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Letters to the Editor

Increase in Resistance of Methicillin-Resistant *Staphylococcus aureus* to β-Lactams Caused by Mutations Conferring Resistance to Benzalkonium Chloride, a Disinfectant Widely Used in Hospitals

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been found worldwide and is one of the major nosocomial agents. When MRSA strains were first discovered in hospitals, resistance of MRSA to low concentrations of β-lactams was evident, but increasing resistance to high concentrations of β-lactams is now common. Most of the key elements responsible for methicillin resistance of MRSA have been well defined; however, mechanisms related to the acquisition of those genetic phenotypes in hospitals remain uncertain (4).

Appearance of MRSA resistant to benzalkonium chloride, a disinfectant widely used in hospitals, has been reported (1). To determine if the disinfectant might be related to increases in resistance to β-lactams, we selected strains of MRSA which showed resistance to low concentrations of oxacillin (MIC, 2 to 32 μg/ml), isolated mutants resistant to benzalkonium, and then monitored the resistance to oxacillin. Approximately 2×10^8 cells of parent MRSA were seeded on a Luria-Bertani (LB) agar plate containing 5 μg of benzalkonium chloride per ml and incubated at 37°C for 3 days. The colonies which appeared were isolated as benzalkonium-resistant mutants. The MIC of benzalkonium for parent MRSA was 5 μg/ml, and the value for the isolated mutants increased to 10 μg/ml. Surprisingly, for 15 to 55% of benzalkonium chloride-resistant mutants from these parental strains the MICs of oxacillin were over eightfold higher than the MICs for the parent strains. MRSA strains highly resistant to oxacillin were also obtained with benzethonium chloride, another cationic detergent (data not shown).

Most benzalkonium chloride-resistant mutants with resistance to high concentrations of oxacillin formed small colonies on LB agar. Revertants which formed large colonies showed sensitivity to both benzalkonium chloride and oxacillin, as did the parent MRSA (data not shown). Thus, resistance to oxacillin and resistance to benzalkonium chloride are closely related and may be caused by a single mutation.

Isolation of *S. aureus* mutants resistant to benzalkonium chloride has been reported (1, 5–7), but a related increase in resistance to β-lactam antibiotics has not been documented. We introduced into a wild type of *S. aureus* plasmid pTZ20 (6) containing the *qacC* gene, which is shown to cause resistance to disinfectants. The *qacC* gene alone increased resistance of *S. aureus* to benzalkonium chloride but not to β-lactams (data not shown).

MRSA mutants resistant to benzalkonium chloride showed a higher resistance than parent strains to various β-lactam antibiotics including cloxacillin, moxalactam, flomoxef, and cefmetazole (Table 1), as seen in the case of oxacillin. These mutants showed no remarkable increase in resistance to cefazolin and cephalothin (narrow-spectrum cephems), chloramphenicol (an inhibitor of protein synthesis) or ampicillin but did show resistance to ofloxacin, the lethal mechanisms of which differ from those of β-lactams. It is possible that the mutation may affect the efficiency of uptake or may activate an efflux pump of drugs, in a nonspecific manner. Another possibility is that mutation of genes encoding elements regulating the expression of methicillin resistance, such as the *femA* gene (2), is responsible for the phenotype. Mutations responsible for the concomitant increase in resistance to benzalkonium chloride and β-lactams will need to be identified.

TABLE 1. Antibiotic resistance in MRSA and derived benzalkonium chloride-resistant mutants

Antibiotic	MIC (μg/ml)[a]						
	MRSA[b]						MSSA[c]
	Parent	BZ-R-1	BZ-R-2	BZ-R-3	BZ-R-4	BZ-R-5	
Oxacillin	16	512	512	128	64	64	0.3
Cloxacillin	0.5	256	512	128	0.5	8	0.3
Moxalactam	64	256	1024	512	256	256	8
Flomoxef	8	128	128	64	16	16	0.5
Cefmetazole	8	128	64	64	32	32	1
Cefazolin	64	128	128	64	128	128	0.5
Cephalothin	64	128	128	128	128	128	0.5
Ampicillin	16	32	32	16	32	16	0.5
Chloramphenicol	4	4	4	4	4	4	4
Ofloxacin	8	32	32	16	16	16	0.3
Tetracycline	128	128	128	32	128	128	ND[d]
Kanamycin	256	512	512	512	256	256	ND
Benzalkonium chloride	5	10	10	10	10	10	0.7

[a] Full growth of bacteria in LB liquid medium was appropriately diluted, and the bacterial suspension (2×10^3) was streaked on LB agar plates containing various concentrations of antibiotics, followed by incubation at 37°C for 72 h.
[b] MRSA strains were examined by identifying the *mecA* gene by PCR (3) in strains of *S. aureus* isolated in Kyushu Univeristy Hospital.
[c] MSSA, methicillin-susceptible *S. aureus*.
[d] ND, not determined.

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REFERENCES

1. **Al-Masaudi, S. B., M. J. Day, and A. D. Russell.** 1988. Sensitivity of methicillin-resistant *Staphylococcus aureus* strains to some antibiotics, antiseptics and disinfectants. J. Appl. Bacteriol. **65:**329–337.
2. **Berger-Bachi, B., L. Barberis-Maino, A. Strassle, and F. H. Kayser.** 1989. FemA, a host-mediated factor essential for methicillin resistance in *Staphylococcus aureus*: molecular cloning and characterization. Mol. Gen. Genet. **219:**263–269.
3. **Hiramatsu, K., H. Kihara, and T. Yokota.** 1992. Analysis of border line-resistant strains of methicillin resistant *Staphylococcus aureus* using polymerase chain reaction. Microbiol. Immunol. **36:**445–453.
4. **Hiramatsu, K.** 1995. Molecular evolution of MRSA. Microbiol. Immunol. **39:**531–543.
5. **Paulsen, I. T., M. H. Brown, and R. A. Skurray.** 1996. Proton-dependent multidrug efflux systems. Microbiol. Rev. **60:**575–608.
6. **Sasatsu, M., Y. Shibata, S. Tamura, and M. Kono.** 1990. Drug-resistant plasmids in multiply drug-resistant *Staphylococcus aureus* L20A. Microbios Lett. **43:**105–112.

7. **Sasatsu, M., Y. Shibata, N. Noguchi, and M. Kono.** 1992. High-level resistance to ethidium bromide and antiseptics in *Staphylococcus aureus*. FEMS Microbiol. Lett. **93:**109–114.

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