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Staphylococcus aureus in former Portuguese colonies from Africa and the Far East: missing data to help fill the world map

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Abstract

The aim of the present study was to determine the prevalence and risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage among patients and healthcare workers in Angola (ANG), São Tomé and Príncipe (STP), Cape Verde (CV) and East Timor (ET), and to characterize the antimicrobial susceptibility, virulence content and population structure of all *S. aureus*. Despite the importance of MRSA as a major human pathogen, data from these former Portuguese colonies in Africa and Asia are scarce. A total of 2065 nasal swabs recovered between 2010–14 were included in the study. Antimicrobial susceptibility testing and molecular characterization of *S. aureus* showed: (i) a very high MRSA prevalence in ANG (61.6%), moderate in STP (25.5%), low in CV (5.6%) and null in ET; (ii) a high prevalence of Panton–Valentine leukocidin in STP (36.8%), ET (29.2%) and CV (28.3%) contrasting with ANG (7.9%); (iii) ST5-SCC*mec*IVa, ST8-IV/V and ST5-VI were the major MRSA clones in ANG (65.2%), STP (44.8%) and CV (50%), respectively; (iv) a high resistance to trimethoprim-sulfamethoxazole in ANG (66.5%) and STP (50.9%), to rifampin in ANG (77.3%), and to tetracycline in STP (26.3%) and ET (20.8%); (v) three major methicillin-susceptible *S. aureus* clones (ST15, ST508, ST152) were present in all four countries. Age <18 years (OR 2.03, 95% CI 1.24–3.31), previous surgery (OR 2.45, 95% CI 1.24–4.83), no smoking (OR 4.04, 95% CI 1.05–15.50), and longer hospitalization (OR 2.53, 95% CI 1.49–4.28) were risk factors for MRSA carriage. This study provided the first comprehensive overview on MRSA in former Portuguese colonies in Africa and Asia, missing data in the world map.
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major threat to public health not only in the developed world, but in developing regions as well where human immunodeficiency virus (HIV)/AIDS, malaria, malnutrition, crowded living conditions, high temperatures and humidity additionally contribute to the increased risk of bacterial infections [1,2]. However, in many developing regions, research protocols do not include major bacterial pathogens such as MRSA and funding for health and research is mainly directed to HIV/AIDS, malaria and tuberculosis programmes, namely in Sub-Saharan Africa [3]. In many developing and low-income countries there is a lack of microbiological diagnostic facilities and patients with signs of infection are often treated empirically; as a result, the assessment of MRSA rates and antimicrobial resistance profiles is of major importance.

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During the last 10 years Portugal has been reporting one of the highest rates of MRSA in Europe (around 50%) [4]. However, *S. aureus* epidemiological surveillance studies in former Portuguese colonies in Africa and Asia, with close demographic relationships with Portugal, remain scarce. Data from Angola and São Tomé and Príncipe are limited to single studies from our group [5,6]. The unique *S. aureus* surveillance study conducted in Cape Verde included isolates from 1997 and at that time MRSA was not detected [7,8]. In Mozambique a recent study assessed exclusively the antimicrobial susceptibility of *S. aureus* and the MRSA prevalence in a single hospital [9]. Studies from East Timor are inexistent so far. Moreover, no single work provided an overview on MRSA in former Portuguese colonies in Africa and Asia, which is essential to help defining local and global public health priorities, and to contribute for the global picture of MRSA epidemiology.

The aim of the present study was to assess rates of MRSA nasal carriage among patients and healthcare workers (HCW) in Angola, São Tomé and Príncipe, Cape Verde and East Timor, to determine risk factors associated with MRSA colonization, and to characterize the antimicrobial susceptibility, virulence content and population structure of *S. aureus*.

Materials and methods

Hospitals

Ten hospitals participated in the surveillance study (see Supporting information, Table S1): (i) Angola: Hospital Pediátrico David Bernardino (HPDB), Hospital Américo Boavida (HAB), Clínica Sagrada Esperança (CSE), Hospital Maria Pia (HMP) and Clínica Girassol (CG); (ii) São Tomé and Príncipe: Hospital Ayres de Menezes (HAM); (iii) Cape Verde: Hospital Agostinho Neto (HAN) and Hospital Baptista de Sousa (HBS); and (iv) East Timor: Hospital Guido Valadares (HGV) and Clínica do Bairro Pité (CBP).

Bacterial isolates

From November 2010 to June 2014, a total of 2065 individuals (1267 inpatients and 798 HCW) from Angola (n = 893), São Tomé and Príncipe (n = 490), Cape Verde (n = 515) and East Timor (n = 167) were screened for *S. aureus* nasal carriage (see Supporting information, Table S1).

Sampling included all patients admitted in the main services where the risk for *S. aureus* infection is usually high (intensive care and burn units, medicine, surgery, paediatrics and orthopaedics) and all HCW from the same wards, active at the time of sampling. HCW included doctors (n = 144), nurses (n = 395), nurse-aids (n = 147), cleaners (n = 54) and technicians (n = 53).

The protocol was approved by the institutional review boards. An informed consent was obtained from each participant, or from the guardians in the case of children, after a verbal presentation of the purpose, method and design of the study. A brief questionnaire was administered verbally by the participating author (MAS) to inpatients, guardians in the case of children, and HCW to collect data concerning their living conditions at home (number of household members, running water facilities and contact with animals) and smoking behaviour. Demographic data as well as admission date, clinical diagnosis, previous surgery and antibiotic usage history were registered after on-site consultation of the clinical records. Exclusion criteria included inpatients admitted for less than 48 h, individuals with inaccessible nares due to the presence of medical devices, children being breastfed at the time of screening, and individuals who refused to participate in the study.

Sampling and *S. aureus* identification

Sampling was performed by a trained nurse and participating author during a 2- or 3-day period in each hospital. Cultures were obtained by rotating a sterile cotton swab into both anterior nares several times before returning the swab to the transport tube, which contained Stuart's medium. The swabs were transported within 10 days at room temperature to the Laboratory of Molecular Genetics, Instituto de Tecnologia Química e Biológica in Portugal, where they were inoculated on Tryptic Soy Agar (Becton Dickinson, Franklin Lakes, New Jersey, USA) and on a selective chromogenic medium Chromagar Staph aureus (ChromAgar; Paris, France). All presumptive *S. aureus* colonies were tested for coagulase by latex agglutination test Staphaurex (Remel, Lenexa, Kansas, USA) or by agglutination of rabbit plasma in tubes (Becton Dickinson) in the case of previously ambiguous results. The *S. aureus* species was confirmed by PCR amplification of the *nuc* [10] or *spa* [11] genes.

Antimicrobial susceptibility testing and confirmative identification of MRSA

Antimicrobial susceptibility testing was performed on all isolates by the disc diffusion method for a panel of 16 antibiotics: penicillin, cefoxitin, erythromycin, gentamicin, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, rifampin, tetracycline, fusidic acid, mupirocin, teicoplanin, vancomycin, linezolid and quinupristin-dalfopristin. Standard published CLSI breakpoints were used for interpretation, except for fusidic acid and mupirocin for which breakpoints from the European Committee on Antimicrobial Susceptibility Testing and the British Society for Antimicrobial Chemotherapy (http://bsac.org.uk/susceptibility/) guidelines were respectively applied. Strain *S. aureus* ATCC25923 was used as quality

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control. Confirmatory identification of MRSA was performed on all *S. aureus* isolates by PCR amplification of the *mecA* gene [12]. Vancomycin MICs were determined for MRSA isolates using E-test strips, according to the manufacturer's instructions (Biomérieux, Marcy l'Etoile, France).

Molecular typing and virulence determinants

Four typing methods—pulsed-field gel electrophoresis (PFGE), *spa* typing, multilocus sequence typing (MLST) and staphylococcal cassette chromosome *mec* (SCC*mec*) typing—were used in the present study. PFGE was performed on all *S. aureus* isolates as described by Chung et al. [13] and the resulting band profiles were analysed by visual inspection, followed by automated analysis with the BioNumerics software version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium) as previously described [14]. *spa* typing was performed on at least one representative of each PFGE subtype, and *spa* types were assigned through the Ridom web server (http://spaserver.ridom.de) [11]. MLST was performed on representative isolates of each PFGE type [15] and the allelic profiles and sequence types (ST) were defined using the MLST online database (http://www.mlst.net). SCC*mec* was characterized by multiplex PCR [16] in all MRSA isolates and SCC*mec* type IV isolates were further subtyped [17]. SCC*mec* type VII was identified by PCR amplification of *mec* complex C1 and *ccrC* [18].

Detection of exotoxins, arginine catabolic mobile element and *agr* type

The presence of 11 specific staphylococcal exotoxin genes, including three leukocidins (*lukS-lukF* (Panton–Valentine leukocidin (PVL) determinant), *lukD-lukE*, *lukM*), three haemolysins (*hlb*, *hlg*, *hlgv*) and five super-antigenic toxins (*eta*, *etb*, *etd*, *sel*, *sep*) were determined by PCR for all *S. aureus* isolates [19]. MRSA isolates belonging to ST8 were screened for the presence of the arginine catabolic mobile element by PCR amplification of *arcA* and *opp3* [20,21]. Subtypes of the accessory gene regulator (*agr* I–IV) were determined for the entire collection by multiplex PCR [22].

Statistical analysis

Risk factors were assessed using SPSS software, version 21.0. Categorical variables were summarized using the frequency and percentage. The chi-squared test was used to identify variables associated with MRSA carriage. Odds ratio (OR) was calculated for all variables found to be statistically significant at a level of significance of 0.05. All significant variables detected in the univariate analyses were introduced into the final multivariate model. Variables were considered to be significantly associated to MRSA carriage if the 95% CI of the OR did not contain 1.

Results

Population description

A total of 2065 individuals (61.4% inpatients and 38.6% HCW) participated in the study. The characteristics of the population are displayed in the Supplementary material (Table S2). Briefly, the demographic data showed that 55.6% of the participants were female and 73.5% were adults. Most of the individuals (67.0%) were not previously submitted to surgery and 66.4% were under current or previous antibiotherapy (mainly β-lactams). In addition, the majority (93.7%) were non-smokers, had contact with animals (62.0%—mainly with chicken, cats and pigs), had no running water at home (54.1%) and lived with more than three householders (77.0%).

Concerning exclusively the patients, 46.5% were admitted to the hospital the week preceding sampling (between 48 h and 8 days), and the main reasons for admission were orthopaedic (16.9%), respiratory (10.7%) and gastrointestinal (9.8%).

MRSA prevalence

Among the 2065 participants who underwent nasal screening, 424 were *S. aureus* carriers (20.5%). Although we found a global MRSA prevalence of 34.8% among *S. aureus* carriers (148 out of 424 *S. aureus*) there were significant inter-country variations: the prevalence was very high in Angola (61.6%; 114/185), moderate in São Tomé and Príncipe (25.5%; 28/110), low in Cape Verde (5.6%; 6/107) and null in East Timor (0/22) (see Supplementary material, Table S1).

Considering the ten hospitals individually, we did not detect MRSA among nasal samples in the hospital on the island of São Vicente in Cape Verde (HBS) nor in the two hospitals in East Timor (see Supplementary material, Table S1). In Angola, with the exception of hospital CG where the single *S. aureus* isolate recovered was methicillin resistant, the MRSA prevalence was higher in the largest public hospital (HMP, 70.6%) and lower in the smallest private hospital (CSE, 40.6%). Interestingly, in this hospital the MRSA prevalence was significantly higher among HCW (76.9%) compared with patients; whereas usually (and as happened in Angola and São Tomé and Príncipe) higher MRSA rates were found among patients (74.6% and 71.4%, respectively) (see Supplementary material, Table S1). Among a total of 40 HCW that were MRSA carriers, the overwhelming majority (32; 80%) were nurses (23; 57.5%) and doctors (9; 22.5%).

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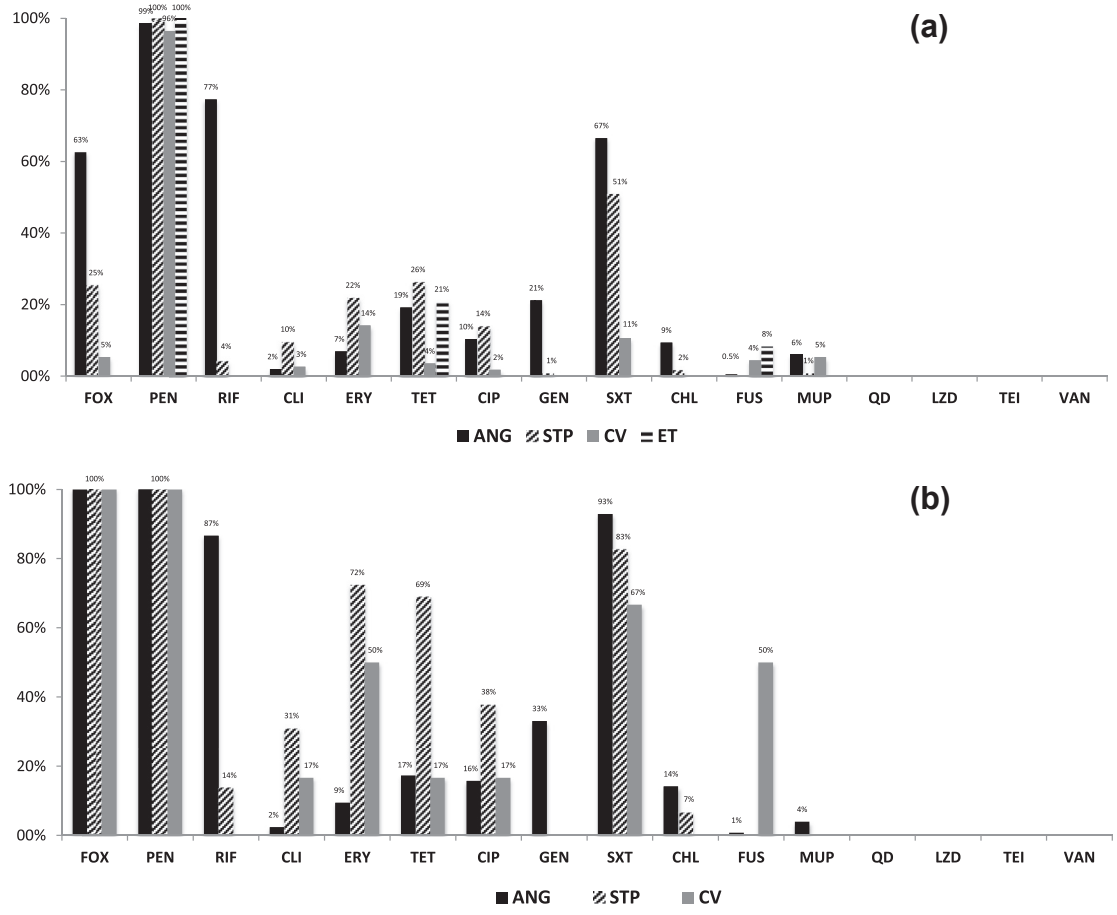

FIG. 1. Antimicrobial resistance in Angola (ANG), São Tomé and Príncipe (STP), Cape Verde (CV) and East-Timor (ET) among (a) *Staphylococcus aureus* isolates and among (b) methicillin-resistant *Staphylococcus aureus* (MRSA). FOX, cefoxitin; PEN, penicillin; RIF, rifampin; CLI, clindamycin; ERY, erythromycin; TET, tetracycline; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; FUS, fusidic acid; MUP, mupirocin; QD, quinupristin-dalfopristin; LZD, linezolid; TEI, teicoplanin; VAN, vancomycin.

As 30 individuals were co-colonized with two *S. aureus* isolates (differing by PFGE type or subtype or an MRSA/methicillin-susceptible *S. aureus* (MSSA) pair), the whole collection comprised a total of 454 *S. aureus* (162 MRSA and 292 MSSA) that were subsequently characterized.

Risk factors for MRSA carriage

The variables significantly associated with MRSA carriage in univariate analysis were the following: being a patient (p 0.001), age <18 years (p < 0.001), surgery in the previous 6 months (p 0.001), current or previous antibiotherapy (p < 0.001), being a non-smoker (p 0.022), having no contact with animals (p 0.001), no running water at home (p 0.020) and being hospitalized for more than 30 days (p 0.001) (see Supplementary material, Table S3).

After multivariate analysis, being <18 years old (OR 2.03, 95% CI 1.24–3.31), having been submitted to a surgery in the previous 6 months (OR 2.45, 95% CI 1.24–4.83), being a non-smoker (OR 4.04, 95% CI 1.05–15.50), and being hospitalized for longer periods (OR 2.53, 95% CI 1.49–4.28) remained as risk factors for MRSA carriage. In contrast, having no contact with animals (OR 0.38, 95% CI 0.18–0.83) decreased the risk of MRSA carriage (see Supplementary material, Table S3).

Antimicrobial resistance

Among the 454 *S. aureus* isolates, we observed resistance to penicillin (98.5%), trimethoprim-sulfamethoxazole (45.2%), rifampin (35.7%), cefoxitin (35.7%), tetracycline (17.2%), erythromycin (12.1%), gentamicin (9.7%), ciprofloxacin (8.6%), chloramphenicol (4.6%), mupirocin (4.2%), clindamycin (4.0%) and fusidic acid (1.8%). However, besides resistance to β-lactams, we found significant differences between countries (Fig. 1a): (i) in Angola we highlight the very high levels of resistance to rifampin (77.3%) and trimethoprim-

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sulfamethoxazole (66.5%); (ii) in São Tomé and Príncipe we observed not only high levels of resistance to trimethoprim-sulfamethoxazole (50.9%), but considerable resistance to tetracycline (26.3%) and erythromycin (21.9%) as well; (iii) in Cape Verde we found an important prevalence of erythromycin-resistant isolates (14.2%); and (iv) in East Timor the isolates were resistant to tetracycline (20.8%) and fusidic acid (8.3%) only, but the latter showed the highest value among the four countries. None of the 454 isolates was resistant to quinupristin-dalfopristin, linezolid, teicoplanin and vancomycin.

Among the MRSA isolates we emphasize the following (Fig. 1b): (i) in Angola an extremely high resistance to trimethoprim-sulfamethoxazole (92.9%) and rifampin (86.6%); (ii) in São Tomé and Príncipe the resistance to multiple classes of antimicrobials in addition to β-lactams (trimethoprim-sulfamethoxazole 82.8%; erythromycin 72.4%; tetracycline 69%; ciprofloxacin 37.9%; clindamycin 31%); and (iii) in Cape Verde the resistance to trimethoprim-sulfamethoxazole (66.7%), erythromycin and fusidic acid (50% each).

Molecular epidemiology

Although the 162 MRSA belonged to 14 different clonal types, there were three major clones: clones A (n = 85; 52.5%), B (n = 32; 19.8%) and C (n = 21; 13.0%) with a different distribution between the three Portuguese-speaking African countries (Table 1). In Angola, clone A (PFGE A-ST5-SCC*mec*IVa-*agr*II, mainly associated with *spa* type t105) included most of the isolates (65.4%), followed by clone B (PFGE B-ST88-IVa-*agr*III, mainly associated with *spa* type t786) represented by 15.0% of the isolates. In São Tomé and Príncipe, clone C (PFGE C-ST8-IV/V-*agr*I, mainly associated with *spa* type t451) was the major clone (44.8%) followed by clone B (37.9%). In Cape Verde, half (n = 3) of the MRSA isolates belonged to PFGE ABB-t002-ST5-VI-*agr*II. Interestingly, there was a higher diversity of the SCC*mec* types and subtypes in clone C-ST8 and of the *spa* types associated with clone B-ST88 (Table 1).

The 292 MSSA were distributed over 30 different clonal types, of which three were prevalent (Table 2): clone K (20.2%)—PFGE type K-ST15-*agr*II, mainly associated with *spa* type t084; clone L (17.8%)—PFGE type L-ST508-*agr*I, mainly associated with *spa* type t861; and clone M (16.1%)—PFGE type M-ST152-*agr*I, mainly associated with *spa* type t355. All three clones were predominant in the four countries, with the exception of clone M-ST152, which was a minor clone in Angola.

Exotoxins

The presence of genes encoding leukocidins, haemolysins, exfoliative toxins and enterotoxins is shown in Table 3. Among the whole collection, 91.5% of the isolates (85.2% of the MRSA and 94.2% of the MSSA) harboured at least one exotoxin gene. Leukocidin DE and γ-variant haemolysin were the most frequent exotoxins in the whole collection, in particular among MRSA isolates (83.3% and 82.7%, respectively), whereas leukocidin M and exfoliative toxins B and D were absent or rare among the 454 isolates (0%, 0.2% and 1.5%, respectively) and were not detected at all among MRSA.

PVL was found in 95 isolates (20.9%) but its prevalence varied considerably between countries: 6.9% in Angola, 28.3%

TABLE 1. Genotypic properties of methicillin-resistant *Staphylococcus aureus* isolates from Angola, São Tomé and Príncipe, and Cape Verde

Country	CC	ST	*spa* types[a]	SCC*mec*	PFGE	*agr*	No. of isolates[b]	PVL+	ACME+
Angola (n = 127)	5	5	**t105**/ t311/ t1657	IVa	A	II	83 (65.4%)		
	88	88	t186/ t325/ **t786**/ t1951/ t3869	IVa	B	III	19 (15.0%)		
	8	8	t064/ t104/ t1771	IVc/IVd/V	C	I	8 (6.3%)		
	72	72	t3092	V	D	I	4 (3.2%)		
	72	72	t148	V	E	I	1 (0.8%)		
	7	789	t091	V	J	I	1 (0.8%)		
	5	5/2629	t6065	V	U	II	2 (1.6%)		
	30	30	t6278	V	X	III	2 (1.6%)	1	
	22	22	t005	IVc	AN	I	1 (0.8%)	1	
	8	8	t1476	VII	AQ	I	6 (4.8%)		
São Tomé & Príncipe (n = 29)	5	5	t105/ t14047	IVa	A	II	2 (6.9%)		
	88	88	**t186**/ t786/ t1814	IVa	B	III	11 (37.9%)		
	8	8	t064/ **t451**	IVg/V	C	I	13 (44.8%)		
	1	1	t590	V	H	III	2 (6.9%)	2	
	5	105	t002	II	ABA	II	1 (3.4%)		
Cape Verde (n = 6)	88	88	t186/ t12827	IVa	B	III	2 (33.3%)		
	8	8	t121	IVa	N	I	1 (16.7%)	1	
	5	5	t002	VI	ABB	II	3 (50.0%)		

Clonal complexes (CC) were defined using the eBURST algorithm (assessed on 23 April 2015) and restricted to single locus variant (SLV) and double locus variant from each group founder or subfounder, inside each clonal group); ST, sequence type defined by multilocus sequence typing; PVL, Panton–Valentine leukocidin; ACME, arginine catabolic mobile element.
[a]Major *spa* types are displayed in bold.
[b]Percentage relative to the total number of methicillin-resistant *Staphylococcus aureus* in each country.

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TABLE 2. Genotypic properties of methicillin-susceptible *Staphylococcus aureus* isolates from Angola, São Tomé and Príncipe, Cape Verde and East-Timor

Country	CC	ST	*spa* types[a]	PFGE	*agr*	No. of isolates[b]	PVL+
Angola (*n* = 76)	5	5	t002/ t1215/ t6071/ t9921	A	II	4 (5.3%)	1
	8	8	t064	C	I	1 (1.3%)	
	72	72	t148/ t1346	E	I	5 (6.6%)	
	15	15	**t084**/ t346/ t774	K	II	9 (11.8%)	1
	45	508	t050/ t095/ t861/ **t1574**/ t1346/ t2626/ t12218	L	I	18 (23.7%)	
	152	152	t355	M	I	4 (5.3%)	2
	8	8	t008/ t1476	N	I	2 (2.6%)	
	121	121	t314/ t1077	O	IV	3 (4.0%)	1
	707	2367	t1458/ t12259	P	III	3 (4.0%)	
	45	45	**t939**/ t11656	Q	IV	9 (11.9%)	
	1	1	t127/ t693	R	III	2 (2.6%)	
	25	25	t349/ t1350/ t6205	W	I	5 (6.6%)	4
	30	30	t1202/ **t9118**	X	III	7 (9.2%)	3
	672	672	t12219	AA	I	1 (1.3%)	
	Singleton	601	t957	AF	II	1 (1.3%)	
	Singleton	2728	t5187	AG	III	1 (1.3%)	
	5	2229	t1476	AK	I	1 (1.3%)	
São Tomé & Príncipe (*n* = 85)	5	5	t311/ t319/ t14190	A	II	3 (3.5%)	1
	88	88	t4195	B	III	1 (1.2%)	
	8	8	t064/ t1774/ t13119	C	I	4 (4.7%)	
	72	72/2448	t148/ t324/ t11082	E	I	3 (3.5%)	
	97	97	t521	G	I	1 (1.2%)	
	15	15	t084	K	II	29 (34.1%)	19
	45	508/2446	t635/ **t861**/ t2771/ t5602/ t10763	L	I	13 (15.3%)	
	152	152	**t355**/ t1299/ t9564	M	I	12 (14.1%)	12
	121	121	t159/ t2304	O	IV	6 (7.1%)	6
	707	2367	t1458	P	III	1 (1.2%)	
	45	45/2447	**t939**/ t14189	Q	na	4 (4.7%)	
	1	1	t1931	R	III	1 (1.2%)	
	25	25	t078	W	I	1 (1.2%)	1
	30	3161	t017	X	III	1 (1.2%)	
	5	6	t701	Z	I	2 (2.4%)	
	718	718	t11083	AC	II	1 (1.2%)	
	8	94	t008	AD	I	1 (1.2%)	
	80	80	t934	AJ	III	1 (1.2%)	1
Cape Verde (*n* = 107)	5	5	t002	A	II	8 (7.5%)	1
	72	72	**t148**/ t3169	E	I	6 (5.6%)	
	97	669	t2734	F	I	1 (0.9%)	
	97	97	t267/ t521	G	I	4 (3.7%)	
	15	15	**t084**/ t3262	K	II	16 (15.0%)	2
	45	508	t589/ t861/ t1510/ t2771/ t3216/ t12615/ t12826	L	I	16 (15.0%)	
	152	152	**t355**/ t774/ t1172	M	I	27 (25.2%)	22
	121	121	t314	O	IV	1 (0.9%)	1
	45	45	t939	Q	na	3 (2.8%)	1
	1	1	t127	R	III	6 (5.6%)	2
	22	22	t223/ t4640/ t5146/ t8934	T	I	8 (7.5%)	
	1	188	t189	V	I	3 (2.8%)	
	25	25	t078	W	I	1 (0.9%)	
	30	1472	t318/ t12808/ t12828	X	III	5 (4.7%)	2
	5	6	t701	Z	I	1 (0.9%)	
	398	398	t571	AL	I	1 (0.9%)	
East Timor (*n* = 24)	5	5	t105	A	II	1 (4.2%)	1
	7	7	t091	J	I	1 (4.2%)	
	1	15	t084/ t335	K	II	5 (20.8%)	3
	45	508	t050/ t13455	L	I	5 (20.8%)	
	152	1633	t355	M	I	4 (16.7%)	3
	121	2782	t159	O	IV	1 (4.2%)	
	1	1	t127	R	III	2 (8.3%)	
	1	2784	t189	V	I	3 (12.5%)	
	398	2783	t13181	Y	I	1 (4.2%)	
	2483	SLV2483[c]	NT	AH	na	1 (4.2%)	

Clonal complexes (CC) were defined using the eBURST algorithm (assessed on 23 April 2015) and restricted to single locus variant (SLV) and double locus variant from each group founder or subfounder, inside each clonal group); ST, sequence type defined by multilocus sequence typing; PVL, Panton–Valentine leukocidin; NT, non-typeable.; na, no amplification.
[a]Major *spa* types are displayed in bold.
[b]Percentage relatively to the total number of methicillin-susceptible *Staphylococcus aureus* in each country.
[c]Allelic profile 151-NA-215-34-175-180-169.

in Cape Verde, 29.2% in East Timor and 36.8% in São Tomé and Príncipe (Table 3).

Only five MRSA harboured PVL and belonged to four different clonal types (Table 1). The single MRSA isolate related to clone USA300 (PFGE N-t121-ST8-IVa-*agr*I) was confirmed to be PVL and arginine catabolic mobile element positive.

PVL was detected in 90 MSSA isolates (30.8%) and was found in isolates belonging to nine different clones (Table 2).

TABLE 3. Distribution of virulence determinants among methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* isolates from Angola, São Tomé and Príncipe, Cape Verde and East-Timor

Genes	Toxin	Angola			São Tomé and Príncipe			Cape Verde			East-Timor	All countries		
		MRSA	MSSA	Total	MRSA	MSSA	Total	MRSA	MSSA	Total	MSSA	MRSA	MSSA	Total
Leukocidins														
lukS-lukF	Panton–Valentine leukocidin	2 (1.6%)	12 (15.8%)	14 (6.9%)	2 (6.9%)	40 (47.1%)	42 (36.8%)	1 (16.7%)	31 (29.0%)	32 (28.3%)	7 (29.2%)	**5 (3.1%)**	**90 (30.8%)**	**95 (20.9%)**
lukD-lukE	Leukocidin DE	101 (79.5%)	35 (46.1%)	136 (67.0%)	29 (100%)	54 (63.5%)	83 (72.8%)	5 (83.3%)	47 (43.9%)	52 (46.0%)	13 (54.2%)	**135 (83.3%)**	**149 (51.0%)**	**284 (62.6%)**
lukM	Leukocidin M	0	0	0	0	0	0	0	0	0	0	**0**	**0**	**0**
Haemolysins														
hlb	β-haemolysin	10 (7.9%)	9 (11.8%)	19 (9.4%)	10 (34.5%)	16 (18.8%)	26 (22.8%)	0	33 (30.8%)	33 (29.2%)	10 (41.7%)	**20 (12.3%)**	**68 (23.3%)**	**88 (19.4%)**
hlg	γ-haemolysin	5 (3.9%)	14 (18.4%)	19 (9.4%)	2 (6.9%)	10 (11.8%)	12 (10.5%)	0	36 (33.6%)	36 (31.9%)	6 (25.0%)	**7 (4.3%)**	**66 (22.6%)**	**73 (16.1%)**
hlgv	γ-haemolysin variant	100 (78.7%)	37 (48.7%)	137 (67.5%)	29 (100%)	53 (62.4%)	82 (71.9%)	5 (83.3%)	53 (49.5%)	58 (51.3%)	8 (33.3%)	**134 (82.7%)**	**151 (51.7%)**	**285 (62.8%)**
Exfoliative toxins														
etA	Staphylococcal exfoliative A	6 (4.7%)	7 (9.2%)	13 (6.4%)	0	9 (10.6%)	9 (7.9%)	2 (33.3%)	5 (4.7%)	7 (6.2%)	3 (12.5%)	**8 (4.9%)**	**24 (8.2%)**	**32 (7.0%)**
etB	Staphylococcal exfoliative B	0	0	0	0	0	0	0	1 (0.9%)	1 (0.9%)	0	**0**	**1 (0.3%)**	**1 (0.2%)**
etD	Staphylococcal exfoliative D	0	4 (5.3%)	4 (2.0%)	0	2 (2.4%)	2 (1.8%)	0	1 (0.9%)	1 (0.9%)	0	**0**	**7 (2.4%)**	**7 (1.5%)**
Enterotoxins														
sel	Staphylococcal enterotoxin L	4 (3.1%)	20 (26.3%)	24 (11.8%)	5 (17.2%)	24 (28.2%)	29 (25.4%)	0	34 (31.8%)	34 (30.1%)	8 (33.3%)	**9 (5.6%)**	**86 (29.5%)**	**95 (20.9%)**
sep	Staphylococcal enterotoxin P	2 (1.6%)	13 (17.1%)	15 (7.4%)	2 (6.9%)	15 (17.6%)	17 (14.9%)	0	15 (14.0%)	15 (13.3%)	7 (29.2%)	**4 (2.5%)**	**50 (17.1%)**	**54 (11.9%)**
At least one toxin		103 (81.1%)	66 (86.8%)	169 (83.3%)	29 (100%)	85 (100%)	114 (100%)	6 (100%)	101 (94.4%)	107 (94.7%)	23 (95.8%)	**138 (85.2%)**	**275 (94.2%)**	**413 (91.0%)**

Discussion

To our knowledge, this is the first global survey of *S. aureus* colonization in four former Portuguese colonies, including Angola, São Tomé and Príncipe, Cape Verde and East Timor. Although several efforts were made to include isolates from Mozambique, the institutional review board never approved the study. For a different reason (war) we were not able to include Guinea Bissau. Significant differences in the MRSA rates were identified in the four countries that participated in the study. A very high MRSA prevalence was reported in Angola (61.6%) contrasting with a low prevalence in Cape Verde and complete absence in both hospitals in East Timor. In a study performed in 1997 in Cape Verde [8] we found no MRSA among nasal samples recovered from the same hospitals screened in this study, and therefore this is the first report of MRSA in this African archipelago. The different rates of MRSA seem to be in agreement with the antibiotic availability and usage in each country.

In our population, being a non-smoker was a relatively high risk factor for MRSA colonization, which is in agreement with a previous study from Taiwan [23]. Moreover, we found that the risk of being an MRSA carrier was higher among younger people (<18 years old) and in individuals previously submitted to surgery or in hospital for longer periods (>30 days). A recent cross-sectional survey of *S. aureus* nasal carriage in Gabonese Babongo Pygmies reported an age-related carriage pattern with a peak colonization of 53.9% in participants between 10 and 20 years of age and a decreasing prevalence in subsequent age groups [24]. In a healthy Chinese population, young age ($\leq$24 years old) was shown to be a risk factor for *S. aureus* nasal carriage [25]. Young age as a risk factor for MRSA should be interpreted with caution as high *S. aureus* nasal colonization rates in Africa are higher immediately after birth and in teenagers [26]. Although HIV infection and administration of multiple antibiotics were identified as risk factors for *S. aureus* and MRSA colonization [27], this was not the case in our study. By contrast, we observed that having no contact with animals seemed to decrease the risk of carrying MRSA. Isolates of MRSA belonging to clones ST5-IV and ST88-IV have already been described in African livestock, in Senegal [28].

Another important clinically relevant finding from this surveillance study was the baseline information concerning *S. aureus* antimicrobial susceptibility in former Portuguese colonies that we were able to obtain. These data are of major importance as a guide for implementation of adequate empiric therapies for *S. aureus* infections and to prevent treatment failures in the absence of information on antibiotic susceptibility testing. We described a global resistance to trimethoprim-sulfamethoxazole

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in the three African countries. Prophylactic use of trimethoprim-sulfamethoxazole in resource-constrained areas is widely recommended for HIV-infected or HIV-exposed children, for everyone with CD4 cell counts below 350, and for those with stage III and IV disease [29]. Moreover, trimethoprim-sulfamethoxazole is inexpensive, orally administered and usually available without prescription in these countries and therefore widely used. Resistance to trimethoprim-sulfamethoxazole confers cross-resistance to sulfadoxine/pyrimethamine, which is used to treat malaria, so it may pose an additional concern in these countries besides its limited utility as an anti-staphylococcal agent. We also found an extremely high preponderance of rifampin-resistant *S. aureus* in Angola, which may be explained by the important prevalence of tuberculosis in the country [30] and subsequent widespread use of rifampin as the first therapeutic option in the treatment of this disease.

Also, MRSA isolates from São Tomé and Príncipe showed resistance to multiple classes of antimicrobials (erythromycin, tetracycline, ciprofloxacin and clindamycin) in addition to resistance to β-lactams and trimethoprim-sulfamethoxazole. Antibiotic availability in São Tomé and Príncipe is fluctuates greatly and prescription is mainly dependent on the antibiotics that are available at the time of prescribing, resulting in selective pressure on different classes of antimicrobial agents.

In East Timor the isolates were almost exclusively resistant to penicillin and tetracycline. The wide clinical application of tetracycline, ease of administration and low price makes it an antibiotic of choice in low-income countries.

Molecular analysis showed that the clonal distribution of MRSA differed in the three Portuguese-speaking African countries (PALOP). Of great interest was the massive prevalence of PFGE A-ST5-IVa in Angolan hospitals. ST5-IVa has been described for the first time as the major clone in a paediatric hospital in Portugal [31]. Although currently it is not a major clone in Portuguese hospitals, ST5-IVa was recently described in 19.4% of elderly living in retirement homes [32]. Portugal, which shows one of the highest rates of MRSA in Europe [4], has close demographic relationships with Angola. Although a recent review suggested that the African ST5-MRSA evolved in Africa from ST5-MSSA through acquisition of the SCC*mec* element [33], MRSA dissemination between Portugal and Angola should not be excluded. However, the major clones presently circulating in Portugal [34,35], i.e. EMRSA-15 (ST22-IVh) and the New York/Japan related clone (ST105-II) were exclusively represented by single isolates in the present study. As ST5-MRSA is currently widespread in Central and West Africa, dissemination within the continent is probably the major reason for the high prevalence of this clone in Angola.

PFGE B-ST88-IVa was the second major clone in the three PALOP countries. Although ST88-IV has been only sporadically reported around the world, with the exception of China where the prevalence rates reached 16% [36], it was described as predominant in West, Central and East Africa and so already deserved the suggestion of using the name of 'African clone' to describe ST88-IV [26,33].

PFGE C-ST8-IV/V was the major clone in São Tomé and Príncipe and the third most prevalent in Angola. However, among ST8 MRSA isolates, there was only one closely related to the highly virulent community-acquired MRSA clone USA300 (ST8-IVa, PVL-positive, arginine catabolic mobile element-positive), curiously found among the few MRSA isolates in Cape Verde. So far USA300 MRSA has only been described among sporadic isolates in the African continent, namely in Central Africa [37], Gabon [38] and Ghana [39].

Three MSSA clones—ST15, ST508 and ST152—were dominant in all four former Portuguese colonies. The backgrounds of the major MRSA clones in PALOP countries were not coincident with the major MSSA clones, which supports MRSA clonal dissemination rather than acquisition of the SCC*mec* element.

Although ST15 and ST152 have been previously reported as prevalent in several countries in West Africa [26], to the best of our knowledge, this is the first report showing a wide prevalence of MSSA-ST508 not only in different countries in Africa but also in a country in Asia. This intercontinental spread might be related to the exchange of populations between former Portuguese colonies. More recently ST152 was also described in Haiti [40] and ST15 is currently the second most frequent clone among MSSA isolates collected in 25 European countries [41].

Since we have conducted a previous surveillance study in 1997 in Cape Verde [7], in the same hospitals, we could note the clonal evolution of the major MSSA clones over time: ST669, ST683 and ST30 were replaced by ST152, ST15 and ST508.

The analysis of the virulence determinants demonstrated a considerable proportion of PVL-positive isolates, namely among MSSA (30.8%), which is in agreement with other studies reporting Africa as an endemic region for PVL-positive *S. aureus* [7,9,39,42,43]. The high prevalence of PVL in the African continent is unexplained, but might be due to alterations in PVL targets of the host (C5a receptors) [26,44]. Interestingly, the total proportion of PVL-positive isolates in Angola was low (6.9%), compared with the remaining countries, namely São Tomé and Príncipe (36.8%). It has been suggested that the acquisition of antibiotic resistance in *S. aureus* involves changes in virulence factor secretion due to the fitness cost associated with the expression of resistance, which is reflected in decreased toxin expression [45,46], and could explain the low prevalence of PVL in Angola, a country that shows higher antimicrobial resistance levels.

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In conclusion, this study provided the first comprehensive overview of the prevalence of MRSA, antibiotic resistance, virulence content and genetic diversity of the *S. aureus* population in former Portuguese colonies in Africa and the Far East. The PALOP nations and East Timor now possess factual data that would help to define public heath priorities. Angola showed an extremely high prevalence of MRSA contrasting with Cape Verde and East Timor. Although the main MRSA lineages differed between countries, three MSSA clones were present in all the studied geographic sites. The high levels of antimicrobial resistance or virulence content warrant continued surveillance and antimicrobial stewardship programmes to promote judicious use of antimicrobials in these countries.

Transparency declaration

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.05.010.

References

[1] Berkley JA, Bejon P, Mwangi T, Gwer S, Maitland K, Williams TN, et al. HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. Clin Infect Dis 2009;49:336–43.

[2] Wang X, Towers S, Panchanathan S, Chowell G. A population based study of seasonality of skin and soft tissue infections: implications for the spread of CA-MRSA. PLoS One 2013;8:e60872.

[3] Olesen OF, Parker MI. Health research in Africa: getting priorities right. Trop Med Int Health 2012;17:1048–52.

[4] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2013. Annual report of the European antimicrobial resistance surveillance network (EARS-Net). Stockholm. 2014. Available online at: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2013.pdf [last accessed 25.03.15].

[5] Conceição T, Coelho C, Santos-Silva I, de Lencastre H, Aires-de-Sousa M. Epidemiology of methicillin-resistant and -susceptible *Staphylococcus aureus* in Luanda, Angola: first description of the spread of the MRSA ST5-IVa clone in the African continent. Microb Drug Resist 2014;20:441–9.

[6] Conceição T, Santos-Silva I, de Lencastre H, Aires-de-Sousa M. *Staphylococcus aureus* nasal carriage among patients and health care workers in São Tomé and Príncipe. Microb Drug Resist 2013;20:57–66.

[7] Aires-de-Sousa M, Conceição T, de Lencastre H. Unusual high prevalence of nosocomial PVL positive *Staphylococcus aureus* isolates in Cape Verde islands. J Clin Microbiol 2006;44:3790–3.

[8] Aires-de-Sousa M, Santos Sanches I, Ferro ML, de Lencastre H. Epidemiological study of staphylococcal colonization and cross-infection in two West African hospitals. Microb Drug Resist 2000;6:133–41.

[9] van der Meeren BT, Millard PS, Scacchetti M, Hermans MH, Hilbink M, Concelho TB, et al. Emergence of methicillin resistance and Panton–Valentine leukocidin positivity in hospital- and community-acquired *Staphylococcus aureus* infections in Beira, Mozambique. Trop Med Int Health 2014;19:169–76.

[10] Poulsen AB, Skov R, Pallesen LV. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the evigene MRSA detection kit. J Antimicrob Chemother 2003;51:419–21.

[11] Aires-de-Sousa M, Boye K, de Lencastre H, Deplano A, Enright MC, Etienne J, et al. High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. J Clin Microbiol 2006;44:619–21.

[12] Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J Clin Microbiol 2002;40:4289–94.

[13] Chung M, de Lencastre H, Matthews P, Tomasz A, Adamsson I, Aires de Sousa M, et al. Molecular typing of methicillin-resistant *Staphylococcus aureus* by pulsed-field gel electrophoresis: comparison of results obtained in a multilaboratory effort using identical protocols and MRSA strains. Microb Drug Resist 2000;6:189–98.

[14] Faria NA, Carriço JA, Oliveira DC, Ramirez M, de Lencastre H. Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J Clin Microbiol 2008;46:136–44.

[15] Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;38:1008–15.

[16] Milheiriço C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. Antimicrob Agents Chemother 2007;51:3374–7.

[17] Milheiriço C, Oliveira DC, de Lencastre H. Multiplex PCR strategy for subtyping the staphylococcal cassette chromosome *mec* type IV in methicillin-resistant *Staphylococcus aureus*: 'SCC*mec* IV multiplex'. J Antimicrob Chemother 2007;60:42–8.

[18] Ito T, Ma XX, Takeuchi F, Okuma K, Yuzawa H, Hiramatsu K. Novel type V staphylococcal cassette chromosome *mec* driven by a novel cassette chromosome recombinase, *ccrC*. Antimicrob Agents Chemother 2004;48:2637–51.

[19] Lina G, Boutite F, Tristan A, Bes M, Etienne J, Vandenesch F. Bacterial competition for human nasal cavity colonization: role of staphylococcal *agr* alleles. Appl Environ Microbiol 2003;69:18–23.

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[20] Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet 2006;367:731–9.

[21] Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, et al. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 2008;197:1523–30.

[22] Gilot P, Lina G, Cochard T, Poutrel B. Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of *Staphylococcus aureus* strains isolated from cows with mastitis. J Clin Microbiol 2002;40:4060–7.

[23] Wang JT, Liao CH, Fang CT, Chie WC, Lai MS, Lauderdale TL, et al. Prevalence of and risk factors for colonization by methicillin-resistant *Staphylococcus aureus* among adults in community settings in Taiwan. J Clin Microbiol 2009;47:2957–63.

[24] Schaumburg F, Kock R, Friedrich AW, Soulanoudjingar S, Ngoa UA, von Eiff C, et al. Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. PLoS Negl Trop Dis 2011;5:e1150.

[25] Yan X, Song Y, Yu X, Tao X, Yan J, Luo F, et al. Factors associated with *Staphylococcus aureus* nasal carriage among healthy people in northern China. Clin Microbiol Infect 2015;21:157–62.

[26] Schaumburg F, Alabi AS, Peters G, Becker K. New epidemiology of *Staphylococcus aureus* infection in Africa. Clin Microbiol Infect 2014;20:589–96.

[27] Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997;10:505–20.

[28] Fall C, Seck A, Richard V, Ndour M, Sembene M, Laurent F, et al. Epidemiology of *Staphylococcus aureus* in pigs and farmers in the largest farm in Dakar, Senegal. Foodborne Pathog Dis 2012;9:962–5.

[29] World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2010. Available online at: http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf?ua=1 [last accessed 20.03.15].

[30] World Health Organization. Global tuberculosis report 2014. 2014. Available online at: http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf?ua=1 [last accessed 20.03.15].

[31] Sá-Leão R, Santos Sanches I, Dias D, Peres I, Barros RM, de Lencastre H. Detection of an archaic clone of *Staphylococcus aureus* with low-level resistance to methicillin in a pediatric hospital in Portugal and in international samples: relics of a formerly widely disseminated strain? J Clin Microbiol 1999;37:1913–20.

[32] Almeida ST, Nunes S, Paulo AC, Faria NA, de Lencastre H, Sá-Leão R. Prevalence, risk factors, and epidemiology of methicillin-resistant *Staphylococcus aureus* carried by adults over 60 years of age. Eur J Clin Microbiol Infect Dis 2014;34:593–600.

[33] Schaumburg F, Pauly M, Anoh E, Mossoun A, Wiersma L, Schubert G, et al. *Staphylococcus aureus* complex from animals and humans in three remote African regions. Clin Microbiol Infect 2014;5:e1150.

[34] Aires-de-Sousa M, Correia B, de Lencastre H. Changing patterns in frequency of recovery of five methicillin-resistant *Staphylococcus aureus* clones in Portuguese hospitals: surveillance over a 16-year period. J Clin Microbiol 2008;46:2912–7.

[35] Faria NA, Conceição T, Miragaia M, Bartels MD, de Lencastre H, Westh H. Nasal carriage of methicillin resistant staphylococci. Microb Drug Resist 2014;20:108–17.

[36] Qiao Y, Ning X, Chen Q, Zhao R, Song W, Zheng Y, et al. Clinical and molecular characteristics of invasive community-acquired *Staphylococcus aureus* infections in Chinese children. BMC Infect Dis 2014;14:582.

[37] Breurec S, Zriouil SB, Fall C, Boisier P, Brisse S, Djibo S, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. Clin Microbiol Infect 2011;17:160–5.

[38] Ateba Ngoa U, Schaumburg F, Adegnika AA, Kösters K, Möller T, Fernandes JF, et al. Epidemiology and population structure of *Staphylococcus aureus* in various population groups from a rural and semi urban area in Gabon, Central Africa. Acta Trop 2012;124:42–7.

[39] Egyir B, Guardabassi L, Sorum M, Nielsen SS, Kolekang A, Frimpong E, et al. Molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. PLoS One 2014;9:e89716.

[40] Rosenthal ME, Mediavilla J, Chen L, Sonnenfeld J, Pierce L, Shannon A, et al. Molecular epidemiology of *Staphylococcus aureus* in post-earthquake northern Haiti. Int J Infect Dis 2014;29:146–51.

[41] Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, et al. The dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: results of a second structured survey. Euro Surveill 2014;19.

[42] Ruimy R, Maiga A, Armand-Lefevre L, Maiga I, Diallo A, Koumare AK, 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;190:3962–8.

[43] Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene-Sarr F, et al. Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton–Valentine leukocidin genes. Clin Microbiol Infect 2011;17:633–9.

[44] Spaan AN, Henry T, van Rooijen WJ, Perret M, Badiou C, Aerts PC, et al. The staphylococcal toxin Panton–Valentine leukocidin targets human c5a receptors. Cell Host Microbe 2013;13:584–94.

[45] Collins J, Rudkin J, Recker M, Pozzi C, O'Gara JP, Massey RC. Off-setting virulence and antibiotic resistance costs by MRSA. ISME J 2010;4:577–84.

[46] Otto M. Basis of virulence in community-associated methicillin-resistant *Staphylococcus aureus*. Annu Rev Microbiol 2010;64:143–62.

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