ISOLATION FREQUENCIES OF STRAINS RESISTANT TO 16 ANTIMICROBIAL AGENTS AMONG METHICILLIN-SUSCEPTIBLE AND LOW- AND HIGH-METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Tetsuro Muratani, Masato Watanabe, Masuhito Yoshida and Susumu Mitsuhashi

Episome Institute, 2220 Kogure, Fujimi-mura, Seta gun, Gunma 371-01, Japan

(Received May 10, 1991 • Accepted September 17, 1991)

The antibacterial activities of 16 antistaphylococcal agents against 351 *Staphylococcus aureus* strains isolated from 1988—1989 were determined. The MIC distribution of methicillin against these strains divided into three groups: methicillin-susceptible *S. aureus* (MICs of methicillin, ≤6.25 μg/ml), low-methicillin-resistant *S. aureus* (L-MRSA, MICs of methicillin, 12.5 to 100 μg/ml) and high-methicillin-resistant *S. aureus* (H-MRSA: MICs of methicillin, ≥200 μg/ml) The antibacterial activities of glycopeptides, tosufloxacin, arbekacin, and minocycline were superior to those of the other agents against methicillin-resistant *S. aureus* isolates. The isolation frequency of H-MRSA strains less-susceptible to β-lactams, fluoroquinolones and erythromycin were significantly higher than the corresponding values of L-MRSA strains. But the isolation frequency of strains less-susceptible to gentamicin, arbekacin and minocycline were almost the same for H-MRSA and L MRSA strains.

Key words: MRSA, *S. aureus*, multi-resistant

It has been reported that the isolation frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) strains increases from year to year, and that most MRSA isolates are multiply resistant to various antimicrobial agents[1~6] Therefore, MRSA strains have become a serious problem in the clinical setting. We investigated the methicillin resistance levels of *S. aureus* strains isolated from 1988—1989 and divided these strains into three groups according to the MIC distribution of methicillin (DMPPC), The isolation frequency of strains less-susceptible to various antistaphylococcal agents were also compared among the three groups.

Against 351 strains of *S. aureus*, isolated in various hospitals throughout Japan from 1988—1989, the MICs of 16 antimicrobial agents were determined by twofold serial agar dilution with Sensitivity Disk Agar-N (Nissui Pharmaceutical Co., Ltd., Tokyo).

The antibacterial activity of DMPPC against *S. aureus* is shown in Fig. 1. Three peaks of DMPPC resistance are clearly evident. Accordingly, we divided the strains into three groups: DMPPC-susceptible *S. aureus* (MSSA, MIC of DMPPC, ≤

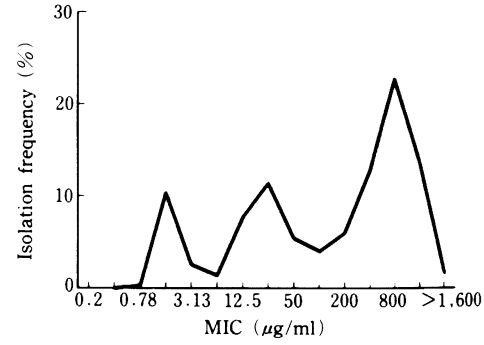

Fig. 1. Distribution of methicillin resistance among *Staphylococcus aureus* strains.

6.25 μg/ml), low-DMPPC-resistant *S. aureus* (L-MRSA: MICs of DMPPC, 12.5 to 100 μg/ml) and high-DMPPC-resistant *S. aureus* (H-MRSA: MIC of DMPPC, ≥200 μg/ml). Murakami et al.[7] divided MRSA (cefazolin-resistant *S. aureus*) isolates from 1980—1984 into two groups based on the MIC of flomoxef, and reported on PBP-2' induction of β-lactams. In our study the rate of high-resistant MRSA isolates was higher than Murakami's value (67% vs. 17%), and the MIC distribution of flomoxef was not clearly bimodal. In contrast, that of DMPPC was clearly bimodal. We therefore divided MRSA isolates into two groups based on the MIC of DMPPC.

The activity distributions of various antistaphylococcal agents against MRSA strains are shown in Fig. 2. All strains were inhibited by vancomycin and teicoplanin at 3.13 μg/ml. The antibacterial activities of tosufloxacin, sparfloxacin, arbekacin, and minocycline were superior to those of other agents and exhibited low MIC values.

These six agents are considered effective against

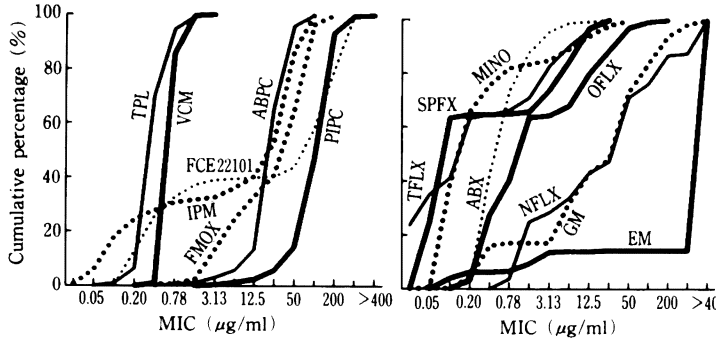

Fig. 2. Activity distributions of antistaphylococcal agents against methicillin-resistant *Staphylococcus aureus* strains.
Abbreviations: ABPC, ampicillin; PIPC, piperacillin; IPM, imipenem; FMOX, flomoxef; NFLX, norfloxacin; OFLX, ofloxacin; TFLX, tosufloxacin; SPFX, sparfloxacin; VCM, vancomycin; TPL, teicoplanin; GM, gentamicin; ABK, arbekacin; MINO, minocycline; EM, erythromycin.

Table 1. Isolation frequency of strains less-susceptible to 13 agents among MSSA, L-MRSA and H-MRSA strains

Group of *S. aureus* isolates[a] (No. of strains)	Isolation frequency (%) of strains less-susceptible to[b]												
	ABPC	PIPC	FMOX	IPM	FCE 22101	EM	NFLX	OFLX	SPFX	TFLX	GM	ABK	MINO
MSSA (51)	2	42	0	0	0	45	22	6	2	2	38	3	0
L-MRSA (100)	83	96	31	4	2	69	59	23	21	19	82	16	15
H-MRSA (200)	100**	100*	98**	94**	90**	94**	83**	43**	41**	34*	83	9	17

[a]MSSA: Methicillin-susceptible *S. aureus* strains (MICs of methicillin, ≤6.25 μg/ml). L-MRSA: group of MRSA with MICs of methicillin from 12.5 to 100 μg/ml. H-MRSA: group of MRSA with MICs of methicillin of ≥200 μg/ml.

[b]MICs of isolates less-susceptible to β-lactams and erythromycin and fluoroquinolones, aminoglycosides, and minocycline were defined as ≥12.5 and ≥3.13 μg/ml, respectively.

Abbreviations, see legend to Fig. 2.

The statistical significance of the difference between L-MRSA and H-MRSA was analyzed by Chisquare test.

* $P<0.01$

** $P<0.001$

MRSA infections. But the isolation frequency of strains resistant to tosufloxacin, sparfloxacin and minocycline increased compared to the corresponding values of isolates from 1984--1985 (data not shown). The isolation of strains resistant to vancomycin and teicoplanin has also been reported[2]. Hence the tendency of resistance must be closely watched from now on.

The isolation frequencies of strains less-susceptible to all the agents tested (except glycopeptides, to which no less-susceptible strains were found), were compared among MSSA, L-MRSA and H-MRSA isolates, and Chi-square analysis was performed (Table 1). The isolation frequency of L-MRSA strains less-susceptible to all 13 agents was significantly higher than the corresponding values for MSSA strains. And the isolation frequency of H-MRSA strains less-susceptible to β-lactams, erythromycin and fluoroquinolones was significantly higher than the corresponding values for L-MRSA strains. But the isolation frequency of strains less-susceptible to gentamicin, arbekacin and minocycline was almost the same for H-MRSA and L-MRSA strains.

It should be noted that although the isolation frequency of strains less-susceptible to aminoglycosides and minocycline was not significantly different for L-MRSA and H-MRSA isolates, the isolation frequency of H-MRSA strains less-susceptible to not only β-lactams but also to fluoroquinolones and erythromycin was significantly higher than the corresponding values for L-MRSA strains.

It has been reported that the production of PBP 2' is involved in the resistance mechanism of MRSA against β-lactams[8,9]. Also that though in some cases the amount of PBP 2' production is associated with the DMPPC resistance level, in other cases it is not[10] It has also been suggested that autolytic enzymes might be involved in the resistance mechanism of MRSA[11,12]. On the other hand, it has been reported that the resistance mechanisms to fluoroquinolones in *S. aureus* are insensitive alteration of DNA gyrase and reduction in uptake[13~15], and that the resistance mechanisms to erythromycin are insensitive alteration of ribosomes and reduction in uptake[16]. Thus though β-lactams, fluoroquinolones, and erythromycin appear to differ in their modes of action and resistance mechanisms, some resistance mechanisms might be common to those compounds, e.g. alteration of autolytic enzymes. Problems in the clinical ward, e.g. kind and amount of drug used, methods of isolating strains, etc., might be mainly associated with this. The reasons are not clear and remain to be investigated in a future study.

ACKNOWLEDGMENTS

We thank Nakanishi N, Higashitani F, Maejima T and Dr. Inoue M for valuable advice and Hamana Y, Sasaki K, Nakane T, Inoue K, Yoshida S, Wakebe H and Wakabayashi E for technical support.

Literature Cited

1) Deguchi K, Yokota N, Koguchi M, Nakane Y, Fukushima Y, Fukayama S, Ishihara R, Oda S, Tanaka S, Sato K: Yearly changes in resistance to new quinolones and multiple drug-regimens in clinical isolates. Chemotherapy 38: 1012 ~ 1019, 1990
2) Goto H, Goto M, Oka S, Shimada K, Shimizu K, Goto S, Ueno K, Hara K: The prevalence of multiply resistant *Staphylococcus aureus* in Japan during 1986 and 1988. Chemotherapy 37: 1334 ~ 1341, 1989
3) Lyon B R, Skurray R: Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiological Reviews 51: 88 ~ 134, 1987
4) Mitsuhashi N, Mizuno M: Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) strains and treatment of in-patients infected with MRSA. Chemotherapy 38: 927 ~ 932, 1990
5) Daum T E, Schaberg D R, Terpenning M S, Sottile W S, Kauffman C A: Increasing resistance of *Staphylococcus aureus* to ciprofloxacin. Antimicrob Agents Chemother 34: 1862 ~ 1863, 1990
6) Kojima K, Inoue M, Mitsuhashi S: In vitro activity of AT-4140 against quinolone- and methicillin-resistant *Staphylococcus aureus*. Antimicrob Agent Chemother 34: 1123 ~ 1127, 1990
7) Murakami K, Nomura K, Doi M, Yoshida T: Production of low-affinity penicillin-binding protein by low- and high-resistance groups of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agent Chemother 31: 1307 ~ 1311, 1987
8) Ubukata K, Yamashita N, Konno M: Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant staphylococci.

Antimicrob Agent Chemother 27: 851～857, 1985
9) Utsui Y, Yokota T: Role of an altered penicillin-binding protein in methicillin- and cephem-resistant *Staphylococcus aureus*. Antimicrob Agent Chemother 28: 397～403, 1985
10) Inoue M, Okubo T, Okamoto R, Hashimoto H: 64th Japanese Society for Bacteriology: Abstract No. D-II-13, 1991
11) Bruns W, Keppeler H, Baucks R: Suppression of intrinsic resistance to penicillins in *Staphylococcus aureus* by polidocanol, a dodecyl polyethyleneoxid ether. Antimicrob Agent Chemother 27: 632～639, 1985
12) Qoronfleh M W, Wilkinson B J: Effects of growth of methicillin-resistant and -susceptible *Staphylococcus aureus* in the presence of β-lactams on peptidoglycan structure and susceptibility to lytic enzymes. Antimicrob Agent Chemother 29: 250～257, 1986
13) Yoshida S, Kojima T, Inoue M, Mitsuhashi S: Uptake of sparfloxacin and norfloxacin by clinical isolates of *Staphylococcus aureus*. Antimicrob Agent Chemother 35: 368～370, 1991
14) Okuda J, Okamoto S, Takahata M, Nishino T: 39th Japanese Society for Chemotherapy, Abstract No. 186, 1991
15) Yoshida S, Wakebe H, Nakanishi N, Kojima T, Inoue M, Mitsuhashi S: 39th Japanese Society for Chemotherapy, Abstract No. 187, 1991
16) Novick P R, Murphy E: MLS-resistance determinants in *Staphylococcus aureus* and their molecular evolution. J Antimicrob Chemother 16: suppl. A, 101～110, 1985

メチシリン感受性，中等度耐性および高度耐性黄色ブドウ球菌に対する16抗菌剤の耐性頻度の比較

村谷　哲郎・渡邊　正人・吉田　益史・三橋　進
エピゾーム研究所*

臨床より分離された黄色ブドウ球菌351株に対する16抗菌剤の感受性を検討した。それらの株をmethicillinの感受性分布により，感受性株（MSSA），中等度耐性株（L-MRSA）および高度耐性株（H-MRSA）の3群に分類したところ，β-lactams, fluoroquinolonesおよびerythromycinのH-MRSAに対する耐性株の分離頻度は，L-MRSA群のそれより有意に高いことが示されたが，その他の薬剤では有意な差は認められなかった。

* 群馬県勢多郡富士見村小暮2220