

## Abstract

**Background:** Infections caused by MRSA occur often in CF patients and there is a lack of new oral agents to treat them. Sodium fusidate has been successfully used in Europe to decolonize the lungs of CF patients (1, 2). This study 1) used MIC to test activity of CEM-102, vancomycin, teicoplanin, daptomycin, tigecycline, azithromycin, clarithromycin, linezolid, quinupristin/dalfopristin and trimethoprim/sulfamethoxazole against a range of recent CF MRSA strains and 2) tested CEM-102 in combination with tobramycin or amikacin by time-kill against two CF MRSA strains. **Methods:** Forty strains of MRSA were isolated from patients at Hershey Med. Ctr. CF clinic during the past 12 months. The variable number of tandem repeats (VNTR, formerly MLVA) method was done on all strains to ensure only one strain per patient. CLSI microdilution was done using commercially, or in-house, prepared trays (TREK, Inc., Cleveland, OH). Vancomycin MICs were read after 24 h. Subinhibitory concentrations of CEM-102 were combined with subinhibitory concentrations of each aminoglycoside to look for synergy or antagonism by time-kill. **Results:** MICs ( $\mu\text{g/mL}$ ) are listed in the Table.

Drug	Range	MIC50	MIC90
CEM-102	0.12-0.5	0.12	0.25
Vancomycin	0.5-1	0.5	1
Teicoplanin	0.25-1	0.5	1
Daptomycin	0.5-1	0.5	1
Tigecycline	0.12-0.25	0.12	0.25
Azithromycin	1- $\geq 32$	$\geq 32$	$\geq 32$
Clarithromycin	0.25- $\geq 32$	$\geq 32$	$\geq 32$
Linezolid	1-4	2	2
Quinupristin/dalfopristin	0.25-1	0.5	1
Trimethoprim/sulfamethoxazole	$\leq 0.5/9.5$	$\leq 0.5/9.5$	$\leq 0.5/9.5$

**Conclusion:** CEM-102, an oral antistaphylococcal agent used in other countries for many years, was very potent against all MRSA strains from CF patients (MIC range: 0.12-0.5  $\mu\text{g/mL}$ ). Vancomycin, teicoplanin, daptomycin, quinupristin/dalfopristin, tigecycline, linezolid and trimethoprim/sulfamethoxazole were very active. All potent drugs tested here except for linezolid and trimethoprim/sulfamethoxazole are only available intravenously. Resistance was found to azithromycin and clarithromycin with MIC<sub>50</sub> and MIC<sub>90</sub> values of  $\geq 32 \mu\text{g/mL}$ . Time-kill studies showed synergy between CEM-102 and tobramycin with 1/2 strains at 24 h.

## Introduction

Cystic fibrosis is a congenital genetic abnormality commonly encountered in the U.S. As a result of this disease patients suffer from recurrent bouts of pneumonia often caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The recurrent nature of these infectious attacks leads to multi-drug-resistance and sometimes pan-resistance, with combination therapy the only therapeutic alternative (1, 3). There is a lack of new experimental agents active against resistant Gram-negative and Gram-positive strains in general, and CF strains in particular (4). Recently, a survival analysis on 19,833 patients with CF in a multicenter study showed that colonization with MRSA is associated with shortened survival, with a risk of death 1.27 times higher than controls (5). Currently trimethoprim/sulfamethoxazole is used to treat these patients but increasing resistance has been noted and treatment alternatives are needed (4).

Fusidic acid is an antibiotic used in the treatment of staphylococcal infections (3, 6). Although it was introduced more than 4 decades ago, fusidic acid remains useful as an antibiotic, because it is not cross-resistant with other antibiotics used to treat staphylococci and has relatively low frequencies of resistance (7).

This study tested activity of CEM-102 [sodium fusidate, a new formulation of fusidic acid (8)] against MRSA strains isolated from cystic fibrosis patients at Hershey Medical Center, alone and in combination with amikacin and tobramycin.

## Materials and Methods

**Strains.** Forty MRSA strains (only one strain with golden yellow colonies) isolated within the past 12 months from patients at our cystic fibrosis clinic, were tested. All strains were identified by standard methods (9). Only one strain per patient was tested. The variable number of tandem repeats (VNTR, formerly MLVA) typing was done for all strains, to examine clonality and ensure that single clones were tested (10).

**Susceptibility testing.** Original MICs of each strain to CEM-102 and other comparators was tested by CLSI microdilution methodology (11). Trays were obtained from Trek, Inc., Cleveland, OH or prepared in-house. Time-kill macrobroth MIC dilution by CLSI (11) was performed for all synergy testing.

**Synergy testing.** Two of the MRSA strains were chosen and tested for synergy time-kill testing. Broth microdilution formed the basis of MICs used in time-kill experiments, as detailed below. The kill kinetics of each drug were tested alone by incubating an initial inoculum of  $5 \times 10^6$  to  $5 \times 10^8$  cfu/mL with drug concentrations at the MIC, three dilutions above and three dilutions below the MIC (1/2, 1/4 and 1/8  $\times$  MIC). Viability counts were performed after 0, 3, 6, 12 and 24 h incubations at 37°C in a shaking water bath by plating onto Trypticase soy-5% sheep blood agar plates (12).

After initial time-kills with drugs alone had been done, CEM-102 was tested in combination with amikacin and tobramycin. Combinations were tested 1-2 dilutions below the MIC (1/2  $\times$  MIC and 1/4  $\times$  MIC) of each drug. Inocula and time-kill methodology were performed as described above. Concentrations in synergy time-kill tests were selected such that one of the two drugs yields a growth curve similar to that of the drug-free control, while the other drug was more active (6).

MICs were achieved by standard methodology (11). Synergy was defined as a  $\geq 2 \log_{10}$  decrease in cfu/mL between the combination and its most active constituent after 3, 6, 12 and 24 h, with the number of surviving organisms in the presence of the combination  $\geq 2 \log_{10}$  cfu/mL below the starting inoculum. At least one of the drugs in the combination was present in a concentration which did not significantly affect the growth curve of the organism when used alone. Antagonism was defined as a  $\geq 2 \log_{10}$  increase in cfu/mL between the combination and its most active constituent after 3, 6, 12 and 24 h, with the number of surviving organisms in the presence of the combination  $\geq 2 \log_{10}$  cfu/mL above the starting inoculum (12).

## Results

Each individual strain tested proved to be an individual clone. *S. aureus* MICs ( $\mu\text{g/mL}$ ) are listed in Table 1. CEM-102 was very potent with MICs between 0.125 and 0.5 against all strains tested. Vancomycin and teicoplanin were also active at MICs 0.25-1  $\mu\text{g/mL}$ , linezolid at MICs 1-4  $\mu\text{g/mL}$ , quinupristin/dalfopristin at MICs 0.25-1  $\mu\text{g/mL}$  and trimethoprim/sulfamethoxazole at MICs  $\leq 0.5/9.5 \mu\text{g/mL}$ . Most strains (38 of 40) were resistant (MIC  $\geq 32 \mu\text{g/mL}$ ) to azithromycin and clarithromycin.

Time-kill macrobroth MICs ( $\mu\text{g/mL}$ ) can be found in Table 2. Synergy time-kill data (Table 3) showed that synergy between CEM-102 and tobramycin was noted at one (0.125/1  $\mu\text{g/mL}$ ) concentration at 24 h for one MRSA strain. All other time points and combinations were indifferent for all MRSA strains tested. One strain of MRSA was not tested with tobramycin in combination because of its very high MIC against tobramycin ( $>512 \mu\text{g/mL}$ ). No correlation between the presence of pigment and any susceptibility result was found.

**Table 1.** Microdilution MICs ( $\mu\text{g/mL}$ ) of all compounds against 40 MRSA strains from cystic fibrosis patients.

Drug	Range	MIC50	MIC90
CEM-102	0.12-0.5	0.12	0.25
Vancomycin	0.5-1	0.5	1
Teicoplanin	0.25-1	0.5	1
Daptomycin	0.5-1	0.5	1
Tigecycline	0.12-0.25	0.12	0.25
Azithromycin	1- $\geq 32$	$\geq 32$	$\geq 32$
Clarithromycin	0.25- $\geq 32$	$\geq 32$	$\geq 32$
Linezolid	1-4	2	2
Quinupristin/dalfopristin	0.25-1	0.5	1
Trimethoprim/sulfamethoxazole	$\leq 0.5/9.5$	$\leq 0.5/9.5$	$\leq 0.5/9.5$

**Table 2.** Time-kill macrobroth MICs ( $\mu\text{g/mL}$ ) of all compounds against 2 MRSA strains from cystic fibrosis patients.

Strain	MIC ( $\mu\text{g/mL}$ ) <sup>a</sup>		
	CEM-102	Tobramycin	Amikacin
SA 2230	0.5	4.0	32.0
SA 2232	0.25	NT <sup>b</sup>	64.0

<sup>a</sup> determined by broth microdilution, read after 24h incubation.

<sup>b</sup> NT; not tested

**Table 3.** Synergy time-kill results of *in vitro* antimicrobial combinations with CEM-102.

Combination and time points <sup>a</sup>	CEM-102/Tobramycin				CEM-102/Amikacin			
	3h	6h	12h	24h	3h	6h	12h	24h
SA2230	IND	IND	IND	SYN <sup>b</sup> (0.125/1 $\mu\text{g/mL}$ )	IND	IND	IND	IND
SA2232	NT <sup>c</sup>	NT	NT	NT	IND	IND	IND	IND

<sup>a</sup> time-point (hours)

<sup>b</sup> IND, indifference; SYN, synergy

<sup>c</sup> NT; not tested (MIC  $>512 \mu\text{g/mL}$ )

## Conclusions

- CEM-102 (sodium fusidate) was very potent against all MRSA strains tested.
- For MRSA, clinically achievable synergy was observed in strain SA 2230, with CEM-102 combined with tobramycin.

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