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"Cephem-Induced Vancomycin Resistance" Observed in Phenotypically Methicillin-Sensitive *Staphylococcus aureus* Isolated from a Patient Treated with Vancomycin and Cephems

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A patient with recurrent transitional cell bladder carcinoma was operated on three times in succession. First, resection of the recurrent lesion was performed, then radical cystectomy, and finally debridement of the surgical wound. The last operation was necessitated by an infection by *Staphylococcus aureus*. Both methicillin-sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated during the course of clinical treatment (Fig. 1). In this study MSSA and MRSA were classified according to their sensitivity to oxacillin (MPIPC); isolates whose minimum inhibitory concentration of MPIPC was less than 4 μg/ml were judged to be MSSA.

"Cephem-induced vancomycin resistance" in *S. aureus* (ClVRSA) was defined previously (1) and detected as follows. Fifty microliters of overnight culture of *S. aureus* whose OD_{578} nm was adjusted to 0.3 were plated evenly on MU3/VAN agar (Becton Dickinson Co., Paramus, N.J., USA) which contained 4 μg/ml of vancomycin (VCM). Disks dipped in

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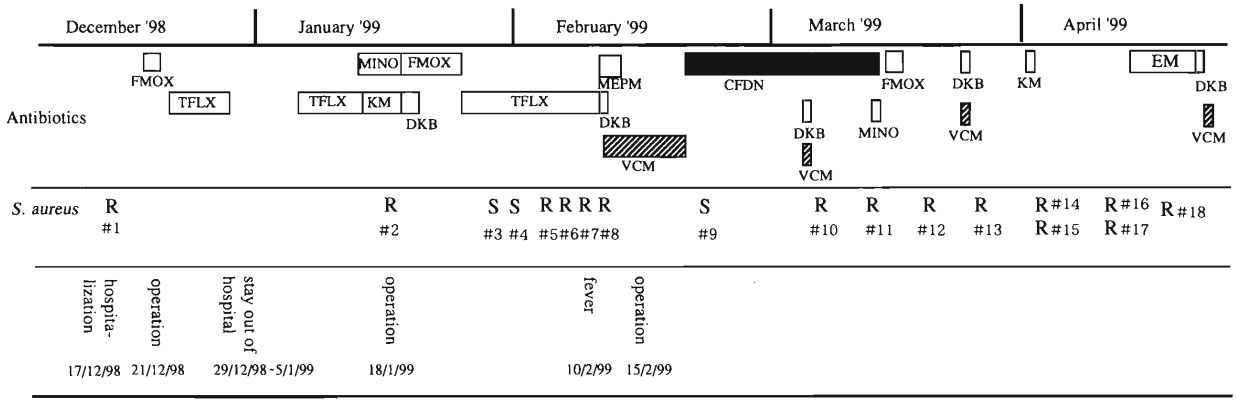

R : MRSA S : MSSA

Fig. 1. Clinical course of the patient.
Antibiotics administered are shown in the upper part of the figure. Bacterial isolates are shown in the middle (R: MRSA; S: MSSA), and treatments and symptoms at the bottom. The dates are indicated as date/month/year. FMOX, flomoxef; KM, kanamycin; CFDN, cefdinir; MINO, minocycline; DKB, dibekacin, VCM, vancomycin; TFLX, tosufloxacin; MEPM, meropenem; EM, erythromycin.

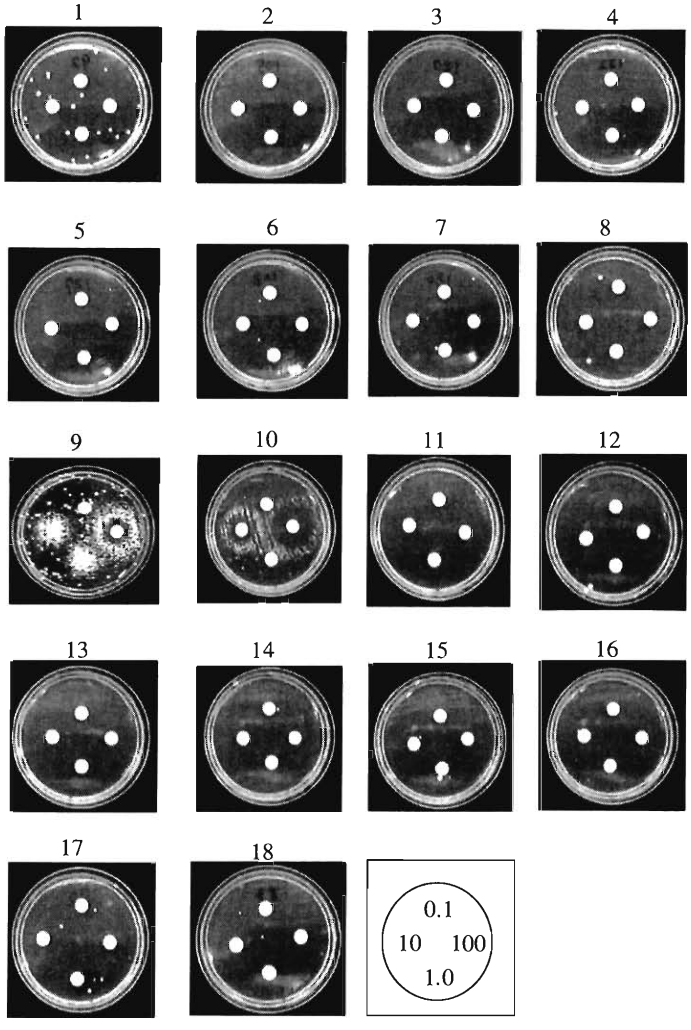

Fig. 2. Assay for CIVR.
The numbers indicate isolate numbers. Four disks on each plate had been dipped in ceftizoxime dissolved at a concentration of 0.1, 1.0, 10, or 100 μg/ml (see diagram at the right end of the bottom).

ceftizoxime (CZX) solutions at concentrations of 0.1, 1.0, 10, and 100 μg/ml were placed on the culture. Isolates giving rise to doughnut-shaped growths around the disks were defined as CIVRSA. Figure 2 shows the results of these assays. Among 18 isolates, isolate #9 and #10 showed doughnut-shaped growths, i.e., they were CIVRSA.

Table. Sensitivity of isolates to antibiotics

Isolates	MPIPC	ABPC	CEZ	CMZ	IPM	GM	ABK	EM	CLDM	MINO	VCM	LVFX	TFLX	β-lactamase
#1	≧8	≧16	≧32	≧64	≧16	≧16	≦4	≦0.5	≦0.5	≦4	≦0.5	≧8	n.t.	+
#2	≧16	16	≧32	≧32	≧16	≧16	16	≧16	≦0.25	1	1	n.t.	≧16	n.t
#3	2	≧16	≧32	≧64	4	≧16	16	≧16	≦0.25	4	1	n.t.	≧16	n.t.
#4	2	≧16	≧32	≧32	4	≧16	16	≧16	≧16	≦0.25	1	n.t.	≧16	n.t.
#5	≧16	16	≧32	≧32	≧16	≧16	16	≧16	≦0.25	≦0.5	1	n.t.	≧16	n.t.
#6	≧16	16	≧32	≧32	≧16	≧16	16	8	≦0.25	≦0.5	2	n.t.	≧16	n.t.
#7	≧16	16	≧32	≧32	≧16	≧16	16	≧16	≦0.25	≦0.5	1	n.t.	≧16	n.t.
#8	≧16	8	≧32	8	1	≧16	4	≧16	0.25	0.5	0.5	n.t.	≧16	n.t.
#9*	≦0.25	≦0.25	≦0.5	2	≦0.25	16	≧16	≦0.25	≦0.25	≦0.5	≦0.5	n.t.	≧16	n.t.
#10*	≧16	16	≧32	≧32	≧16	≧16	≧16	≧16	≦0.25	8	2	n.t.	≧16	n.t.
#11	≧16	≧16	≧32	≧32	4	≧16	≧16	≦0.25	4	≧16	2	n.t.	n.t.	n.t.
#12	≧16	16	≧32	≧32	≧16	≧16	16	≧16	≦0.25	1	1	n.t.	≧16	n.t.
#13	≧8	≧16	≧32	≧64	≧16	≧16	≦4	≦0.5	≦0.5	≦4	≦0.5	≧8	n.t.	+
#14	≧8	≧16	≧32	≧64	≧16	≧16	≦4	≦0.5	≦0.5	≦4	≦0.5	≧8	n.t.	+
#15	≧8	≧16	≧32	≧64	≧16	≧16	≦4	1	≦0.5	≦4	≦0.5	≧8	n.t.	+
#16	≧8	≧16	≧32	≧64	≧16	≧16	≦4	≦0.5	≦0.5	≦4	1	≧8	n.t.	+
#17	≧8	≧16	≧32	≧64	≧16	≧16	≦4	1	≦0.5	≦4	1	≧8	n.t.	+
#18	≧8	≧16	≧32	≧64	≧16	≧16	≦4	1	≦0.5	≦4	1	≧8	n.t.	+

The figures indicate minimum inhibitory concentration (μg/ml).
MPIPC, oxacillin; ABPC, ampicillin; CEZ, cefazolin; CMZ, cefmetazole; IPM, imipenem; GM, gentamycin; ABK, arbekacin; EM, erythromycin; CLDM, clindamycin; MINO, minocycline; LVFX, levofloxacin; TFLX, tosufloxacin.
*"Cephem-induced vancomycin-resistant *S. aureus*" (CIVRSA)

The Table shows the antibiotic sensitivity of these isolates. All were sensitive to VCM. Isolate #9 was MSSA (MIC ≦ 0.25 μg/ml), while #10 was MRSA (MIC ≧ 16 μg/ml). However, a PCR amplification assay revealed that both #9 and #10 had a *mec*A gene. The reason why #9 was phenotypically MSSA remains unknown. It was noted that #9 was generally more sensitive to the tested antibiotics than other isolates.

Isolate #9 was derived from a sample resected on February 22 and #10 from samples resected on March 9. Cefdinir (CFDN) was continuously administered during this period. VCM was administered just before isolation of #9 and #10 (on February 19 and March 8). The timing of the combined antibiotic administration may have influenced the generation of CIVRSA.

REFERENCE

1. Hanaki, H., Inaba, Y., Sasaki, K. and Hiramatsu, K. (1998): A novel method of detecting *Staphylococcus aureus* heterogenously risistant to vancomycin. Jpn. J. Antibiot., 51, 521-530.