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CHALLENGING CLINICAL CASE IN ANTIMICROBIAL RESISTANCE

Imipenem plus Fosfomycin as Salvage Therapy for Vertebral Osteomyelitis

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This Journal section presents a real, challenging case involving a multidrug-resistant organism. The case authors present the rationale for their therapeutic strategy and discuss the impact of mechanisms of resistance on clinical outcome. An expert clinician then provides a commentary on the case.

ABSTRACT We applied combination antibiotic therapy to treat vertebral osteomyelitis and a psoas abscess caused by glycopeptide-intermediate (MIC, 2 μg/ml) and daptomycin-nonsusceptible (>2 μg/ml) methicillin-resistant *Staphylococcus aureus*. The Etest synergy test showed the largest synergistic effects for imipenem/cilastatin and fosfomycin. Whole-gene sequencing showed amino acid changes in SA0802, SA1193 (*mprF*), and SA1531 (*ald*). Four weeks of combination treatment using imipenem/cilastatin (1.5 g per day) and fosfomycin (4.0 g per day) resulted in clinical improvement.

KEYWORDS antibiotic combination, fosfomycin, imipenem, Etest synergy test, whole genome analysis

Vancomycin (VAN) and daptomycin (DAP) have been widely used as preferred treatments for methicillin-resistant *Staphylococcus aureus* (MRSA) infections (1). However, the effectiveness of these treatments is often not ideal, and the mortality of MRSA-mediated bacteremia ranges from approximately 20% to 60% (2, 3). Additionally, emerging resistance against these key MRSA drugs has already been reported (1, 4–6). Therefore, we must develop additional drug treatment options for cases associated with MRSA strains that present reduced antibiotic treatment efficacy.

CASE PRESENTATION

An 84-year-old male, with chronic kidney disease, type II diabetes mellitus, and chronic heart failure associated with mitral stenosis presented with fever, backache, and walking difficulties that lasted for 4 days. Magnetic resonance imaging (MRI) on admission showed L2-3 vertebral osteomyelitis and a bilateral psoas abscess, and the blood culture was positive for MRSA. Transthoracic echocardiography indicated no findings of vegetation; however, transesophageal echocardiography was not performed. Two weeks of VAN treatment for MRSA osteomyelitis was started, followed by 2 weeks of DAP treatment. The MIC of VAN for the MRSA isolates derived from the blood culture at this time was determined to be 1 μg/ml, whereas the MIC of DAP was not measured. The patient was transferred to Tokyo Medical University Hospital (TMUH, Tokyo, Japan) because blood cultures remained MRSA positive for 1 month and the psoas abscess deteriorated. Upon his arrival at TMUH, computed tomography (CT)-assisted drainage of the psoas abscess was performed because both blood cultures and the psoas abscess remained MRSA positive. The MICs for the isolates from the psoas abscess (TUM19798) and the blood culture (TUM19799) were determined and are shown in Table 1.

We switched to high-dose VAN treatment, combined with CT-assisted drainage, with a trough concentration of 15.0 to 20.0 μg/ml due to DAP resistance in the blood culture

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TABLE 1 MICs for TUM19798 and TUM 19799

Antibiotic agent	MIC (μg/ml) for[a]: TUM19798	TUM19799
CFZ	>16	≤8
GEN	≤2	≤2
ABK	≤1	2
ERY	>4	>4
CLI	≤2	≤2
LVX	>4	>4
SXT	≤19/1	≤19/1
MIN	≤2	≤2
IPM/CS	>8	≤1
FOF	≤4	≤4
VAN	1	2
DAP	0.25	>2
LZD	2	2

[a]Determined by the microdilution method. CFZ, cefazolin; GEN, gentamicin; ABK, arbekacin; ERY, erythromycin; CLI, clindamycin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; MIN, minocycline; IPM/CS, imipenem/cilastatin; FOF, fosfomycin; VAN, vancomycin; DAP, daptomycin; LZD, linezolid.

isolate. However, C-reactive protein (CRP) levels fluctuated, with high positive values ranging from 6.5 to 10.5 mg/dl, despite becoming negative in blood cultures on day 3 after transfer to TMUH. The treatment timeline can be summarized as 2 weeks of VAN and 2 weeks of DAP, administered at the previous hospital, followed by 4 weeks of high-dose VAN combined with abscess drainage, performed at TMUH. Despite 8 total weeks of antibiotic treatment and pus drainage, enhanced CT revealed no improvements in the remaining abscess or vertebral osteomyelitis. The CRP value was 8.4 mg/dl. Additionally, pus from the second CT-assisted drainage remained MRSA positive. Although we considered performing a more invasive surgical intervention, the patient was deemed to have a low tolerance for such intervention. We considered other antibiotic treatment regimens and the discontinuation of VAN treatment.

CHALLENGE QUESTION

What is the next option without progressing to a more invasive surgical intervention?

A. High-dose DAP
B. Linezolid
C. Fosfomycin
D. Imipenem
E. Antibiotic combination of fosfomycin and imipenem

TREATMENT AND OUTCOME

We selected a combination treatment of imipenem/cilastatin (IPM/CS, 1.5 g per day) and fosfomycin (FOF, 4.0 g per day), based on the results of the Etest synergy test, withdrawing VAN treatment. Despite existing reports regarding the efficacy of various antibiotic combinations and because the methods for selecting ideal antibiotic combination regimens can be time-consuming, determining significant treatments can be difficult in the clinical setting. The effectiveness of each potential combination regimen depends on the response of specific MRSA isolates, which makes determining optimal antibiotic regimens difficult. The Etest synergy test has been reported to represent a practical method for assessing the efficacy of antibiotic combination regimens, without requiring the performance of time-consuming techniques (7, 8). The Etest synergy test is a prediffusion technique, in which the first or second Etest strip is removed after 1 h and replaced directly with the second or third strip (7, 8). Each Etest strip was placed so that the positions of the MICs of the drugs, which were measured *a priori*, overlapped.

Etest synergy tests were carried out using FOF, IPM/CS, DAP, VAN, and linezolid (LZD), in the following combinations: IPM/CS+FOF, IPM/CS+VAN, FOF+VAN, IPM/

TABLE 2 MICs of the last antibiotic agent and distance between a reference point, equidistant from each MIC, and the last inhibitory line

Antibiotic agent[a]	MIC[b] (μg/ml)	Distance between MIC lines in combination therapy vs. single therapy (mm)
	Single agent	
FOF	3	
VAN	3	
DAP	3	
LZD	2	
	2nd agent	
IPM/CS+FOF	0.38	8
IPM/CS+VAN	1.5	3
FOF+VAN	2	2
IPM+DAP	1.5	3
FOF+DAP	1.5	3
IPM+LZD	0.75	4
FOF+LZD	1	3
	3rd agent	
IPM+FOF+VAN	0.75	6
IPM+FOF+DAP	0.75	6
IPM+FOF+LZD	0.38	7

[a]IPM/CS, imipenem/cilastatin; FOF, fosfomycin; VAN, vancomycin; DAP, daptomycin; LZD, linezolid.
[b]Determined by Etest.

CS+DAP, FOF+DAP, IPM/CS+LZD, FOF+LZD, IPM/CS+FOF+VAN, IPM/CS+FOF+DAP, and IPM/CS+FOF+LZD. The combination effects were measured based on the distance (in millimeters) from the MIC line to the reference point. If a combination has no effect, the reference point and the MIC of each antibiotic agent will be the same, and the distance will be 0 mm. The distance of each combination regimen was evaluated to determine the effect size of each combination.

The MICs of the drugs indicated that TUM19799 was more likely to be resistant than TUM19798; therefore, an Etest synergy test was performed for TUM19799. The results of the Etest synergy test (Table 2) suggested that triple antibiotic combination regimens would be effective; however, the risks of side effects increase as the number of antibiotic agents increase. The MICs of the last antibiotic agents (in micrograms per milliliter) and the largest distances from the reference point (in millimeters) were observed for the following combinations: IPM+FOF (MIC of last antibiotic agent/distance = 0.38/8), IPM+FOF+LZD (0.38/7), IPM+FOF+VAN (0.75/6), and IPM+FOF+DAP (0.75/6); these results indicated that these combinations were more effective than any single drug and all other combination regimens.

Two weeks after the initial administration of IPM/CS and FOF, the abscess improved dramatically, and CRP values fell within the normal range, with the lowest value reported at 0.18 mg/dl. CT showed improvement of the psoas abscess, which almost disappeared. However, despite 4 weeks of successful combination therapy, the patient died of end-stage heart failure.

To elucidate the mechanisms underlying the differences in antimicrobial susceptibility between isolates, TUM19798 and TUM19799 were subjected to whole-genome sequencing using MiSeq (Illumina, Inc., CA, USA) and MinION (Oxford Nanopore Technologies, Oxford, UK), as previously described (9). Furthermore, the MiSeq reads from TUM19799 were mapped to the complete genome sequence of TUM19798, and mutations in each isolate were identified using CLC Genomics Workbench software (Qiagen). Pathogen protocols were approved by the Toho University Safety Committee for Pathogens (approval number 20-53-102).

The sequence type of each strain was ST1/SCC*mec* type IVa, and the *spa* type was t1784. The virulence genes detected were *lukD*, *lukE*, *sea*, *seh*, *sek*, *seq*, and *cna*. We detected several acquired resistance genes, including *blaZ*, *mecA*, *ant*(*9*)-*Ia*, and *erm*(*A*), and we detected single nucleotide polymorphisms (SNPs) in *grlA* (S80F and E84G) and

TABLE 3 Mutations according to comparisons between whole-genome sequence mapping results and N315 (NC_002745)

ORF ID in NC_002745	Gene name	Gene product	Nucleotide change (region in NC_002745)	Amino acid change (compared with NC_002745)	Amino acid in:			
					N315 (NC_002745)	MW2 (NC_003923)	TUM19798	TUM19799
SA0802	SA0802	Hypothetical protein	G907002A	Glu344Lys	Glu	Glu	Glu	Lys
SA1193	*mprF*	MprF	T1364633C	Leu341Ser	Leu	Leu	Leu	Ser
SA1531	*ald*	Alanine dehydrogenase	T1750854C	Asp47Gly	Asp	Asp	Asp	Gly

gyrA (S84L). The acquired resistance genes and the SNPs resulted in resistance to β-lactams, aminoglycosides, macrolides, and quinolones. Compared to a reference *S. aureus* strain, MW2, two SNPs were identified in genes associated with VAN resistance, which resulted in the nonsynonymous substitutions Ile8Thr, located in *prsA*, and Ala8Thr, located in *sigB*. No SNPs were identified in genes associated with LZD or FOF resistance. The comparison between TUM19798 and TUM19799 showed highly similar overall genomic structures, with only eight distinguishing SNPs identified in the whole-genome sequences. Three detected SNPs were associated with the nonsynonymous substitutions Glu344Lys, Leu341Ser, and Asp47Gly, located in SA0802, *mprF*, and *ald*, respectively (Table 3). SNPs in *mprF* have previously been shown to affect the resistance of MRSA to DAP (4–6). Mutations in *mprF* can confer reduced susceptibility not only to exogenously administered DAP but also to endogenous cationic antimicrobial peptides; therefore, these mutations can potentially be selected without DAP selection pressure (10).

The sequencing data for TUM19798 and TUM19798 were deposited in the DNA Data Bank of Japan (DDBJ), under BioProject accession number PRJDB10142.

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We declare that we have no competing interests.

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