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Activity of Oxacillin versus That of Vancomycin against Oxacillin-Susceptible *mecA*-Positive *Staphylococcus aureus* Clinical Isolates Evaluated by Population Analyses, Time-Kill Assays, and a Murine Thigh Infection Model

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We compared the activity of dicloxacillin with that of vancomycin against 15 oxacillin-susceptible, methicillin-resistant *Staphylococcus aureus* (OS-MRSA) clinical isolates. By population analyses, we found that 6 OS-MRSA isolates were able to grow in the presence of up to 8 μg/ml dicloxacillin and 9 isolates were able to grow in 12 to >32 μg/ml dicloxacillin; all isolates grew in up to 2 μg/ml vancomycin. Both drugs exhibited similar bactericidal activities. In experimental infections, the therapeutic efficacy of dicloxacillin was significant ($P < 0.05$ versus untreated controls) in 10 OS-MRSA isolates and vancomycin was effective ($P < 0.05$) against 12 isolates; dicloxacillin had an efficacy that was comparable to that of vancomycin ($P > 0.05$) in 8 isolates. The favorable response to dicloxacillin treatment might suggest that antistaphylococcal penicillins could be used against OS-MRSA infections.

Staphylococcus aureus isolates that carry and express the *mecA* gene are considered methicillin resistant (MRSA) but may exhibit oxacillin MICs ranging from the susceptible range (≤2 μg/ml) to >1,000 μg/ml (8, 16, 20). It was generally believed that most *mecA*-positive *S. aureus* strains, including those appearing oxacillin susceptible (OS-MRSA), exhibit a degree of oxacillin heteroresistance and the use of β-lactams might lead to treatment failure. However, OS-MRSA isolates with no oxacillin heteroresistance (truly oxacillin susceptible) also appeared (9).

In a previous study, we reported that the activity of oxacillin against four OS-MRSA isolates was intermediate between that against *mecA*-negative *S. aureus* and highly resistant MRSA isolates (9). It was subsequently found that the OS-MRSA isolates of that study harbored specific mutations in their Fem proteins that probably conferred atypical oxacillin responsiveness (6). To further investigate these preliminary observations, we tested and report herein the *in vitro* and *in vivo* activities of oxacillin compared with those of vancomycin (treatment of choice for most MRSA infections) against a larger collection of OS-MRSA. The aim of this study was to investigate whether antistaphylococcal β-lactams, which were previously shown to exhibit superior activity than vancomycin against methicillin-susceptible *S. aureus* (MSSA) (11), retain activity against MRSA isolates that appear phenotypically susceptible to oxacillin. To the best of our knowledge, the activity of β-lactams has not been tested against OS-MRSA isolates.

Bacterial strains and susceptibility testing. Fifteen vancomycin-susceptible OS-MRSA clinical isolates, collected during 2006 and 2007, were studied. A high-level MRSA isolate (isolate 7263; oxacillin MIC, 256 μg/ml) and the *mecA*-negative strain *S. aureus* ATCC 29213 were included as controls. Isolates were stored at −80°C in brain heart infusion broth with 15% glycerol before testing. MIC testing of oxacillin and vancomycin was performed by agar dilution according to CLSI guidelines (4).

Detection of PBP2a and the *mecA* gene and MLST. The study isolates were tested for the *mecA* gene by PCR (12) and for PBP2a production by the Slidex MRSA agglutination test (bioMérieux, Marcy l'Etoile, France). Pulsed-field gel electrophoresis (PFGE) was performed as previously described (21), and banding patterns were compared visually. All isolates were tested for the Panton-Valentine leukocidin (PVL)-encoding genes *lukS-lukF* (14). Multilocus sequence typing (MLST) was also performed (http://www.mlst.net) (5).

Population analysis assays. Isolates were tested by population analyses (PAs) for dicloxacillin and vancomycin. Approximately 10^8 CFU were spread on Mueller-Hinton (MH) agar plates (2% NaCl) containing 0.125 to 32 μg/ml dicloxacillin or vancomycin (9). Colonies were counted after 48 h of growth at 35°C. Analyses were performed in triplicate, and mean CFU counts were plotted on a semilogarithmic graph.

Time-kill assays. Time-killing curves were performed in triplicate by inoculating approximately 10^6 CFU into MH broth containing 20 μg/ml dicloxacillin or 10 μg/ml vancomycin (9, 19). Aliquots were removed at 0, 6, 24, and 48 h postinoculation at 35°C and plated on MH agar plates for CFU enumeration. Bactericidal activity was defined as a ≥3-$\log_{10}$ reduction, and bacteriostatic activity was defined as the maintenance of, or a <3-$\log_{10}$ reduction of, the total number of CFU/ml in the original inoculum (15).

Murine infection model. An experimental murine thigh infection model was used to test the *in vivo* activity of dicloxacillin versus that of vancomycin. Animal studies were approved by the

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TABLE 1 Characteristics of the study isolates[a]

Isolate	PVL	MLST type	PFGE type	OXA MIC (μg/ml)	VAN MIC (μg/ml)	$\Delta\log_{10}$ CFU reduction at: 6 h in dicloxacillin time-kill assays	24 h in dicloxacillin time-kill assays	6 h in vancomycin time-kill assays	24 h in vancomycin time-kill assays
446	+	80	Ia	0.5	0.5	3.8	4.2	3.8	5.8
959	+	80	Ia	0.25	2	4.6	5.8	3.5	5.1
970	+	728	Ib	0.25	1	4.9	5.9	3.5	4.7
1117	+	80	Ib	0.5	1	2.4	4.4	3.4	4.9
1512	+	30	Ic	0.25	1	5.4	5.4	4.4	5.9
1546	+	80	Ic	0.25	1	5	5.8	3.6	5.0
2629	+	80	Ib	0.25	1	4.9	6	3.8	6
4324	+	80	Ib	0.25	1	3.6	4.7	3.7	3.8
5014	−	80	II	0.5	1	4.2	5.7	2.7	4.2
5505	+	80	Ib	1	1	3.6	4.4	3	5.4
6036	+	80	Ia	0.5	1	5.1	6	3.8	5.8
6601	+	80	Ib	0.25	1	5	5.4	4	5.2
7059	+	80	Ib	0.25	1	4.9	5.0	3.9	6
9131	+	80	Ib	0.25	1	3.5	4.3	3.9	4.7
9833	+	80	Ib	0.5	1	2.7	4.8	3.3	5.5
ATCC 29213	−	ND	III	0.25	1	5	6	3.5	6
7263	+	ND	IV	256	1	2	2	4.5	5.8

[a] OXA, oxacillin; VAN, vancomycin; ND, not determined.

Greek Veterinary Authorities and conformed to the Protocol on the Protection and Welfare of Animals. Six-week-old, specific-pathogen-free, female BALB/c mice (Harlan, Indianapolis, IN) weighing 23 to 27 g were used (7). Mice were rendered neutropenic by injecting cyclophosphamide intraperitoneally on day 4 (150 mg/kg) and day 1 (100 mg/kg) preinoculation (1, 12). Thigh infections were performed in triplicate by injecting approximately 10^6 CFU, and the mice were treated with either dicloxacillin at 500 mg/kg/12 h intraperitoneally or vancomycin at 180 mg/kg/12 h subcutaneously (7, 12, 18) or they were left untreated; animals were euthanized after 24 h. Thigh muscles were aseptically excised, homogenized, serially diluted and plated on antibiotic-free plates for CFU enumeration. Thigh CFU titer was expressed as log 10 CFU/thigh muscle. A *t* test was used for statistical analysis using Minitab software (version 13.31); a *P* value of ≤0.05 was considered statistically significant.

Results. All 15 OS-MRSA isolates carried the *mecA* gene and produced PBP2a; MICs of oxacillin and vancomycin were 0.25 to 1 μg/ml and ≤1 μg/ml, respectively. Two unrelated PFGE types were identified, with the predominant type including 14 isolates and exhibiting three subtypes that differed by 1 or 2 bands from each other. Fourteen isolates were PVL positive and one was PVL negative. MLST results showed that 13 isolates belonged to ST80, one isolate to ST728, and one to ST30. Characteristics of isolates are shown in Table 1.

PAs showed that 6 OS-MRSA isolates grew at up to 8 μg/ml oxacillin and 9 isolates at oxacillin concentrations of 12 to >32 μg/ml; all isolates were clearly susceptible to vancomycin. Results of the PAs are presented in Fig. 1.

Time-killing kinetics showed a ≥3 log 10 reduction of CFU/ml, indicating efficient bactericidal activity of dicloxacillin and vancomycin at 24 h for all OS-MRSA. In 10 isolates, the bactericidal activity of dicloxacillin after 6 h of incubation was higher than that of vancomycin. Overall, vancomycin exhibited low killing activity, eliminating most bacterial populations at 24 to 48 h compared with dicloxacillin, which eliminated most populations at 6 to 24 h in 10 isolates and at 24 to 48 h in 5 isolates. The *mecA*-negative control ATCC 29213 was rapidly killed, and the highly resistant MRSA control remained unaffected by dicloxacillin. The results of the bactericidal assays at 6 and 24 h are shown in Table 1.

The therapeutic efficacy of dicloxacillin, reflected by comparing the number of colonies grown from thighs of treated animals with the number in untreated animals, was significant ($P < 0.05$) in 10 of the 15 OS-MRSA isolates. Similarly, vancomycin was effective ($P < 0.05$ versus untreated controls) against infections caused by 12 OS-MRSA isolates. Interestingly, animals infected by 3 isolates, where vancomycin did not have significant efficacy, responded favorably to dicloxacillin treatment. When directly comparing the efficacy of dicloxacillin with that of vancomycin, a significant difference was not observed ($P > 0.05$) in 8 OS-MRSA infections, while vancomycin was significantly more effective ($P < 0.05$) in the remaining 7 isolates. Vancomycin treated significantly more efficiently than dicloxacillin the infections caused by the high-level MRSA control isolate, while the susceptible ATCC 29213 control responded slightly better to dicloxacillin than vancomycin. The results of the experimental infections are shown in Table 2. It should be noted that colonies yielded by infected thighs were tested again and found to carry and express the *mecA* gene.

Discussion. OS-MRSA clinical isolates have been increasingly reported in several countries (2, 8, 17) and in distant Greek regions (3, 9, 19). It has been shown previously that OS-MRSA may respond to oxacillin (9), possibly providing treatment alternatives. Recently, it has also been implied that some mutant MRSA isolates with relatively low oxacillin MICs may respond to oxacillin treatment in murine infections (10). However, it is generally believed that heterogeneous MRSA isolates may become homogeneously resistant under oxacillin exposure, resulting in treatment failure. In that respect, it has been suggested that oxacillin activity against such isolates should be compared with that of vancomycin, as most clini-

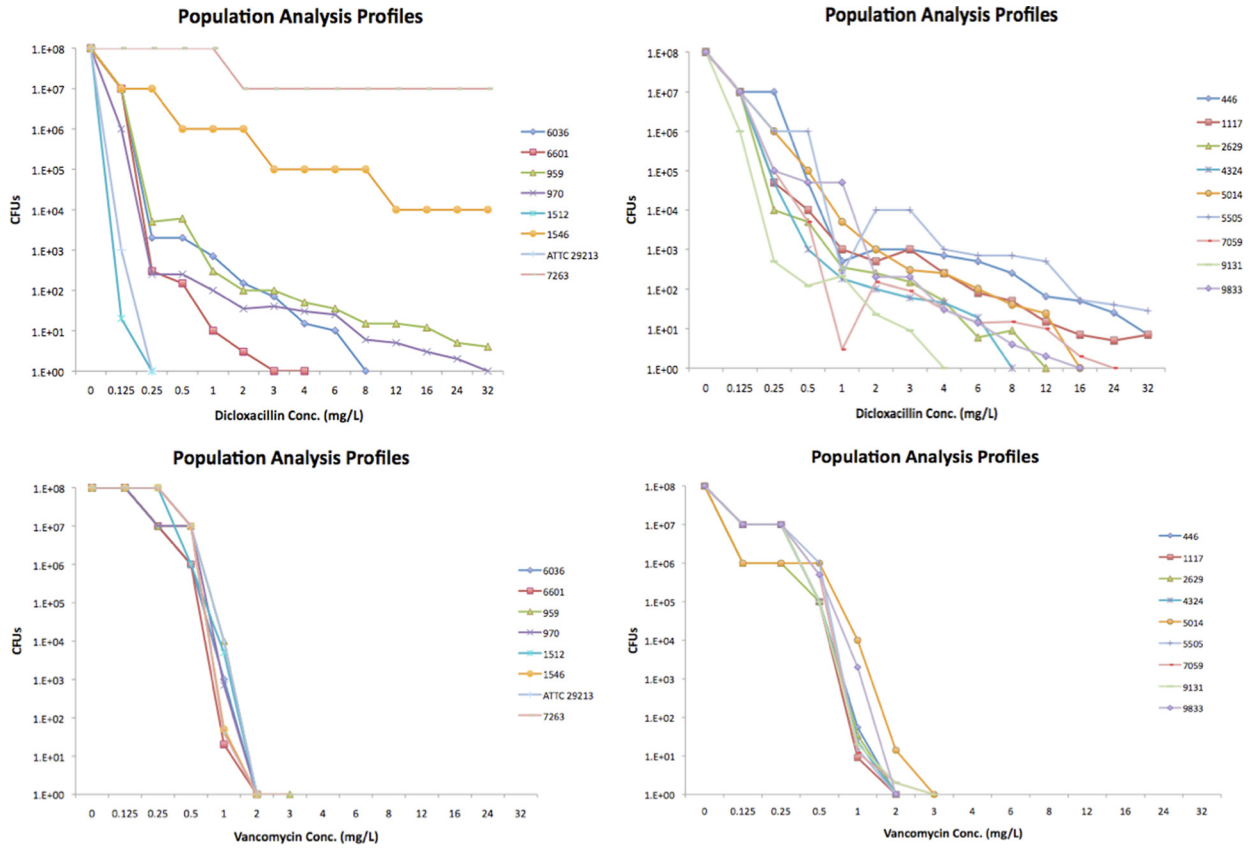

FIG 1 Population analysis assays of the 15 OS-MRSA study isolates and the control isolates using dicloxacillin and vancomycin.

cians who knowingly encounter such strains would utilize vancomycin (9).

In the current study, we directly compared the *in vitro* and *in vivo* activities of dicloxacillin versus vancomycin against 15 OS-MRSA clinical isolates. In time-kill assays vancomycin exhibited lower bactericidal activity than dicloxacillin in most isolates. It has to be noted that vancomycin was also previously shown to kill MSSA less rapidly than antistaphylococcal penicillins (20). This

TABLE 2 Therapeutic efficacies in murine infections of dicloxacillin and vancomycin versus untreated controls and vancomycin versus dicloxacillin[a]

Isolate	Avg log CFU ± SD per thigh muscle in untreated controls	Avg log CFU ± SD per thigh muscle in DCX treatment	DCX treatment efficiency (*P* value)	Avg log CFU ± SD per thigh muscle in VAN treatment	VAN treatment efficiency (*P* value)	DCX vs VAN activity in mouse thigh infections (*P* value)
446	7.6 ± 0.4	5.9 ± 0.4	0.016	6.3 ± 0.4	0.058	0.776
959	7.6 ± 0.3	6.9 ± 1.9	0.502	3.6 ± 1.8	0.011	0.009
970	8.7 ± 0.2	6.0 ± 0.6	0.037	5.8 ± 1.9	0.021	0.815
1117	7.9 ± 0.5	6.7 ± 0.4	0.062	6.3 ± 0.4	0.003	0.157
1512	8.7 ± 0.2	6.7 ± 0.6	0.034	4.3 ± 0.4	0.007	0.007
1546	8.9 ± 0.3	6.7 ± 0.8	0.208	3.9 ± 0.5	0.006	0.009
2629	8.3 ± 0.7	6.3 ± 1.1	0.058	3.4 ± 0.4	<0.001	0.015
4324	8.8 ± 0.2	4.1 ± 0.01	0.001	3.6 ± 0.4	<0.001	0.078
5014	7.2 ± 0.4	6.8 ± 0.8	0.488	4.0 ± 0.5	0.001	0.023
5505	7.7 ± 0.4	4.0 ± 1.1	0.04	3.5 ± 1.1	<0.001	0.981
6036	8.8 ± 0.1	6.7 ± 0.3	0.033	5.7 ± 0.3	0.025	0.014
6601	8.8 ± 0.4	7.4 ± 0.3	0.015	4.2 ± 0.8	0.003	0.005
7059	8.9 ± 0.2	5.0 ± 1.9	0.026	3.2 ± 0.3	<0.001	0.208
9131	8.7 ± 0.1	3.4 ± 0.4	0.001	3.3 ± 0.4	<0.001	0.273
9833	8.9 ± 0.2	6.2 ± 1.4	0.035	5.1 ± 1.6	0.020	0.476
ATCC 29213	8.7 ± 0.1	3.6 ± 0.6	0.001	3.8 ± 0.5	<0.001	0.771
7263	8.6 ± 0.1	8.2 ± 0.2	0.33	3.3 ± 0.5	<0.001	<0.001

[a] *P* value of <0.05 represents significant difference in CFU grown from infected thighs; DCX, dicloxacillin; VAN, vancomycin.

low *in vitro* bactericidal activity of vancomycin probably caused the suboptimal results observed in the treatment of serious *S. aureus* infections (13, 20). It has also been reported that the bactericidal activity of β-lactams against MSSA may be superior to that of vancomycin (11). In that respect, it is not surprising that our *mecA*-carrying isolates, which are functionally oxacillin susceptible, responded sufficiently to oxacillin treatment. In particular, dicloxacillin successfully treated 66.7% of mouse infection due to OS-MRSA isolates, similar to vancomycin, which succeeded against 75% of the isolates, while dicloxacillin was efficient in infections where vancomycin failed. Furthermore, the direct comparison of the activities of dicloxacillin and vancomycin showed that, interestingly, dicloxacillin was similarly efficient with vancomycin in more than half of the OS-MRSA isolates tested.

Overall, our findings suggest that the use of antistaphylococcal penicillins could still be considered when treating OS-MRSA infections. Should OS-MRSA be more common in the future, this observation could have significant implications for the treatment of the respective infections.

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