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In Vivo Effect of Flucloxacillin in Experimental Endocarditis Caused by *mecC*-positive *Staphylococcus aureus* Showing Temperature-Dependent Susceptibility *In Vitro*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the *mecC* gene (*mecC*-MRSA) exhibited at 37°C MICs of oxacillin close to those of methicillin-susceptible *S. aureus* (MSSA). We investigated whether at this temperature, *mecC*-MRSA strains respond to flucloxacillin treatment like MSSA strains, using a rat model of endocarditis. Flucloxacillin (human-like kinetics of 2 g intravenously every 6 h) cured 80 to 100% of aortic vegetations infected with five different *mecC*-MRSA strains. These results suggest that *mecC*-MRSA infections may successfully respond to treatment with β-lactams.

Methicillin-resistant *Staphylococcus aureus* (MRSA), isolated for the first time in the 1960s (1), developed resistance against methicillin and other β-lactam antibiotics through the acquisition of *mecA*, a gene encoding the penicillin binding protein 2a (PBP2a) (2). In 2011, a novel *mecA* homologue, *mecC* (3), was identified in bovine and human MRSA isolates (4–8). Although the incidence of *mecC*-MRSA is low (8–10), these strains cause severe infections in humans (11, 12).

Most *mecC*-MRSA isolates exhibit MICs of oxacillin less than or slightly greater than the MIC breakpoint of a susceptible strain (≤2 mg/liter), and no clear distinction from methicillin-susceptible *S. aureus* (MSSA) isolates can be made (13, 14). Furthermore, the activity of the *mecC* product, namely, PBP2c (15), appears to be thermosensitive, with a decline at 37°C (16). This raises the question as to whether *mecC*-MRSA infections should be considered like *mecA*-MRSA infections and treated with vancomycin (17) or like MSSA infections, generally treated with β-lactams, such as flucloxacillin (18). In this study, we investigated the *in vivo* activity of flucloxacillin on *mecC*-MRSA strains using a rat model of endocarditis.

Five *mecC*-MRSA strains, two of animal origin (NCTC 13552 and 1100) and three of human origin (strain 820, from an infected knee [6], and the urine and screening strains S090 and S129 [19]), were used. *S. aureus* strains ATCC 29213 (MSSA) and Mu50 (*mecA*-MRSA) were used as controls. The MICs of oxacillin and cefoxitin were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20). The MICs were interpreted according to the CLSI guidelines after incubation at 30°C and 37°C for 24 h. Population analysis profiles (PAPs) were performed on agar plates containing 2-fold serial dilutions of oxacillin and on oxacillin-free plates, as described previously (21). Colony numbers were enumerated after 48 h of incubation at 30°C and 37°C. Oxacillin-induced killing was assessed by time-kill assays using strains NCTC 13552 and S129. The flasks containing Mueller-Hinton broth supplemented with 2% NaCl were inoculated with 10^6 CFU/ml of bacteria, exposed to oxacillin (10 mg/liter), and incubated at 30°C or 37°C. The concentration of oxacillin approximated trough levels in the sera of rats and was either less than or greater than the MICs for the organisms when tested at a temperature of 30°C or 37°C, respectively (see below).

The production of catheter-induced aortic vegetations and the installation of the infusion pump device to deliver flucloxacillin were performed in rats as described previously (22, 23). Endocarditis was induced 24 h after catheterization by intravenous (i.v.) challenge of the animals with 10^5 CFU of each test *mecC*-MRSA strain and the *mecA*-MRSA Mu50 strain. Therapy with flucloxacillin (human-like kinetics of 2 g i.v. every 6 h) was started 12 h later and lasted for 3 days. Control rats were sacrificed at the onset of treatment and treated rats at 8 h after the end of the last antibiotic dose. A group of 3 to 4 control animals was also sacrificed at the same time as the treated animals. After the sacrifice, aortic valve vegetations were removed and processed as described previously (24) for colony counts. All protocols for animal studies were reviewed and approved by the Cantonal Committee on Animal Experiments of the State of Vaud, Switzerland (permit number 879.9).

MICs are shown in Table 1. All of the *mecC*-MRSA isolates were formally resistant to oxacillin at 30°C and susceptible at 37°C (threshold, ≤2 mg/liter). All but the S820 isolate were resistant to cefoxitin (threshold, ≥8 mg/liter) at both 30°C and 37°C. Figure 1 shows that upon exposure to oxacillin at 30°C, *mecC*-MRSA isolates exhibited subpopulations of resistant bacteria at concentra-

Received 6 November 2014 **Returned for modification** 3 December 2014
Accepted 15 January 2015

Accepted manuscript posted online 20 January 2015

Citation Mancini S, Laurent F, Veloso TR, Giddey M, Vouillamoz J, Vandenesch F, Moreillon P, Entenza JM. 2015. *In vivo* effect of flucloxacillin in experimental endocarditis caused by *mecC*-positive *Staphylococcus aureus* showing temperature-dependent susceptibility *in vitro*. Antimicrob Agents Chemother 59: 2435–2438. doi:10.1128/AAC.04733-14.

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doi:10.1128/AAC.04733-14

TABLE 1 MICs of oxacillin and cefoxitin[a]

Strain	MIC (mg/liter)			
	Oxacillin		Cefoxitin	
	30°C	37°C	30°C	37°C
NCTC 13552	8–16	0.5–1	64	8
1100	16	0.5–2	32	8
820	4	0.5–1	8	4
S090	16	1	32	16
S129	16–32	0.5–2	64	16–32

[a] MICs were determined at least three times independently at the indicated temperatures in Mueller-Hinton broth supplemented with 2% NaCl.

tions up to 32 mg/liter. However, these oxacillin-resistant subpopulations virtually disappeared at 37°C. Moreover, with the exception of strain NCTC 13552, for which resistant subpopulations emerged on 8-mg/liter oxacillin plates, the remaining four tested *mecC*-MRSA isolates displayed a profile similar to that of MSSA ATCC 29213 (growth at up to 4 mg/liter oxacillin). The *mecA*-MRSA Mu50 strain displayed homogeneous resistance to oxacillin, as reported previously (25). The rate of killing by oxacillin of the selected isolates NCTC 13552 and S129 was also affected by temperature (Fig. 2). After 24 h of incubation at 30°C, oxacillin did not display any antibacterial activity (Fig. 2A). In contrast, after 24 h of incubation at 37°C, oxacillin caused a 2-$\log_{10}$ CFU/ml decrease in cell viability (Fig. 2B). The results of experimental endocarditis are shown in Table 2. Vegetations of

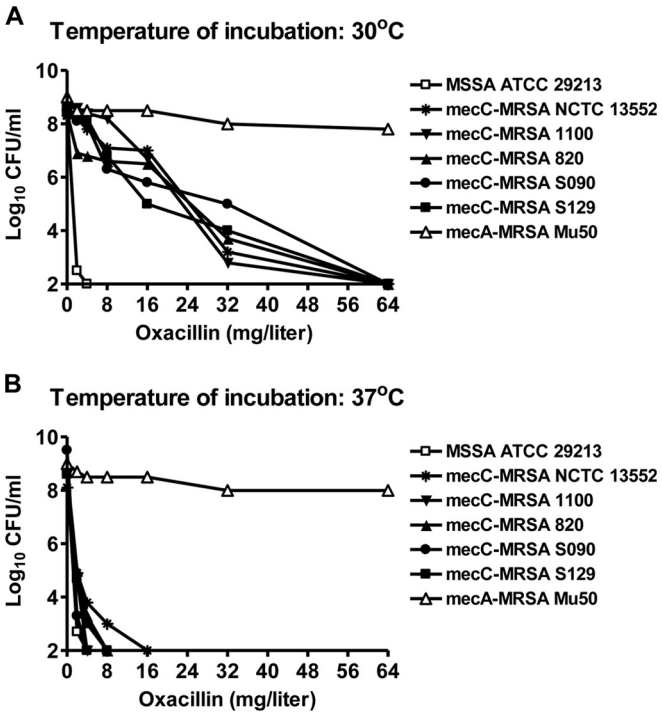

FIG 1 Phenotypic expression of oxacillin resistance in *mecC*-MRSA strains assessed by population analysis. Large inocula (ca. 10^9 CFU) of the test strains were spread onto Mueller-Hinton agar plates supplemented with 2% NaCl and containing increasing concentrations of oxacillin and on oxacillin-free plates. The plates were incubated at 30°C (A) or 37°C (B) for 48 h, and then the CFU were enumerated. Each assay was performed on two to three independent occasions.

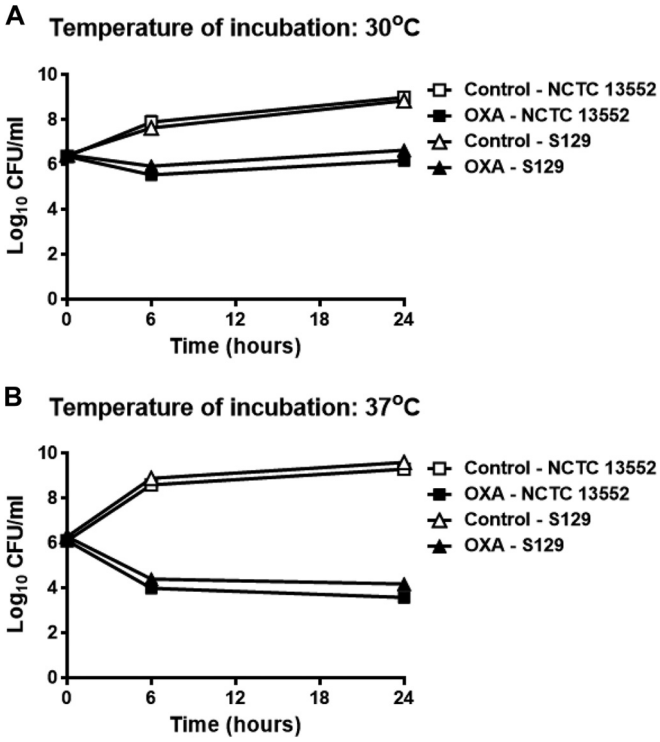

FIG 2 Killing curves of *mecC*-MRSA isolates NCTC 13552 and S129 by oxacillin (OXA) at a concentration of 10 mg/liter, simulating antibiotic levels achieved at trough in rat or human serum. The flasks containing the test strains not exposed (controls) or exposed to OXA were incubated at 30°C (A) or 37°C (B) for 24 h. Results are representative of two independent experiments.

control animals sacrificed at the start of treatment were all infected and contained high (median, 8.5 $\log_{10}$ CFU/g) bacterial counts. The peak (30 min), the trough (6 h) (mean ± standard deviation), and the area under the curve values for flucloxacillin in rat serum were 174 ± 50 mg/liter, 16 ± 5 mg/liter, and 213 mg · h/liter, respectively. The half-life was ca. 1 h. These values were close to those reported in humans, i.e., 125 to 154 mg/liter, 14 mg/liter, 178.6 mg · h/liter, and 1 h, respectively (26, 27). Flucloxacillin sterilized 80 to 100% of vegetations infected with *mecC*-MRSA strains and reduced the vegetation counts by >6 log10 CFU/g compared to those of controls ($P < 0.005$). Bacteria recovered from vegetations showed unchanged oxacillin susceptibility. All of

TABLE 2 Outcome of 3-day treatment with flucloxacillin of experimental endocarditis caused by *mecC*-MRSA isolates

Strain	No. of sterile vegetations/total no. of vegetations (%; median [range] $\log_{10}$ CFU/g of vegetation) for treatment group:	
	Control	Flucloxacillin[a]
NCTC 13552	0/5 (0; 8.5 [6.7–8.9])	10/11 (91[b]; 2.0 [2.0–2.7])[c]
1100	0/5 (0; 8.8 [8.1–9.1])	9/9 (100); 2.0 [2.0–2.0])
820	0/5 (0; 7.7 [6.4–9.0])	6/8 (75); 2.0 [2.0–4.1])[c]
S090	0/5 (0; 8.0 [7.6–9.4])	9/10 (90[b]; 2.0 [7.0–3.2])[c]
S129	0/5 (0; 8.7 [8.3–9.1])	8/10 (80[b]; 2.0 [2.0–6.0])[c]

[a] Simulation in rats of human pharmacokinetics following 2 g i.v. every 6 h.
[b] $P < 0.05$ compared to untreated controls by Fisher's exact test.
[c] $P < 0.005$ compared to untreated controls by Mann-Whitney test.

the vegetations from control animals sacrificed at the end of treatment were heavily infected (median, 9.8 $\log_{10}$ CFU/g) (data not shown). Flucloxacillin was totally ineffective against the *mecA*-MRSA Mu50 isolate (0/6 [0%] sterile valves).

In the current study, we observed a clear effect of the temperature on the activity of oxacillin against *mecC*-MRSA isolates *in vitro*. Indeed, all five tested *mecC*-MRSA isolates showed resistance to oxacillin in assays performed at 30°C but were susceptible at 37°C. These results are in agreement with those of previous studies with the NCTC 13552 (formerly LGA251) strain (16). Temperature is known to have an impact *in vitro* on the susceptibility of heterogeneous *mecA*-MRSA strains to oxacillin (28–30), possibly due to PBP2a downregulation (31) or enzymatic inactivation at ≥37°C (2). Our results indicate that thermosensitive heterogeneous expression of oxacillin resistance is also present in *mecC*-MRSA isolates. However, in contrast with *mecA*-MRSA isolates, where resistant subpopulations grew on plates containing >1,000 mg/liter oxacillin at 37°C (29), *mecC*-MRSA isolates showed no subpopulations emerging on plates containing >32 mg/liter oxacillin. Furthermore, rats with endocarditis induced by *mecC*-MRSA were successfully treated with human-like kinetics of flucloxacillin. We attribute these positive outcomes to the body temperature (≥37°C) present in animals with endocarditis (32). Indeed, at this temperature, the MICs of oxacillin for the tested isolates were ≤2 mg/liter, and plasma concentrations of flucloxacillin were greater than the MIC of the organisms throughout the dosing interval.

These data suggest consideration of β-lactams as an option for the treatment of *mecC*-MRSA infections. However, due to the presence of heterogeneous oxacillin-resistant subpopulations, albeit at low frequency, caution is advised in the use of these drugs. Indeed, in an analogous situation, resistant subpopulations in *mecA*-MRSA strains negatively impacted the β-lactam therapy of MRSA infections (33).

ACKNOWLEDGMENTS

This work was supported in part by grant 310030-143799/1 from the Swiss National Science Foundation.

We thank Ariane Deplano, Université Libre de Bruxelles, Brussels, Belgium, for sending us *mecC S. aureus* isolates S090 and S129.

We declare no conflicts of interest.

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