from each site (228 in total) were randomly selected for molecular studies. The number of isolates selected per site is shown in Table 1. The isolates were mainly associated with skin and soft tissue infections (SSTIs) and surgical site infections at all the sites except in Dakar, where all types of infection were noted. The lack of routine blood culture probably explains the small number of isolates associated with bacteraemia/septicaemia and osteomyelitis. The 86 unique MRSA isolates collected simultaneously have been already described elsewhere [4]. They belonged mainly to three major clones (defined by their sequence type (ST) and SCC*mec* type), namely ST239/241-III (n = 34, 40%), a well-known pandemic clone, and two atypical clones, ST88-IV (n = 24, 28%) and ST5-IV (n = 18, 21%). Almost all of the isolates (94%) belonging to the latter clone were PVL-positive, suggesting that the spread of MRSA carrying *luk*-PV genes is a cause for concern, especially in Dakar and possibly throughout Africa.

The 228 MSSA isolates belonged to 87 *spa* types. The BURP algorithm separated 222 isolates into 16 *spa* CCs and 19 singletons (groups represented by a single type). Four *spa* types (six strains) shorter than five repeats were excluded from the clustering. A total of 44 *spa* types assigned to eight *spa* CCs were investigated by MLST, and 16 STs were found. Each *spa* CC corresponded to one MLST CC, confirming the excellent concordance of these typing methods (Table 2) [12]. More than half of the isolates (n = 132, 58%) belonged to only five MLST CCs: CC121 (19%), CC15 (12%), CC1 (9%), CC152 (9%), and CC30 (9%). The remaining isolates belonged to CC8 (6%), CC5 (4%), and CC88 (3%), as well as eight *spa* CCs (13%) with no founder and 19 singletons (13%) (Tables 2 and S1).

The five major CCs recovered in our study shared significant features (Table 2): (i) an unusually high prevalence of *luk*-PV genes encoding PVL; (ii) detection in at least three participating centres, indicating their wide geographical distribution; (iii) presence in both the hospital and community settings; and (iv) susceptibility to most of the antibiotics tested, except for penicillin (91% of resistant isolates), tetracycline (46%), and co-trimoxazole (22%).

The first two major CCs—CC121 (*agr*4, *spa* type t314 or related, ST121) and CC15 (*agr*1, *spa* type t084 or related, mainly ST15 (96%))—accounted for 44 (19%) and 27 (12%) of the 228 isolates, respectively (Table 2). The main toxin genes were the *luk*-PV (93%) and *sem* genes (98%), found in isolates belonging to ST121, and *luk*-PV (27%), *eta* (23%) and *sea* (n = 6, 23%) in ST15 isolates. These two clones (each defined by an ST) shared the following features: (i) they have already been extensively described as major MSSA clones in many countries in the Americas, Asia, and Europe [10,14,15], but have also been found in Algeria, Cape Verde Islands,

TABLE 1. Distribution of 228 methicillin-susceptible *Staphylococcus aureus* isolates obtained in five African towns (Antananarivo, Casablanca, Dakar, Niamey, and Yaounde) according to the presence of Panton–Valentine leukocidin (PVL) and the types of infection[a]

	Antananarivo		Casablanca		Dakar		Niamey		Yaounde		Total	
	N	PVL+, *n* (%)	*N*	PVL+, *n* (%)	*N*	PVL+, *n* (%)	*N*	PVL+, *n* (%)	*N*	PVL+, *n* (%)	*N*	PVL+, *n* (%)
Bacteraemia	0	0	0	0	17	8 (47)	0	0	0	0	17	8 (47)
Myositis	0	0	0	0	25	23 (92)	0	0	0	0	25	23 (92)
Osteomyelitis	2	0	0	0	11	8 (73)	5	3 (60)	1	1 (100)	19	12 (63)
Pulmonary infection	0	0	0	0	6	3 (50)	0	0	0	0	6	3 (50)
Skin and soft tissue infection	24	8 (33)	18	3 (17)	47	34 (72)	13	10 (77)	17	12 (71)	119	67 (56)
Surgical site infection	12	5 (42)	0	0	7	1 (14)	14	7 (50)	3	3 (100)	36	16 (44)
Genitourinary tract infection	2	0	0	0	2	0	0	0	0	0	4	0
Unknown type of infection	0	0	0	0	0	0	0	0	2	1 (50)	2	1 (50)
Total	40	13 (33)	18	3 (17)	115	77 (67)	32	20 (63)	23	17 (74)	228	130 (57)

N, total number of strains isolated.

TABLE 2. Molecular characteristics of community-associated methicillin-susceptible *Staphylococcus aureus* (CA-MSSA) and hospital-acquired methicillin-susceptible *S. aureus* (HA-MSSA) isolates obtained in five African towns (Antananarivo, Casablanca, Dakar, Niamey, and Yaounde)

spa CCs	CC	ST	Total (*n*)	*spa* types (no.)	Toxin genes[a] (no.)	*luk*-PV (%)	Location (no.) HA-MSSA (*n* = 159)	Location (no.) CA-MSSA (*n* = 69)
314	121	121	44	t314 (37), t317 (1), t4499 (1), t159 (4), t645 (1)	*sem* (43), *luk*-PV (41), *hlb* (5), *sec* (1), *selr* (1)	93	D (19), N (6), A (4), Y (3)	D (9), N (1), Y (2)
084	15	15	26	t084 (25), t346 (1)	*luk*-PV (7), *eta* (6), *sea* (6), *hlb* (1)	27	D (12), N (4), Y (2), A (1),	C (3), A (2), D (1), Y (1)
		1403	1	t3733 (1)		0	D (1)	
127	1	852	19	t127 (19)	*seh* (16), *luk*-PV (12), *sea* (11), *sec* (3), *sem* (2), *hlb* (2), *tst* (1), *selp* (1)	63	D (10), C (4), A (1), Y (1), A (1), C (1)	D (3)
		1402	2	t948 (2)	*sea* (2), *sec* (1), *seh* (1), *sem* (1)	0		
355	152	152	15	t1299 (2), t1096 (1), t355 (12)	*luk*-PV (14), *hlb* (14), *sem* (2)	93	N (6), D (5) Y(1), A (1), D (1), A (1)	D (3), D (2)
		377	4	t5129 (1), t5047 (1), t4687 (1), t468 (1)	*luk*-PV (4), *hlb* (4)	100		
		1471	1	t5127 (1)	*luk*-PV (1), *hlb* (1)	100		
021	30	30	12	t318 (6), t342 (2), t021 (2), t5134 (1), t3646 (1)	*sem* (11), *luk*-PV (11), *sea* (2), *hlb* (1)	92	D (5), Y (1), A (1), N (1), D (4)	D (3), Y (1), D (4)
		535	4	t433 (4)	*sea* (4), *sem* (4), *luk*-PV (4), *hlb* (1)	100		
		1472	4	t665 (4)	*sem* (4), *luk*-PV (4)	100		
064	8	8	11	t064 (3), t008 (2), t451 (2), t301 (1), t1705 (1), t1476 (1), t4688 (1)	*sea* (5), *hlb* (2), *selp* (2), *seh* (1), *selr* (1), *tst* (1), *luk*-PV (1)	9	D (3), N (3), C (1), D (2)	D (3), A (1)
		1404	2	t1617 (2)	*selp* (2)	0		
002	5	5	9	t002 (4), t570 (1), t311 (3), t4681 (1)	*sem* (9), *luk*-PV (3), *sed* (3), *selr* (3), *sea* (2), *sec* (2), *selp* (2), *tst* (1), *hlb* (1)	33	Y (3), A (3), N (1), N (1)	A (1), D (1)
		730	1	t4590 (1)	*sem* (1), *selp* (1)	0		
186	88	88	7	t186 (3), t325 (2), t448 (1) t786 (1)	*hlb* (2), *sea* (1), *tst* (1)	0	A (3), N (1), D (1), Y (1)	A (1)
spa CCs with no founder[b]			66		*luk*-PV (28), *sem* (25), *sec* (6), *seh* (11), *hlb* (10), *sea* (7), *tst* (4), *selp* (3), *eta* (2), *selr* (1)	42	D (11), A (8), Y (8), N (7), C (5)	D (12), A (8), C (5), Y (2)
Singleton *spa* types shorter than five repeats								
Total			228		*luk*-PV (130), *sem* (104), *hlb* (44), *sea* (40), *seh* (29), *sec* (23), *selp* (11), *eta* (8), *tst* (8), *selr* (6), *sed* (3)	57	D (74), N (30), A (25), Y (19), C (11)	D (41), A (13), C (8), Y (6), N (1)

A, Antananarivo; C, Casablanca; D, Dakar; N, Niamey; Y, Yaounde; *spa* CCs, staphylococcal protein A clonal complexes; CC, multilocus sequence typing clonal complex; ST, sequence type; *eta*, exfoliatin A; *etb*, exfoliatin B; *etd*, exfoliatin D; *hlb*, β-haemolysin; *lukM*, staphylococcal leukocidin; *luk*-PV, Panton–Valentine leukocidin; *se*, staphylococcal enterotoxin; *tst*, toxic shock syndrome toxin.

[a]*etb*, *etd*, *sep*, *ser* and *lukM* genes were not found in any of the isolates.

[b]Eight *spa* CCs with no founder (30 isolates), 19 singletons (30 isolates), and six isolates with *spa*-types shorter than five repeats.

Mali, Nigeria, and South Africa [5,8–10]; and (ii) these two genetic backgrounds have rarely (CC15) or never (CC121) been identified in MRSA isolates [3], suggesting that they may not provide a stable genetic environment for SCC*mec* integration.

The third and fourth major CCs—CC1 (*agr*3, *spa* type t127 or related, mainly ST852 (90%)) and CC152 (agr1, *spa* type t355 or related, mainly ST152 (75%))—corresponded to 21 (9%) and 20 (9%) isolates, respectively. In ST852, the predominant toxin genes were *seh* (84%), *luk*-PV (63%), and *sea* (58%). This clone has rarely been described, with one isolate having been detected in Germany [16] and two in Mali [5]. However, CC1 is a major worldwide MSSA clonal lineage, and the putative founder, ST1, which was not found in our study, is a successful CA-MRSA genetic background [3]. Similarly, ST152 has been associated sporadically with a CA-MRSA genetic background in several countries throughout Europe (Denmark, Germany, Austria, and Slovenia) [3], and at a high frequency with an MSSA background in Mali [5] and Nigeria [7]. The *hlb* and *luk*-PV genes were usually present (93%). The fifth CC, CC30 (*agr*3 and *spa* type t318 or related), a successful MRSA lineage [3], corresponded to 20 isolates (9%) belonging mainly to ST30 (60%). The *luk*-PV and *sem* genes were found in the vast majority of isolates (92%).

The absence of common genetic backgrounds between major MSSA and MRSA clones is one important finding in this study. Only 7% of MSSA isolates (16 of the 228 isolates) had a genetic background corresponding to the major MRSA clones found in the same study population during the same period [4], namely ST239/241-III, ST88-IV, and ST5-IV. Only the latter two genetic backgrounds were present in MSSA and MRSA lineages (Table 2). The best strategy with which

to determine whether MRSA clones arise locally via SCC*mec* acquisition by successful MSSA clones would have been to analyse MRSA isolates and a representative MSSA collection during a period preceding the emergence of MRSA, with the use of high-resolution genotyping methods. Despite the absence of such data, our results suggest various hypotheses. The main MRSA clone, ST239/241-III, a well-known pandemic clone [3], probably did not originate via the introduction of an SCC*mec* element into locally dominant MSSA backgrounds, but was probably imported from abroad, as previously described [17]. Although most of the MRSA isolates with the ST5 genetic background were detected in Dakar, only one MSSA isolate was obtained there, but this does not exclude an evolutionary link. It has recently been reported that geographical spread of MRSA isolates with the ST5 genetic background over long distances is rare in comparison with SCC*mec* element acquisition [18]. ST88 MSSA isolates were detected in Antananarivo (n = 4), Dakar (n = 1), Niamey (n = 1), and Yaounde (n = 1), matching the distribution of ST88-IV MRSA isolates. This latter clone is an atypical successful clone found mainly in Africa, suggesting its emergence from native MSSA. Further studies based on high-resolution genotyping methods are needed to investigate the evolutionary history of this clone [18]. Finally, the high prevalence of PVL-positive MSSA isolates belonging to CC152, CC30 and CC1—genetic backgrounds associated with successful CA-MRSA [3] and possibly representing a stable genetic environment for SCC*mec* integration—could pose a serious public health threat in future.

The second major finding in this study is the high prevalence and high genetic diversity of PVL-positive isolates; *luk*-PV genes were detected in 130 (57%) of the 228 isolates tested, belonging to 39 (45%) of the 87 *spa* types and to all the major STs identified here (Table 2). Interestingly, a large proportion of the PVL-positive genetic backgrounds described worldwide were observed in our study [3,9,10]. The *luk*-PV genes encoding PVL have been linked in epidemiological studies to deep abscesses, severe necrotizing pneumonia, and severe bone and joint infections [19,20], and recent experimental models support a significant pathological contribution of PVL [21,22]. This pore-forming toxin targets human immune system cells, such as polymorphonuclear neutrophils, monocytes, and macrophages [23]. Depending on the concentration, PVL pores cause cytokine release and cell death by apoptosis or necrosis, which undoubtedly contributes to the pathogenicity of PVL-producing *S. aureus* isolates [24]. As the MSSA isolates obtained in the participating centres, except for Dakar, were mainly associated with SSTIs (Table 1), infections in which PVL has been strongly implicated [25], a recruitment bias cannot be excluded. Nevertheless, our data for Dakar, showing a high prevalence of *luk*-PV genes (>47%) regardless of the type of infection, except for surgical site infections, suggest that PVL-positive isolates are extensively distributed in Senegal (West Africa). Despite the different distributions of the types of infection across the study sites, it is noteworthy that the prevalence of PVL-positive isolates in SSTIs was significantly higher in Dakar (72%), Niamey (77%) and Yaounde (71%) (West and Central Africa) than in Antananarivo (Madagascar) (33%) and Casablanca (Maghreb) (17%) (p <0.01). Furthermore, the high prevalence of nasal carriage of PVL-positive MSSA described in Cape Verde [9] and Mali [5] (West Africa) (>35%) contrasts with the low prevalence reported in industrialized countries (0.5–1.4%) [26–28]. All of these data suggest that West and Central Africa have a particularly high prevalence of PVL-positive MSSA isolates, although further investigations on *S. aureus* from nasal sources are needed to confirm this high prevalence. Finally, no significant difference in the prevalence of PVL genes was observed between the hospital and community settings (p 0.34). When the prevalence of PVL-positive isolates in the community is high, these isolates are able to spread in hospitals [29], particularly in Africa, where the permanent presence of friends and relatives in hospitals, and the transfer of some healthcare responsibilities to these persons, increases the risk of isolate circulation between the community and hospital settings [4].

Even though the reasons for this very high prevalence of PVL-positive MSSA isolates are currently unclear, its implications in terms of the evolutionary history of *S. aureus* in Africa should be investigated. The *luk*-PV genes are carried on mobile genetic elements (prophages), implying that PVL-positive MSSA isolates may represent a reservoir for PVL genes. These genes could subsequently be incorporated into *S. aureus* lineages through horizontal transfer, either before or after acquisition of the *mecA* gene [30]. This is a major concern. Conditions in Africa that increase the risk of inter-individual transmission (poor access to healthcare services, poor hygienic and sanitary conditions, overcrowding, etc.), combined with the high prevalence of PVL-positive *S. aureus* isolates, has the potential to lead to massive emergence and spread of successful and highly virulent PVL-positive MRSA clones, a phenomenon that has already been described in Algeria, Cameroon, Morocco, Nigeria, and Senegal [4,6,8].

In conclusion, this study provides an unique overview of MSSA clones circulating in seven hospitals in five major African towns. We studied isolates from only one or two centres in each country, which may not be representative of the overall situation. However, the healthcare institutions studied here were among the main tertiary hospitals in each of the five countries studied. The high prevalence of *luk*-PV genes

among MSSA strains is a major concern, both as a source of severe infections and as a potential reservoir that could accelerate the emergence of successful clones that are both PVL-positive and resistant to methicillin. Moreover, human mobility may hasten the spread of native African clones, and thus have a significant future impact on *S. aureus* epidemiology in other regions of the world.

Acknowledgements

We thank F. B. Badji and F. B. Dieye (Institut Pasteur, Dakar, Senegal) and H. Meugnier (National Reference Centre for Staphylococci, Lyon, France) for their technical help, and all the clinicians involved in this study. We also thank M. Bes (National Reference Centre for Staphylococci, Lyon, France) and C. Bouchiat (Institut Pasteur, Dakar, Senegal) for helpful discussions, and I. Eronini and D. Young for editorial assistance.

Transparency Declaration

This study was supported by grants from Institut Pasteur (Grant PTR 197) and by Institut de Veille Sanitaire (InVS, Saint-Maurice, France), and was performed at institutes belonging to the Institut Pasteur International Network. All of the authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Molecular characteristics of CA-MSSA and HA-MSSA.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Parker MT, Jevons MP. A survey of methicillin resistance in *Staphylococcus aureus*. *Postgrad Med J* 1964; 40: 170–178.
2. Ellis MW, Hospenthal DR, Dooley DP *et al.* Natural history of community-acquired methicillin-resistant *Staphylococcus aureus* colonization and infection in soldiers. *Clin Infect Dis* 2004; 39: 971–979.
3. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol* 2008; 8: 747–763.
4. Breurec S, Zriouil SB, Fall C *et al.* Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. *Clin Microbiol Infect* 2010; doi: 10.1111/j.1469-0691.2010.03219.x [Epub ahead of print]
5. Ruimy R, Maiga A, Armand-Lefevre L *et al.* The carriage population of *Staphylococcus aureus* from Mali is composed of a combination of pandemic clones and the divergent Panton–Valentine leukocidin-positive genotype ST152. *J Bacteriol* 2008; 190: 3962–3968.
6. Ramdani-Bouguessa N, Bes M, Meugnier H *et al.* Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton–Valentine leukocidin genes in an Algiers hospital. *Antimicrob Agents Chemother* 2006; 50: 1083–1085.
7. Okon KO, Basset P, Uba A *et al.* Cooccurrence of predominant Panton–Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 *Staphylococcus aureus* clones in Nigerian hospitals. *J Clin Microbiol* 2009; 47: 3000–3003.
8. Ghebremedhin B, Olugbosi MO, Raji AM *et al.* Emergence of a community-associated methicillin-resistant *Staphylococcus aureus* with unique resistance profile in Southwest of Nigeria. *J Clin Microbiol* 2009; 47: 2975–2980.
9. Aires-de-Sousa M, Conceicao T, de Lencastre H. Unusually high prevalence of nosocomial Panton–Valentine leukocidin-positive *Staphylococcus aureus* isolates in Cape Verde Islands. *J Clin Microbiol* 2006; 44: 3790–3793.
10. Goering RV, Shawar RM, Scangarella NE *et al.* Molecular epidemiology of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from global clinical trials. *J Clin Microbiol* 2008; 46: 2842–2847.
11. Jarraud S, Mougel C, Thioulouse J *et al.* Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. *Infect Immun* 2002; 70: 631–641.
12. Strommenger B, Kettlitz C, Weniger T *et al.* Assignment of Staphylococcus isolates to groups by *spa* typing, SmaI macrorestriction analysis, and multilocus sequence typing. *J Clin Microbiol* 2006; 44: 2533–2540.
13. Enright MC, Day NP, Davies CE *et al.* Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; 38: 1008–1015.
14. Deurenberg RH, Beisser PS, Visschers MJ *et al.* Molecular typing of methicillin-susceptible *Staphylococcus aureus* isolates collected in the Yogyakarta area in Indonesia, 2006. *Clin Microbiol Infect* 2010; 16: 92–94.
15. Vorobieva V, Bazhukova T, Hanssen AM *et al.* Clinical isolates of *Staphylococcus aureus* from the Arkhangelsk region, Russia: antimicrobial susceptibility, molecular epidemiology, and distribution of Panton–Valentine leukocidin genes. *APMIS* 2008; 116: 877–887.
16. Strommenger B, Braulke C, Heuck D *et al. spa* Typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. *J Clin Microbiol* 2008; 46: 574–581.
17. Harris SR, Feil EJ, Holden MT *et al.* Evolution of methicillin-resistant *Staphylococcus aureus* during hospital transmission and intercontinental spread. *Science* 2010; 327: 469–474.
18. Nubel U, Roumagnac P, Feldkamp M *et al.* Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. *Proc Natl Acad Sci USA* 2008; 105: 14130–14135.
19. Lina G, Piemont Y, Godail-Gamot F *et al.* Involvement of Panton–Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999; 29: 1128–1132.
20. Gillet Y, Issartel B, Vanhems P *et al.* Association between *Staphylococcus aureus* strains carrying gene for Panton–Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002; 359: 753–759.

21. Cremieux AC, Dumitrescu O, Lina G *et al.* Panton–Valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. *PLoS ONE* 2009; 4: 7204–7211.
22. Labandeira-Rey M, Couzon F, Boisset S *et al. Staphylococcus aureus* Panton–Valentine leukocidin causes necrotizing pneumonia. *Science* 2007; 315: 1130–1133.
23. Szmigielski S, Sobiczewska E, Prevost G *et al.* Effect of purified staphylococcal leukocidal toxins on isolated blood polymorphonuclear leukocytes and peritoneal macrophages in vitro. *Zentralbl Bakteriol* 1998; 288: 383–394.
24. Genestier AL, Michallet MC, Prevost G *et al. Staphylococcus aureus* Panton–Valentine leukocidin directly targets mitochondria and induces Bax-independent apoptosis of human neutrophils. *J Clin Invest* 2005; 115: 3117–3127.
25. Del Giudice P, Blanc V, de Rougemont A *et al.* Primary skin abscesses are mainly caused by Panton–Valentine leukocidin-positive *Staphylococcus aureus* strains. *Dermatology* 2009; 219: 299–302.
26. Prevost G, Couppie P, Prevost P *et al.* Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J Med Microbiol* 1995; 42: 237–245.
27. Kuehnert MJ, Kruszon-Moran D, Hill HA *et al.* Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001–2002. *J Infect Dis* 2006; 193: 172–179.
28. Melles DC, van Leeuwen WB, Boelens HA *et al.* Panton–Valentine leukocidin genes in *Staphylococcus aureus*. *Emerg Infect Dis* 2006; 12: 1174–1175.
29. Carleton HA, Diep BA, Charlebois ED *et al.* Community-adapted methicillin-resistant *Staphylococcus aureus*: population dynamics of an expanding community reservoir of MRSA. *J Infect Dis* 2004; 190: 1730–1738.
30. O'Hara FP, Guex N, Word JM *et al.* A geographic variant of the *Staphylococcus aureus* Panton–Valentine leukocidin toxin and the origin of community-associated methicillin-resistant *Staphylococcus aureus* USA300. *J Infect Dis* 2008; 197: 187–194.