JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2009, p. 3000–3003 Vol. 47, No. 9
0095-1137/09/$08.00+0 doi:10.1128/JCM.01119-09

Cooccurrence of Predominant Panton-Valentine Leukocidin-Positive Sequence Type (ST) 152 and Multidrug-Resistant ST 241 *Staphylococcus aureus* Clones in Nigerian Hospitals▽

Kenneth O. Okon,[1,3]† Patrick Basset,[2]† Auwalu Uba,[3] Johnson Lin,[4] Bukola Oyawoye,[3] Adebayo O. Shittu,[5] and Dominique S. Blanc[2]*

Department of Medical Microbiology, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria[1]; Service of Hospital Preventive Medicine, University Hospital, Lausanne, Switzerland[2]; Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria[3]; School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Durban, Republic of South Africa[4]; and Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria[5]

Received 8 June 2009/Returned for modification 8 July 2009/Accepted 10 July 2009

Ninety-six clinical isolates of *Staphylococcus aureus* from Nigeria were characterized phenotypically and genetically. Twelve multidrug-resistant methicillin (meticillin)-resistant *S. aureus* (MRSA) isolates carrying a new staphylococcal cassette chromosome *mec* element and a high proportion of Panton-Valentine leukocidin (PVL)-positive methicillin-susceptible *S. aureus* (MSSA) isolates were observed. The cooccurrence of multidrug-resistant MRSA and PVL-positive MSSA isolates entails the risk of emergence of a multidrug-resistant PVL-positive MRSA clone.

Staphylococcus aureus is a major cause of both hospital- and community-acquired infections. In particular, methicillin (meticillin)-resistant *S. aureus* (MRSA) strains have been detected worldwide (15), and the prevalence of MRSA varies among countries and health institutions (2, 4, 27). The emergence of MRSA strains resistant to glycopeptides, as well as the increasing prevalence in the community (7), highlights the need for worldwide epidemiological studies of this pathogen. However, data about the epidemiology and prevalence of staphylococcal infections in Africa are scarce compared to information about such infections in the rest of the world. Studies have indicated low prevalences of MRSA in Nigeria, Somalia, and Tanzania (1), but high prevalences in South Africa, Zimbabwe, Kenya, Ethiopia, Egypt, Senegal, and the Ivory Coast have been reported (2, 9, 18). In addition, a recent study of the genetic diversity of *S. aureus* strains in a carriage population from Mali showed a high frequency of a Panton-Valentine leukocidin (PVL)-positive clone (25). The mechanisms for the emergence and spread of *S. aureus* clones in Africa are largely unknown; hence, the characterization of isolates may provide baseline information needed in establishing effective infection control measures in Nigeria.

In this study, a total of 96 *S. aureus* isolates obtained between January and December 2007 from clinical specimens in six tertiary-care hospitals located in northeastern Nigeria were characterized. The isolates were identified based on standard bacteriological procedures (i.e., Gram staining and catalase, tube coagulase, and DNase testing), and susceptibilities to 12 antibiotics (Table 1) were determined by the disk diffusion method according to the CLSI guidelines. All the isolates were susceptible to vancomycin, fusidic acid, and mupirocin, and 12 (12.5%) were resistant to methicillin (i.e., oxacillin and cefoxitin resistant) (Table 1). The MRSA isolates were multidrug resistant (i.e., resistant to beta-lactams, along with at least three other classes of antibiotics), a finding similar to previously reported findings in other African countries like Morocco, Kenya, Cameroon, and South Africa (17). MRSA resistance to non-beta-lactams may further increase the medical expenses and the complexity of patient management, as well as morbidity and mortality rates since alternative antibiotics may not be affordable in many African countries.

The genetic diversity of the *S. aureus* population was assessed by the highly discriminatory double-locus sequence typing (DLST) method as described previously (20). This method is based on the analysis of partial sequences (about 500 bp) of the variable *clfB* and *spa* genes. A total of 41 *clfB* and 46 *spa* alleles were observed among the 96 *S. aureus* isolates evaluated by DLST, and these alleles represented 53 different DLST types. The eBURST software was used to cluster DLST types with identical sequences of at least one allele. Cluster analysis showed a low level of diversity among the 12 MRSA isolates, which belonged to a single cluster, while a high level of diversity among the methicillin-susceptible *S. aureus* (MSSA) isolates (i.e., 10 single-locus variant clusters and 23 singletons) was observed (Fig. 1). However, one cluster (DLST type 48-43) was predominant among the MSSA isolates. To confirm the relationship between *S. aureus* genotypes from Nigeria and worldwide clonal complexes (CCs), multilocus sequence typing (MLST) of at least one representative strain from each of the main DLST clusters (Table 2) was performed as described earlier (10). A total of 12 sequence types (STs) were observed among the 16 isolates analyzed by MLST. The MRSA cluster belonged to ST 241, while the predominant MSSA cluster was grouped into ST 152. With the exception of the genetically

* Corresponding author. Mailing address: Service of Hospital Preventive Medicine, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland. Phone: 41 21 314 02 59. Fax: 41 21 314 02 62. E-mail: Dominique.Blanc@chuv.ch.
† These authors contributed equally to this study.
▽ Published ahead of print on 15 July 2009.

TABLE 1. Frequency of resistance of *S. aureus* (MSSA and MRSA) isolates to antibiotics

Antibiotic	% of resistant isolates among:		
	MSSA isolates (n = 84)	MRSA isolates (n = 12)	All isolates (n = 96)
Penicillin	91.6	100	92.7
Oxacillin	0	100	12.5
Cefoxitin	0	100	12.5
Gentamicin	2.4	100	14.6
Erythromycin	3.6	100	15.6
Clindamycin	0	75	9.4
Co-trimoxazole	8.3	100	19.8
Ciprofloxacin	3.6	100	15.6
Rifampin	2.4	0	2.1
Vancomycin	0	0	0
Fusidic acid	0	0	0
Mupirocin	0	0	0

divergent ST 152, all the STs belonged to one of eight internationally recognized *S. aureus* CCs: CC1, CC5, CC8, CC9, CC15, CC30, CC80, and CC121.

The clonality of MRSA strains was further confirmed by the typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) elements observed in these isolates. Using the multiplex PCRs described by Kondo et al. (19) and Milheiriço et al. (23), we found that all the Nigerian isolates carried *ccr* type 5 and *mec* class A, as well as a J2 region similar to SCC*mec* type III. So far, the combination of these elements had been observed only in strains simultaneously carrying two SCC*mec* elements: SCC*mec* type III and SCC*mercury* (6, 19). However, we did not detect the presence of the mercury operon, suggesting that the Nigerian cassette does not carry SCC*mercury* and that it is a new SCC*mec* element. Recombination between different SCC*mec* types and/or local acquisitions may explain the emergence of new resistance elements (5, 12, 13). Recent data indicated that the local acquisition of SCC*mec* elements is a frequent phenomenon (24), highlighting the need to compare the molecular epidemiologies of MSSA and MRSA. However, we were not able to establish a link between these two categories since the genetic background of MRSA was clearly distinct from that of MSSA. SCC*mec* elements are often associated with resistance to multiple classes of antibiotics (8). However, resistance determinants may also be carried on other mobile elements, such as plasmids, transposons, and phages (22), and further investigations are needed to characterize this new cassette and unambiguously link the multidrug resistance pattern with this element.

PVL is a toxin responsible for skin and soft-tissue infections and is often associated with community-acquired MRSA infections. All isolates were tested for the presence of PVL genes as described elsewhere (21). Among the 96 isolates, 41 (42.7%) were PVL positive, but the MRSA isolates were PVL negative (Fig. 1). The prevalence of PVL-positive *S. aureus* isolates in this study was high compared with the data in recent reports indicating prevalences of less than 10% in several European countries (16, 26). The Nigerian PVL-positive MSSA isolates were well distributed among the hospitals, and more (39%) were recovered from wound specimens than from any other source. Interestingly, the PVL genes were noted to be present in almost all the MSSA isolates in the predominant group (DLST cluster 48-43). This observation supports the finding of a high prevalence of PVL-positive *S. aureus* isolates (ST 152) in a carriage population from Mali (25). Furthermore, a PVL-

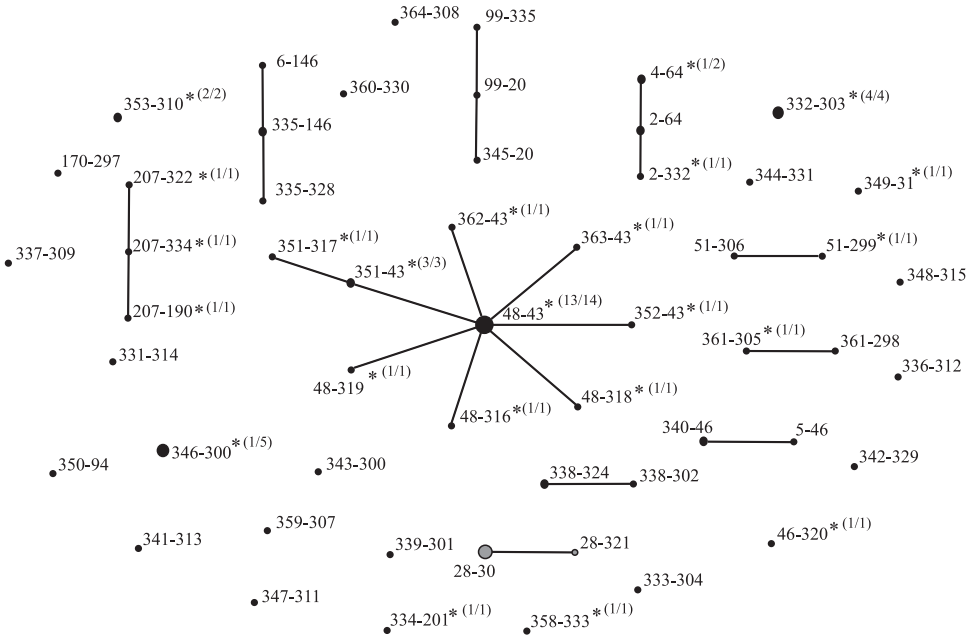

FIG. 1. DLST single-locus variant clustering of 96 *S. aureus* isolates from northeastern Nigeria by using eBURST. Each circle represents one DLST type, and the diameter of the circle reflects the frequency (i.e., the number of isolates) of that type. Linked DLST types differ at one of the two loci (*clfB* or *spa*). DLST types represented by only MSSA or MRSA isolates are indicated in black or gray, respectively. DLST types including PVL-positive isolates are indicated by asterisks (values in parentheses indicate the number of PVL-positive isolates/total number of isolates of that DLST type).

TABLE 2. Multilocus STs of representative isolates of the major *S. aureus* DLST clones observed in hospitals in northern Nigeria

Strain no.	Identification	PVL status[a]	DLST type	MLST profile	ST	CC[b]
H18134	MSSA	Pos	361-305	10-1-1-1-1-1-1	New	1
H18192	MSSA	Pos	340-46	1-new-1-1-1-1-1	New	1
H18109	MSSA	Pos	2-64	10-8-1-4-12-1-10	5	5
H18105	MRSA	Neg	28-30	2-3-1-1-4-4-30	241	8
H18113	MRSA	Neg	28-321	2-3-1-1-4-4-30	241	8
H18132	MSSA	Pos	346-300	3-3-1-1-4-4-3	8	8
H18196	MSSA	Pos	99-20	3-3-1-1-4-4-3	8	8
H18166	MSSA	Pos	338-324	3-3-1-1-1-1-10	9	9
H18127	MSSA	Pos	335-146	New-13-1-1-12-11-13	New	15
H18118	MSSA	Pos	353-310	2-2-2-7-6-3-2	30	30
H18165	MSSA	Pos	46-320	1-3-1-14-11-51-10	80	80
H18129	MSSA	Pos	332-303	6-5-6-new-7-14-5	New	121
H18101	MSSA	Pos	207-334	6-5-6-2-7-14-5	121	121
H18100	MSSA	Pos	48-43	46-75-49-44-13-68-60	152	NA
H18106	MSSA	Pos	48-316	46-75-49-44-13-68-60	152	NA
H18172	MSSA	Pos	51-299	46-75-49-44-13-68-60	152	NA

[a] Pos, positive; neg, negative.
[b] NA, not applicable.

positive community-acquired MRSA clone (ST 152) has been observed in the Balkans and Central Europe (3, 11, 14). The presence of PVL-positive MSSA isolates (ST 152) in Nigeria and Mali supports the hypothesis that the MRSA clone originated in Africa, migrated throughout central Europe, and acquired methicillin resistance (25).

In conclusion, our analysis of isolates from northeastern Nigeria indicated a high number of PVL-positive MSSA isolates, along with a multidrug-resistant MRSA clone carrying a novel SCC*mec* element. The cooccurrence of multidrug-resistant MRSA and PVL-positive MSSA highlights the risk for the emergence of a multidrug-resistant PVL-positive MRSA clone. This point further underlines the need for surveillance studies in Africa and the enforcement of antibiotic stewardship and infection control to prevent further dissemination of epidemic clones.

We are grateful to Caroline Choulat for technical assistance in Switzerland and to Ahmadu Danhajja, Victoria Bitrus, and Saleh Harun for their help in the collection of Nigerian *S. aureus* isolates. We thank Valérie Vogel and Giorgio Zanetti for insightful comments on the manuscript.

The stay of K.O.O. in the Lausanne laboratory was supported by Swiss National Science Foundation grant no. IZKOBO-121266.

REFERENCES

1. **Adesida, S., H. Boelens, B. Babajide, A. Kehinde, S. Snijders, W. van Leeuwen, A. Coker, H. Verbrugh, and A. van Belkum.** 2005. Major epidemic clones of *Staphylococcus aureus* in Nigeria. Microb. Drug Resist. **11:**115–121.
2. **Bell, J. M., and J. D. Turnidge.** 2002. High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998–1999. Antimicrob. Agents Chemother. **46:**879–881.
3. **Blanc, D. S., C. Petignat, A. Wenger, G. Kuhn, Y. Vallet, D. Fracheboud, S. Trachsel, A. Reymond, N. Troillet, H. H. Siegrist, S. Oeuvray, M. Bes, J. Etienne, J. Bille, P. Francioli, and G. Zanetti.** 2007. Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a small geographic area over an eight-year period. J. Clin. Microbiol. **45:**3729–3736.
4. **Bouchillon, S. K., B. M. Johnson, D. J. Hoban, J. L. Johnson, M. J. Dowzicky, D. H. Wu, M. A. Visalli, and P. A. Bradford.** 2004. Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int. J. Antimicrob. Agents **24:**119–124.
5. **Branger, C., C. Gardye, J. O. Galdbart, C. Deschamps, and N. Lambert.** 2003. Genetic relationship between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* strains from France and from international sources: delineation of genomic groups. J. Clin. Microbiol. **41:**2946–2951.
6. **Chongtrakool, P., T. Ito, X. X. Ma, Y. Kondo, S. Trakulsomboon, C. Tiensasitorn, M. Jamklang, T. Chavalit, J. H. Song, and K. Hiramatsu.** 2006. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. Antimicrob. Agents Chemother. **50:**1001–1012.
7. **Deresinski, S.** 2005. Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. Clin. Infect. Dis. **40:**562–573.
8. **Deurenberg, R. H., and E. E. Stobberingh.** 2008. The evolution of *Staphylococcus aureus*. Infect. Genet. Evol. **8:**747–763.
9. **El Kholy, A., H. Baseem, G. S. Hall, G. W. Procop, and D. L. Longworth.** 2003. Antimicrobial resistance in Cairo, Egypt 1999–2000: a survey of five hospitals. J. Antimicrob. Chemother. **51:**625–630.
10. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.
11. **Faria, N. A., D. C. Oliveira, H. Westh, D. L. Monnet, A. R. Larsen, R. Skov, and H. de Lencastre.** 2005. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. J. Clin. Microbiol. **43:**1836–1842.
12. **Feil, E. J., J. E. Cooper, H. Grundmann, D. A. Robinson, M. C. Enright, T. Berendt, S. J. Peacock, J. M. Smith, M. Murphy, B. G. Spratt, C. E. Moore, and N. P. Day.** 2003. How clonal is *Staphylococcus aureus*? J. Bacteriol. **185:**3307–3316.
13. **Fitzgerald, J. R., D. E. Sturdevant, S. M. Mackie, S. R. Gill, and J. M. Musser.** 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc. Natl. Acad. Sci. USA **98:**8821–8826.
14. **Harbarth, S., P. Francois, J. Schrenzel, C. Fankhauser-Rodriguez, S. Hugonnet, T. Koessler, A. Huyghe, and D. Pittet.** 2005. Community-associated methicillin-resistant *Staphylococcus aureus*, Switzerland. Emerg. Infect. Dis. **11:**962–965.
15. **Hiramatsu, K., L. Cui, M. Kuroda, and T. Ito.** 2001. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol. **9:**486–493.
16. **Holmes, A., M. Ganner, S. McGuane, T. L. Pitt, B. D. Cookson, and A. M. Kearns.** 2005. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J. Clin. Microbiol. **43:**2384–2390.
17. **Kesah, C., S. Ben Redjeb, T. O. Odugbemi, C. S. B. Boye, M. Dosso, J. O. N. Achola, S. Koulla-Shiro, M. Benbachir, K. Rahal, and M. Borg.** 2003. Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. Clin. Microbiol. Infect. **9:**153–156.
18. **Klugman, K. P.** 1998. Emerging infectious diseases—South Africa. Emerg. Infect. Dis. **4:**517–520.
19. **Kondo, Y., T. Ito, X. X. Ma, S. Watanabe, B. N. Kreiswirth, J. Etienne, and K. Hiramatsu.** 2007. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrob. Agents Chemother. **51:**264–274.

20. **Kuhn, G., P. Francioli, and D. S. Blanc.** 2007. Double-locus sequence typing using *clfB* and *spa*, a fast and simple method for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **45:**54–62.
21. **Lina, G., Y. Piemont, F. Godail-Gamot, M. Bes, M. O. Peter, V. Gauduchon, F. Vandenesch, and J. Etienne.** 1999. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin. Infect. Dis. **29:**1128–1132.
22. **Lindsay, J. A., and M. T. Holden.** 2006. Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. Funct. Integr. Genomics **6:**186–201.
23. **Milheiriço, C., D. C. Oliveira, and H. de Lencastre.** 2007. Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **51:**4537.
24. **Nübel, U., P. Roumagnac, M. Feldkamp, J. H. Song, K. S. Ko, Y. C. Huang, G. Coombs, M. Ip, H. Westh, R. Skov, M. J. Struelens, R. V. Goering, B. Strommenger, A. Weller, W. Witte, and M. Achtman.** 2008. Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **105:**14130–14135.
25. **Ruimy, R., A. Maiga, L. Armand-Lefevre, I. Maiga, A. Diallo, A. K. Koumare, K. Ouattara, S. Soumare, K. Gaillard, J. C. Lucet, A. Andremont, and E. J. Feil.** 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.
26. **Wannet, W. J., E. Spalburg, M. E. Heck, G. N. Pluister, E. Tiemersma, R. J. Willems, X. W. Huijsdens, A. J. de Neeling, and J. Etienne.** 2005. Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. J. Clin. Microbiol. **43:** 3341–3345.
27. **Zinn, C. S., H. Westh, and V. T. Rosdahl.** 2004. An international multicenter study of antimicrobial resistance and typing of hospital *Staphylococcus aureus* isolates from 21 laboratories in 19 countries or states. Microb. Drug Resist. **10:**160–168.