

Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant *Staphylococcus aureus*

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Compared with ampicillin, oxacillin, cefmetazole and imipenem, the combination of ampicillin and sulbactam at a constant ratio of 2:1 showed the greatest effect against 28 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), but MICs of ampicillin/sulbactam were still above the resistance breakpoint. When ampicillin/sulbactam was further combined with epigallocatechin gallate (EGCg, a main constituent of tea catechins), the MIC₉₀ of ampicillin/sulbactam was reduced to 4 mg/L, the susceptibility breakpoint. The fractional inhibitory concentration indices were between 0.19 and 0.56 in combination with 6.25 and 25 mg/L EGCg, respectively, indicating that ampicillin/sulbactam and EGCg combination may be effective against MRSA infections.

Introduction

Ampicillin has good bactericidal activity; however, it can be degraded by β -lactamases produced by various bacteria, including some of the methicillin-susceptible *Staphylococcus aureus* strains (MSSA) and most of the methicillin-resistant strains (MRSA). Sulbactam is a β -lactamase inhibitor and acts primarily by irreversible inactivation of β -lactamases. The combination of ampicillin/sulbactam inhibits β -lactamase activity and increases the spectrum of activity. According to previous reports and our *in vitro* experiments, the combination is effective, but is not powerful enough against MRSA, including strains producing β -lactamase.^{1,2}

Epigallocatechin gallate (EGCg; a main constituent of tea catechins) is synergic with β -lactams against MRSA.^{3–5} We hypothesized that ampicillin/sulbactam could be effective against MRSA if combined with EGCg.

Materials and methods

Antibiotics and EGCg

Nitrocefin was purchased from Oxoid (Basingstoke, UK). Oxacillin, methicillin, ampicillin, imipenem, cefmetazole and ampicillin/sulbactam were purchased from Sigma (St Louis, MO, USA). EGCg was extracted from green tea

with a purity of 98% as confirmed by high-performance liquid chromatography analysis.

Bacterial strains and media

Twenty-eight clinical isolates of MRSA were obtained from Fujigaoka and Hatanodai Hospitals of Showa University. MSSA ATCC 25923 and β -lactamase-producing MSSA 226 were used as controls. Cation-adjusted Mueller–Hinton broth and agar (Becton Dickinson, Cockeysville, MD, USA) were used for susceptibility tests. Heart infusion broth (HIB; Difco Laboratories, Detroit, MI, USA) was used for β -lactamase tests.

β -Lactamase tests and *mecA* gene detection

For β -lactamase tests, an inoculum of 10⁴ bacteria/well was incubated at 35°C overnight in 100 μ L HIB in the absence/ or presence of 0.5 mg/L methicillin, which was used to induce the β -lactamase production. Fifty microlitres of nitrocefin 500 mg/L was then added to the cultures. β -Lactamase broke down the ring of nitrocefin resulting in a colour change from yellow to red. The colours were graded from no change (–) to dark red (3+) at 5, 30, 60 and 120 min, respectively. All the strains were also identified by PCR analysis for *mecA* gene expression as reported previously.⁶

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Susceptibility tests

MICs were determined using the broth dilution and checkerboard methods at a final inoculum $>5 \times 10^5$ cfu/mL as reported previously.⁶ Ampicillin/sulbactam combination was tested at a constant ratio of 2:1 as used in the clinically available formulation. MICs of ampicillin/sulbactam were presented as the concentrations of ampicillin. Synergy between ampicillin/sulbactam and EGCg was evaluated by a fractional inhibitory concentration (FIC) index. FIC was calculated as the MIC of ampicillin/sulbactam EGCg in combination divided by the MIC of ampicillin/sulbactam or EGCg alone, and the FIC index was obtained by adding the FICs. If an FIC index was ≤ 0.5 , the combination was defined as synergic. Synergy was also determined by time-kill assay and defined as a reduction of $\geq 2 \log_{10}$ of the initial inoculum (10^6 cfu/mL) at 24 h by the combination of ampicillin/sulbactam and EGCg at sub-MICs. The time-kill assays were performed in triplicate and the data presented are mean \pm S.D.

Results

All 28 isolates were resistant to the tested β -lactams, except for F-98, which was susceptible to imipenem. Sixty-eight per cent (19/28) of the strains produced β -lactamase. Compared with ampicillin, oxacillin, cefmetazole and imipenem, ampicillin/sulbactam showed the strongest anti-MRSA effect, especially against the β -lactamase-producing strains of MRSA and MSSA 226. The MIC range of ampicillin against β -lactamase-producing MRSA changed from 64–256 to 16–32 mg/L in the presence of sulbactam, but no change was observed against β -lactamase-non-producing MRSA. Nevertheless, MICs of ampicillin/sulbactam were still above its resistant breakpoint (≥ 16 mg/L). EGCg also showed anti-MRSA effect with an MIC at 100 mg/L. When ampicillin/sulbactam at sub-MICs was further combined with EGCg at sub-MICs, potent synergy and dose-dependent reversion of the resistance against ampicillin/sulbactam were observed (Table). The MIC₅₀ of ampicillin/sulbactam decreased to 8 mg/L in the presence of 6.25 mg/L EGCg, and the MIC₉₀ of ampicillin/sulbactam decreased to 4 mg/L in the presence of 25 mg/L EGCg. The synergic effect between ampicillin/sulbactam and EGCg was very similar for the β -lactamase-producing and β -lactamase-non-producing MRSA isolates.

Time-kill curves show the synergic bactericidal effects of ampicillin/sulbactam and EGCg against two typical strains of the β -lactamase-producing and β -lactamase-non-producing isolates (Figure). The combinations resulted in a reduction of $\geq 2 \log_{10}$ of the initial inoculum at 24 h.

Discussion

Although MRSA is resistant to almost all β -lactams, ampicillin in combination with β -lactamase inhibitors may

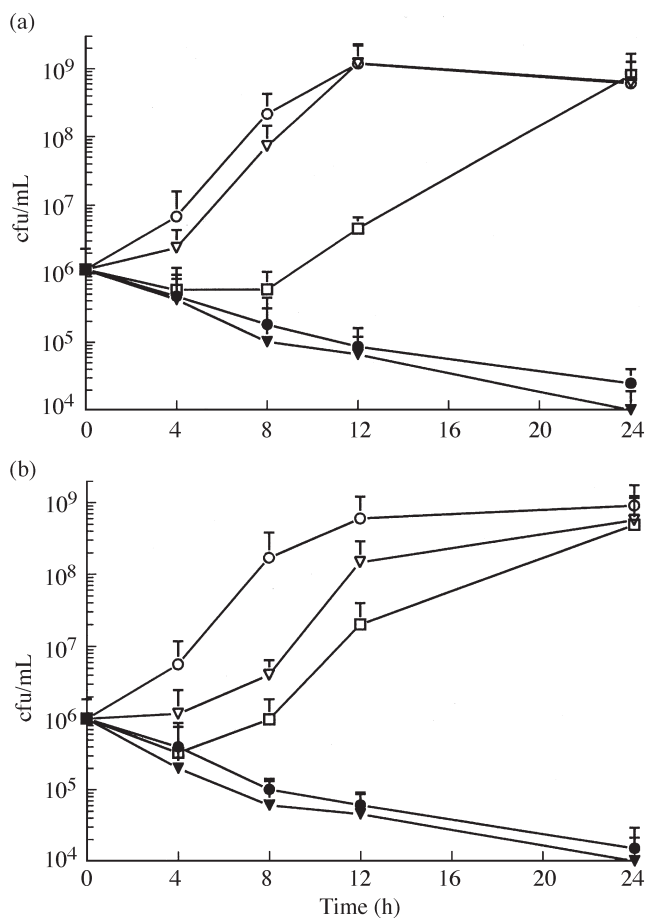


Figure. Time-kill curves of the synergic activity of the combination of ampicillin/sulbactam and EGCg. (a) F-10, β -lactamase-producing MRSA. (b) F-56, β -lactamase-non-producing MRSA. \circ , no addition; ∇ , ampicillin/sulbactam alone at $0.5 \times \text{MIC}$ (8 mg/L for F-10 and 16 mg/L for F-56); \square , EGCg alone at 25 mg/L ($0.25 \times \text{MIC}$); \bullet , EGCg at 6.25 mg/L plus ampicillin/sulbactam at $0.5 \times \text{MIC}$; \blacktriangledown , EGCg at 25 mg/L plus ampicillin/sulbactam at $0.25 \times \text{MIC}$.

be effective, because most MRSA produce β -lactamase, and ampicillin has a relative affinity for PBP2' 10–15 times greater than that of penicillinase-resistant β -lactams.⁷ Recently, an ampicillin and sulbactam combination (UNASYN) has been approved by US Food and Drug Administration for intravenous administration in the treatment of various infections.

About 10–15% of the dry weight of green tea is made up of catechins, including catechin, epicatechin, epigallocatechin, epicatechin gallate and EGCg. EGCg, the main constituent (60%) of tea catechins, has the best antibacterial activity. Interestingly, catechins demonstrate synergy with β -lactams for MRSA.^{3–5} We recently confirmed that EGCg is synergic with β -lactams owing to interference with the integrity and biosynthesis of the bacterial cell wall through direct binding to peptidoglycan.⁶

Synergy between ampicillin/sulbactam and EGCg against MRSA

Table. MICs of β -lactams and FIC indices of ampicillin/sulbactam in combination with EGCg against 28 isolates of MRSA

Strain	β -Lactamase ^a	<i>mecA</i> ^b	MIC (mg/L) ^c							FIC index	
			MPIPC	CMZ	IPM	AMP	AMP/SUL ^d			B'	C'
							A	B	C		
F-8	+	+	256	64	64	64	16	4	1	0.31	0.31
F-10	+++	+	256	32	32	256	16	4	2	0.31	0.38
F-49	+	+	512	128	64	64	32	8	4	0.31	0.38
F-68	+	+	512	128	128	64	32	8	4	0.31	0.38
F-74	+	+	512	128	128	64	32	8	4	0.31	0.38
F-84	+	+	512	128	64	64	32	8	4	0.31	0.38
F-87	+++	+	256	128	128	256	16	4	2	0.31	0.38
F-94	+	+	512	128	128	64	32	8	4	0.31	0.38
F-96	+	+	512	128	128	64	32	8	2	0.31	0.31
F-97	+	+	512	128	128	64	32	8	4	0.31	0.38
F-98	+++	+	32	32	<1	256	16	2	0.5	0.19	0.28
H-5	+	+	256	64	64	64	16	4	2	0.31	0.38
H-7	+	+	256	128	128	64	16	4	2	0.31	0.38
H-9	+	+	256	64	64	64	32	8	2	0.31	0.31
H-13	++	+	256	64	128	64	32	8	2	0.31	0.31
H-15	+	+	256	64	64	64	32	8	4	0.31	0.38
H-20	+++	+	512	128	64	256	16	4	2	0.31	0.38
H-29	+	+	256	64	128	64	16	4	2	0.31	0.38
H-49	+	+	512	128	128	64	32	8	4	0.31	0.38
F-34	-	+	1024	128	256	64	64	16	4	0.31	0.31
F-36	-	+	256	64	32	16	16	4	2	0.31	0.38
F-38	-	+	1024	256	128	32	32	16	8	0.56	0.5
F-41	-	+	128	64	64	16	16	2	2	0.19	0.38
F-47	-	+	1024	256	256	64	64	16	4	0.31	0.31
F-56	-	+	1024	256	256	32	32	16	4	0.56	0.38
F-61	-	+	1024	128	256	64	32	16	4	0.56	0.38
F-69	-	+	1024	256	128	32	32	16	8	0.56	0.5
F-100	-	+	512	128	128	32	32	8	2	0.31	0.31
#226	+++	-	2	2	0.125	256	4				
25923	-	-	2	2	0.125	1	1				

^a+++ , colour changed in 5 min; ++ , colour changed in 30 min; + , colour changed in 60 min; - , no colour change in 120 min.

^b+, *mecA* gene positive; -, *mecA* gene negative.

^cMPIPC, oxacillin; CMZ, cefmetazole; IPM, imipenem; AMP, ampicillin; SUL, sulbactam.

^dA, ampicillin/sulbactam alone; B and B', ampicillin/sulbactam in combination with EGCg 6.25 mg/L; C and C', ampicillin/sulbactam in combination with EGCg 25 mg/L.

The potent synergy between ampicillin/sulbactam and EGCg suggests a possible clinical use for MRSA infection. Tea, one of the most popular beverages, is consumed every day by billions of people. Capsules of tea catechins and EGCg are also becoming available for research and pre-clinical trial. Usually, EGCg concentration in tea beverage is 2–3 g/L. EGCg is absorbed through digestive tract and distributed to many organs of animals and humans.^{8–10} In rat blood plasma, EGCg at 5.6 mg/L was detected after oral

administration of EGCg at 500 mg/kg body weight,⁸ and total catechins at 15–112 mg/L were detected at 2 h after oral dosing with catechins at 5 g/kg body weight.⁹ In human blood plasma, EGCg at 2 mg/L was detected 3 h after taking 525 mg EGCg capsules.¹⁰ Therefore, further experiments are needed to confirm the possibility of using ampicillin/sulbactam and EGCg combination against MRSA infections.

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