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Susceptibility of Methicillin-resistant *Staphylococcus aureus* Clinical Isolates to Various Antimicrobial Agents

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ABSTRACT

Resistance patterns against 23 antimicrobial agents were examined for 42 strains of methicillin-resistant *Staphylococcus aureus* (MRSA). Thirty-four strains were isolated at Hiroshima University Hospital during 1984-1990 and 8 strains were collected in Tokushima city in 1986. Overall resistance to the antimicrobial agents in clinical use is summarized as follows: methicillin 100%, flomoxef 93% (β-lactams); kanamycin 98%, tobramycin 88%, amikacin 83%, isepamicin 81%, gentamicin 60%, dibekacin 64%, arbekacin 0% (aminocyclitol aminoglycosides); ofloxacin 31%, TA-167 33% (fluoroquinolones); erythromycin 100%, clarithromycin 100%, josamycin 71% (macrolides); vancomycin 0% (glycopeptide); tetracycline 43%, minocycline 31% (tetracyclines); fosfomycin 93%. The MRSA strains remained susceptible to the non-clinical peptide group of antibiotics except for mikamycin B: mikamycin A 2%, mikamycin B 69%, nosiheptide 0%, bottromycin A2 0%, bottromycin D-1 0%, bottromycin D-2 0%.

Since April 1990, the MRSA strains isolated at Hiroshima University Hospital showed a tendency to acquire resistance to tetracyclines and fluoroquinolones and to lose mikamycin B-resistance.

As of August 1990, none of the MRSA strains isolated at Hiroshima University Hospital was resistant to vancomycin and arbekacin.

Key words: *Methicillin-resistant Staphylococcus aureus (MRSA), Antibiotic resistance, Vancomycin, Arbekacin*

Methicillin (DMPPC) is a semisynthetic β-lactam antibiotic resistant to β-lactamase. Soon after DMPPC became available, DMPPC-resistant *Staphylococcus aureus* (MRSA) was reported in 1961[12]. In 1960s and 1970s, outbreaks of hospital infections caused by MRSA were sporadic. Since 1980, MRSA has caused increasing problems in hospitals worldwide[18,23,25].

The low-affinity penicillin binding protein (PBP), designated PBP 2'[27], PBP 2a[10] or MRSA PBP[22], is encoded by the DMPPC-resistance determinant *mec*A, which is a 2,130-bp segment of foreign DNA[2]. This PBP is responsible for the intrinsic resistance to β-lactams. Furthermore, many MRSA strains are resistant to a variety of antibiotics including kanamycin (KM), tobramycin (TOB),gentamicin (GM), erythromycin (EM), clindamycin and tetracycline (TC)[18]. In the absence of susceptibility data or serious infections due to MRSA, vancomycin (VCM) is usually regarded as the antibiotic of choice for treatment[9]. However, resistance to VCM has been reported in enterococci and coagulase-negative staphylococci[15,21]. In clinical isolates of *Enterococcus faecium*, VCM-resistance was mediated by plasmids which were self-transferable to the other *E. faecium* strains[16]. The plasmids could also conjugate to *Enterococcus faecalis*, *Streptococcus sanguis*, *Streptococcus pyogenes*, *Streptococcus lactis* and *Listeria monocytogenes*, but not to *S. aureus*[16]. Since certain plasmids of enterococci are transmissible to *S. aureus* by conjugation[8], there is a potential risk of future spread of vancomycin resistance to *S. aureus*.

This study aimed to examine the incidence of multi-drug resistance in MRSA isolated at Hiroshima University Hospital during the period September 1984 to August 1990. The future prospect for chemotherapy in MRSA infections will be discussed.

MATERIALS AND METHODS

The 42 MRSA strains used in this study were as follows: 8 strains isolated in Tokushima city in 1986, 22 strains in Hiroshima University Hospital between September 1984 and March 1990 and 12

strains at the same hospital between April 1990 and August 1990. DMPPC-sensitive *S. aureus* FDA 209P was used as a reference.

The antibiotics used and their manufacturers or distributors are as follows: DMPPC (Banyu Pharmaceutical Co., Ltd.); flomoxef (FMOX) and VCM (Shionogi & Co., Ltd.); isepamicin (ISP) (Toyo Jozo Co., Ltd.); EM and clarithromycin (CAM) (Taisho Pharmaceutical Co., Ltd.); josamycin (JM) (Yamanouchi Pharmaceutical Co., Ltd.); TC and minocycline (MINO) (Lederle Japan, Ltd.); fosfomycin (FOM) (Meiji Seika Kaisha, Ltd.); nosiheptide (NH) (Mitsubishi Kasei Corporation); ofloxacin (OFLX) and DR-3355 (Daiichi Pharmaceutical Co., Ltd.); TA-167 (Tanabe Seiyaku Co., Ltd.); mikamycins A and B (MKM-A, MKM-B) (Kanegafuchi Chemical Ind. Co., Ltd.); KM, TOB, dibekacin (DKB), amikacin (AMK), GM and arbekacin (ABK) (Inst. Microb. Chem.). Bottromycin A2 and its derivatives D-1 and D-2 were obtained as described previously[20].

The minimum inhibitory concentration (MIC) was measured by two-fold agar dilution method with Mueller-Hinton agar (DIFCO Laboratories). Test strains grown overnight at 37°C in 5 ml of Meuller-Hinton broth (MHB) (DIFCO Laboratories) were diluted 10^2-fold with fresh MHB, and about 5×10^4 CFU was applied with multipoint plating apparatus on the surface of agar plates. The plates were incubated at 37°C for 18 hr.

The production of β-lactamase by individual MRSA strains was monitored by using BBL cefinase (Becton Dickinson Microbiology Systems).

RESULTS

Forty-two MRSA strains were classified as: resistant, moderately resistant or susceptible to each antimicrobial agent depending on their MICs according to the definitions of Maple et al[18] and the British Society for Antimicrobial

Table 1. Incidence of antibiotic resistance in 42 MRSA strains isolated at Hiroshima University Hospital (34 strains) and in Tokushima city (8 strains)

Antimicrobial agent	Resistant strains		Moderately resistant strains		Sensitive strains		*S. aureus*
	No. of strains (MIC, μg/ml)	%	No. of strains (MIC, μg/ml)	%	No. of strains (MIC, μg/ml)	%	FDA 209P
Methicillin (DMPC)	42 (≧25)	100	0	0	0	0	(1.56)
Flomoxef (FMOX)	39 (≧12.5)	93	3 (3.13-6.25)	7	0	0	(0.20)
Kanamycin (KM)	41 (≧25)	98	1 (3.13)	2	0	0	(0.78)
Tobramycin (TOB)	37 (≧25)	88	0	0	5 (0.39-1.56)	12	(0.10)
Dibekacin (DKB)	27 (≧12.5)	64	13 (1.56-6.25)	30	2 (0.10-0.20)	6	(0.20)
Amikacin (AMK)	35 (6.25-50)	83	—		7 (1.56-3.13)	16	(0.39)
Gentamicin (GM)	25 (≧25)	60	0	0	17 (0.10-1.56)	40	(0.10)
Isepamicin (ISP)	34 (12.5-50)	81	—		8 (1.56-6.25)	19	(1.56)
Arbekacin (ABK)	0	0	3 (3.13-6.25)	7	39 (0.05-1.56)	93	(0.20)
Erythromycin (EM)	42 (≧12.5)	100	0	0	0	0	(0.20)
Clarithromycin (CAM)	42 (≧3.13)	100	0	0	0	0	(0.05)
Josamycin (JM)	30 (≧100)	71	1 (25)	2	11 (0.78)	27	(0.39)
Tetracycline (TC)	18 (≧25)	43	0	0	24 (0.10-0.78)	57	(0.10)
Minocycline (MINO)	13 (12.5-50)	31	4 (0.78)	9	25 (0.05-0.39)	60	(0.05)
Fosfomycin (FOM)	39 (≧50)	93	0	0	3 (6.25)	7	(1.56)
Vancomycin (VCM)	0	0	—		42 (0.39-3.13)	100	(0.39)
Ofloxacin (OFLX)	13 (12.5-50)	31	0	0	29 (0.20-3.13)	69	(0.20)
DR-3355[a]	13 (6.25-12.5)	31	0	0	29 (0.20-1.56)	69	(0.10)
TA-167	14 (3.13-12.5)	33	0	0	28 (0.10-0.78)	67	(0.10)
Mikamycin A (MKM-A)	1 (>100)	2	2 (25-50)	5	39 (1.56-12.5)	93	(1.56)
Mikamycin B (MKM-B)	29 (≧100)	69	0	0	13 (6.25-25)	31	(3.13)
Bottromycin A_2	0	0	—		42 (0.20-1.56)	100	(0.39)
Bottromycin D-1[b]	0	0	—		42 (1.56-12.5)	100	(1.56)
Bottromycin D-2[c]	0	0	—		42 (0.78-12.5)	100	(0.39)
Nosiheptide (NH)	0	0	—		42 (0.006-0.05)	100	(0.006)

[a] L-Ofloxacin.
[b] Bottromycin A_2 N-α-iminoisobutyl hydrazide.
[c] Bottromycin A_2 N-α-iminobenzo hydrazide.

Chemotherapy[3]. Resistance patterns of all the MRSA strains against 23 antimicrobial agents as well as the MIC distribution of individual compounds are shown in Table 1.

Many of the MRSA strains showed resistance to more than 10 antibiotics. The antibiotics, to which more than 80% strains were resistant, included DMPPC, FMOX, KM, TOB, AMK, ISP, EM, CAM and FOM, whereas the development of resistance to ABK; VCM; bottromycin A2, D-1 or D-2; or NH was not observed. Only a few strains showed resistance to MKM-A.

The efficacy of TC, MINO, OFLX, DR-3355, TA-167 or MKM-B was intermediate and the frequency of resistance to these compounds changed remarkably in April 1990. The % of resistance to tetracyclines and fluoroquinolones increased significantly after this point and, in contrast, MKM-B became more effective than before (Figs. 1, 2).

The MIC distributions for the MRSA strains isolated at Hiroshima University Hospital before March 1990 (22 strains; terminated group A) and after April 1990 (12 strains; group B) are shown in Tables 2 and 3, respectively. Table 4 shows the MIC distribution for 8 strains isolated in Tokushima city in 1986 (group C).

The resistance of MRSA to the aminocyclitol aminoglycoside antibiotics was determined by three inactivating enzymes: bifunctional 6'-acetyltransferase/2"-phosphotransferase AAC(6')/APH(2"), 4'-adenyltransferase AAD(4') and 3'-phosphotransferase APH(3')[26]. The divergent phenotypes with respect to the resistance pattern to KM, TOB, GM and AMK were accounted for by the expression of these inactivating enzymes in individual MRSA strains: $KM^sTOB^sGM^sAMK^s$, no enzyme; $KM^rTOB^sGM^sAMK^s$, APH(3'); $KM^rTOB^rGM^sAMK^r$, AAD(4'); $KM^rTOB^rGM^rAMK^s$, AAC(6')/APH(2") or AAC(6')/APH(2") + APH(3'); $KM^rTOB^rGM^rAMK^r$, AAC(6')/APH(2") + AAD(4') or AAC(6')/APH(2") + AAD(4') + APH(3'). As can be seen in Table 5, most MRSA strains isolated at Hiroshima University Hospital expressed AAD(4') alone or in combination with AAC(6')/APH(2"). The MRSA strains in group C are heterogeneous in this criteria; three strains expressed APH(3'), two strains AAD(4'), one strain AAC(6')/APH(2") and two strains AAC(6')/APH(2") + AAD(4'). Among 42 MRSA strains tested, only one strain in group A did not express any inactivating enzymes. At Hiroshima Unversity Hospital, the MRSA strains producing AAC(6')/APH(2") + AAD(4') were dominant, as of August 1990.

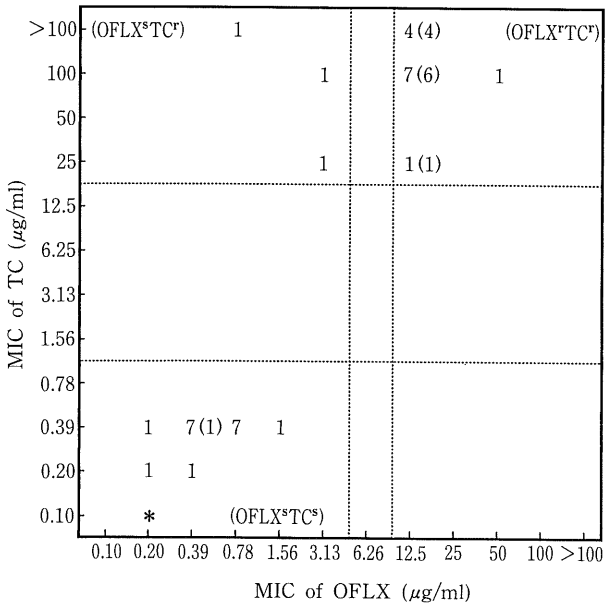

Fig. 1. Relationship between resistance to TC and OFLX of the MRSA strains isolated at Hiroshima University Hospital

Figures represent total number of MRSA strains isolated at Hiroshima University Hospital from 1984 to 1990 (groups A and B) with the corresponding MICs. The number of MRSA strains isolated since April 1990 (group B) are given in parenthesis.

*: MICs of OFLX and TC for *S. aureus* FDA 209P.

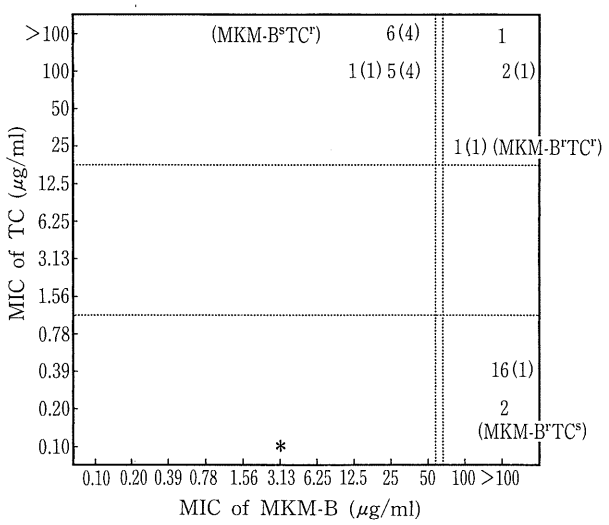

Fig. 2. Relationship between resistance to TC and MKM-B of the MRSA strains isolated at Hiroshima University Hospital

Figures represent the number of MRSA strains in groups A and B with corresponding MICs. The number of MRSA strains in group B are given in parenthesis.

*: MICs of OFLX and TC for *S. aureus* FDA 209P.

DISCUSSION

All the MRSA strains harbor *mec*A gene which encodes low-affinity PBP responsible for their intrinsic resistance to β-lactams (data not shown), whereas the β-lactamase is thought to contribute to borderline resistance to β-lactams. The high incidence of β-lactamase-positive strains was observed in groups A and C but not in those of group B: 14 out of 22 (64%), 7 out of 8 (87.5%), and 1 out of 12 (8%), respectively.

The resistance patterns of MRSA to aminocyclitol

Table 2. MICs against the MRSA strains isolated at Hiroshima University Hospital from September 1984 to March 1990 (22 strains, group A)

Antimicrobial agent	MIC (μg/ml)			
	Range	50%	90%	FDA 209P
DMPPC	25 - >100	>100	>100	1.56
FMOX	3.13 - >100	50	100	0.20
KM	3.13 - >100	>100	>100	0.78
TOB	0.39 - >100	50	>100	0.10
DBK	0.39 - >100	12.5	100	0.20
AMK	3.13 - 50	12.5	25	0.39
GM	0.10 - >100	0.78	>100	0.10
ISP	3.13 - 50	25	50	1.56
ABK	0.05 - 6.25	0.39	3.13	0.20
EM	>100	>100	>100	0.20
CAM	25 - >100	>100	>100	0.05
JM	0.78 - >100	>100	>100	0.39
TC	0.20 - >100	0.39	100	0.10
MINO	0.10 - 12.5	0.20	6.25	0.05
FOM	6.25 - >100	>100	>100	1.56
VCM	0.78 - 3.13	1.56	3.13	0.39
OFLX	0.20 - 50	0.78	3.13	0.20
DR-3355	0.20 - 6.25	0.39	1.56	0.10
TA-167	0.10 - 12.5	0.39	3.13	0.10
MKM-A	1.56 - 50	6.25	6.25	1.56
MKM-B	12.5 - >100	>100	>100	3.13
A:B (2:1)	0.39 - 6.25	3.13	6.25	0.20
A:B (1:1)	0.78 - 12.5	3.13	12.5	0.10
A:B (1:2)	0.39 - 3.13	1.56	3.13	0.20
Bottromycin A_2	0.78 - 1.56	1.56	1.56	0.39
Bottromycin D-1	3.13 - 12.5	6.25	12.5	1.56
Bottromycin D-2	1.56 - 12.5	3.13	6.25	0.39
NH	0.025 - 0.05	0.025	0.05	0.00625

aminoglycoside antibiotics can be conveniently accounted for by the function of three different inactivating enzymes, AAD(4'), APH(3') and AAC(6')/APH(2'') (Table 5) encoded by *aad*D, *aph*A and *aac*A-*aph*D, respectively. The feature of MRSA strains isolated at Hiroshima University Hospital is the close association between *mec*A and *aad*D (94%) but is not so evident among those isolated in Tokushima city (50 %). The linkage between *mec*A and *aad*D in MRSA was proved by coordinate elimination with growth at a high temperature[27]. This was further confirmed by the same authors by cloning chromosomal BamHI DNA fragments of MRSA strains[29]. Two types of MRSA strains were recognized differing in the length of HindIII fragments carrying *mec*A gene: 4.3- and 4.0-kb fragments. The HindIII fragment of TOB-resistant MRSA strains containing both *mec*A and *aad*D genes was confined to the longer 4.3-kb fragment[29] and this type of MRSA had rapidly become dominant in Japan since its first report in 1983[13].

AAC(6')/APH(2'') is also frequently detected in the MRSA strains either alone or together with APH(3') and/or AAD(4'); the incidence of coexpression of AAC(6')/APH(2'') and AAD(4') has been extremely high in the MRSA strains isolated at Hiroshima University Hospital since April 1990. APH(3') is rarely detected independently from the other enzymes in the MRSA strains isolated at Hiroshima University Hospital (1 out of 34 strains), whereas 3 out of 8 strains isolated in Tokushima city showed $KM^rTOB^sGM^sAMK^s$ phenotype. One strain in group A with $KM^sTOB^sGM^sAMK^s$ phenotype did not express any aminocyclitol aminoglycoside-modifying enzymes. In Australian strains of *S. aureus*, AAC(6')/APH(2'') has been shown to be encoded on a transposon, Tn*4001*[17]. Tn*4001* is commonly found on members of the pSK1 family of multiresistance plasmids found in Australian clinical strains of staphylococci. Many of these plasmids are conjugative and can also specify resistance due to AAD(4') and APH(3')[1]. The conjugative and nonconjugative plasmids isolated in North American clinical strains of *S. aureus* con-

Table 3. MICs against the MRSA strains isolated at Hiroshima University Hospital from April to August 1990 (12 strains, group B)

Antimicrobial agent	MIC (μg/ml)			
	Range	50%	90%	FDA 209P
DMPPC	100 - >100	>100	>100	1.56
FMOX	12.5 - 100	100	100	0.20
KM	25 - >100	>100	>100	0.78
TOB	1.56 - >100	50	>100	0.10
DKB	1.56 - 100	25	50	0.20
AMK	3.13 - 25	12.5	12.5	0.39
GM	1.56 - >100	50	100	0.10
ISP	6.25 - 50	25	50	1.56
ABK	0.39 - 0.78	0.78	0.78	0.20
EM	12.5 - >100	>100	>100	0.20
CAM	3.13 - >100	>100	>100	0.05
JM	0.78 - >100	0.78	>100	0.39
TC	0.39 - >100	100	>100	0.10
MINO	0.20 - 25	12.5	12.5	0.05
FOM	≧100	>100	>100	1.56
VCM	0.78 - 1.56	0.78	1.56	0.39
OFLX	0.39 - 12.5	12.5	12.5	0.20
DR-3355	0.20 - 12.5	6.25	12.5	0.10
TA-167	0.10 - 12.5	12.5	12.5	0.10
MKM-A	3.13 - 25	6.25	25	1.56
MKM-B	12.5 - >100	25	>100	3.13
A:B (2:1)	0.39 - 6.25	0.78	3.13	0.20
A:B (1:1)	0.78 - 3.13	1.56	1.56	0.10
A:B (1:2)	0.39 - 3.13	0.78	3.13	0.20
Bottromycin A_2	0.20 - 1.56	1.56	1.56	0.39
Bottromycin D-1	1.56 - 12.5	6.25	12.5	1.56
Bottromycin D-2	0.78 - 3.13	3.13	3.13	0.39
NH	0.00625 - 0.05	0.025	0.05	0.00625

tained the same *aac*A-*aph*D determinant as found in Tn*4001*[4]. In addition to *aac*A-*aph*D, *aad*D was carried on large conjugative plasmids as can been seen in the pSK1 family of plasmids. Tn*4001* and the related elements have been detected on plasmids and chromosomes of *S. aureus* strains, including MRSA, from several European countries. Although the genetic analysis of Japanese clinical strains of *S. aureus* has not yet been reported, the widespread resistance to aminocyclitol aminoglycoside antibiotics could be attributed to the similar multiresistant plasmids as found in the other nations.

Twenty-nine MKM-B-resistant MRSA strains were unexceptionally resistant to macrolide antibiotics, EM, CAM and JM. Macrolide-lincosamide-streptogramin B (MLS) resistance was first described in *S. aureus*[5] and is now common in this and other species of staphylococci. MKM-B is structurally related with streptogramin B and this is the basis of the high incidence of resistance to this antibiotic, though it has never been in clinical use. MLS-resistance is due to the function of methylase which converts an adenosine residue of 23S ribosomal RNA to 6-*N*-dimethyladenosine, thereby reducing the affinity of the ribosome for all MLS antibiotics[14]. The methylase is encoded in *S. aureus* by two distinct prototypes of *erm* genes, *erm*A and *erm*C, of which the former is by far more common in MRSA strains than the latter[24]. The *erm*A and *erm*C are, respectively, often located in the chromosome (invariably as a part of transposon Tn*554*)[19] and on plasmids[11]. Tn*554* has an insertion site on *mec*-associated DNA (terminated as *att*155) in addition to the highly specific chromosomal attachment site *att*554. The classical MLS-resistance is inducible. In contrast, when Tn*554* is inserted on *mec*A-related DNA at *att*155, MLS-resistance becomes constitutive. Furthermore, Tillotson et al[24] has suggested competition between Tn*554* and *tet* (TC-resistance determinant)-containing plasmid pT181 for the insertion site on *mec*A-related DNA. The MRSA strains in groups A and B with inducible MLS-

Table 4. MICs against the MRSA strains isolated in Tokushima city in 1986 (8 strains, group C)

Antimicrobial agent	MIC (μg/ml)			
	Range	50%	90%	FDA 209P
DMPPC	25 - >100	100	>100	1.56
FMOX	12.5 - 100	50	100	0.20
KM	≧100	>100	>100	0.78
TOB	0.39 - 100	25	100	0.10
DKB	0.20 - 50	6.25	50	0.20
AMK	1.56 - 25	3.13	25	0.39
GM	0.20 - 50	0.39	50	0.10
ISP	1.56 - 25	6.25	25	1.56
ABK	0.39 - 0.78	0.39	0.78	0.20
EM	12.5 - >100	>100	>100	0.20
CAM	12.5 - >100	>100	>100	0.05
JM	0.78 - >100	>100	>100	0.39
TC	0.10 - >100	0.39	>100	0.10
MINO	0.05 - 0.78	0.10	0.78	0.05
FOM	6.25 - >100	100	>100	1.56
VCM	0.78 - 3.13	1.56	3.13	0.39
OFLX	0.39 - 1.56	0.39	1.56	0.20
DR-3355	0.20 - 0.39	0.20	0.39	0.10
TA-167	0.10 - 0.39	0.20	0.39	0.10
MKM-A	1.56 - 6.25	3.13	6.25	1.56
MKM-B	6.25 - >100	100	>100	3.13
A:B (2:1)	0.39 - 3.13	0.78	3.13	0.20
A:B (1:1)	0.39 - 1.56	0.78	1.56	0.10
A:B (1:2)	0.39 - 1.56	0.78	1.56	0.20
Bottromycin A_2	0.39 - 1.56	0.78	1.56	0.39
Bottromycin D-1	1.56 - 6.25	6.25	6.25	1.56
Bottromycin D-2	0.78 - 3.13	1.56	3.13	0.39
NH	0.025 - 0.05	0.05	0.05	0.00625

Table 5. Distribution of aminocyclitol aminoglycoside inactivating enzymes in the MRSA strains

Phenotype	Aminocyclitol aminoglycoside-modifying enzyme	Number of strains		
		group A	group B	group C
$KM^sTOB^sGM^sAMK^sABK^s$	—	1	0	0
$KM^rTOB^sGM^sAMK^sABK^s$	APH(3')	0	1	3
$KM^rTOB^rGM^sAMK^rABK^s$	AAD(4')	10	0	2
$KM^rTOB^rGM^rAMK^sABK^s$	AAC(6')/APH(2'') or AAC(6')/APH(2'')+APH(3')	0	0	1
$KM^rTOB^rGM^rAMK^rABK^s$	AAC(6')/APH(2'')+AAD(4') or AAC(6')/APH(2'')+AAD(4')+APH(3')	11	11	2
	Total ----------	22	12	8

resistance showed TC-resistance; on the other hand, the incidence of TC-resistant strains in the MRSA strains expressing MLS-resistance constitutively is very low (Table 6). Thus, the loss of MKM-B-resistance by the MRSA strains isolated at Hiroshima University Hospital seems to result from their acquisition of TC-resistance gene on *mec*A-related DNA. (Table 6, Fig. 2)

MKM-A is unaffected by MLS-resistance and synergy between two components of MKM is maintained (Tables 2, 3, 4), as in the case of streptogramins A and B[6].

The incidence of TC- or OFLX-resistance was very low before March 1990 (group A), and since April 1990 (group B) they increased simultaneously (Fig. 1). The *nor*A gene responsible for fluoroquinolone resistance in *S. aureus* TK2566 was cloned and partially characterized by Ubukata et al[28]. The *nor*A-containing 5.5-kb HindIII fragment showed homology to DNA fragment from a sensi-

Table 6. Type distribution of MLS-resistance in the MRSA strains and the incidence of simultaneous expression of TC-resistance

Phenotype	Type of resistance	Number of strains		
		group A	group B	group C
$EM^sCAM^sJM^sMKM\text{-}B^s$	none	0	0	0
$EM^rCAM^rJM^sMKM\text{-}B^s$	MLS-resistance/inducible	1(1)	9(9)	1(0)
$EM^rCAM^rJM^rMKM\text{-}B^s$	macloride specific resistance	1(1)	0	1(0)
$EM^rCAM^rJM^rMKM\text{-}B^r$	MLS-resistance/constitutive	20(3)	3(2)	6(2)
	Total ----------	22(5)	12(11)	8(2)

The figures in the parenthesis represent the numbers of TC-resistant strains. When MLS-resistance is inducible, the strains are resistant to 14-membered macrolides (e.g., EM, CAM), but sensitive to 16-membered maclorides (e.g., JM), lincosamides and streptogramin B-type antibiotics (e.g., MKM-B).

tive strain with the same size. It seemed to hybridize with the DNA fragments containing *gyr*A and *gyr*B genes from *Escherichia coli*. Taking these findings into consideration, fluoroquinolone-induced mutation on the chromosome of MRSA might result in the development of resistance to the fluoroquinolone group of compounds. The coexpression of resistance to fluoroquinolones and tetracyclines in the MRSA strains, especially in group B, implies the possibility that *nor* determinant is accidentally being carried by a *tet*-containing plasmid. Likewise, resistance to rifampicin and to fucidic acid is due to chromosomal mutations followed by selection[18]. When mutation to resistance is likely, use of appropriate drug combinations is recommended.

FOM is effective against a broad spectrum of gram-positive and -negative bacteria. In combination with other antimicrobial agents, FOM was synergistic. Due to the structural uniqueness, the absence of cross-resistance with other antibiotics in clinical use was one of the features of FOM. Further, FOM can be administered orally or parenterally and can protect against renal damage caused by VCM.

According to the results of a three-year worldwide survey, covering 28 centers in 21 countries, on antibiotic resistance in MRSA strains[18], 83% still remained sensitive to FOM. However, it was not unexpected that FOM-resistance was shown by 93% of the MRSA strains isolated at Hiroshima University Hospital and in Tokushima city, because plasmid-carried *fos*A determinant encoding intracellular FOM-modifying enzyme has been spreading rapidly both geographically and biologically (even to gram-positive bacteria). However, the identification of FOM-resistance determinant in our MRSA strains with *fos*A is not yet completed.

The risk of VCM-resistance was aforementioned. ABK was recently introduced into clinical trials and as of August 1990, the incidence of resistance was 0% (Table 1). However, MRSA would become resistant to ABK by the acquisition of aminocyclitol aminoglycoside modifying enzymes not yet found in *S. aureus*, e.g., AAC(2') which was discovered in *Providencia*[7].

All the MRSA strains isolated at Hiroshima University Hospital and in Tokushima city are susceptible to peptide antibiotics, NH and bottromycin A_2; these antibiotics have never been in clinical use. Bottromycin A_2, however, failed to protect mouse against staphylococcal infection owing to ease of metabolization. To overcome this defect of bottromycin A_2, various derivatives of bottromycin A_2 were synthesized[20]. Among them two derivatives, bottromycin A_2 *N*-α-iminoisobutyl hydrazide (D-1) and *N*-α-iminobenzo hydrazide (D-2), were tested for their MICs against the MRSA strains. The results shown in Tables 2, 3 and 4 showed that all the MRSA strains were susceptible to bottromycin A_2 and its derivatives to the same extent. The peptide group of antibiotics would be candidates for the alternative chemotherapeutics against MRSA infections which were no longer treatable with glycopeptide antibiotics such as VCM and teicoplanin, and ABK.

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REFERENCES

1. **Archer, G.L. and Johnston, J.L.** 1983. Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. Antimicrob. Agents Chemother. **24:** 70–77.
2. **Beck, W.D., Berger-Bächi, B. and Kayser, F.H.** 1986. Additional DNA in methicillin-resistant *Staphylococcus aureus* and molecular cloning of *mec*-specific DNA. J. Bacteriol. **165:** 373–378.
3. **British Society for Antimicrobial Chemotherapy.** 1988. Break-points in in-vitro antibiotic sensitivity testing. J. Antimicrob. Chemotherapy **21:** 701–710.
4. **Byrne, M.E., Gillespie, M.T. and Skurray, R.A.** 1990. Molecular analysis of a gentamicin resistance transposonlike element on plasmids isolated from North American *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **34:** 2106–2113.
5. **Chabbert, Y.A.** 1956. Antagonisme in vitro entre l'erythromycine et la spiramycine. Ann. Inst. Pasteur (Paris) **90:** 787–790.
6. **Chabbert, Y.A. and Courvalin, P.** 1971. Synergie des composants des antibiotiques du groupe de la streptogramine. Pathol. Biol. **19:** 613–619.
7. **Chevereau, M., Daniels, P.J.L., Davies, J. and Le Goffic, F.** 1974. Aminoglycoside resistance in bac-

teria mediated by gentamicin acetyltransferase II, an enzyme modifying the 2'-amino group of aminoglycoside antibiotics. Biochemistry **13:** 598–603.

8. **Engel, H.W.B., Soedirman, N., Rost, J.A., Van Leewen, W.J. and Van Embden, J.D.A.** 1980. Trasferability of macrolide, lincomycin and streptogramin resistance between group A, B and D streptococci, *Streptococcus pneumoniae* and *Staphylococcus aureus*. J. Bacteriol. **142:** 407–413.
9. **Hackbarth, C.J. and Chambers, H.F.** 1989. Methicillin-resistant staphylococci: detection methods and treatment of infections. Antimicrob. Agents Chemother. **33:** 995–999.
10. **Hartman, B.J. and Tomasz, A.** 1984. Low-affinity penicillin- binding protein associated with β-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:** 513–516.
11. **Horinouchi, S. and Weisblum, B.** 1980. Post-transcriptional modification of RNA conformation: mechanism that regulates erythromycin-induced resistance. Proc. Natl. Acad. Sci. USA **7:** 7079–7083.
12. **Jevons, M.P.** 1961. Celbemin resistant *Staphylococcus aureus*. Br. Med. J. **1:** 124–125.
13. **Konno, M., Ubukata, K., Yamashita, N., Matsushita, M., Kawakami, S., Masuda, M. and Nonoguchi, N.** 1985. Relationship between resistance pattern for antimicrobial agents and phage type of methicillin-resistant *Staphylococcus aureus*. J. Jpn. Assoc. Infect. Dis. **159:** 1029–1040. (in Japanese)
14. **Lai, C.J. and Weisblum, B.** 1971. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **68:** 856–860.
15. **LeClercq, R., Derlot, E., Duval, J. and Courvalin, P.** 1988. Plasmid mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:** 157–161.
16. **LeClercq, R., Derlot, E., Weber, M., Duval, J. and Courvalin, P.** 1989. Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. Antimicrob. Agents Chemother. **33:** 10–15.
17. **Lyon, B.R., May, J.W. and Skurray, R.A.** 1984. Tn*4001*: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. Mol. Gen. Genet. **193:** 554–556.
18. **Maple, P.A.C., Hamilton-Miller, J.M.T. and Brumfitt, W.** 1989. Worldwide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Lancet **i:** 537–540.
19. **Murphy, M.** 1985. Nucleotide sequence of *erm*A, a macrolide- lincosamide-streptogramin B determinant in *Staphylococcus aureus*. J. Bacteriol. **162:** 633–640.
20. **Nakamura, S., Omura, S., Nishimura, T., Tanaka, N. and Umezawa, H.** 1967. Derivatives of bottromycin A_2 and their biological activity. J. Antibiotics Ser. A. **20:** 162–166.
21. **Schwalbe, R.S., Stapleton, J.T. and Gilligan, P.H.** 1987. Emergence of vancomycin-resistance in coagulase negative staphylococci. N. Engl. J. Med. **316:** 927–931.
22. **Song, M.D., Wachi, M., Doi, M., Ishino, F. and Matsuhashi, M.** 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. FEBS Lett. **221:** 167–171.
23. **Thompson, R.L., Cabezudo, I. and Wenzel, R.P.** 1982. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococuus aureus*. Ann. Intern. Med. **97:** 309–317.
24. **Tillotson, L.E., Jenssen, W.D., Moon-McDermott, L. and Dubin, D.T.** 1989. Characterization of a novel insertion of the macrolides-lincosamides-streptogramin B resistance transposon Tn*554* in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **33:** 541–550.
25. **Townsend, D.E., Ashdown, N., Bolton, S., Bradley, J., Duckworth, G., Moorhouse, E.C. and Grubb, W.B.** 1987. The international spread of methicillin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **9:** 60–71.
26. **Ubukata, K., Yamashita, N., Gotoh, A. and Konno, M.** 1984. Purification and characterization of aminoglycoside-modifying enzymes from *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **25:** 754–759.
27. **Ubukata, K., Yamashita, N. and Konno, M.** 1985. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **27:** 851–857.
28. **Ubukata, K., Itoh-Yamashita, N. and Konno, M.** 1989. Cloning and expression of the *nor*A gene for fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **33:** 1535–1539.
29. **Ubukata, K., Nonoguchi, R., Matsuhashi, M., Song, M.D. and Konno, M.** 1989. Restriction maps of the regions coding for methicillin and tobramycin resistances on chromosomal DNA in methicillin-resistant staphylococci. Antimicrob. Agents Chemother. **33:** 1624–1626.