Epidemiology of methicillin-resistant *Staphylococcus aureus* lineages in five major African towns: emergence and spread of atypical clones

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Abstract

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in Africa is poorly documented. From January 2007 to March 2008, we collected 86 MRSA isolates from five African towns, one each in Cameroon, Madagascar, Morocco, Niger and Senegal. Although one or two major clones, defined by the sequence type and staphylococcal cassette chromosome *mec* type, predominated at each site, genetic diversity (ten clones) was relatively limited in view of the large geographical area studied. Most of the isolates (n = 76, 88%) belonged to three major clones, namely ST239/241-III, a well-known pandemic clone (n = 34, 40%), ST88-IV (n = 24, 28%) and ST5-IV (n = 18, 21%). The latter two clones have only been sporadically described in other parts of the world. The spread of community-associated MRSA carrying the Panton–Valentine leukocidin genes is a cause for concern, especially in Dakar and possibly throughout Africa.

Keywords: Africa, clones, community-acquired infections, hospital infections, methicillin-resistant *Staphylococcus aureus*, Panton–Valentine leukocidin
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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have spread throughout the world, first in the hospital setting and more recently in the community. Only five major ancestral hospital clones of MRSA are recognized, suggesting that acquisition of the staphylococcal cassette chromosome (SCC)*mec*, the genetic element harboring the *mec*A gene that confers methicillin resistance, has been a rare event. Most hospital-associated MRSA (HA-MRSA) clones are found worldwide, although their frequencies vary from country to country. For example, the major HA-MRSA clone (Lyon) found in France is neither dominant nor even frequent in neighboring countries [1].

Initially, community-associated MRSA (CA-MRSA) differed from HA-MRSA by their continental distribution but travel has rapidly blurred this distinction. For example, the CA-MRSA clone USA300, initially restricted to the USA, is now detected in many other countries [2], albeit at a low frequency. Likewise, all the most common CA-MRSA clones (USA 300, the European sequence type (ST) 80 clone and the Oceania ST30 clone) have been detected in Singapore, a major intercontinental travel hub [3]. Almost a quarter of a century elapsed between the emergence of MRSA in industrialized countries in the 1960s and its first description in Africa in 1988 [4]. However, data on the epidemiology of MRSA in Africa remain scarce [5–9].

We collected MRSA isolates from five major African towns in Morocco (North Africa), Senegal (West Africa), Niger (West Africa), Cameroon (Central Africa) and Madagascar (East Africa), and performed accessory gene regulator (*agr*) typing, multilocus sequence typing (MLST),

staphylococcal protein A (*spa*) typing, and toxin profiling. Antimicrobial susceptibility was also evaluated.

Materials and methods

Study population

All patients with suspected staphylococcal infection were pre-included in seven major tertiary care centres located in five major African towns, namely Antananarivo (Madagascar), Casablanca (Morocco), Niamey (Niger), Dakar (Senegal) and Yaounde (Cameroon), between January 2007 and March 2008 (January 2007 to June 2007 in Casablanca). They were included in the study only if their *S. aureus* isolate was confirmed to be resistant to methicillin. A standardized specific medical record was filled out during the hospitalization. If more than one *S. aureus* isolate with the same toxin type was recovered from the same patient, only the first was included. Isolates were considered to be community-acquired if recovered by culture from a sample obtained within 48 h after admission in a patient with no risk factors for nosocomial acquisition in the previous year, namely hospitalization or surgery, use of an indwelling catheter or a percutaneous device, or frequent exposure to healthcare facilities for an underlying chronic disorder. All other isolates were considered hospital-acquired. The study protocol was approved by local ethics committees.

Microbiological analysis

S. aureus identification was based on Gram staining, morphology, catalase positivity (ID color Catalase; bioMérieux, Marcy l'Etoile, France), agglutination in the Pastorex Staph Plus® test (Bio-Rad, Marnes la Coquette, France) and free coagulase production (lyophilized rabbit plasma; bioMerieux).

DNA extraction, *mecA* detection, *agr* typing and SCC*mec* typing

Genomic DNA was extracted with a standard phenol–chloroform procedure [10]. Species identification was confirmed by multiplex PCR amplification of the *agr* locus [10], allowing concomitant determination of the *agr* allelic group. All isolates were screened for genes encoding methicillin resistance (*mec*A), staphylococcal enterotoxins (se) A, B, C, D, H, K, L, M, O, P, Q, and R (*sea-d*, *seh*, *sek-m*, *seo-r*), toxic shock syndrome toxin 1 (*tst*), exfoliative toxins A, B and D (*eta*, *etb*, *etd*), Panton–Valentine leukocidin (*luk*-PV), class F *Luk*M leukocidin (*lukM*), β-haemolysin (*hlb*) and epidermal cell differentiation inhibitor (*edinA/B/C*), as previously described [10,11]. SCC*mec* types were determined by PCR with a simplified version of Kondo's typing system, including M-PCR-1 and M-PCR-2, without considering the structure of the junkyard region [12].

Spa typing and MLST

Spa sequence typing was performed with the Ridom Staph Type standard protocol (http://www.3.ridom.de.doc/Ridom_spa_sequencing.pdf) and the Ridom *Spa*Server, which automatically analyzes *spa* repeats and assigns *spa* types (http://spa.ridom.de/index.shtml). MLST was performed as described previously [13]. MLST and *spa* typing were performed on 23 representative isolates of all toxin types within each *agr* type.

Antimicrobial susceptibility

Susceptibility to penicillin, oxacillin, cefoxitin, gentamicin, kanamycin, tobramycin, tetracycline, erythromycin, lincomycin, pristinamycin, fosfomycin, fusidic acid, rifampicin, pefloxacin, co-trimoxazole and vancomycin was determined by each participating laboratory, in accordance with the guidelines of the French Society for Microbiology [14]. *S. aureus* strain ATCC 25923 was used for quality control. External quality control was ensured by the French National Antibiotic Reference Center (Pasteur Institute, Paris, France).

Results and Discussion

In total, 542 patients were included during the study period. Six hundred and nine biological samples were taken and 555 *S. aureus* strains were collected, of which 86 were resistant to methicillin. The low prevalence of MRSA (15%), combined with the lack of systematic samples for bacteriological culture and frequent initiation of antibiotic therapy before biological sampling, explains the small number of MRSA isolates. Nevertheless, this represents the largest collection of clinical MRSA isolates ever studied on the African continent, excluding Nigeria and South Africa. Isolates were recovered mainly from male patients (64%). The patients' median age was 29.0 years (mean 32.4 years; range 2 months to 84 years). Nine isolates (10%) were recovered in Yaounde (Cameroon), 11 (13%) in Niamey (Niger), 16 (19%) in Antananarivo (Madagascar), 21 (24%) in Casablanca (Morocco) and 29 (34%) in Dakar (Senegal) (Fig. 1). Forty-four isolates (51%) were associated with skin and soft-tissue infections, 27 (31%) with surgical wound infections, nine (10%) with bacteraemia/septicaemia, four (5%) with urinary/genital tract infections and two (2%) with osteomyelitis/myositis. The small number of isolates associated with bacteraemia/septicaemia reflects the lack of routine blood culture in the participating centres.

The 86 isolates belonged to seven STs and five clonal complexes (CCs). The isolates were assigned to ten different