IN VITRO ACTIVITY OF ARBEKACIN AGAINST CLINICAL ISOLATES OF METHICILLIN-RESISTANT *Staphylococcus aureus* IN A HOSPITAL

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The frequency of isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) strains from patients and hospital environments has becoming higher and higher in recent years[1,2]. MRSA is now one of the most important pathogens of the nosocomial infections in Japan[3,4]. No doubt, this is ascribed to the very low susceptibility of MRSA strains to various chemotherapeutic agents[3~6]. Arbekacin (ABK) [1-*N*-(S)-4 amino-2-hydroxybutyryl-3′,4′-dideoxykanamycin B], a semisynthetic aminoglycosidic antibiotic has recently become into clinical use, and its efficacy has been reported[7~9].

This led us to re-evaluate the *in vitro* activity of the ABK against MRSA isolates together with other chemotherapeutic agents in current use for comparison, as the ABK is now considered to be one of the few drugs effective for the treatment of patients with MRSA infection.

MRSA Strains Examined

A total of 496 MRSA strains, isolated during the years 1989 to 1992 from clinical specimens submitted to our laboratory, was used in this study. They were comprised of 82 strains from pharyngeal swab, 84 from urine, 185 from pus, 90 from nose, 25 from sputum, and 30 from miscellaneous materials which included the 16 from blood specimens. Only one strain from each patient was served for the examination. These strains were identified as *S. aureus* with the Vitek GPI Cards (Vitek Systems, BioMerieux Vitek, Inc., Hazelwood, Mo., U.S.A.) and/or ID-Test SP-18 System (Nissui Pharmaceuticals Co., Ltd., Tokyo, Japan).

Susceptibility Tests

This was carried out by the method as recommended by Japanese Association of Chemotherapy (Chemotherapy, 29: 76~77, 1981). The minimal inhibitory concentration (MIC, defined here as the lowest concentration of an agent that inhibited visible growth at the cultural condition given below) was determined against the following ten chemotherapeutic agents, *i.e.*, arbekacin, gentamicin (GM), amikacin (AMK), fosfomycin (FOM), chloramphenicol, minocycline, imipenem (IPM), methicillin (DMPPC), vancomycin (VCM), and ofloxacin. In a few instances, resistances to clindamycin, ribostamycin (RSM), lividomycin (LVD), butyrocin (BUTY), neomycin (NM), tobramycin (TOB), sisomicin (SISO), astromycin (ASTM), netilmicin (NTL), micronomicin (MCR), kanamycin (KM), dideoxykanamycin B (DKB), and isepamicin (ISP) were also tested. The medium used was the cation-supplemented Mueller-Hinton agar (MMH; Eiken Chemical Co., LTD., Tokyo, Japan). For FOM, glucose-6-phosphate was added to the medium at the concentration of 25 μg/ml, and as for IPM, 2% sodium chloride was incorporated into the medium, respectively, as recommended by THORNSBERRY *et al.*[10,11]. Saline cell suspension for the inoculation was prepared from the growth on Heart Infusion (HI) agar (Eiken Chemical Co., Ltd., Tokyo, Japan) incubated at 35°C overnight. Turbidity of the suspension was adjusted to 0.5 McFarland standard. A loopful of the suspension (15 μl) was streaked on the agar plates. The results were read after incubation at 35°C for 48 hours.

Resistance of the MRSA Strains

Results of the tests are summarized in Table 1. Presented in the Table were the MICs that inhibited the growth of the 50% (MIC50) and 90% (MIC90) strains from each of the sources. No MRSA strain was inhibited its growth by DMPPC at the concentration of lower than 12.5 μg/ml and MIC90 of this drug against the strains tested were higher than 800 μg/ml. Both ABK and VCM were more active than any other drugs examined. Two strains were demonstrated to be resistant to ABK. The MICs of the resistant strains were 12.5 μg/ml and 25 μg/ml, respectively. The former was isolated from pus of otitis media and the latter came from urine of a patient with urinary tract infection. The two strains were also resistant to KM, DKB, AMK, TOB, GM, SISO, NTL, MCR, ISP, NM, RSM, LVM, BUTY, ASTM but sensitive to streptomycin. This result

Table 1. Susceptibility of 496 MRSA strains against 10 chemotherapeutic agents.

Drug	MIC range (μg/ml) Among a total of 496 strains	Modal MIC (μg/ml)	MIC (μg/ml) required to inhibit following (%) of 82 isolates from pharyngeal swab specimens		MIC (μg/ml) required to inhibit following (%) of 84 isolates from urine specimens		MIC (μg/ml) required to inhibit following (%) of 185 isolates from pus specimens	
			MIC50[a]	MIC90[a]	MIC50[a]	MIC90[a]	MIC50[a]	MIC90[a]
Arbekacin	0.01～25	0.78	0.78	1.56	0.78	1.56	0.78	1.56
Gentamicin	0.05～≥800	50	50	200	50	200	50	200
Amikacin	12.5～≥800	≥800	≥800	≥800	25	100	25	100
Fosfomycin	0.39～≥800	≥800	≥800	≥800	≥800	≥800	25	100
Chloramphenicol	1.56～100	12.5	12.5	100	25	100	12.5	100
Minocycline	0.02～50	12.5	12.5	25	12.5	25	25	50
Imipenem	0.01～≥200	25	25	50	25	50	25	50
Methicillin	12.5～≥800	≥800	≥800	≥800	≥800	≥800	≥800	≥800
Vancomycin	≤0.2～3.13	0.78	0.78	1.56	0.78	3.13	0.78	1.56
Ofloxacin	≤0.01～≥100	6.25	6.25	≥100	≥100	≥100	6.25	≥100

Drug	MIC (μg/ml) required to inhibit following (%) of 90 isolates from nose specimens		MIC (μg/ml) required to inhibit following (%) of 25 isolates from sputum specimens		MIC (μg/ml) required to inhibit following (%) of 30 isolates from miscellaneous specimens	
	MIC50[a]	MIC90[a]	MIC50[a]	MIC90[a]	MIC50[a]	MIC90[a]
Arbekacin	0.78	1.56	0.78	1.56	0.78	1.56
Gentamicin	25	100	50	200	50	200
Amikacin	25	100	25	25	12.5	25
Fosfomycin	≥800	≥800	400	≥800	≥800	≥800
Chloramphenicol	12.5	100	12.5	50	25	100
Minocycline	25	50	12.5	25	12.5	25
Imipenem	25	50	25	50	25	50
Methicillin	≥800	≥800	≥800	≥800	≥800	≥800
Vancomycin	0.78	1.56	0.78	1.56	0.78	1.56
Ofloxacin	6.25	≥100	6.25	≥100	6.25	≥100

[a] The concentrations of the drugs required to inhibit the growth of 50% (MIC50) and 90% (MIC90) of the total number of MRSA strains tested.

suggested the involvement of, at least, more than one aminoglycoside modifying enzymes with the ABK resistance. Genetic analysis of the ABK resistance for the two strains are currently in progress. None of the MRSA strains examined was resistant to VCM. Along with DMPPC, FOM exhibited the lowest degree of activity among the drugs examined except for the strains from pus materials. This was also the case for AMK, that is, the drug exhibited the lowest activity on the strains from pus when compared with the strains from the other sources. We have been unable to clarify why only the strains from pus exhibited significantly higher suscetibility to the two drugs.

Our results have clearly shown that ABK is one of the few potent chemotherapeutic agents against MRSA, that is, although two (0.4%) ABK-resistant strains have been found among 496 strains, its modal MIC of the drug was 0.78 μg/ml. As expectedly, another effective agent among the drugs tested was VCM and its modal MIC was also 0.78 μg/ml. For all of the other eight kinds of chemotherapeutic agents, their modal MICs ranged from 12.5 μg/ml to more than 800 μg/ml. ABK would be an advantageous drug because, in contrast to VCM which is active only on Gram-positive bacteria, antimicrobial spectrum of ABK is not confined to only *Staphylococcus aureus* strains but it can inhibit growth of diverse species of both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa* strains[12,13].

A criticism to our results would be that, as the strains examined had been isolated from a limited environment, that is, only in our hospital, hence the results may not represent the susceptibility of MRSA strains now widely distributed in Japan. However, the number of resistant pattern of our strains for the ten agents tested counted twelve, but none of them was predominant (data not shown). We have also classified approximately one hundred isolates among the strains tested into the eleven types by Pulsed Field Gel Electrophoresis for the genomic DNAs digested with *Sma*I and in this case, none of the type was significantly prevalent nor associated with a particular drug resistant pattern (data not shown). We since did not consider that the strains used represented only a few number of clones of the MRSA strains that were distributed in our hospital during the period we isolated them.

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