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ORIGINAL ARTICLE

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Inhibitory action of clarithromycin on glycocalyx produced by MRSA

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Abstract
We determined whether clarithromycin (CAM) had the ability to eliminate glycocalyx and biofilm produced by methicillin-resistant *Staphylococcus* aureus (MRSA) using an in-vitro experimental system (consisting of a bladder model and a kidney model) simulating complicated urinary tract infection (UTI). We also examined whether a combination of CAM and vancomycin (VCM) was effective for eliminating the MRSA biofilm. VCM (urinary concentration simulating drip infusion of 500 mg twice a day for 5 days; minimum inhibitory concentration (MIC) 0.5 μg/ml) eliminated MRSA from the bladder model medium at 48 h, but reproliferation occurred immediately after withdrawal of the agent. No disappearance of MRSA biofilm was noted, and this appeared to be the cause of the bacterial regrowth. CAM (urinary concentration simulating oral administration of 200 mg twice a day for 5 days; MIC, 128 μg/ml) allowed microbial recovery to the initial level within 48 h, but led to the disappearance of the glycocalyx-forming biofilm. A combination of VCM and CAM caused microbial elimination from the bladder model medium at 46 h with no regrowth after withdrawal of the antimicrobial agents. Scanning electron microscopy confirmed that the MRSA biofilm disappeared completely and no microbial adhesion was noted. These results suggest that CAM has an inhibitory action on glycocalyx and biofilm of MRSA, and that the combined use of VCM and CAM may be efficacious for the treatment of MRSA UTI.

Key words MRSA · Biofilm · Clarithromycin · Vancomycin

Introduction

Complicated urinary tract infection (UTI) is often refractory to antibacterial therapy. One of the reasons for this is that the infection involves biofilm formation on the surface of the bladder mucosa or a foreign body.[1–3] Researchers have studied biofilm infections caused by *Pseudomonas aeruginosa*, which is frequently isolated from complicated UTI.[1–6] In biofilm infections caused by *P. aeruginosa*, clarithromycin (CAM), a macrolide antimicrobial agent, has been reported not to have any antiproliferative effect, but to eliminate the glycocalyx component of the biofilm, which results in reduced biofilm formation, suggesting the possibility of CAM employment for the treatment of respiratory infections.[7] We have already confirmed this action of the drug against *P. aeruginosa* in an in-vitro experimental model simulating complicated cystitis.[4–6] However, biofilm infections caused by Gram-positive cocci such as methicillin-resistant *Staphylococcus aureus* (MRSA) have not been sufficiently investigated.

Hospital-acquired MRSA infection, including urinary MRSA infection, has recently become an issue of clinical importance.[8–10] Most urinary infections are associated with complicated UTIs related to an indwelling catheter in the urinary tract, and thus may involve biofilm formation on the catheter surface by MRSA. In this study, we determined whether CAM has an inhibitory action on glycocalyx production by MRSA as well as by *P. aeruginosa* using an in-vitro experimental system simulating complicated UTI. We also investigated whether a combination of CAM and vancomycin (VCM; which has an antimicrobial effect on MRSA), has a more favorable therapeutic effect on MRSA UTI than CAM alone.

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Materials and methods

Bacteria and antimicrobial agents

MRSA isolated from urine (coagulase type II) was used in the experiment. The minimum inhibitory concentrations (MIC) of VCM and CAM against MRSA were 0.5 μg/ml and 128 μg/ml, respectively.

Table 1. Pharmacokinetic parameters

Drug and dose	Parameter				
	Ka (h^{-1})	Kel (h^{-1})	Vd (I)	Clt (ml/min)	CIR (ml/min)
One-compartment model CAM (200 mg)	2.29	0.174	136.0	393.0	151.0

Drug and dose	Parameter					
	α (h^{-1})	β (h^{-1})	K_{10} (h^{-1})	K_{21} (h^{-1})	Clt (ml/min)	CIR (ml//min)
Two-Compartment model VCM (500 mg)	2.1	0.167	0.476	0.731	101.0	91.3

Ka, absorption rate constant; Kel, elimination rate constant; Vd, volume of distribution; Clt, total clearance; CIR, renal clearance; α, $t_{1/2}(\alpha)$; β, $t_{1/2}(\beta)$; K_{10}, rate constant of compartment 1; K_{21}, rate constant of compartment 2.
CAM, clarithromycin; VCM, vancomycin.

Concentrations of antimicrobial agents used in the experiment

As previously reported,[4–6] the in-vitro urinary concentrations of the antimicrobial agents were calculated from pharmacokinetic parameters based on measured urinary concentrations in healthy adult volunteers.[11–14] Pharmacokinetic parameters for CAM were calculated using a one-compartment model, while those for VCM were calculated with a two-compartment model (Table 1). The parameters for VCM were obtained after drip infusion of a dose of 500 mg given twice daily in healthy adults, and those for CAM were obtained by the oral administration of 200 mg twice daily in healthy adults. By inputting the values into a computer-regulated system, changes in serial in-vitro urinary concentrations of VCM and CAM were obtained. Changes in the in-vitro urinary concentrations of both VCM and CAM were measured when the drug combination was used.

In-vitro experimental model of complicated urinary tract infection

Our experimental system has been reported previously in detail.[4–6] The urinary concentration of each antimicrobial agent in the kidney model was changed serially under computer control to simulate the profile in the clinical situation. A liquid culture medium (Antibiotic medium 3, Difco Laboratories, Detroit, MI, USA) containing antimicrobial agents flowed from the kidney model into the bladder model at 0.5 ml/min. The liquid medium was automatically evacuated from the bladder model every 2 h, seven times a day , and 10 ml was left in a diverticulum of the model as residual urine after each evacuation. After 14 h of this sequence, evacuation was withheld for 10 h, and then evacuation was resumed every 2 h. Bacteria were exposed to CAM, VCM, or a combination of the two drugs for 5 days in the in-vitro experimental system, and the exposure was terminated by replacing the medium with one that did not contain a drug or drugs.

Biofilm formation and bacterial count in the bladder model

As previously reported,[4–6] MRSA was cultured for 48 h in a diverticulum containing small glass balls (diameter, 4 mm) in the bladder model to form MRSA biofilm on the glass balls. The initial bacterial count in the bladder model was then adjusted at 10^7 CFU/ml. For determining bacterial count, the medium in the bladder model was sampled every h.

Scanning electron microscopy (SEM) for MRSA biofilm

Prior to the administration of the antimicrobial agent, and as a control (without antimicrobial agents on day 5 and after treatment for 5 consecutive days) a few glass balls were removed from the diverticulum, and fixed with 2.5% glutaraldehyde by a method reported previously.[4–6] After dehydration in ethanol and critical point drying , the glass balls were observed by SEM.

Results

Changes in MRSA count in the bladder model caused by treatment with antimicrobial agents

VCM alone eliminated MRSA from the bladder model within 48 h, and there was no bacterial proliferation during VCM application. However, on day 5 (120 h), when the medium was replaced with a new liquid culture medium containing no antimicrobial agent, bacteria immediately began to reproliferate (Fig. 1). CAM alone had no antimicrobial effect on MRSA. The bacterial count was transiently reduced to 10^5 CFU/ml, but then rapidly increased to more than 10^7 CFU/ml within 48 h (Fig. 2). The combined application of the two agents eliminated bacteria from the bladder model after 46 h, as with VCM application. No bacterial reproliferation was observed after withdrawal of

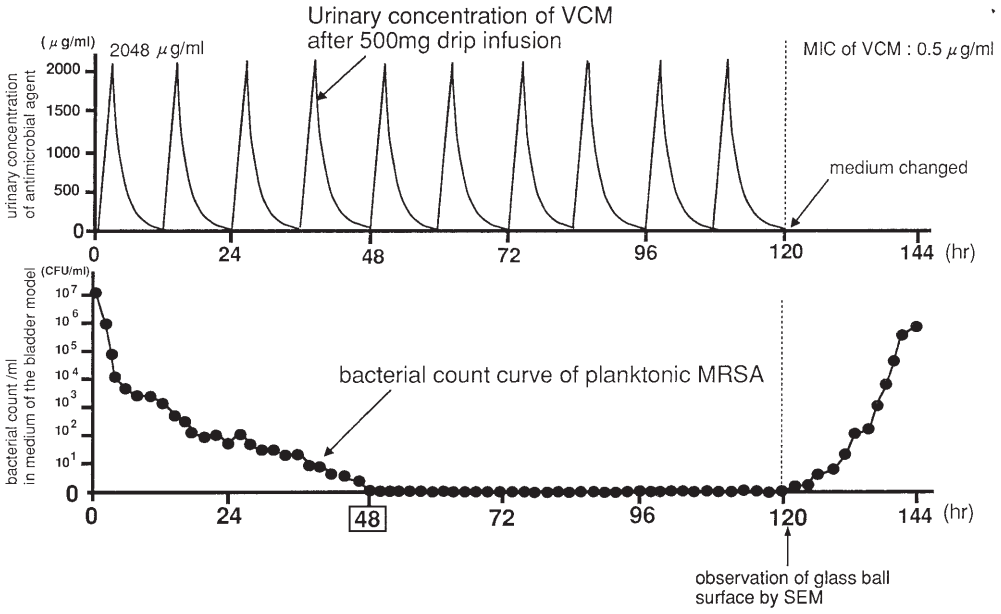

Fig. 1. Bactericidal effects of Vancomycin (*VCM*) in medium of the bladder model simulating complicated urinary tract infection. *MRSA*, Methicillin-resistant *Staphylococcus aureus*; *SEM* scanning electron microscope; MIC, minimum inhibitory concentration

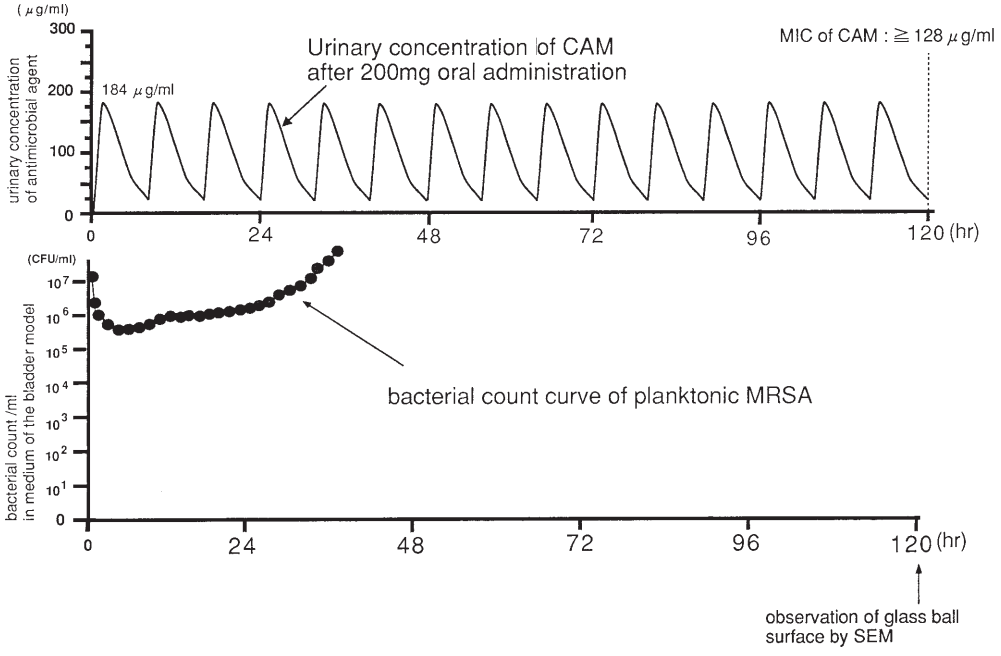

Fig. 2. Bactericidal effects of clarithromycin (*CAM*) in medium of the bladder model

both antimicrobial agents by replacing the medium with one not containing the drugs (Fig. 3).

Morphological changes in MRSA biofilm caused by treatment with antimicrobial agents

Without antimicrobial agents, SEM revealed that MRSA were almost all embedded in a glycocalyx (Fig. 4). With VCM alone, MRSA were attached to each other by the glycocalyx, and a thick biofilm covered the glass balls around the bacteria (Fig. 5). This SEM finding was similar to that in the experiment without antimicrobial agents. In contrast, CAM alone almost totally eliminated the glycocalyx forming the biofilm on the glass balls. However, CAM had no antimicrobial effect on MRSA, as demonstrated by the finding that the bacteria did not disappear and remained attached to the balls as numerous single cells (Fig. 6). The combined application of VCM and CAM led to the almost complete elimination of both the glycocalyx and the bacteria, thus not allowing biofilm formation and bacterial growth (Fig. 7).

Discussion

In recent years, the increase in nosocomial MRSA infections has presented serious problems in the clinical setting, although the frequency of MRSA isolation varies depend-

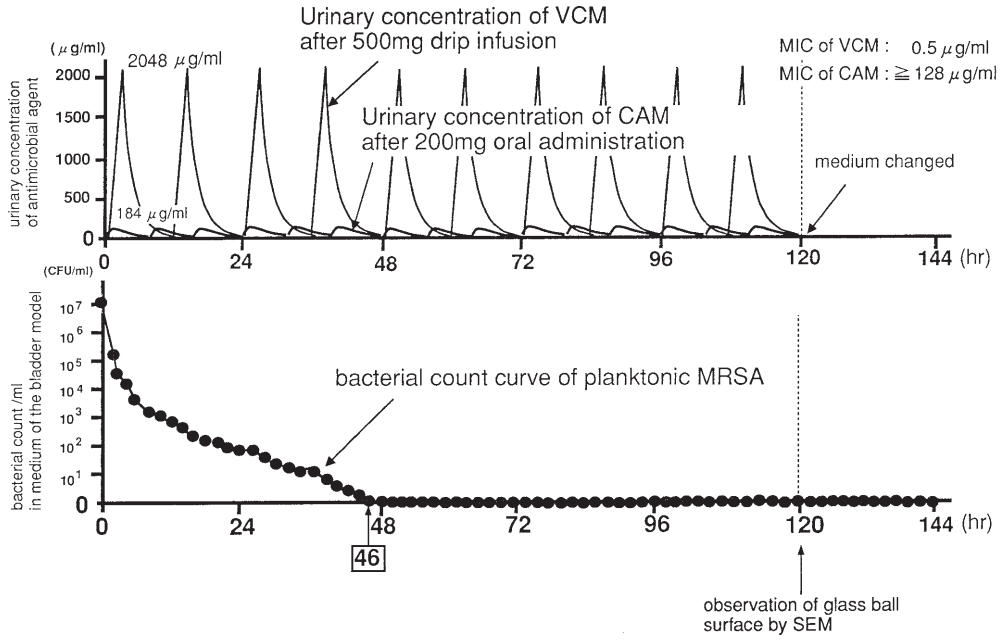

Fig. 3. Bactericidal effects of a combination of VCM and CAM in medium of the bladder model

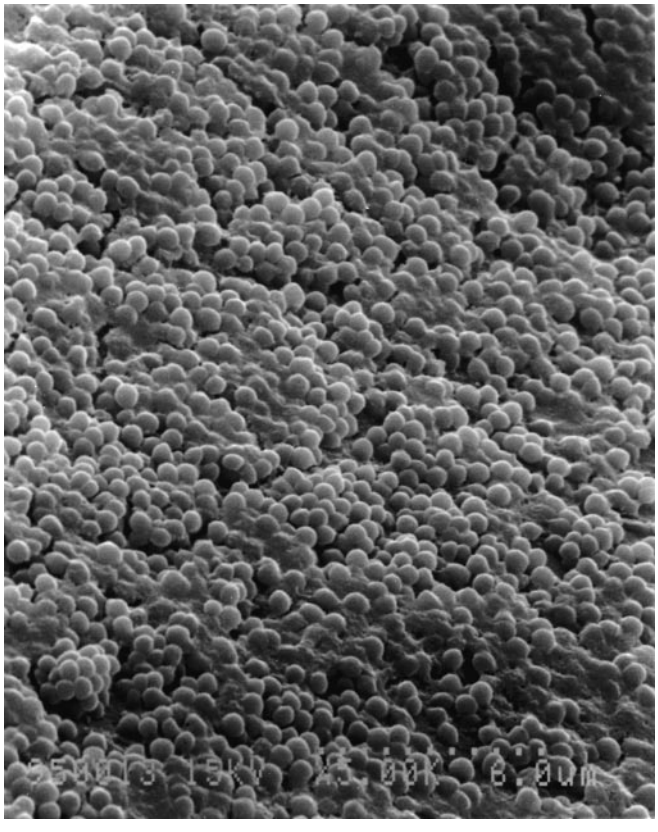

Fig. 4. SEM findings of glass beads in the diverticulum of the bladder model without antimicrobial agents. ×5000

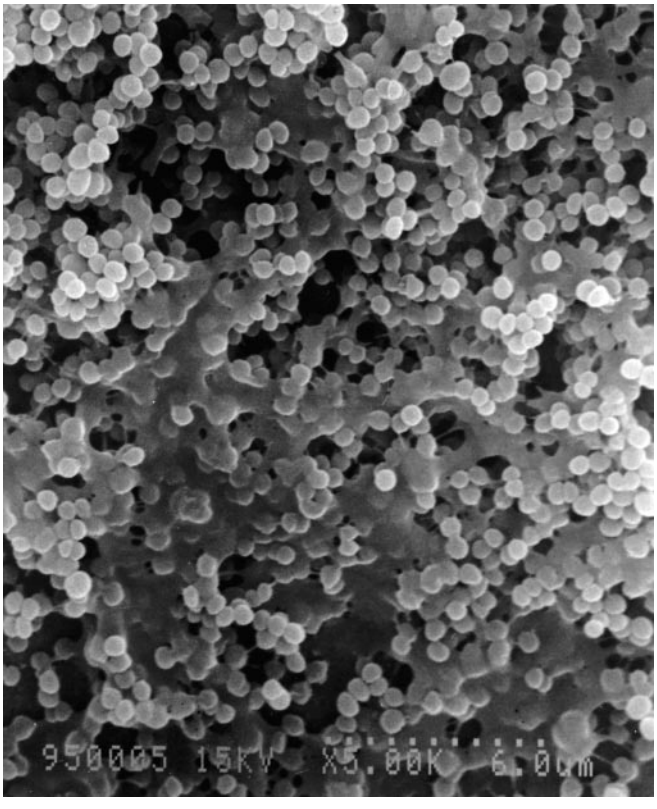

Fig. 5. SEM findings of glass beads in the diverticulum of the bladder model after VCM treatment. ×5000

ing on the institution. In urology practice, MRSA is a cause of UTI, and urinary MRSA is attributed to wound infections following open surgery on the urinary tract.[8–10] The frequency of isolation of *S. aureus* from UTIs had always been low, with reports of an incidence of about 5.0%. However, from the latter half of the 1980s, the isolation rate of *S. aureus* has increased, and MRSA has shown an almost parallel increase.[8–10] This trend indicates that the increase in the

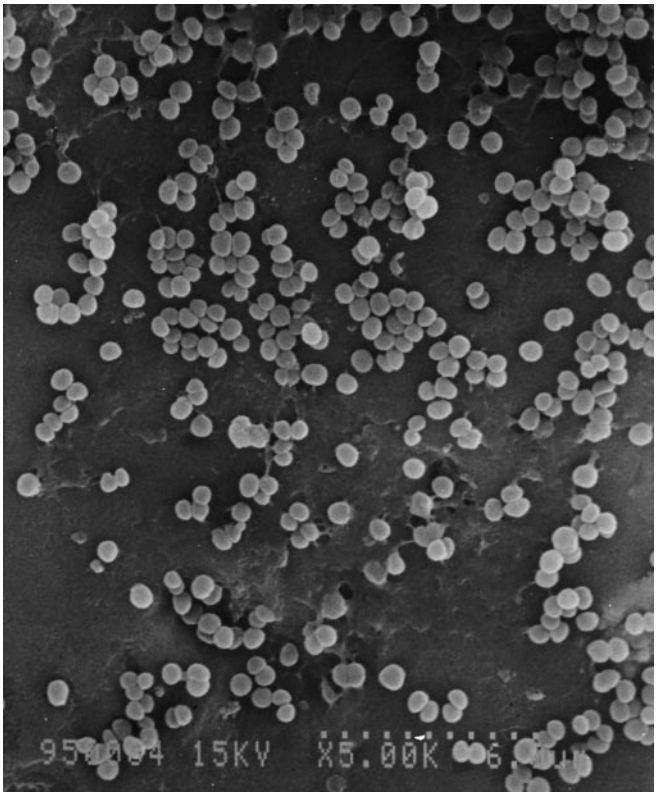

Fig. 6. SEM findings of glass beads in the diverticulum of the bladder model after CAM treatment. ×5000

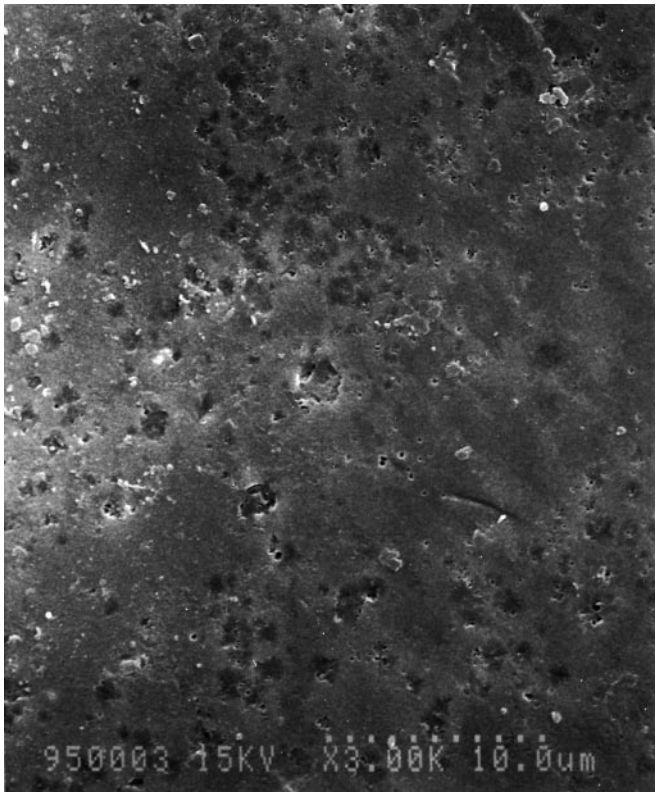

Fig. 7. SEM findings of glass beads in the diverticulum of the bladder model after combination VCM and CAM treatment. ×3000

isolation rate of *S. aureus* has been caused by an increase in the frequency of MRSA isolation.

Many patients with MRSA in urine have had polymicrobial UTIs, and they also often have an indwelling urinary catheter. Therefore, indwelling medical devices are thought to be one of the main causes of MRSA infections.[8–10] Such infections are also known as biofilm infections. After the bacteria adhere to the surface of a foreign object such as a catheter, a glycocalyx, consisting mainly of polysaccharides, is produced outside the bacterial cells, resulting in biofilm formation. The formation of this biofilm is considered to provide resistance, allowing bacteria to escape the host defense against infection and also to escape the action of antimicrobial agents.[1–3,7]

Biofilm infections include those associated with cardiac pacemakers,[15] endocarditis,[16] osteomyelitis,[17] and cystic fibrosis.[18] It has also been reported that a biofilm is present not only on the surface of urinary stones and indwelling catheters in UTI patients, but also on the surface of the mucosa of the urinary tract after endoscopic surgery.[1] Recent studies of respiratory infections have suggested that macrolide antimicrobial agents such as erythromycin and CAM have an anti-biofilm action.[7] In this context, we have reported that CAM, at concentrations clinically achievable in the urine, suppressed the glycocalyx production of *P. aeruginosa*, thus eliminating biofilm formation.[4–6]

The present study of MRSA biofilm showed that VCM alone eliminated planktonic bacteria, but had no effect on bacteria in the biofilm, with bacteria from the biofilm showing early reproliferation when treatment with this antimicrobial agent was terminated. CAM alone caused some decrease in the planktonic bacterial count, probably because the drug killed a small population of cells with an MIC below the peak urinary concentration of CAM (184 μg/ml). Thus , CAM reduced the planktonic bacterial count, but immediate reproliferation occurred when there were no longer any bacteria sensitive to this agent. However, our SEM study clearly showed that CAM suppressed glycocalyx production and, thus, biofilm formation, thereby leaving the bacteria exposed as single cells on the glass balls. This situation may be more favorable for VCM to eradicate MRSA. Indeed, the combined use of VCM and CAM eliminated MRSA even after the drugs were with drawn. In infections in areas other than the urinary tract, it has been suggested that CAM has an anti-biofilm action against methicillin-sensitive *S. aureus* (MSSA) biofilm infections,[19] and that the drug can eliminate not only the glycocalyx formed by *P. aeruginosa* but also that formed by *Staphylococcus epidermidis*.[20]

It has been suggested that CAM inhibits the production of alginic acid, the main glycocalyx component of the *P. aeruginosa*[7] biofilm, and CAM may also suppress the synthesis of monosaccharides such as hexose (a component of

the *S. epidermidis* glycocalyx).[20] However, it is not yet sufficiently clear whether glycocalyx components differ among bacterial species and strains, and, to our knowledge, there have been no reports on the components of the MRSA glycocalyx. Further studies are thus necessary for the better understanding of biofilm infections.

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