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ORIGINAL ARTICLE

Characterisation of *Staphylococcus aureus* isolates from bloodstream infections, Democratic Republic of the Congo

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Abstract *Staphylococcus aureus* is known worldwide as an invasive pathogen, but information on *S. aureus* from bloodstream infections in Central Africa remains scarce. A collection of *S. aureus* blood culture isolates recovered from hospitals in four provinces in the Democratic Republic of the Congo (2009–2013) was assessed. A total of 27/108 isolates were methicillin-resistant *S. aureus* (MRSA), of which >70% were co-resistant to aminoglycosides, tetracyclines, macrolides and lincosamides. For MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates, resistance to chloramphenicol and trimethoprim–sulphamethoxazole (TMP-SMX) was <10%. However, 66.7% (72/108) of all isolates harboured the trimethoprim resistance gene *dfrG*. More than three-quarters (84/108, 77.8%) of isolates belonged to CC5, CC8, CC121 or CC152. Genetic diversity was higher among MSSA (31 *spa* types) compared to MRSA (four *spa* types). Most MRSA (23/27, 85.2%) belonged to CC8-*spa* t1476-SCC*mec* V and 17/23 (73.9%) MRSA ST8 were oxacillin susceptible but cefoxitin resistant. Among MRSA and MSSA combined, 49.1% (53/108) and 19.4% (21/108) contained the genes encoding for Panton–Valentine leucocidin (*lukS-lukF PV*, PVL) and toxic shock syndrome toxin-1 (*tst*, TSST-1), respectively. PVL was mainly detected among MSSA (51/53 isolates harbouring PVL were MSSA, 96.2%) and associated with CC121, CC152, CC1 and CC5. TSST-1 was associated with CC8-*spa* t1476-SCC*mec* V. The immune evasion cluster (IEC) genes *scn*, *sak* and *chp* were detected in 81.5% of isolates (88/108, equally represented among MSSA and MRSA). The present study confirms the occurrence of MRSA with high levels of multidrug co-resistance and PVL-positive MSSA among invasive *S. aureus* isolates in Central Africa.

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Introduction

Staphylococcus aureus features among the most important human pathogens worldwide and is capable of causing a variety of infections in healthcare facilities, as well as in the community. *Staphylococcus aureus* is well known for its adaptive versatility and ability to acquire various resistance and virulence genes, the most famous example being its ability to acquire beta-lactam resistance due to uptake of the *mec* gene (methicillin-resistant *S. aureus*, MRSA). Numerous studies have investigated the clinical and molecular epidemiology of *S. aureus* and MRSA. However, data about the molecular epidemiology of *S. aureus* have long remained inadequate for the African continent. The present study reports on a collection of *S. aureus* isolates recovered from blood cultures from patients admitted to healthcare facilities in the

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Democratic Republic of the Congo (DRC), Central Africa. The objectives were to: (1) assess the proportion and genetic diversity of methicillin-susceptible *S. aureus* (MSSA) and MRSA isolates, (2) investigate their phenotypic and genotypic antibiotic resistance profiles and (3) determine the presence of virulence factors and immune evasion cluster (IEC) genes in these isolates.

Materials and methods

Strain collection and identification

A total of 108 *S. aureus* isolates from blood cultures were analysed, all of which were collected within the framework of the Microbiological Surveillance Network of the National Institute of Biomedical Research (INRB, Kinshasa, DRC) and the Institute of Tropical Medicine (ITM, Antwerp, Belgium), which studies bloodstream infections and antibiotic resistance [1]. Patients recruited were adults and children admitted to the participating healthcare facilities. Indications for blood culture sampling after the neonatal period (>28 days old) were: (i) a body temperature ≥38 °C or ≤35.5 °C; (ii) clinical signs of severe localised infections [pneumonia, meningitis, complicated urinary tract infection, arthritis and osteomyelitis, severe skin and soft tissue infections (SSTI), gynaecological infections, peritonitis]; (iii) suspicion of sepsis, typhoid fever or severe malaria. In the neonatal period, criteria included premature rupture of membranes, intra-partum fever, low Apgar score and symptoms such as tachypnoea, cyanosis and lethargy. Basic demographic data (age, gender and geographic origin) were recorded from the laboratory request form. Neonatal sepsis was defined by an infection during the first 28 days of life, and was classified as very early onset (occurring within the first 72 h of life), early onset (occurring within the first week) and late onset (occurring after the first week until 28 days of life) [2].

Isolates were collected between June 2009 and December 2013 and originated from 13 healthcare facilities located in four (out of 11) provinces of the DRC: Bas-Congo ($n = 58$), Kinshasa ($n = 25$), Oriental Province ($n = 22$) and Équateur ($n = 3$). *Staphylococcus aureus* were initially recovered and identified by standard techniques (Gram staining, catalase test, coagulase and DNase production). Only the first *S. aureus* isolate per patient was considered. Isolates were stored in tubes of tryptone soya agar (Oxoid, Basingstoke, UK) and shipped to the ITM for confirmation and antibiotic resistance testing.

Diagnosis of MSSA and MRSA: genetic diversity

At the Belgian National Reference Centre (NRC) for *S. aureus*, all isolates were confirmed as MSSA or MRSA by a 16S rRNA-*nuc*-*mecA* triplex polymerase chain reaction (PCR) [3]. *spa* typing and clustering into *spa* clonal complexes (*spa* CCs) were performed using default parameters set by the Ridom StaphType software (exclusion if <5 repeats; clustering if cost is ≤4), as previously described [3]. Multilocus sequence typing (MLST) was performed for all *spa* types detected among MRSA ($n = 4$) and for one Panton–Valentine leucocidin (PVL)-positive ($n = 9$) or toxic shock syndrome toxin (TSST)-positive ($n = 1$) *spa* type per *spa* CC for MSSA [3]. For those *spa* CCs which contained only MSSA isolates negative for PVL and TSST, MLST was derived from the Ridom SpaServer if available (http://www.spaserver.ridom.de/). Sequence types (STs) were clustered in MLST CCs by the Based Upon Related Sequence Types (BURST) algorithm using default parameters (http://eburst.mlst.net/). The staphylococcal cassette chromosome *mec* (SCC*mec*) type was determined by multiplex PCR assay for all MRSA by determination of the type of *ccr* and *mec* gene complex [3].

Assessment of phenotypic and genotypic antibiotic resistance

Susceptibility to 13 antibiotics [penicillin, cefoxitin, gentamicin, kanamycin, tobramycin, erythromycin, tetracycline, ciprofloxacin, clindamycin, trimethoprim–sulphamethoxazole (TMP-SMX), chloramphenicol, linezolid and mupirocin] was tested for all isolates by the disc diffusion method (Rosco Diagnostica, Taastrup, Denmark), according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [4]. Vancomycin minimum inhibitory concentration (MIC) values were determined by the Etest macromethod (bioMérieux, Marcy l'Etoile, France) [5]. For all MRSA (*mec*A-positive), cefoxitin and oxacillin MIC values were determined by broth microdilution (Sensititre, TREK Diagnostic Systems, England) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, clinical breakpoints version 7.0). For all experiments, *S. aureus* ATCC® 25923, ATCC® 25213 and ATCC® 43300 were used as quality control strains.

Resistance genes for macrolides and/or lincosamide antibiotics [*erm*(A), *erm*(B), *erm*(C) and *erm*(T)], for aminoglycosides [*aac*(6′)-*aph*(2″), *aph*(3′) and *ant*(4′)] and for tetracyclines [*tet*(M) and *tet*(K)] were determined by PCR for isolates showing resistance to the respective antibiotics. The presence of trimethoprim resistance genes *dfrG* and *dfrK* was assessed by multiplex PCR for all isolates [3, 6].

Virulence factors and immune evasion cluster genes

For all *S. aureus* isolates, the presence of the genes *lukS-lukF PV*, *tst*, *eta* and *etb*, encoding Panton–Valentine leucocidin

(PVL), toxic shock syndrome toxin-1 (TSST-1) and exfoliative toxins A (ETA) and B (ETB), respectively, was determined by PCR as previously described [3, 5].

The presence of IEC genes *scn*, *sak* and *chp*, encoding the human-specific immune evasion proteins SCIN (staphylococcal complement inhibitor), staphylokinase and CHIPS (chemotaxis inhibitory protein of *S. aureus*), respectively, was determined by an in-house developed multiplex PCR using the following primers *scn2-Fw*: TTTAGTGCTTCGTCAATTTC, *scn1-Rev*: CTAGCACAAGCTTGCCAACA (amplification product of 247 bp), *sak1-Fw*: TGGGACAACAAAAC CTTTTTC, *sak1-Rev*: CGATGACGCGAGTTATTTTG (307 bp product), *chp1-Fw*: ACGGCAGGAAGTACACA, *chp1-Rev*: TTCATTCGTTTTTCCAGGACCA (393 bp product), *16S rRNA1*: GTTATTAGGGAAGAACATATGTG, *16S rRNA2*: CCACCTTCCTCCGGTTTGTCACC (750 bp product, internal control gene). MRSA252 and a strain from the Belgian NRC for *S. aureus*, 2010S029 [7], were used as positive controls, and MRSA COL was used as a negative control. The PCR program was as follows: 15 min at 95 °C; 25 cycles of denaturation for 30 s at 95 °C, annealing for 90 s at 57 °C and elongation for 90 s at 72 °C; and a final elongation step of 10 min at 72 °C.

Results

Suspected focus of *S. aureus* bacteraemia

For 95 (88.0%) out of 108 isolates, information about the suspected clinical source of bacteraemia was registered on the laboratory request form. For many patients, more than one source was registered, giving a total of 128 sources registered (Table 1). The most frequent sources included suspicion of severe malaria (for 29.5% of isolates), meningitis (15.8%), complicated urinary tract infection (14.7%) and pneumonia (11.6%, of which one with empyema); skin infections were registered in only 2.1% of isolates (both PVL positive). Detailed results can be consulted in Table 1.

Table 1 Suspected focus of bacteraemia for 108 *Staphylococcus aureus* isolates (Democratic Republic of the Congo, DRC), 2009–2013)

	Number	Proportion (%) of the total number of diagnoses ($n = 128$)	Proportion (%) of the total number of isolates with data available ($n = 95$)
Sepsis	1	0.8	1.1
Pneumonia	11	8.6	11.6
Meningitis	15	11.7	15.8
Other	52	40.6	54.7
No data	13		
Malaria	28	21.9	29.5
Skin	2	1.6	2.1
Endocarditis	1	0.8	1.1
Typhoid fever	3	2.3	3.2
Complicated urinary tract infection	14	10.9	14.7
Neonatal infection	1	0.8	1.1
Total number of diagnoses of anticipated focus of bacteraemia[a]	128	100.0	

Cases with human immunodeficiency virus (HIV) were not counted as an anticipated focus of bacteraemia

[a] The total number of diagnoses exceeds the total number of isolates because, for several isolates, more than one focus of bacteraemia was registered

Proportion of MSSA and MRSA: genetic diversity

Out of the collection of 108 *S. aureus* strains, 27 (25.0%) were identified as MRSA. The demographic characteristics of patients with MRSA and MSSA bacteraemia are shown in Table 2. Overall, patients' median age was 4 years (range: 1 day to 92 years) and 53.9% ($n = 55$) were female. No significant differences were observed in the MRSA/MSSA rates between gender, age groups or geographic origin. Nearly one-third (30.5%) of the *S. aureus* isolates were recovered from children <1 year old and over half (55.2%) from children <5 years old, corresponding to the higher number of blood cultures sampled in these age groups (respectively, 27.6% and 56.9% of all blood cultures).

The molecular characteristics of MRSA and MSSA isolates are displayed in Tables 3 and 4, respectively. Overall, 32 *spa* types were found, corresponding to at least 11 STs. Sixty-eight percent of all strains belonged to five major *S. aureus* lineages: CC8 ($n = 35$), CC152 ($n = 24$), CC5 ($n = 15$), CC1 ($n = 10$) and CC121 ($n = 9$). The overwhelming majority of MRSA isolates (23/27, 85.2%) belonged to CC8-*spa* t1476-SCC*mec* V, while the remaining MRSA were assigned to CC88-*spa* t186-SCC*mec* IV ($n = 1$) and SCC*mec* V ($n = 1$), CC152-*spa* t5691-SCC*mec* V ($n = 1$) and CC5-*spa* t311-SCC*mec* IV ($n = 1$). Although MSSA were genetically more diverse than MRSA, 85.2% (69/81) of MSSA isolates clustered into five major lineages only: CC152 (29.6% of MSSA isolates), CC5 (17.3%), CC8 (14.8%), CC1 (12.3%) and CC121 (11.1%).

Antibiotic susceptibility testing and detection of antibiotic resistance genes

For all MRSA, oxacillin MIC values by broth microdilution ranged from 0.5 to 64 mg/L and cefoxitin MIC values ranged from 8 to 32 mg/L. Of the MRSA ST8, 17/23 (73.9%) had an

Table 2 Demographic characteristics of the 108 patients with *S. aureus* bloodstream infection (DRC, 2009–2013)

Total number of patients	MSSA 81	% 75.0	MRSA 27	% 25.0	Proportion of MRSA/total *S. aureus* (%)
Female[a] (%)	41	53.9	14	53.8	25.0
Age[b] (range)	4 (0–67)		1 (0–92)		
<1 year	22	28.2	10	37.0	
Neonatal, very early (<72 h)	3		2		
Neonatal, early (<7 days)	0		0		
Neonatal, late onset (<28 days)	5		1		
Geographic origin					
Kinshasa (capital)	16	19.8	9	33.3	36.0
Bas-Congo (west)	45	55.6	10	48.1	22.4
Oriental (centre)	17	21.0	3	18.5	22.7
Équateur (north)	3	3.7	0	0	0.0

[a] For five patients, data were not available; 41/76 (MSSA) and 14/26 (MRSA) were female, respectively

[b] For three patients, data were not available

oxacillin MIC value in the susceptible range: 0.5 mg/L ($n = 1$) or 2 mg/L ($n = 16$). All MRSA ST8 were classified as resistant using cefoxitin MIC values, while two were categorised as susceptible when performing cefoxitin disc diffusion (two isolates had a diameter of 22 mm). MRSA non-ST8 were determined as resistant, regardless of the method used.

All isolates were susceptible to vancomycin, mupirocin and linezolid. MRSA were characterised by a multidrug resistance profile, with high resistance rates to aminoglycosides [92.6%, associated with the *aac*(6′)-*aph*(2″) gene], tetracycline [85.2%, associated with *tet*(K)], macrolides–lincosamides (ML) [74.1%, associated with *erm*(C)] and ciprofloxacin (59.3%) (Table 3). By contrast, MSSA isolates were less resistant: the highest resistance rates were found for penicillin (74.1%) and tetracycline (46.9%), but resistance rates for ML, aminoglycosides, ciprofloxacin, TMP-SMX and chloramphenicol were below 10% (Table 4). Although only four isolates (one MRSA and three MSSA) were resistant to TMP-SMX, almost two-thirds (72/108, 66.7%) of all isolates harboured the trimethoprim resistance gene *dfrG*; *dfrK* was not detected. Likewise, the resistance rates to chloramphenicol were below 10% among both MSSA and MRSA isolates.

Virulence factors and immune evasion cluster genes

Overall, the genes encoding PVL or TSST-1 were detected in 53 (49.1%) and 21 (19.4%) *S. aureus* isolates, respectively. *tst* was mainly detected among MRSA CC8-*spa* t1476-SCC*mec* V (18/21 of TSST-1-positive isolates were MRSA, 85.7%). By contrast, PVL genes were mainly detected among MSSA (51/53 strains harbouring PVL were MSSA, 96.2%) and associated with CC121 (9/9 CC121 isolates harboured PVL genes), CC152 (23/24), CC1 (9/10) and CC5 (6/14) (Table 4). None of the MRSA or MSSA isolates carried the genes encoding ETA or ETB. Overall, IEC genes were detected in 81.5% of all *S. aureus*; no difference was observed for MRSA (77.8% of isolates harboured IEC genes) compared to MSSA (82.7%). The detailed distribution of IEC genes according to genotype is displayed in Tables 3 and 4 for MRSA and MSSA, respectively. The isolates that lacked IEC genes belonged to CC1 (MSSA), CC8 (MRSA and MSSA), CC9 (MSSA), CC25 (MSSA), CC88 (MRSA), CC121 (MSSA) and CC152 (MSSA).

Discussion

Recent studies from various countries have generated information about *S. aureus* as a clinically relevant pathogen in Africa, but most studies originated from Northern, Western or Southern Africa. Only in the last several years has information on *S. aureus* as a pathogen in Central Africa become available [8–10]. As to the present study setting and surveillance network, *S. aureus*, however, ranked only sixth among the causative organisms of bloodstream infections, preceded by, ranked in decreasing frequency, *Salmonella* Typhi, the non-typhoidal *Salmonella*, *Klebsiella* spp., *Escherichia coli* and *Enterobacter* spp. [1]. As a comparison, a review on bloodstream infections in sub-Saharan Africa ranked *S. aureus* as the fourth most common, accounting for 9.5% of BSI isolates [11].

Consistent with reports from other African countries, the proportion of MRSA in the present study was 25.0% [8, 12–14], with no major differences between age groups and geography. The vast majority of MRSA isolates was assigned to a single MLST CC8-*spa* CC1476-SCC*mec* V PVL-negative MRSA clone, which has been detected previously in the DRC [15], Sao Tome and Principe (STP) [16] and at low frequency in South Africa [17]. It has also been reported as MSSA in Senegalese patients [18] and pigs [19]. In the

Table 3 Molecular characteristics and antibiotic resistance profile of methicillin-resistant *S. aureus* (MRSA) isolates from blood cultures, DRC

MLST				No. of isolates	No. of MRSA			No. of MRSA resistant to and harbouring resistance genes[a]									
CC	ST	*spa* type	SCC*mec* type		PVL	TSST-1	IEC+	AG	*aac*(6')-*aph*(2'')	ML[b]	*erm*(C)	TET	*tet*(K)	CIP	CHL	TMP-SMX	*dfrG*	PEN
5	5	t311	IV(2B)	1	1	0	1	1	1	0	0	1	1	0	0	0	0	1
8	8	t1476	V(5C2)	23	0	18	18	23	23	19	19	20	20	16	2	0	23	23
88	88	t186	IV(2B)	1	0	0	0	0	0	1	1	1	1	0	0	1	1	1
			V(5C2)	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1
152	152	t5691	V(5C2)	1	1	0	1	1	1	0	0	1	1	0	0	0	0	1
Total				27	2 (7%)	18 (67%)	21 (78%)	25 (93%)	25 (93%)	20 (74%)	20 (74%)	23 (85%)	23 (85%)	16 (59%)	2 (7%)	1 (4%)	24 (89%)	27 (100%)

For ease of interpretation and to compare with previously used nomenclature of major *S. aureus* lineages/clones, CC5 and CC8 are here considered as distinct CCs, although eBURST analysis with all STs previously submitted to the MLST database indicate that they are now all incorporated into CC5

Abbreviations: IEC, immune evasion cluster genes; AG, aminoglycosides; ML, macrolides–lincosamides; TET, tetracyclines; CIP, ciprofloxacin; CHL, chloramphenicol; ; TMP-SMX, sulphamethoxazole–trimethoprim; PEN, penicillin

[a] All MRSA isolates were susceptible to mupirocin and linezolid. The resistance genes *ant*(4'), *aph*(3'), *erm*(A), *erm*(B), *erm*(T), *tet*(M), *tet*(K) and *dfrK* were not detected

[b] All isolates that were resistant to erythromycin showed inducible resistance to clindamycin

present study, MRSA CC8 were characterised by a multidrug resistance profile on the one hand, and atypical oxacillin susceptibility on the other hand. The dissociation between oxacillin resistance and presence of the *mec*A gene has been reported previously for MRSA ST8 from the DRC, Angola and STP [15]. In settings where direct testing for *mec*A or *PBP2a* is not available, cefoxitin susceptibility testing should be performed. According to the present findings, cefoxitin disc diffusion will identify most MRSA. Cefoxitin MIC determination by the Etest or broth microdilution is, however, recommended for the identification of MRSA isolates with a diameter just above the cut-off of 21 mm. Both MSSA and MRSA t1476 were frequent among the present collection, suggesting that MRSA CC8-*spa* CC1476-SCC*mec* V might have locally arisen through acquisition of the SCC*mec* V element, followed by clonal spread within the DRC.

Two of the remaining MRSA isolates were assigned to a typical 'African' MRSA clone CC88-SCC*mec* IV/V, which appears to be a predominant MRSA clone in Western, Central and Eastern Africa [8]. One PVL-positive MRSA isolate belonged to ST152-SCC*mec* V, a genetic background that has frequently been detected among MSSA from Mali, Gabon, Nigeria, STP and Cape Verde [16, 20–22]. To the best of our knowledge, this clone has been reported as MRSA only once from Africa, in Nigeria [13]. By contrast, it appears to be more frequent as a PVL-positive CA-MRSA clone that circulates in the Balkan region and in Central Europe [23–25]. Phylogenetically, ST152 is a divergent genotype that does not cluster within one of the ten major *S. aureus* lineages (http://eburst.mlst.net/). It has been suggested that this lineage originated in Africa, migrated throughout Central Europe and acquired methicillin resistance. Of note, because of its nearly absolute association with PVL genes (also confirmed for both MSSA and MRSA isolates in the present study), ST152 has been suggested as the original lineage that acquired PVL, after which the genes disseminated throughout the *S. aureus* population [21, 26].

It is interesting to compare the present results to those obtained in a cross-sectional study of nasal carriage among healthcare workers in Kisangani (Oriental Province of the DRC) [27]. In this study (conducted in 2011), the MRSA rate was 15.6% (10 out of 63 *S. aureus* isolates). As was the case for the blood culture isolates, two-thirds of nasal carriage *S. aureus* isolates was assigned to four clonal complexes, i.e. CC5, CC8, CC121 and CC152. Genetic diversity was higher among MSSA (27 *spa* types) compared to MRSA (four *spa* types); among the latter and in line with the present findings, ST8-*spa* t1476-SCC*mec* V was the most frequent. MRSA nasal carriage isolates had high proportions of multidrug resistance (similar to those recorded for the blood culture isolates), whereas MSSA had lower resistance rates. Although the proportion of PVL genes was lower (28.5% among nasal carriage isolates vs. 49.1% among blood culture isolates), the

Table 4 Molecular characteristics and antibiotic resistance profile of methicillin-susceptible *S. aureus* (MSSA) isolates ($n = 81$) from blood cultures, DRC

MLST		*spa*-		No. of MSSA				No. of MSSA resistant to and harbouring resistance genes[b]										
CC	ST	CC[a]	Types	No. of isolates	PVL	TSST-1	IEC+	AG	*aac*(6′)-*aph*(2″)	ML[c]	*erm*(C)	TET	*tet*(K)	*tet*(M)	CIP	CHL	SXT	*dfrG*	PEN
1	1	NF1	t590, t2907	10	9	0	8	0	ND	0	ND	10	10	0	0	0	0	4	6
5	2975	311	t002, t311, t568, t5576, t12741, t12743	13	5	0	13	1	1	2	2	4	1	3	1	1	0	2	10
	5	Si1	t1154	1	1	0	1	0	ND	0	ND	0	ND	ND	0	0	0	0	1
8	8	1476	t498, t1476, t6940, t701	10	1	2	8	0	ND	2	2	7	7	0	2	0	0	9	9
	8[d]	NF2	t064[d], t1774	2	0	0	2	0	ND	0	ND	1	1	0	0	0	2	2	2
15	15[d]	NF3	t084[d], t279[d]	4	0	0	4	0	ND	1	1	4	0	4	0	0	0	4	3
25	25	Si2	t078	1	1	0	0	0	ND	0	ND	1	1	0	0	0	0	0	1
30	30	Si3	t12742	1	1	0	1	0	ND	0	ND	0	ND	ND	0	0	0	0	1
121	121	NF4	t314, t8330	7	7	0	5	0	ND	0	ND	2	2	0	0	1	1	5	6
	2976	Si5	t645	1	1	0	1	0	ND	0	ND	0	ND	ND	0	0	0	1	1
	121	Si6	t12744	1	1	0	1	0	ND	0	ND	1	1	0	0	0	0	1	1
152	152	355	t355, t1096, t5691	24	23	0	21	0	ND	2	2	7	7	0	0	3	0	18	16
9	9	Si7	t9793	1	0	1	0	0	ND	0	ND	0	ND	ND	0	0	0	1	1
	9	Si8	t1430	1	0	0	0	0	ND	0	ND	0	ND	ND	0	0	0	0	0
ND	ND	Si9	t939	2	0	0	0	0	ND	0	ND	0	ND	ND	0	0	0	0	2
ND	ND	Si10	t5585	1	0	0	1	0	ND	1	0[e]	0	ND	ND	0	0	0	0	0
ND	ND	Si11	t4190	1	1	0	1	0	ND	0	ND	1	1	0	1	0	0	1	0
Total				81	51 (63%)	3 (4%)	67 (83%)	1 (1%)	1 (1%)	8 (10%)	7 (7%)	38 (47%)	31 (38%)	7 (9%)	4 (5%)	5 (6%)	3 (4%)	48 (59%)	60 (74%)

For ease of interpretation and to compare with previously used nomenclature of major *S. aureus* lineages/clones, CC1, CC5, CC8, CC9 and CC15 are here considered as distinct CCs, although eBURST analysis with all STs previously submitted to the MLST database indicate that they are now all incorporated into CC5

Abbreviations: IEC, immune evasion cluster genes; AG, aminoglycosides; ML, macrolides–lincosamides; TET, tetracyclines; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, sulphamethoxazole; PEN, penicillin; ND, not determined

[a] Abbreviations used in column displaying *spa*-CC (terminology used in Ridom StaphType software): NF, no founder, a cluster without an *spa* type founder; Si, singleton, a cluster containing only one *spa* type

[b] All MSSA isolates were susceptible to cefoxitin, mupirocin and linezolid. The resistance genes *ant*(4′), *aph*(3′), *erm*(A), *erm*(B), *erm*(T) and *dfrK* were not detected

[c] All isolates that were resistant to erythromycin showed inducible resistance to clindamycin

[d] MLST derived from Ridom SpaServer (http://www.spaserver.ridom.de/)

[e] For this MSSA isolate, the observed resistance to erythromycin/clindamycin was not due to *erm*(A), *erm*(B), *erm*(C) or *erm*(T)

proportion of TSST-1-encoding genes was similar (17.5% vs. 19.4%, respectively), as were the associations with particular lineages: in the nasal carriage group, PVL was also restricted to MSSA and associated with ST152, ST121 and ST5, whereas TSST-1 mainly occurred among MRSA and was associated with ST8-*spa* t1476-SCC*mec* V.

Among the present MRSA isolates, there were high multidrug resistance rates, particularly among the CC8-t1476-SCC*mec* V clone. This is of great concern, since microbiological facilities and second-line antibiotics are rarely available in the DRC. As to serious MRSA infections such as bacteraemia, glycopeptides are the treatment of choice, and besides availability, costs and need for parenteral use, there are issues of toxicity requiring therapeutic drug monitoring. Of note, TMP-SMX and chloramphenicol had maintained activity, with less than 10% resistance among MRSA as well as MSSA isolates in the present series. TMP-SMX is a broad-spectrum antibiotic with excellent bioavailability and tissue penetration, which has currently been assessed as an alternative treatment option for invasive MRSA infections [28]. The percentage of TMP-SMX resistance observed in our study (3.7%) is lower than the resistance rate of 30% reported for *S. aureus* isolated from SSTI in travellers returning from Africa [29]. This percentage is, however, in line with other studies investigating TMP-SMX resistance among *S. aureus* from Africa, notably Gabon, Tanzania and Cape Verde [16, 30]. The fact that nearly one-third of the *S. aureus* isolates were recovered from children <1 year old and over half from children <5 years old could also have influenced the observed resistance rate. In line with findings for isolates from Gabon, Namibia, Nigeria and Tanzania, we did observe a high frequency of the *dfrG* gene encoding for trimethoprim resistance [30]. This is a striking but alarming observation, as concurrent resistance to the sulphamethoxazole component may arise quickly and preclude the use of TMP-SMX for the empirical treatment of *S. aureus* infections [29]. In their study, Nurjadi et al. warn that susceptibility testing against the combination TMP-SMX (instead of testing individual compounds) does not allow to detect emerging resistance [30].

As observed for other countries in sub-Saharan Africa, we found a prevalence of PVL genes that is markedly high compared to *S. aureus* isolates from Europe, Australia or the USA [31]. As reported previously from elsewhere in Africa, they were mainly associated with specific MSSA lineages: CC1, CC121 and CC152 [13, 21, 22, 27, 32]. Although it has been suggested that SSTI in Africa would be more frequent and invasive than elsewhere, partly due to the high prevalence of PVL, skin infections were registered as the suspected clinical source of bacteraemia in only 2.1% of the isolates in this study (both PVL positive). Further research is required to elucidate the relationship between PVL and invasive *S. aureus* disease in Africa.

The IEC genes *sak*, *chp* and *scn* encode the immune evasion proteins staphylokinase, CHIPS and SCIN that act specifically against human cells [33, 34]. They are highly prevalent (90%) in *S. aureus* isolated from humans but (nearly) absent in animal-derived *S. aureus* strains [34]. The prevalence of IEC genes observed in the present study (81.5%) is not significantly different to that typically detected among human isolates. Several recent articles point to the occurrence of human-to-animal transfer of *S. aureus* in Africa, followed by loss of IEC genes as a mean of host adaptation [34]. In our study, the absence of IEC genes was not linked to a specific genotype, and was observed for MRSA as well as MSSA. *Staphylococcus aureus* isolates with a genetic background also observed in the present study, and belonging to genetic lineages that are frequently encountered among human *S. aureus*, have, meanwhile, been recovered from multiple animal species in Africa, both domestic and wildlife: cattle in Uganda (MSSA ST121-t645 [35]), pigs in Senegal (MSSA ST8-t1476, MRSA ST5-t311-IV and MRSA ST88-t3489-IV [19]), dogs (MSSA ST152-t11375 [36]) and monkeys (ST15 and CC152 [34]) in Zambia and wildlife in the DRC (ST1 and ST15 [37]).

Conclusion

In conclusion, the present study adds information from Central Africa to the microbiological map of *Staphylococcus aureus* as an invasive pathogen in humans. It confirms the occurrence of methicillin-resistant *S. aureus* (MRSA) with high levels of multidrug co-resistance as well as Panton–Valentine leucocidin (PVL)-positive invasive isolates in the Democratic Republic of the Congo (DRC). The impact of this information calls for the availability of microbiological surveillance as well as appropriate antibiotics.

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Compliance with ethical standards

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Conflict of interest None to declare.

Ethical approval The present project was carried out in the scope of a project of Antimicrobial Surveillance in Tropical Settings, for which ethical clearance had been obtained at the Institutional Review Board of the Institute of Tropical Medicine (ref. IRB/AB/ec/56), as well as from the University Hospital of Antwerp (ref. 8/20/96) and the Ministry of Health, DRC.

References

1. Lunguya O, Phoba MF, Mundeke SA, Bonebe E, Mukadi P, Muyembe JJ, Verhaegen J, Jacobs J (2012) The diagnosis of typhoid fever in the Democratic Republic of the Congo. Trans R Soc Trop Med Hyg 106:348–355
2. Zaidi AK, Thaver D, Ali SA, Khan TA (2009) Pathogens associated with sepsis in newborns and young infants in developing countries. Pediatr Infect Dis J 28:S10–S18
3. Vandendriessche S, Vanderhaeghen W, Soares FV, Hallin M, Catry B, Hermans K, Butaye P, Haesebrouck F, Struelens MJ, Denis O (2013) Prevalence, risk factors and genetic diversity of methicillin-resistant *Staphylococcus aureus* carried by humans and animals across livestock production sectors. J Antimicrob Chemother 68: 1510–1516
4. Clinical and Laboratory Standards Institute (CLSI) (2013) Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement. CLSI document M100-S23. CLSI, Wayne
5. Nonhoff C, Denis O, Struelens MJ (2005) Low prevalence of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to glycopeptides in Belgian hospitals. Clin Microbiol Infect 11(3):214–220
6. Argudín MA, Tenhagen BA, Fetsch A, Sachsenröder J, Käsbohrer A, Schroeter A, Hammerl JA, Hertwig S, Helmuth R, Bräunig J, Mendoza MC, Appel B, Rodicio MR, Guerra B (2011) Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. Appl Environ Microbiol 77:3052–3060
7. McCarthy AJ, van Wamel W, Vandendriessche S, Larsen J, Denis O, Garcia-Graells C, Uhlemann AC, Lowy FD, Skov R, Lindsay JA (2012) *Staphylococcus aureus* CC398 clade associated with human-to-human transmission. Appl Environ Microbiol 78(24): 8845–8848
8. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP (2013) MRSA in Africa: filling the global map of antimicrobial resistance. PLoS One 8:e68024
9. Herrmann M, Abdullah S, Alabi A, Alonso P, Friedrich AW, Fuhr G, Germann A, Kern WV, Kremsner PG, Mandomando I, Mellmann AC, Pluschke G, Rieg S, Ruffing U, Schaumburg F, Tanner M, Peters G, von Briesen H, von Eiff C, von Müller L, Grobusch MP (2013) Staphylococcal disease in Africa: another neglected 'tropical' disease. Future Microbiol 8:17–26
10. Schaumburg F, Alabi AS, Peters G, Becker K (2014) New epidemiology of *Staphylococcus aureus* infection in Africa. Clin Microbiol Infect 20:589–596
11. Reddy EA, Shaw AV, Crump JA (2010) Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis 10:417–432
12. Kesah C, Ben Redjeb S, Odugbemi TO, Boye CS, Dosso M, Ndinya Achola JO, Koulla-Shiro S, Benbachir M, Rahal K, Borg M (2003) Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin Microbiol Infect 9:153–156
13. Shittu A, Oyedara O, Abegunrin F, Okon K, Raji A, Taiwo S, Ogunsola F, Onyedibe K, Elisha G (2012) Characterization of methicillin-susceptible and -resistant staphylococci in the clinical setting: a multicentre study in Nigeria. BMC Infect Dis 12:286
14. Shittu AO, Lin J (2006) Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. BMC Infect Dis 6:125
15. Phaku P, Lebughe M, Strauß L, Peters G, Herrmann M, Mumba D, Mellmann A, Muyembe-Tamfum JJ, Schaumburg F (2016) Unveiling the molecular basis of antimicrobial resistance in *Staphylococcus aureus* from the Democratic Republic of the Congo using whole genome sequencing. Clin Microbiol Infect 22(7):644.e1–644.e5
16. Conceição T, Coelho C, Silva IS, de Lencastre H, Aires-de-Sousa M (2015) *Staphylococcus aureus* in former Portuguese colonies from Africa and the Far East: missing data to help fill the world map. Clin Microbiol Infect 21(9):842.e1–842.e10
17. Oosthuysen WF, Orth H, Lombard CJ, Sinha B, Wasserman E (2014) Population structure analyses of *Staphylococcus aureus* at Tygerberg Hospital, South Africa, reveals a diverse population, a high prevalence of Panton–Valentine leukocidin genes, and unique local methicillin-resistant *S. aureus* clones. Clin Microbiol Infect 20:652–659
18. Fall C, Richard V, Dufougeray A, Biron A, Seck A, Laurent F, Breurec S (2014) *Staphylococcus aureus* nasal and pharyngeal carriage in Senegal. Clin Microbiol Infect 20:O239–O241
19. Fall C, Seck A, Richard V, Ndour M, Sembene M, Laurent F, Breurec S (2012) Epidemiology of *Staphylococcus aureus* in pigs and farmers in the largest farm in Dakar, Senegal. Foodborne Pathog Dis 9:962–965
20. Okon KO, Basset P, Uba A, Lin J, Oyawoye B, Shittu AO, Blanc DS (2009) 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 47:3000–3003
21. Ruimy R, Maiga A, Armand-Lefevre L, Maiga I, Diallo A, Koumaré AK, Ouattara K, Soumaré S, Gaillard K, Lucet JC, Andremont A, Feil EJ (2008) 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 190:3962–3968
22. Schaumburg F, Ngoa UA, Kösters K, Köck R, Adegnika AA, Kremsner PG, Lell B, Peters G, Mellmann A, Becker K (2011) Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. Clin Microbiol Infect 17: 1507–1513
23. Garnier F, Tristan A, François B, Etienne J, Delage-Corre M, Martin C, Liassine N, Wannet W, Denis F, Ploy MC (2006) Pneumonia and new methicillin-resistant *Staphylococcus aureus* clone. Emerg Infect Dis 12:498–500
24. Krziwanek K, Metz-Gercek S, Mittermayer H (2011) Trends in the occurrence of MRSA strains in Upper Austria from 2006 to 2009. Clin Microbiol Infect 17:920–923
25. Müller-Premru M, Strommenger B, Alikadic N, Witte W, Friedrich AW, Seme K, Kucina NS, Smrke D, Spik V, Gubina M (2005) New strains of community-acquired methicillin-resistant *Staphylococcus aureus* with Panton–Valentine leukocidin causing an outbreak of severe soft tissue infection in a football team. Eur J Clin Microbiol Infect Dis 24:848–850
26. Monecke S, Slickers P, Ellington MJ, Kearns AM, Ehricht R (2007) High diversity of Panton–Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S. aureus*. Clin Microbiol Infect 13:1157–1164
27. De Boeck H, Vandendriessche S, Hallin M, Batoko B, Alworonga JP, Mapendo B, Van Geet C, Dauly N, Denis O, Jacobs J (2015)

Staphylococcus aureus nasal carriage among healthcare workers in Kisangani, the Democratic Republic of the Congo. Eur J Clin Microbiol Infect Dis 34:1567–1572

28. Goldberg E, Paul M, Talker O, Samra Z, Raskin M, Hazzan R, Leibovici L, Bishara J (2010) Co-trimoxazole versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteraemia: a retrospective cohort study. J Antimicrob Chemother 65:1779–1783
29. Nurjadi D, Friedrich-Jänicke B, Schäfer J, Van Genderen PJ, Goorhuis A, Perignon A, Neumayr A, Mueller A, Kantele A, Schunk M, Gascon J, Stich A, Hatz C, Caumes E, Grobusch MP, Fleck R, Mockenhaupt FP, Zanger P (2015) Skin and soft tissue infections in intercontinental travellers and the import of multi-resistant *Staphylococcus aureus* to Europe. Clin Microbiol Infect 21(6):567.e1–567.e10
30. Nurjadi D, Olalekan AO, Layer F, Shittu AO, Alabi A, Ghebremedhin B, Schaumburg F, Hofmann-Eifler J, Van Genderen PJ, Caumes E, Fleck R, Mockenhaupt FP, Herrmann M, Kern WV, Abdulla S, Grobusch MP, Kremsner PG, Wolz C, Zanger P (2014) Emergence of trimethoprim resistance gene *dfrG* in *Staphylococcus aureus* causing human infection and colonization in sub-Saharan Africa and its import to Europe. J Antimicrob Chemother 69:2361–2368
31. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, Liassine N, Bes M, Greenland T, Reverdy ME, Etienne J (2003) Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton–Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9:978–984
32. Shittu AO, Okon K, Adesida S, Oyedara O, Witte W, Strommenger B, Layer F, Nübel U (2011) Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. BMC Microbiol 11:92
33. Rooijakkers SH, Wu J, Ruyken M, van Domselaar R, Planken KL, Tzekou A, Ricklin D, Lambris JD, Janssen BJ, van Strijp JA, Gros P (2009) Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. Nat Immunol 10:721–727
34. Senghore M, Bayliss SC, Kwambana-Adams BA, Foster-Nyarko E, Manneh J, Dione M, Badji H, Ebruke C, Doughty EL, Thorpe HA, Jasinska AJ, Schmitt CA, Cramer JD, Turner TR, Weinstock G, Freimer NB, Pallen MJ, Feil EJ, Antonio M (2016) Transmission of *Staphylococcus aureus* from humans to green monkeys in the Gambia as revealed by whole-genome sequencing. Appl Environ Microbiol 82(19):5910–5917
35. Kateete DP, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML (2013) Prevalence and antimicrobial susceptibility patterns of bacteria from milkmen and cows with clinical mastitis in and around Kampala, Uganda. PLoS One 8:e63413
36. Youn JH, Park YH, Hang'ombe B, Sugimoto C (2014) Prevalence and characterization of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from companion animals and environment in the veterinary teaching hospital in Zambia, Africa. Comp Immunol Microbiol Infect Dis 37:123–130
37. Schaumburg F, Pauly M, Anoh E, Mossoun A, Wiersma L, Schubert G, Flammen A, Alabi AS, Muyembe-Tamfum JJ, Grobusch MP, Karhemere S, Akoua-Koffi C, Couacy-Hymann E, Kremsner PG, Mellmann A, Becker K, Leendertz FH, Peters G (2015) *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clin Microbiol Infect 21(4): 345.e1–345.e8