Short Communication

In Vitro Activity of Novel Oxazolidinone Analogs and 13 Conventional Antimicrobial Agents against Clinical Isolates of *Staphylococcus aureus* in Beijing, China

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SUMMARY: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a public health concern since it was first identified in the United Kingdom in 1961. In this study, we investigated the prevalence of MRSA in the PLA 309 Hospital located in Beijing, China, and we determined the susceptibility of *S. aureus* to novel oxazolidinone analogs and 13 conventional antimicrobial agents. It was shown that drug resistant patterns were severe among the clinical isolates, and the average rate of MRSA among the *S. aureus* strains was 56.3%. All the *S. aureus* strains tested were completely susceptible to linezolid, but the minimum inhibitory concentration distribution drifted upwards. Among the novel oxazolidinone analogs, **sy142** exhibited more potent antimicrobial activity than linezolid against both the standard and clinical *S. aureus* strains. This observation provides an impetus for the development of potential novel agents based on **sy142** in the near future to combat MRSA and linezolid-resistant *S. aureus*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged rapidly since it was first identified in the United Kingdom in 1961 and has currently become a challenging problem worldwide (1). Infections due to MRSA, which usually increases the use of medical resources and the treatment cost, cause considerable morbidity and mortality. According to previous reports, in China, about 35%–80% of *S. aureus* strains isolated from patients are MRSA (2,3). In general, patients who suffer from MRSA infections are treated with glycopeptides such as vancomycin and teicoplanin and the oxazolidinone of linezolid, however, vancomycin-resistant *S. aureus* (VRSA) has started to emerge, and the duration of treatment with linezolid is limited because of potentially serious neurologic, ophthalmologic, and hematologic toxicities. Therefore, we need an alternative, novel effective antibiotic to cure MRSA. In our previous work, we identified a series of novel 4-substituted piperazinyl phenyl oxazolidinone analogs; however, data on these analogs against MRSA are limited. In the present study, we aimed to summarize the in vitro efficacy of these compounds against MRSA strains isolated from the PLA 309 Hospital located in Beijing, China, which is the treatment center for tuberculosis and respiratory tract infections in the Chinese army.

The bacterial strains used in this study were obtained from the Chinese PLA 309 Hospital from January 2010 to December 2012. A total of 623 *S. aureus* strains isolated from various clinical specimens of patients were

included in this study. All the isolates were independent isolates, and duplicate isolates from the same patient were excluded. The isolates were identified using the MicroScan Walk Away 96 System, and antimicrobial susceptibilities were determined by the broth liquid microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (4). Minimum inhibitory concentration (MIC) was defined as the lowest antimicrobial concentration that completely inhibited bacterial growth. Linezolid was obtained from PROMEGA (Part Number: PZ0014). The novel oxazolidinone analogs were synthesized as described previously (5) at the School of Pharmaceutical Engineering, Shenyang Pharmaceutical University. The structures of the novel oxazolidinone analogs are shown in Fig. 1. The conventional antimicrobial agents used in this study were penicillin, gentamycin, rifampicin, ciprofloxacin, levofloxacin, moxifloxacin, trimethoprim-sulfamethoxazole, clindamycin, erythromycin, linezolid, vancomycin, quinupristin-dalfopristin, and tetracycline. Methicillin-resistant strains were identified using a 1 µg oxacillin disc on Mueller-Hinton agar according to CLSI guidelines (6). *S. aureus* ATCC 29213 was used as the control.

The antimicrobial susceptibilities of *S. aureus* determined according to CLSI guidelines (4) are summarized in Table 1. Of 623 isolates studied, 351 (56.3%) were confirmed to be MRSA strains. Penicillin had the lowest activity, followed by erythromycin, levofloxacin, tetracycline, clindamycin, and ciprofloxacin, all of which showed resistance rates that were higher than 60%. Trimethoprim-sulfamethoxazole and quinupristin-dalfopristin exhibited high activity. It must be noted that the activity of erythromycin, rifampicin, and gentamycin significantly decreased in the last 3 years. Although no strains were identified to be resistant to linezolid

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(for staphylococci, MIC of $\geq 8 \mu\text{g/ml}$ was defined as nonsusceptibility), a significant upwards drift of MIC was observed for this antibiotic in the present study. When compared with previous reports, a larger number of isolates could not be completely inhibited in this study until the linezolid concentration reached $4 \mu\text{g/ml}$ (2).

The novel oxazolidinone compounds were found to exhibit excellent activity against the standard *S. aureus*

strain ATCC 29213, with MIC values ranging from 0.5 to $8 \mu\text{g/ml}$ (Table 2). In particular, **sy142** demonstrated better antimicrobial activity than linezolid (**sy142** MIC = $0.5 \mu\text{g/ml}$ vs linezolid MIC = $1 \mu\text{g/ml}$), and it inhibited all the clinical isolates, with MIC values higher than $1 \mu\text{g/ml}$, including the isolates with linezolid MIC that drifted upwards.

Oxazolidinones kill bacteria by blocking the formation of the initiation complex on the ribosome to inhibit bacterial protein synthesis at a site. Linezolid was the first oxazolidinone antibacterial agent approved for clinical use. As this antibiotic acts on a distinctive target, namely 23S ribosomal RNA, many reports have focused on its effect on drug-resistant strains, including MRSA (7,8). Although uncommon, investigators have reported the existence of linezolid-resistant *S. aureus* (LRSA) in some countries. For example, LRSA was detected in the USA soon after linezolid was available in the market in 2001 (9,10). To the best of our knowledge, linezolid has been used in many hospitals in China to treat ICU patients infected with MRSA, because this drug was approved in 2007 by the Chinese government. This might be one of the reasons for the upwards drift of linezolid MICs and for the possibility that LRSA might emerge in China in the near future.

In the recent years, many efforts have been taken to develop second-generation oxazolidinones. The currently developed oxazolidinones such as tedizolid (TR-701), LCB01-0062, DA-7218, and DA-7157 have been reported to be more potent and/or exhibit lower toxicity than linezolid (11–13). On comparing the difference in the

Table 1. Antibiotic susceptibility profile of MRSA and MSSA isolates

Year	2010	2011	2012
No. of total isolates	206	231	186
No. (%) of MRSA isolates	104 (50.5)	139 (60.2)	108 (58.1)
No. (%) resistant rate to			
Penicillin	197 (95.6)	223 (96.5)	180 (96.8)
Gentamycin	123 (59.7)	146 (63.2)	125 (67.2)
Rifampicin	76 (36.9)	124 (53.7)	114 (61.3)
Ciprofloxacin	135 (65.5)	145 (62.8)	128 (68.8)
Levofloxacin	135 (65.5)	150 (65.1)	121 (65.0)
Moxifloxacin	125 (60.7)	141 (61.0)	116 (62.3)
Trimethoprim-sulfamethoxazole	26 (14.1)	38 (16.5)	25 (13.4)
Clindamycin	133 (64.6)	142 (61.5)	130 (69.9)
Erythromycin	141 (68.4)	170 (73.6)	153 (82.2)
Linezolid	0	0	0
Vancomycin	0	0	0
Quinupristin-dalfopristin	2 (0.9)	3 (1.3)	3 (1.6)
Tetracycline	130 (63.1)	147 (63.6)	125 (67.2)

Table 2. Antimycobacterial activities of new oxazolidinone analogs against MRSA and MSSA

	MIC ($\mu\text{g/ml}$) for ATCC 29213	No. of isolates inhibited at MIC ($\mu\text{g/ml}$)							In total strains (No.)
		≤ 0.25	0.5	1	2	4	8	> 8	
Linezolid	1	2	16	142	174	17	0	0	MRSA (351)
		2	23	103	143	1	0	0	MSSA (272)
Sy40	4	0	0	0	12	177	157	5	MRSA (351)
		0	0	0	41	130	98	3	MSSA (272)
Sy41	8	0	0	0	0	7	178	166	MRSA (351)
		0	0	0	0	13	127	132	MSSA (272)
Sy142	0.5	2	221	128	0	0	0	0	MRSA (351)
		2	207	63	0	0	0	0	MSSA (272)
Sy143	2	0	0	0	102	164	80	5	MRSA (351)
		0	0	3	84	172	13	0	MSSA (272)
Sy144	1	0	2	221	128	0	0	0	MRSA (351)
		0	2	207	63	0	0	0	MSSA (272)

Fig. 1. Structures of novel oxazolidinone analog.

structure and activity between **sy142** and linezolid, it could be concluded that the *N*-heteroaryl group at the 4-position of piperazine is favorable for enhancing the activity of **sy142**. This observation provides an impetus for designing a novel series of drugs based on **sy142** in the near future. Furthermore, considering that **sy142** activity was not influenced by the upwards drift of linezolid MIC, novel drugs based on **sy142** might possibly be employed to combat MRSA and LRSA.

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Conflict of interest None to declare.

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