Antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated in 2007 from French patients with bloodstream infections: goodbye hVISA, welcome Geraldine?

Olivier Gallon¹, Brigitte Lamy², Frédéric Laurent³, Marie-Elisabeth Reveryer³, Florence Doucet-Populaire⁵,⁶ and Jean-Winoc Decousser²* on behalf of the Collège de Bactériologie Virologie Hygiène (ColBVH) Study Group†

¹Department of Biology and Infection Control, Centre Hospitalier de Dourdan, 91415 Dourdan, France; ²Department of Biology, Centre Hospitalier du Bassin de Thou, 34200 Sète, France; ³Centre National de Référence des Staphylocoques, Faculté Laennec, INSERM 851, 69372 Lyon, France; ⁴Department of Bacteriology, Hôpital de la Croix Rousse, 69317 Lyon, France; ⁵Department of Microbiology and Infection Control, AP-HP, Centre Hospitalo-Universitaire Antoine Béclère, 92140 Clamart, France; ⁶Department of Microbiology, EA 4065, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, Paris, France

*Corresponding author. Tel: +33-1-45-37-46-26; Fax: +33-1-45-37-48-45; E-mail: jean-winoc.decousser@abc.aphp.fr
†Members are listed in the Acknowledgements section.

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Sir,

In France, the high prevalence of oxacillin-resistant *Staphylococcus aureus* (ORSA) leads to the empirical use of vancomycin in the case of invasive staphylococcal disease.¹,² We previously reported in 2000 the spread of heterogeneous vancomycin-resistant *S. aureus* (hVISA) among bacteria implicated in bacteraemia; 8% of the ORSA showed confirmed heteroresistance to glycopeptides, all of them being gentamicin resistant.³ Recently, the increasing prevalence of hVISA and confirmation of their clinical impact were reported.³ These disturbing reports led us to renew a similar study 7 years later in the same network.

During October 2007, all non-duplicate isolates of *S. aureus* from clinically relevant blood culture were collected in 52 non-teaching hospitals belonging to the College of Bacteriology-Virology-Hygiene.¹,² All isolates were sent to a central laboratory (Department of Biology and Infection Control, Hôpital de Dourdan). Antibiotic susceptibility was determined by the disc diffusion method and interpreted according to the guidelines of the Antimicrobial Committee of the French Society of Microbiology. An additional D-zone test was performed to detect inducible clindamycin resistance. *S. aureus* identification and oxacillin susceptibility were previously confirmed by a specific method.² As in 2000, hVISA was specifically sought by inoculation of 10 μL of broth culture (turbidity equivalent to that of a 2 McFarland standard) on Mueller–Hinton agar (MHA) plates containing 5 mg/L teicoplanin. Additionally, the Macara Etest® method was performed; vancomycin and teicoplanin Etest® (bioMérieux, France) strips were deposited on MHA plates inoculated with a bacterial suspension (turbidity equivalent to that of a 2 McFarland standard) and a positive result was considered when the MIC reached ≥6 mg/L after a 48 h incubation period. Population analysis of the isolates presenting at least one positive screening test was performed at the National Reference Centre of Staphylococci (Lyon, France). Statistical analysis was performed using the Fisher exact test; a *P* value of ≤0.05 was considered significant.

The results of the susceptibility testing according to oxacillin susceptibility are reported in Table I. The 115 oxacillin-susceptible *S. aureus* (OSSA) were mainly multi-susceptible to other antibiotics (≥90% of susceptible isolates), except for erythromycin and clindamycin; for this last antibiotic, the main resistance profile was the erythromycin-inducible clindamycin resistance (D-zone test positive). Among the 51 ORSA, two-thirds presented a dual resistance to kanamycin and tobramycin, but only 6% (3/51) were gentamicin resistant (versus 11.5% in 2000; *P* = 0.37); none of them presented the previously individualized antimicrobial resistance profile of the French VISA clones (co-resistance to macrolides, tetracycline, rifampicin, fosfomycin and fusidic acid).³ One-third of the ORSA were resistant to erythromycin and clindamycin, all but one strain exhibiting a constitutive clindamycin resistance profile. Only 6% of the ORSA (3/51) were fluoroquinolone susceptible (versus 0% in 2000; *P* = 0.048); these three isolates exhibited the typical antibiotic resistance profile of the newly described ORSA clone containing the toxic shock syndrome toxin 1 (TSST-1) gene (the ‘Geraldine clone’ resistant to kanamycin and tobramycin, with intermediate resistance to fusidic acid).³ Theses three isolates originated from distinct areas. Such a profile was absent in 2000. PFGE analysis confirmed the clonal relationship between these strains and the ‘Geraldine clone’ (Figure 1). Additionally, on the basis of a DNA microarray (StaphyType Kit, CLONDIA, Iena, Germany), the genetic contents of these three isolates perfectly matched those of the isolates belonging to the ‘Geraldine clone’, including the accessory gene regulator allele (agr2), SCC mec cassette type I (ccrA1 and ccrB1), the genes encoding virulence factors (especially tst, sec, sed, sel, sem, set, cfrA-B, ebpS, eno and efb) and resistance determinants (mecA, blaR, blaZ, bla2, aadD, tet efflux and fosB).³ Among the 166 isolates, 7 showed at least one positive hVISA screening test (5 OSSA and 2 ORSA); none

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of them was gentamicin resistant. Population analysis did not confirm any hVISA phenotype among ORSA (versus 8% in 2000; $P=0.046$), but identified vancomycin-resistant subpopulations in 1.7% (2/115) of the OSSA. We previously reported the lack of VISA isolates in 2000 and 2007.\textsuperscript{1,2} To date, the reasons for these interesting observations were not clearly elucidated. The use of the same network and the same in vitro screening methods reinforce our findings. In 2007, the prevalence of gentamicin resistance among ORSA was lower than in 2000, but this decrease was not statistically significant. Additionally, the putative impact on hVISA prevalence of the spread of new gentamicin-susceptible ORSA clones exhibiting a better fitness than their gentamicin-resistant counterparts was debatable, because (i) the epidemiological relationship between gentamicin and vancomycin resistance was not clearly established, and (ii) the decrease in the prevalence of gentamicin-resistant strains among ORSA began in 1992, and did not prevent the emergence and spread of hVISA in 2000–01.\textsuperscript{1,6} The emergence of vancomycin-resistant subpopulations was described in isolates with different genetic and antimicrobial resistance profiles, including OSSA; as in the present study, a screening test to detect the presence of VISA and hVISA should be performed independently of oxacillin or other antimicrobial-associated resistance, to estimate their prevalence. One of the main events occurring since 2000 in the area of invasive ORSA disease in France was the spread of a fluoroquinolone-susceptible clone that produced TSST-1. Such ‘superbugs’ were previously reported in France and their increasing prevalence was confirmed by our national network. Even if these trends should be confirmed by larger samples of isolates, the emergence of new virulent ORSA clones seems to counterbalance the relative positive news of the decreasing hVISA prevalence in France in 2007.

### Acknowledgements


### Table 1. Percentage of antimicrobial susceptibility among the 166 $S$. aureus isolates, separated according to oxacillin susceptibility (disc diffusion method)

<table>
<thead>
<tr>
<th></th>
<th>OXA</th>
<th>OFX</th>
<th>KAN</th>
<th>TOB</th>
<th>GEN</th>
<th>ERY</th>
<th>CLI</th>
<th>CLI (D-test neg$^a$)</th>
<th>PRI</th>
<th>TET</th>
<th>MUP</th>
<th>SXT</th>
<th>RIF</th>
<th>FUS</th>
<th>FOF</th>
</tr>
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<tbody>
<tr>
<td>All $S$. aureus ($n=166$)</td>
<td>69</td>
<td>69</td>
<td>76</td>
<td>76</td>
<td>98</td>
<td>77</td>
<td>87</td>
<td>78</td>
<td>99</td>
<td>95</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td>Oxacillin susceptible ($n=115$)</td>
<td>100</td>
<td>97</td>
<td>94</td>
<td>96</td>
<td>100</td>
<td>83</td>
<td>97</td>
<td>86</td>
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<td>98</td>
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<tr>
<td>Oxacillin resistant ($n=51$)</td>
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<td>6</td>
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<td>31</td>
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<td>63</td>
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<td>94</td>
<td>98</td>
<td>100</td>
<td>100</td>
<td>92</td>
<td>98</td>
</tr>
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</table>

OXA, oxacillin; OFX, ofloxacin; KAN, kanamycin; TOB, tobramycin; GEN, gentamicin; ERY, erythromycin; CLI, clindamycin; PRI, pristinamycin; TET, tetracycline; MUP, mupirocin; SXT, trimethoprim/sulfamethoxazole; RIF, rifampicin; FUS, fusidic acid; FOE, fosfomycin.

$^a$The D-test was considered as positive when blunting of the clindamycin zone of inhibition was observed.

![Figure 1. PFGE patterns of the isolates harbouring the ‘Geraldine’ antibiotic susceptibility profile. Lane 1, ATCC 29213 oxacillin-susceptible $S$. aureus control strain; lane 2, ATCC 33591 oxacillin-resistant $S$. aureus control strain; lane 3, ‘Geraldine clone’ control strain; lanes 4–6, clinical strains harbouring the ‘Geraldine’ antibiotic susceptibility profile; lane 7, molecular weight markers; lane 8, NCTC 8325 control strain.](image-url)
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**Transparency declarations**
None to declare.

**References**

**rpoB gene mutation profile in rifampicin-resistant Mycobacterium tuberculosis**
clinical isolates from Guizhou, one of the highest incidence rate regions in China

Ling Chen1, Xin Gan1, Nana Li1, Jianhua Wang1, Kailun Li1 and Hong Zhang1,2*

1Department of Respiratory Medicine, Affiliated Hospital of Zunyi Medical College, Zunyi, Guizhou 563003, China; 2Z-BioMed, Inc., Rockville, MD 20855, USA

*Corresponding author. Tel: +1-301-258-8968; Fax: +1-301-260-0622; E-mail: hzhang@zbiomed.com

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Sir,

Tuberculosis (TB) is the leading single-agent infectious disease killer in the world, especially in Asia and Africa.1 Worldwide, 9.2 million new cases of TB (139 per 100 000 population) and 1.7 million deaths from TB occurred in 2006.2 Ranked first to fifth, respectively, in terms of total numbers of TB cases in the world are India, China, Indonesia, South Africa and Nigeria; and the African region has the highest incidence rate per capita (363 per 100 000 population). China is one of the high-burden countries for TB (99 per 100 000 population)2 and Guizhou Province is one of the highest incidence rate regions in China, with 699 TB cases per 100 000 population (www.gog.com.cn).

A combination of four drugs, rifampicin, isoniazid, pyrazinamide and ethambutol, with or without streptomycin, is recommended for the standard or first-line treatment of TB. Resistance to these drugs has been linked to mutations in at least nine genes; katG, inhA, aphC and kasA for isoniazid resistance, rpoB for rifampicin resistance, rpsL and rrS for streptomycin resistance, embB for ethambutol resistance and pncA for pyrazinamide resistance.3 It is estimated that 4.3% of all new and previously treated TB cases are caused by multidrug-resistant (MDR) strains of Mycobacterium tuberculosis. China, India and the Russian Federation accounted for 62% of the estimated global burden of MDR-TB cases.2 China is ranked number one in terms of the total number of MDR-TB cases.2 Many mutations have been identified in different regions of these drug resistance genes, and resistance to rifampicin has been shown to be associated with mutations in the 81 bp rifampicin resistance-determining region (RRDR) of the RNA polymerase β-subunit (rpoB) gene.4 In today’s world of global travel, infectious diseases such as MDR-TB pass into industrialized countries from the developing world. However, there are ways to combat the problems of MDR-TB, for example early recognition of MDR could lead to effective drug interventions using second-line anti-TB drugs. Recognizing the threat of MDR-TB, the WHO launched The Global Plan to Stop TB 2006–2015 in 20065 and set out how the Stop TB Strategy should be implemented over the decade 2006–2015. The US CDC has emphasized the importance of expeditious detection of drug resistance and the implementation of appropriate therapy.

We report here the determination of the rpoB gene mutation profile in rifampicin-resistant M. tuberculosis clinical isolates from Guizhou Province and the comparison with mutation profiles from different regions of China and nine other countries of Asia, Europe and America in order to select region-specific mutations, which could potentially be used for the development of quick diagnostic tools for MDR-TB suitable for particular regions of the world. One hundred M. tuberculosis isolates were obtained from the Affiliated Hospital of Zunyi Medical College, Guizhou Province, China from March 2007 to September 2008. These clinical isolates were grown on Löwenstein–Jensen agar slants at 37°C for 4 weeks and were examined for microscopic colony morphology. All cultures were used for rifampicin resistance testing by the absolute concentration method, and the isolates were considered resistant to rifampicin when they were resistant to at least 50 mg/L rifampicin. M. tuberculosis DNA

1299

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