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Combination Therapy against Polymicrobial Infection, Including by NDM-1-Producing *Enterobacteriaceae* Resistant to Colistin

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A 49-year-old male presented with continuous recurrent chronic gluteal pyoderma. A total surgical excision of a subcutaneous abscess, followed by skin graft surgery, was performed. Three days postsurgery, skin engraftment failure was observed with local infection signs, including pain and suppuration at the surgical wound site. Subsequent microbiological culture revealed the presence of multiple-organism infections, including extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae*, New Delhi metallo-beta (β)-lactamase 1 (NDM-1)- and ESBL-producing *Escherichia coli*, NDM-1- and ESBL-producing *Citrobacter freundii*, *Proteus vulgaris*, and methicillin-resistant *Staphylococcus aureus* (MRSA). We analyzed whole-genome sequences of *E. coli* and *C. freundii* by next-generation sequencing (NGS) using MiSeq (Illumina), which showed the presence of *blaNDM-1* and *blaCTX-M-55* in both strains and of *blaCMY-48* only in *C. freundii*.

The MICs for organisms detected from the pus culture are shown in Table 1. The breakpoint checkerboard plate method was used because it can simultaneously screen 19 type combinations of eight antibiotic agents at clinically achievable levels (1). Only the combination regimen of colistin (COL) with rifampin (RFP) was found to be effective against NDM-1-producing *E. coli* and *C. freundii* by this method. Further combination regimens were assessed using the Etest synergy test (2). The Etest synergy test was performed as a prediffusion technique where the first or second Etest strip was removed after 1 h, and the second or third strip was then placed directly where the previous strip had been removed. The results of the Etest synergy test against NDM-1-producing *E. coli* and *C. freundii* are shown in Table 1. All COL-based combination regimens resulted in a greater decrease in the MICs than with COL monotherapy. Tigecycline (TGC)-based regimens slightly increased the MICs compared with those of monotherapy, particularly in *E. coli*.

We determined that the optimal antibiotic combination against NDM-1- and/or ESBL-producing Gram-negative organisms was COL (intravenous, 150 mg twice a day) with RFP (oral, 300 mg twice a day) and meropenem (MEPM; intravenous, 1.0 g three times a day) and that the optimal antibiotic against MRSA was daptomycin (DAP; 350 mg once a day). As both reoperation and antibiotic treatment were needed in this case, we administered an 11-day course of the described antibiotic combination regimen. There was no postoperative infection recurrence or deterioration. No NDM-1-producing organisms were detected in recultured wound specimens.

Monotherapy with COL (3), TGC (4), or a chloramphenicol (5), as well as combination therapy (6), has been described for patients infected with NDM-1-producing bacteria. TGC monotherapy has been reported to have poor intrinsic activity, and the possibility of antagonistic effects between COL and TGC has also been described (7). In addition, although we selected regimens without TGC in consideration of the gastrointestinal side effects,

TABLE 1 Identification of bacteria from pus culture and MICs of antibiotic agents[a]

Agent	MIC (μg/ml) for:		
	E. coli with NDM-1 and ESBL	*C. freundii* with NDM-1 and ESBL	*K. pneumoniae* with ESBL
CTX	>32	>32	>32
CAZ	>16	>16	<8
CFPM	>16	>16	>16
AZT	>16	>16	8
IPM-CS	>8	>8	<1
MEPM	>8	2	<1
LVFX	>4	<0.5	2
AMK	>32	>32	<4
RFP	>4	>4	>4
COL	3.0	3.0	<1
COL + RFP	1.0[b]	1.0[b]	
COL + RFP + IPM	1.5[b]	1.0[b]	
COL + RFP + LVFX	1.5[b]	0.064[b]	
COL + TGC + RFP	0.75[b]	0.38[b]	
COL + TGC + IPM	1.0[b]	1.0[b]	
TGC	0.75	0.75	
TGC + COL	1.0[c]	0.75[c]	
TGC + RFP	0.75[c]	1.5[c]	
TGC + LVFX	1.0[c]	0.047[c]	
TGC + IPM	1.0[c]	0.5[c]	

[a] Abbreviations: AMK, amikacin; AZT, aztreonam; CAZ, ceftazidime; CFPM, cefepime; COL, colistin; CTX, cefotaxime; ESBL, extended-spectrum beta-lactamase; IPM-CS, imipenem-cilastatin; LVFX, levofloxacin; MEPM, meropenem; NDM-1, New Delhi metallo-beta-lactamase; TCG, tigecycline. In the Etest synergy test, breakpoint matching at each Etest strip replacement was as follows: LVFX (1 μg/ml), IPM-CS (1 μg/ml), COL (2 μg/ml), TGC (1 μg/ml), and RFP (4 μg/ml).
[b] MIC of COL after preparation of other agents by the Etest.
[c] MIC of TGC after preparation of other agents by the Etest.

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TGC-based regimens might be an option for combination therapy based on the *in vitro* results of this case.

NDM-1-producing organisms and MRSA polymicrobial infection limited the selection of antimicrobial regimens in this case. The findings of the present case report might demonstrate the effectiveness of the combination of COL with RFP and a carbapenem, as this combination has previously shown activity *in vitro* (8). A prospective controlled study is required to examine this further.

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