Epidemiol. Infect. (2014), **142**, 1737–1740. © Cambridge University Press 2013
doi:10.1017/S0950268813003117

SHORT REPORT
Population dynamics of *Staphylococcus aureus* from Northeastern Nigeria in 2007 and 2012

K. O. OKON[1], A. O. SHITTU[2], A. A. KUDI[3], H. UMAR[4], K. BECKER[5] AND F. SCHAUMBURG[5]*

[1] *Department of Medical Laboratory Services, Federal Medical Centre, Makurdi, Nigeria*
[2] *Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria*
[3] *Department of Medical Laboratory Services, Federal Medical Centre, Gombe, Nigeria*
[4] *Department of Medical Laboratory Services, Federal Medical Centre, Nguru, Nigeria*
[5] *Institute of Medical Microbiology, University Hospital Münster, Münster, Germany*

Received 22 October 2013; Final revision 15 November 2013; Accepted 15 November 2013; first published online 4 December 2013

SUMMARY

The population structure of *Staphylococcus aureus* is changing globally but the situation regarding dominant clones in sub-Saharan Africa is not clear. We therefore assessed changes in the population structure of clinical *S. aureus* isolates obtained in 2007 ($n=75$) and 2012 ($n=75$) from Northeastern Nigeria. A reduction in resistance to penicillin, gentamicin, erythromycin and clindamycin was observed in 2012. A decrease of methicillin resistance rates (13·3% to 8·0%) was associated with the decline of the ST241 MRSA clone. The proportion of Panton–Valentine leukocidin (PVL)-positive isolates also decreased from 65·3% to 44%, and was linked with the emergence of PVL-negative ST601 clone in 2012. The significant decline in antibiotic resistance in the study area is in contrast to the worldwide trend of increasing resistance rates.

Key words: Antibiotic resistance, bacterial infections, bacteriology, staphylococcal infections *Staphylococcus aureus*.

Staphylococcus aureus is a causative agent of hospital- and community-associated infections. The population structure of *S. aureus* is changing globally; in particular, Panton–Valentine leukocidin (PVL)-positive community-associated methicillin-resistant *S. aureus* (CA-MRSA) are emerging. Surveillance of antimicrobial resistance of pathogens has been largely neglected in the African continent and the situation regarding dominant resistant and virulent clones such as PVL-positive CA-MRSA in sub-Saharan Africa remains unclear. To assess the population dynamics of *S. aureus* in sub-Saharan Africa, we investigated the resistance pattern, genotypes and virulence factors of *S. aureus* collected during a prospective study in Northeastern Nigeria in 2007 and 2012 [1].

The *S. aureus* isolates were obtained from the medical microbiology laboratories serving the University of Maiduguri Teaching Hospital (Borno State, 500 beds, hospital A), the Federal Medical Centre Gombe (Gombe State, 420 beds, hospital B) and the Federal Medical Centre Nguru (Yobe State, 150 beds, hospital C). Consecutive, non-duplicate clinical *S. aureus* isolates were prospectively collected in 2007 and 2012 up to a total number of 75 isolates in each sampling period. Demographic information (age and sex) were retrieved from the patient's record files. The isolates from 2007 have been described

* Author for correspondence: Dr F. Schaumburg, Institute of Medical Microbiology, University Hospital Münster, Domagkstr. 10, 48149 Münster, Germany.
(Email: frieder.schaumburg@ukmuenster.de)

recently [1]. Ethical clearance was obtained from the University of Maiduguri Teaching Hospital (Adm/th.75/vol. 1). All procedures contributing to this work complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Identification and antibiotic susceptibility testing were performed using Vitek 2 automated systems (bioMérieux, France) and EUCAST clinical breakpoints (version 3.1), respectively. Confirmation of *S. aureus* and methicillin resistance was based on polymerase chain reaction (PCR) detection of *nuc* and *mecA* genes, respectively [2]. SCC*mec* typing was performed for all MRSA isolates [3]. Virulence factors encoding genes for toxic shock syndrome toxin-1 (*tst*), enterotoxins (*sea*, *seb, sec, sed, see, seg, seh, sei, sej*), exfoliative toxins (*eta, etb*) and Panton–PVL (*luk*F-PV/*luk*S-PV) were detected by PCR [2]. *S. aureus* protein A typing (*spa* typing) was performed for each isolate [4]. MRSA isolates belonging to sequence type 8 (ST8) were further screened for arginine catabolic mobile element (ACME) [5]. Multilocus sequence typing (MLST) was conducted for each *spa* type [6]. MLST sequence types were assigned to clonal complexes applying the definition of six (out of seven) shared alleles using eBURST and the MLST database (http://saureus.mlst.net).

Categorical variables were compared using Fisher's exact test or χ^2 test as appropriate. The strength of association was expressed by the odds ratio (OR) and its 95% confidence interval (CI). Mean values of continuous variables (age) were compared using Student's *t* test. The significance level was 0·05. Statistical analysis was performed with 'R' (http://cran.r-project.org, version 2.13.1) and the package 'epicalc'.

The number of the isolates among the three hospitals in 2007 *vs.* 2012 differed in hospital A (68 *vs.* 48 isolates, $P<0·05$) and hospital B (6 *vs.* 24 isolates, $P<0·05$) but was similar in hospital C (1 *vs.* 3 isolates, $p=0·6$).

The patient populations in 2007 and 2012 were comparable in terms of age (25·4 ± 16·3 *vs.* 29·7 ± 16·8 years, $P=0·07$) and proportion of females (46·7% *vs.* 48·0%; OR 0·95, 95% CI 0·48–1·89, $P=0·87$). In addition, the distribution of various clinical samples in the two cohorts was comparable except for blood culture, which was significantly more frequent in 2007 (Table 1).

We observed a decline in *S. aureus* resistance rates to penicillin (2·1-fold), gentamicin (11-fold), erythromycin (12-fold) and clindamycin (10-fold, $P<0·05$, Table 1). The resistance to oxacillin (2007, 13·3%; 2012, 8%) and rifampicin (2007, 5·3%; 2012, 1·3%) declined as well but not significantly (oxacillin, $P=0·3$; rifampicin. $P=0·4$), while co-trimoxazole resistance remained unchanged at 25·3% in 2007 and 2012. Resistance against glycopeptides and linezolid was not detected.

The proportion of PVL-positive isolates decreased from 65·3% ($n=49$) in 2007 to 44% ($n=33$) in 2012 ($P=0·009$, Table 1), and although the decline for *sea* was not significant, the proportion of other pyrogenic toxin superantigens decreased significantly (e.g. *seb, sec, tst*; Table 1). Sub-Saharan Africa is considered to be a PVL-endemic region as many countries such as Nigeria [7], Mali [8], Gabon [2] and Madagascar, Senegal, Niger and Cameroon [9] have reported high proportions of PVL-positive *S. aureus* (33–74%). Other virulence factors had a low prevalence (%) in 2007 and 2012 such as *sed* (1·3% and 0%), *sej* (1·3% and 2·7%), *eta* (2·7% and 5·3%) and *etb* (2·7% and 0%; see Supplementary material).

The most prevalent *spa* type in 2007 was t355 (14·7%, ST152) followed by t037 (12·0%, ST241) and t314 (8·0%, ST15). In 2012, t355 (24·0%, ST152) was dominant, followed by t4684 (12·0%, ST601), t064 (8·0%, ST8) and t084 (8%, ST15). ST152 has been frequently reported from the study area and other African countries [7, 8, 10]. The proportion of two clonal complexes changed significantly: isolates belonging to CC5 decreased from 58·7% ($n=44$) to 40% ($n=30$) in 2012 which was mainly due to the disappearance of MRSA-ST241 (t037, $n=9$; t1152, $n=1$). This clone is predominant in North Africa (Niger, Morocco and the region investigated in this study) [11]. The MRSA-ST241 in 2007 possessed SCC*mec* I and were all resistant to erythromycin, gentamicin and co-trimoxazole. Sequence types associated with MRSA in 2012 were ST8 ($n=3$, ACME negative), ST88 ($n=2$) and ST15 ($n=1$). All MRSA isolates were PVL negative. These findings are in line with other studies which revealed a predominance of ST8 and ST241 in MRSA from 2006 to 2007 (Southwest Nigeria) [12] or ST8 and ST241 in MRSA from 2009 (Southwest and Northeast Nigeria) [13]. Isolates belonging to ST601 were only found in 2012 and were all susceptible to oxacillin, gentamicin, erythromycin, clindamycin and did not harbour any of the virulence factors tested (only one isolate harboured *sei*). However, penicillin resistance in the ST601 lineage was high (77·8%, $n=7$).

https://doi.org/10.1017/S0950268813003117 Published online by Cambridge University Press

Table 1. *Selected characteristics of S. aureus isolates in Nigeria from two collection periods in 2007 and 2012*

	Sampling period, *n* (%)			
	2007 (*n* = 75)	2012 (*n* = 75)	OR (95% CI)	*P* value
Specimen				
Blood culture	14 (18·7)	2 (2·7)	0·1 (0·01–0·6)	< 0·005
Wound swab	24 (32)	31 (41·3)	1·5 (0·7–3·1)	0·24
Urine	12 (16)	22 (29·3)	2·2 (0·9–5·3)	0·05
Ear swab	8 (10·7)	2 (2·7)	0·2 (0·0–1·2)	0·09
Others[a]	17 (22·7)	18 (24)	1·1 (0·5–2·5)	0·85
Resistance				
Penicillin	68 (90·7)	32 (42·7)	13·1 (5·0–37·5)	< 0·005
Oxacillin	10 (13·3)	6 (8)	1·8 (0·5–6·3)	0·30
Gentamicin	11 (14·7)	1 (1·3)	12·7 (1·7–553·2)	0·01
Erythromycin	12 (16)	1 (1·3)	14·1 (2·0–608·7)	< 0·005
Clindamycin	10 (13·3)	1 (1·3)	11·4 (1·5–499·4)	0·01
Co-trimoxazole	19 (25·3)	19 (25·3)	1 (0·5–2·2)	1·00
Rifampicin	4 (5·33)	1 (1·3)	4·2 (0·4–207·8)	0·37
Virulence factors				
PVL	49 (65·3)	33 (44)	0·3 (0·2–0·7)	0·009
sea	18 (24)	12 (16)	0·6 (0·2–1·5)	0·22
seb	36 (48)	10 (13·3)	0·2 (0·1–0·4)	< 0·005
sec	9 (12)	1 (1·3)	0·1 (0·0–0·8)	0·02
see	7 (9·3)	0 (0)	0 (0–0·7)	0·01
seg	45 (60)	24 (32)	0·3 (0·2–0·7)	0·001
seh	17 (22·7)	2 (2·7)	0·1 (0·0–0·4)	< 0·005
sei	52 (69·3)	26 (34·7)	0·2 (0·1–0·5)	< 0·005
tst	15 (20)	4 (5·3)	0·2 (0·1–0·8)	0·01
Clonal complex				
CC5[b]	44 (58·7)	30 (40)	0·5 (0·2–1·0)	0·02
CC30[c]	5 (6·7)	6 (8)	1·2 (0·3–5·3)	0·75
CC152[d]	17 (22·7)	19 (25·3)	1·2 (0·5–2·6)	0·70
CC601[e]	0 (0)	9 (12)	∞ (2·1-∞)	< 0·005
Others[f]	9 (12)	11 (14·7)	1·3 (0·4–3·7)	0·63

OR, Odds ratio; CI, confidence interval; PVL, Panton–Valentine leukocidin.
[a] Sputum, semen, vaginal swab, eye swab.
[b] ST1, ST5, ST8, ST9, ST15, ST25, ST241, ST567, ST1846, ST2355, ST2623, ST2664, ST2701.
[c] ST30, ST2665.
[d] ST152.
[e] ST601.
[f] ST88, ST101, ST121, ST395, ST728, ST789, ST2624, ST2662, ST2663 and non-typable.

The disappearance of the MRSA-ST241 clone and the emergence of isolates belonging to ST601 accounted for the decrease in antibiotic resistance and virulence factors observed in this study (Table 1). Reasons for these changes in the population structure are unknown but might be associated with the reported change in clinical syndromes (e.g. decrease of isolates from blood cultures and increase of isolates from urine, Table 1).

Changes in the population structure might mirror long-term evolution or short-term outbreaks. We observed that the ST601 isolates were obtained from the same hospital. This might indicate an outbreak, although internal laboratory recording did not show an increase of *S. aureus* infections during the sampling period and did not show any epidemiological link between the nine MRSA cases treated in this hospital. To the best of our knowledge, *S. aureus* belonging

https://doi.org/10.1017/S0950268813003117 Published online by Cambridge University Press

to ST601 from sub-Saharan Africa was only found in a human carrier in Mali ($n = 1$) [8] and in chimpanzees living in the wild in Côte d'Ivoire ($n = 3$) [14].

Our study has some limitations. First, the small sample size and varying number of isolates from the study centres might overweigh naturally occurring fluctuations. Continuous regional and national surveillance is therefore needed to confirm our findings. Second, we did not apply other typing methods with higher resolution (e.g. pulsed-field gel electrophoresis) which would have been helpful in assessing the cluster of ST601 in hospital B in more detail.

In conclusion, the decline in antibiotic resistance rates and selected virulence factors in *S. aureus* collected in a defined Nigerian region is in contrast to the worldwide emergence of resistant isolates.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268813003117.

ACKNOWLEDGEMENTS

We thank Damayanti Kaiser, Heike Rengbers and Martina Schulte for excellent technical assistance. K. Okon was supported by Studenteninitiative 'Weitblick' Münster.

The study was funded by the Deutsche Forschungsgemeinschaft (DFG) (EI 247/8-1).

DECLARATION OF INTEREST

None.

REFERENCES

1. **Okon KO, *et al.*** Cooccurrence of predominant Panton-Valentine leukocidin-positive sequence type (ST) 152 and multidrug-resistant ST 241 *Staphylococcus aureus* clones in Nigerian hospitals. *Journal of Clinical Microbiology* 2009; **47**: 3000–3003.
2. **Schaumburg F, *et al.*** Population structure of *Staphylococcus aureus* from remote African Babongo pygmies. *PLoS Neglected Tropical Diseases* 2011; **5**: e1150.
3. **Kondo Y, *et al.*** Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec, ccr* and major differences in junkyard regions. *Antimicrobial Agents and Chemotherapy* 2007; **51**: 264–274.
4. **Mellmann A, *et al.*** Automated DNA sequence-based early warning system for the detection of methicillin-resistant *Staphylococcus aureus* outbreaks. *PLoS Medicine* 2006; **3**: e33.
5. **Diep BA, *et al.*** Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. *Lancet* 2006; **367**: 731–739.
6. **Enright MC, *et al.*** Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of Clinical Microbiology* 2000; **38**: 1008–1015.
7. **Shittu A, *et al.*** Characterization of methicillin-susceptible and -resistant staphylococci in the clinical setting: a multicentre study in Nigeria. *BMC Infectious Diseases* 2012; **12**: 286.
8. **Ruimy R, *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. *Journal of Bacteriology* 2008; **190**: 3962–3968.
9. **Breurec S, *et al.*** Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. *Clinical Microbiology and Infection* 2011; **17**: 633–639.
10. **Schaumburg F, *et al.*** Virulence factors and genotypes of *Staphylococcus aureus* from infection and carriage in Gabon. *Clinical Microbiology and Infection* 2011; **17**: 1507–1513.
11. **Breurec S, *et al.*** Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones. *Clinical Microbiology and Infection* 2011; **17**: 160–165.
12. **Ghebremedhin B, *et al.*** Emergence of a community-associated methicillin-resistant *Staphylococcus aureus* strain with a unique resistance profile in Southwest Nigeria. *Journal of Clinical Microbiology* 2009; **47**: 2975–2980.
13. **Shittu AO, *et al.*** Antibiotic resistance and molecular epidemiology of *Staphylococcus aureus* in Nigeria. *BMC Microbiology* 2011; **11**: 92.
14. **Schaumburg F, *et al.*** Highly divergent *Staphylococcus aureus* isolates from African non-human primates. *Environmental Microbiology Reports* 2012; **1**: 141–146.