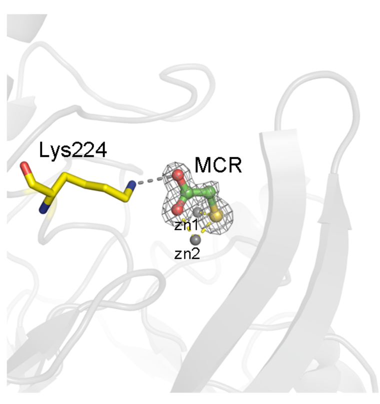

FIG S1 (A) Schematic representation of the structure of IMP-1 in complex with MCR. The |Fo|-|Fc| omit map of MCR contoured at 3.0 σ (gray mesh) is shown. MCR is illustrated using green (carbon), red (oxygen), and yellow (sulfur) sticks. The amino acid Lys224 of IMP-1 is shown as yellow sticks. Zinc ions are illustrated as gray spheres. Gray and yellow dashed lines indicate hydrogen and coordination bonds, respectively.

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                                                    221 223
SMB-1    S.marcescens        GITWTWQSCEQGKCKDVVFADSLTAVSADSYRFSD---HPEVVASLRGSF
AIM-1    P.aeruginosa        GTSWTWRSCEGDDCRQMVYADSLTAISDDVFRYSDDAAHPGYLAAFRNTL
LMB-1    E.cloacae           ALSWQWSSCEAGQCQVLVYADSLSPVSSDSYRFSE---HLTYLNAYRASL
POM-1    P.otitidis          SLSWTWTDRRDGKPLRIAYSDSLSAPGYSLWMNPR---FPKIAEAFRSGF
L1       S.maltophilia       STAWTWTDTRDGKPVRIAYADSLSAPGYQLKDNPR---YPRLIEDYKRTF
GOB-1    E.meningoseptica    SCSFIFETKDEKRKYRVLIANMPSVIVDKKFSEVT--AYPNIQSDYAYTF
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FIG S2 Amino acid alignments of B3 MBLs of human pathogenic bacteria. The GenBank accession numbers of the proteins are as follows: SMB-1 (BAL14456), AIM-1 (CAQ53840), LMB-1 (AMK49163), POM-1 (ADC79563), L1 (ABC02083), and GOB-1 (AAF04458).

Table S1. IC_{50} values of 14 compounds for SMB-1

No.	compounds	IC_{50} (μM)	No.	compounds	IC_{50} (μM)
1		0.6	8		N.D
2		15.0	9		N.D
3		15.6	10		N.D
4		39.4	1a		0.2
5		54.2	1b		54.5
6		125.3	1c		N.D
7		272.7	1d		N.D

N.D.: no apparent deceleration of hydrolyzing velocity in the presence of 200 μM of the compounds.
Measurements were performed using 100 μM nitrocefin as substrate.

Table S2. Bacterial strains and plasmids used in this study

strains	characteristics	sources
E. coli DH5α	strain for cloning β-lactamase gene and its expression	In house
E. coli BL21(DE3)	strain for overexpression of β-lactamases	In house
E. coli BL21(DE3)pLysS	strain for overexpression of β-lactamases	In house
E. coli ATCC 25922	strain for animal experiments	In house
plasmids		
pBC-IMP-1	pBCSK+ vector carrying $bla_{\mathrm{IMP-1}}$	this study
pBC-NDM-1	pBCSK+ vector carrying $bla_{\mathrm{NDM-1}}$	this study
pBC-VIM-2	pBCSK+ vector carrying $bla_{\mathrm{VIM-2}}$	this study
pBC-L1	pBCSK+ vector carrying bla_{L1}	this study
pCL-SMB-1	pCL1920 vector carrying $bla_{\mathrm{SMB-1}}$	(1)
pBC-LMB-1	pBCSK+ vector carrying $bla_{\mathrm{LMB-1}}$	this study
pET9a-ΔIMP-1	IMP-1 expression vector	(2)
pET-dNDM-1	NDM-1 expression vector	this study
pET-VIM-2	VIM-2 expression vector	this study
pET-L1	L1 expression vector	this study
pET-SMB-1	SMB-1 expression vector	(1)
pET-AIM-1	AIM-1 expression vector	this study
pET28a	Plasmid for protein expression	In house
pET29a	Plasmid for protein expression	In house
pBCSK(+)	Plasmid for cloning β-lactamase gene and its expression	In house

1. **Wachino J, Yoshida H, Yamane K, Suzuki S, Matsui M, Yamagishi T, Tsutsui A, Konda T, Shibayama K, Arakawa Y.** 2011. SMB-1, a novel subclass B3 metallo-β-lactamase, associated with IS*CR1* and a class 1 integron, from a carbapenem-resistant *Serratia marcescens* clinical isolate. Antimicrob Agents Chemother **55:**5143-5149.

2. **Yamaguchi Y, Kuroki T, Yasuzawa H, Higashi T, Jin W, Kawanami A, Yamagata Y, Arakawa Y, Goto M, Kurosaki H.** 2005. Probing the role of Asp-120(81) of metallo-β-lactamase (IMP-1) by site-directed mutagenesis, kinetic studies, and X-ray crystallography. J Biol Chem **280:**20824-20832.

Table S3. Primers used in this study

Primer	Sequences[a]
IMP-SK-F	5′-CGGGATCCTGCCCCATTTCGCGCGGATG-3′
IMP-SK-R	5′-GGAATTCAAGTTGCGCGTTGTGGAATAC-3′
VIM2-SK-F	5′-CCGGAATTCCCGTGGGTCGATGGTTTGAT-3′
VIM2-SK-R	5′-CGGGATCCATGCCTAACGCCGAAGTTCA-3′
VIM2-pET-F	5′-GGAATTCCATATGTTCAAACTTTTGAGTAAGTTATT-3′
VIM2-pET-R	5′-CGGGATCCCTACTCAACGACTGAGCGATTTGTGTG-3′
NDM1-SK-F	5′-CCGGAATTCATGGCAGATTGGGGGTGAC-3′
NDM1-SK-R	5′-CGGGATCCCGCCCCATATTTTTGCTACAGT-3′
NDM1-pET-F	5′-GGAATTCCATATGGGTGAAATCCGCCCGACGATTGGC-3′
NDM1-pET-R	5′-CCCAAGCTTTCAGCGCAGCTTGTCGGCCATGC-3′
LMB-SK-F	5′-CCGGAATTCAAACCGTAAACCCGGTAATTA-3′
LMB-SK-R	5′-CGGGATCCTTACCCGCTGGCCAAGTTGCGG-3′
L1-pET-F	5′-GGAATTCCATATGCGTTGTTCCCTGCTCGCCTTCG-3′
L1-pET-R	5′-CGGGATCCTCAGCGGTGTGCCGCAGTTTCCTTGGCC-3′
AIM1-pET-F	5′-GGAATTCCATATGAAACGCCGGTTTACCTTA-3′
AIM1-pET-R	5′-CCCAAGCTTTTACGGACGTGCACCTGACGA-3′

[a]Underlined text indicates the cleavage sites for restriction endonucleases.

Table S4. Structural data

	IMP-1–MCR complex	SMB–TSA complex	SMB–ASB complex
Data collection			
Beam line	NW-12A (PF)	BL2S1 (AichiSR)	BL-5A (PF)
Wavelength (Å)	1.00	1.12	1.00
Resolution range (Å)	56.82–1.57 (1.65–1.57)	40.61–1.39 (1.47–1.39)	50.00–1.17 (1.19–1.17)
Space group	*P*212121	*P*1	*P*1
Cell dimensions			
a **(Å)**	35.9	37.0	40.0
b **(Å)**	56.8	41.6	41.7
c **(Å)**	101.1	45.6	45.7
α (°)	90.0	108.6	108.8
β (°)	90.0	102.8	102.8
γ (°)	90.0	106.1	105.9
No. of unique reflections	29943 (4256)	42083 (5988)	73794 (2884)
Redundancy	11.3 (11.2)	3.7 (3.8)	3.5 (3.2)
Completeness (%)	100.0 (100.0)	90.3 (87.9)	93.1 (71.4)
R_{merge} **(%)**	6.4 (35.4)	5.6 (7.7)	4.1 (8.7)
mean $I/\sigma(I)$	22.2 (6.4)	16.6 (12.2)	37.3 (16.3)
Refinement			
$R_{working}$ **(%)**	16.7	14.8	13.0
R_{free} **(%)**	20.0	17.0	15.1
Average ***B*****-factors (Å^2)**			
Protein	21.6	9.5	8.9
Ligand/ion	29.7	16.2	15.0
Water	32.0	19.6	20.9
r.m.s.d.			
Bond lengths (Å)	0.008	0.008	0.009
Bond angles (°)	1.266	1.304	1.453

Supplementary methods

Purification of MBLs.

IMP-1 expression and purification. The recombinant plasmid (pET9a-ΔIMP-1) was introduced into *Escherichia coli* BL21(DE3). The transformants were grown at 37°C in Luria-Bertani (LB) broth until the OD_{610} was 0.6, at this point isopropyl β-D-1-thiogalactopyranoside (IPTG) was added at a final concentration of 0.5 mM. After incubation for another 3 h at 37°C, bacterial cells were harvested, suspended in 50 mM Tris-HCl buffer (pH 7.5), and disrupted by sonication. The supernatant obtained after ultracentrifugation (100,000 x *g*, 1 h) was loaded onto a HiTrap SP HP column (GE Healthcare) and eluted using a linear gradient of 0 to 0.5 M NaCl in 50 mM Tris-HCl buffer (pH 7.5). The eluted protein was dialyzed against 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl and 2.0 M ammonium sulfate, loaded onto a HiTrap Phenyl HP column (GE Healthcare), and eluted with 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl. The protein was loaded onto a HiLoad 16/60 Superdex 200 pg column (GE Healthcare), and then eluted with 50 mM Tris-HCl (pH 7.5) containing 0.3 M NaCl. Lastly, the protein was buffer-exchanged into 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.5). The purified protein was stored at -80°C until use. The concentration of the eluted protein was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) and its purity was estimated by sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) analysis and Coomassie Brilliant Blue staining.

NDM-1 expression and purification. The recombinant plasmid (pET-dNDM-1) was introduced into *E. coli* BL21(DE3). The transformants were grown at 37°C in LB broth until an OD_{610} of 0.4 was achieved, at this point IPTG was added at a final concentration of 0.5 mM. After incubation at 25°C for another 24 h, bacterial cells were harvested, suspended in 50 mM Tris-HCl buffer [pH 8.0] containing 0.5 M NaCl, 20 mM imidazole, 10 mM β-mercaptoethanol, and 1 mM $ZnCl_2$ (buffer A), and disrupted. The supernatant obtained after ultracentrifugation was loaded onto a HisTrap HP column (GE Healthcare) and eluted using a linear imidazole gradient in the buffer A. The eluted protein was dialyzed against buffer A and digested with thrombin at 20°C for 24 h. The digested protein was loaded onto a HisTrap HP column, and unbounded fractions were collected. The protein was loaded onto a HiTrap Q HP column (GE Healthcare) and eluted using a linear gradient of 0.02 to 0.5 M NaCl in 20 mM Tris-HCl buffer (pH 8.0) containing 2

mM DTT. Finally, the eluted protein was loaded onto a HiLoad 16/60 Superdex 200 pg column and eluted with 20 mM of Tris-HCl (pH 8.0) containing 0.15 M NaCl and 2 mM DTT.

VIM-2 expression and purification. *E. coli* BL21(DE3) carrying pET-VIM-2 were cultured, suspended in 30 mM of Tris-HCl buffer (pH 7.6) containing 0.5 M NaCl, and disrupted. The supernatant after ultracentrifugation was dialyzed against 50 mM of HEPES-NaOH buffer (pH 7.5) containing 50 μM $ZnSO_4$ (buffer B). The crude protein was loaded onto a HiTrap SP HP column and eluted using a linear gradient of 0 to 1.0 M NaCl in buffer B. The eluted protein was dialyzed against 50 mM Tris-HCl buffer containing 0.1 M NaCl, 1.2 M $(NH_4)_2SO_4$, and 50 μM $ZnSO_4$ (buffer C), loaded onto a HiTrap Phenyl HP column, and eluted with buffer C that did not contain $(NH_4)_2SO_4$. The obtained protein was loaded onto a HiLoad 16/60 Superdex 200 pg column and then eluted with 20 mM Tris-HCl (pH 7.5) containing 0.2 M NaCl and 0.1 mM $ZnCl_2$. Finally, the protein was buffer-exchanged into 20 mM Tris-HCl (pH 7.5) buffer containing 0.1 mM $ZnCl_2$, and stored at -80°C until use.

L1 expression and purification. The pET-L1 plasmid was introduced into *E. coli* BL21(DE3). Transformants were grown at 37°C, harvested, and disrupted. The supernatant obtained by ultracentrifugation was loaded onto a HiTrap SP HP column (GE Healthcare) and eluted using a linear gradient of 0 to 0.5 M NaCl in 30 mM MES buffer. The eluted protein was buffer-exchanged against 50 mM HEPES (pH 7.5) containing 2.0 M ammonium sulfate, loaded onto a HiTrap Phenyl HP column, and eluted with 50 mM of HEPES (pH 7.5) buffer. Finally, the protein was loaded onto a HiLoad 16/60 Superdex 200 pg column and then eluted with 50 mM of HEPES-NaOH (pH 7.5) buffer containing 0.2 M NaCl.

AIM-1 expression and purification. pET-AIM-1 was introduced into *E. coli* BL21(DE3). The cells were grown, harvested, suspended with 20 mM Tris-HCl (pH 7.5) buffer, and disrupted by sonication. After ultracentrifugation, the supernatant was loaded onto a HiTrap Q HP column, and eluted with a gradient of 20 mM Tris-HCl (pH 7.5) buffer containing 0.5 M NaCl. The eluted proteins were buffer-exchanged to 20 mM Tris-HCl (pH 7.5) buffer containing 0.3 M NaCl and 1.5 M $(NH_4)_2SO_4$, loaded onto a HiTrap Phenyl HP column, and eluted with 20 mM Tris-HCl (pH 7.5) buffer containing 0.3 M NaCl. Finally, the collected proteins were subjected to size-exclusion chromatography using a Hiload 16/60 Superdex 200 pg column and eluted with 50 mM Tris-HCl (pH 7.5)

buffer containing 0.1 M NaCl.