Supplemental Material

A Simplified Derivative of Human Defensin 5 with Potent Efficiency against Multidrug-resistant *Acinetobacter baumannii*

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TABLE S1 Sequence, purity, and mass of the peptides

Peptide	Sequence	Purity [a]	k' [a]	Theoretical mass [b]	Measured mass [c]
HD5	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	96.0%	3.96	3582.2	3582.3
FITC-HD5	FITC- ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	96.7%	3.83	4090.8	4090.2
HD5d1	ATAYARTGRAATRESLSGVAEISGRLYRLAAR	98.4%	4.34	3395.8	3395.5
HD5d2	ATCYARTGRAATRESLSGVAEISGRLYRLACR	95.4%	4.26	3458.0	3458.4
HD5d3	ATAYCRTGRAATRESLSGVCEISGRLYRLAAR	96.6%	4.68	3458.0	3458.0
HD5d4	ATAYARTGRCATRESLSGVAEISGRLYRLCAR	95.6%	4.27	3458.0	3458.2
HD5d5	ARARCRRGRAARRRRLRGVCRIRGRLRRLAAR	97.1%	3.78	3870.7	3870.9
HD5d6	AKAKCRKGRAAKRKKLKGVCRIKGRLKRLAAR	96.8%	3.85	3618.6	3618.7
FITC-HD5d5	FITC-ARARCRRGRAARRRRLRGVCRIRGRLRRLAAR	96.9%	3.67	4379.3	4379.6

[a] Measured by reverse-phase high-performance liquid chromatography (HPLC). Data were obtained at 25 °C on a Phenomenex/Luna C18 (2) column (5 µm, 4.6 × 150 mm) applying a linear gradient of 20-40% buffer B [buffer A: 0.1% trifluoroacetic acid in water; buffer B: 0.09% trifluoroacetic acid in (80% acetonitrile plus 20% water)] at a flow rate of 1 ml/min over 20 min. k', capacity factor, calculated as the retention time of peptides in stationary phase divided by the retention time of the solvent.

[b] Calculated on http://web.expasy.org/protparam/.

[c] Measured by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF, SHIMADZU, Kyoto, JPN).

FIG S1 Cytotoxic evaluation of HD5, HD5d5, and CIP to erythrocytes and human epithelial HaCaT cells. (A) Hemolytic experiment. Mouse blood was collected and washed with cold PBS, which was then diluted 1:5. The suspension was incubated with peptides at 37 °C for 15 min. The percentage of hemolysis was determined as (A_{pep} − A_{blank}) / (A_{tot} − A_{blank}) × 100%. The total hemolysis (A_{tot}) was actualized by 1% Triton X-100. HD5, HD5d5, and CIP were harmless to erythrocytes, manifested by a hemolysis less than 1%. The results derived from three independent experiments conducted in duplicate are presented as means ± SD. (B) Human epithelial HaCaT cells (CAS, Shanghai, CHN) were cultured in 1640 medium (SH30809.01B, HyClone) and seeded in 96-well plates at a density of 4000 CFU/well. After adherence, cells were washed with PBS and co-incubated with 50, 100, and 150 μg/ml peptides at 37 °C for 24 h. CCK-8 method was used to detect cell survival. The survival rate was calculated as (A_{pep} / A_{blank}) × 100%. This experiment was conducted in duplicate and repeated three times. Results are presented as means ± SD. *, $p < 0.05$, compared to the survival of cells exposed to 50 μg/ml HD5.

Note: The figure is shown in next page.

FIG S1 Cytotoxic evaluation of HD5, HD5d5, and CIP to erythrocytes and human epithelial HaCaT cells.

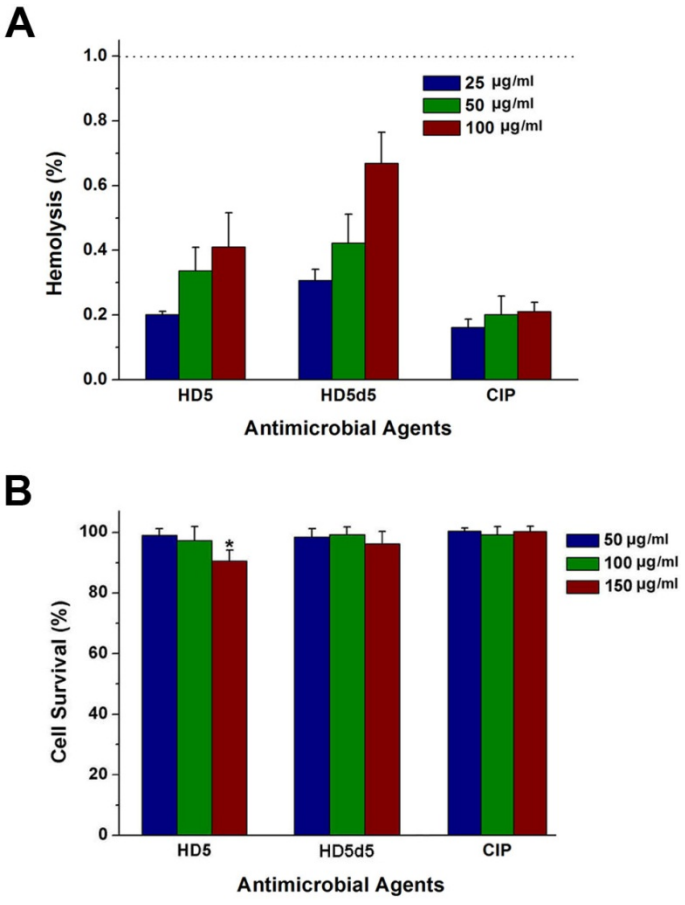

FIG S2 Scanning electron microscopy (SEM) observing the morphological changes of MDR*Ab* exposed to HD5 and HD5d5. MDR*Ab* cells (1×10^8 CFU, 50 μl) cultured to mid-logarithmic phase were co-incubated with 5 μl of peptides (1mg/ml) at 37 ^{o}C for 30 min. Bacteria were coated a coverslip and dried at room temperature. Samples were fixed with 2.5% glutaraldehyde at 4 ^{o}C, dehydrated with gradient concentrations of ethanol, and desiccated using tert-butyl alcohol. A gold coating was used to prevent the accumulation of static electric fields in the specimen. The extra high tension of SEM is 2,000 V. Arrow-pointed cells generally maintained the integrity, indicating that these cells are freed from catastrophic collapse. C.C, catastrophic collapse.

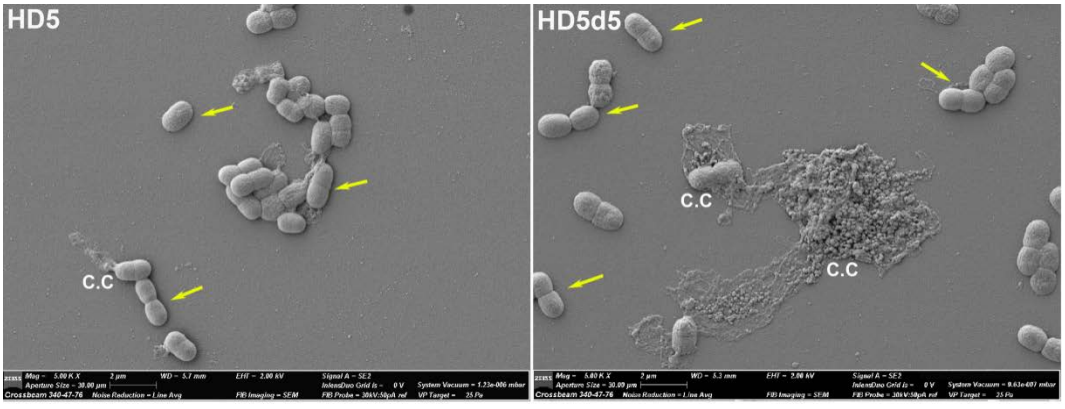

FIG S3 HD5d5 elicits agglutination of the cytoplasmic content of MDR*Ab*. Bacteria cultured to stationary phase were centrifuged and resuspended in 200 μl of 10 mM sodium phosphate (pH 7.4). Cytoplasmic content was obtained and diluted to 1500 μg/ml. A 10 μl aliquot of the content was co-incubated with 100 and 500 μg/ml peptides at room temperature, and 10 μl of the mixture was placed on a glass slide. Based on the changes of the liquid turbidity, we proposed that HD5d5 was stronger than HD5 to cause agglutination of the cytoplasmic content.

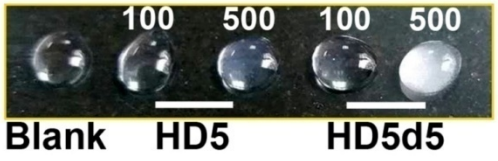

FIG S4 HD5d5 neutralizes *Ab*OmpA in a dose-dependent manner. Human laryngeal epithelial HEp-2 cells were cultured in DMEM supplemented with 10% foetal bovine serum at 37 ^{o}C. Cells were seeded into a 96-well plate at a density of 5000 CFU/well. After adherence, cells were washed with PBS and co-incubated with 5 μg/ml *Ab*OmpA in the absence or presence of peptides at 37 ^{o}C for 9 h. Peptides were prepared in sterile water at concentrations of 10, 30, and 50 μg/ml. Before co-incubation, peptides and *Ab*OmpA were mixed at 37 ^{o}C for 10 min. Absorbance was determined at 450 nm using a microplate reader. Results derived from three independent experiments conducted in duplicate are presented as means ± SD. n.s, not significantly; **, $p < 0.01$, compared to the absorbance of the *Ab*OmpA group.

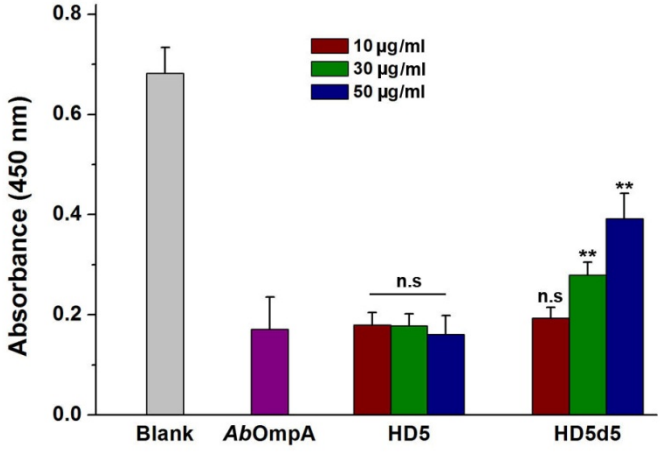

FIG S5 MHB lowers the antibacterial activity of HD5 in a dose-dependent manner. The virtual colony count assay was employed to determine the bactericidal potency of HD5 against MDR*Ab* in 10 mM sodium phosphate buffer (pH 7.4) containing 1%, 5%, 10%, 20%, and 40% MHB. HD5 was prepared in sterile water at concentrations of 12.5 and 6.25 μg/ml. Results derived from three independent experiments conducted in duplicate are shown as means ± SD. n.s, not significantly; **, $p < 0.01$, compared to the bacterial killing of 12.5 μg/ml HD5. $^{\#\#}$, $p < 0.01$, compared to the bacterial killing of 6.25 μg/ml HD5.

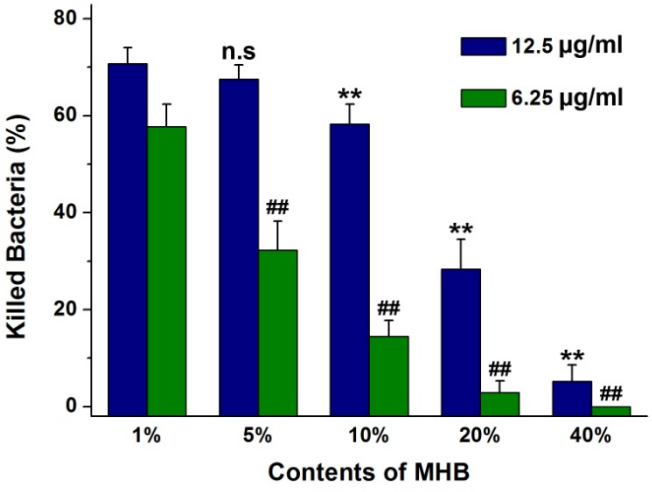

FIG S6 Amino acid sequences of human α- and β-defensins. Cysteines are colored red and box-linked based on the disulfide connectivity. Cys1-6, Cys2-4, and Cys3-5 pairing is highlighted in green, blue, and orange, respectively.

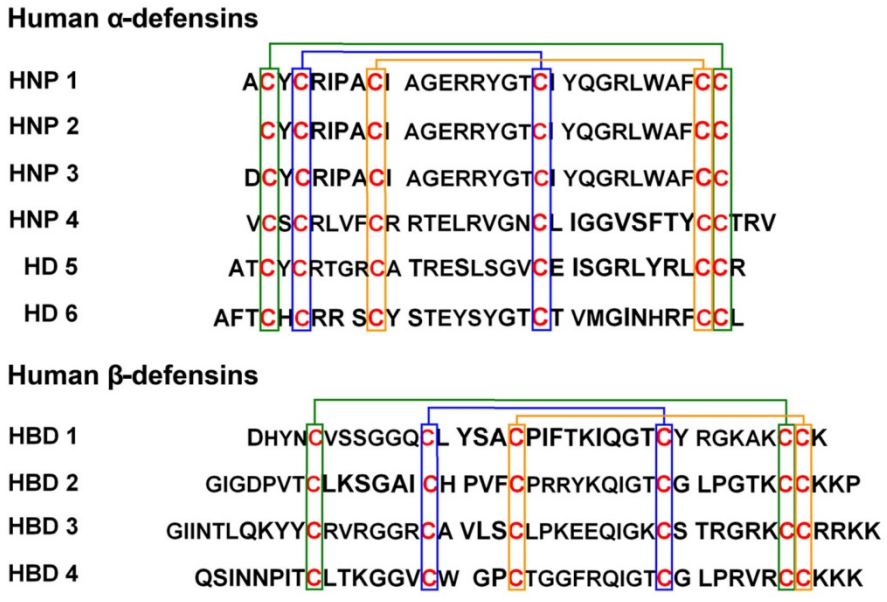

FIG S7 API 20NE and 16S rDNA sequencing confirming microbial species. (A) The isolated strain was cultured in MHB and analysed by API 20NE (Bio-Mérieux, Marcy l'Etoile, France), obtaining a code of 0040437, which is indicative of the being of *A. baumannii*. (B) Bacterial genomic DNA was extracted and sequenced by Beijing Genomics Institute. The data was analysed by the Basic Local Alignment Search Tool (BLAST) of NCBI, demonstrating that the isolate is *A. baumannii*.

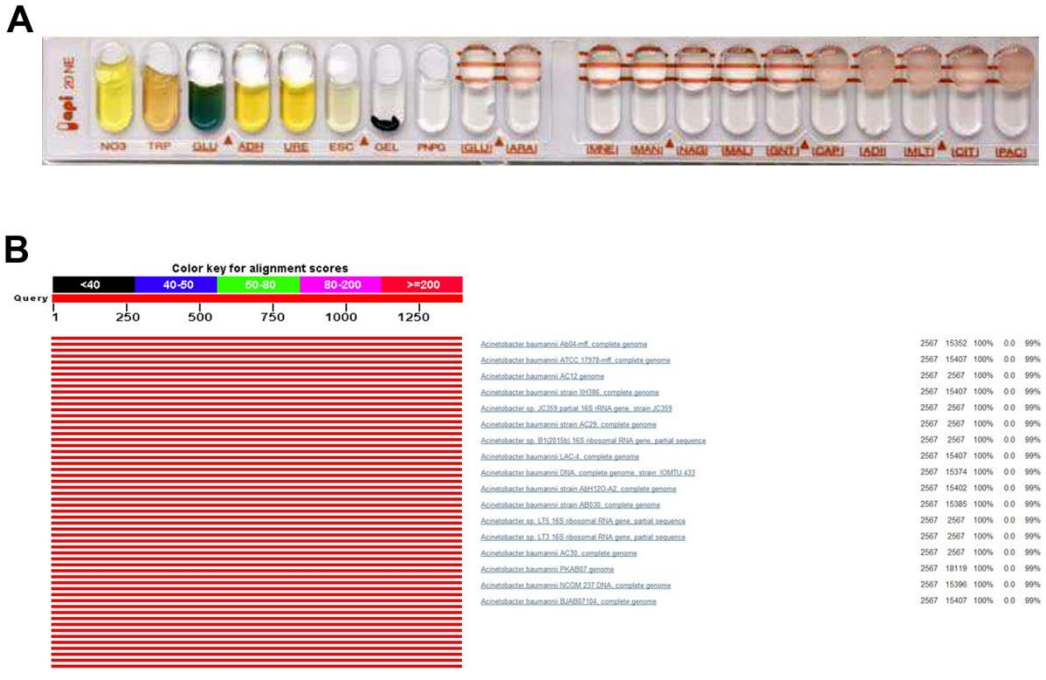

FIG S8 Kirby-Bauer method determining the antibiotic resistance of isolated *A. baumannii*. Isolated *A. baumannii* cultured overnight was coated on the MHB plate, which was then dried at room temperature. Six OXOID antibiotic tablets, specifically, TET, AMK, CTX, CIP, ATM, and SXT, were subsequently attached. Bacteria were incubated at 37 ^{o}C for 16 h. The multi-drug resistance was confirmed by the Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility testing standards.

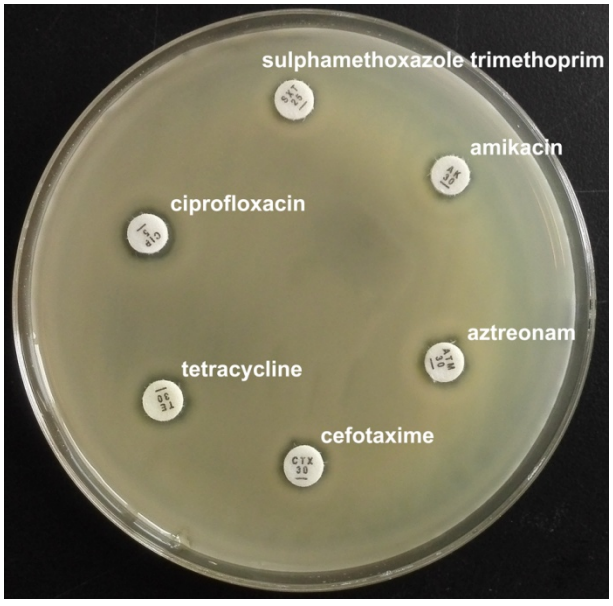

FIG S9 Information on the pET-30a-OmpA expression plasmid. (A) The sequence of the optimized gene encoding *Ab*OmpA, which was inserted into a pET30a vector through NdeI and HindIII. (B) Schematic diagram of the pET-30a-OmpA expression plasmid. (C) SDS-PAGE and Coomassie blue staining analyzing the purified efficiency of *Ab*OmpA. M, marker; 1, lysed supernatant dissolved in 8 M urea solution; 2, lysed supernatant purified by Ni-IDA resin; 3-14, purified product eluted by different concentrations of imidazole.

A

NdeI

CatATGCACCATCACCATCACCAT

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1     CAGGATAGTC AGCACAACAA CGGCGGTAAA GACGGTAATC TGACCAACGG TCCGGAACTG
61    CAGGATGACC TGTTTGTTGG CGCAGCACTG GGTATTGAAC TGACCCCGTG GCTGGGTTTT
121   GAAGCGGAAT ACAACCAGGT TAAAGGCGAC GTTGATGGCG CAAGCGCAGG CGCAGAATAT
181   AAACAGAAAC AGATCAACGG CAACTTCTAC GTCACCAGCG ACCTGATCAC CAAAAACTAC
241   GACAGCAAAA TCAAACCGTA CGTTCTGCTG GGCGCGGGTC ATTACAAATA CGATTTCGAC
301   GGTGTTAATC GCGGTACCCG CGGTACCTCT GAAGAAGGTA CGCTGGGTAA CGCAGGGGTT
361   GGCGCATTTT GGCGTCTGAA CGACGCACTG AGTCTGCGTA CCGAAGCACG CGCAACCTAT
421   AACGCGGACG AAGAGTTCTG GAACTATACC GCACTGGCAG GTCTGAACGT TGTTCTGGGT
481   GGTCATCTGA AACCGGCTGC ACCGGTTGTT GAAGTTGCAC CGGTTGAACC GACCCCGGTT
541   GCACCGCAAC CGCAAGAACT GACCGAAGAT CTGAACATGG AACTGCGCGT CTTCTTCGAC
601   ACCAACAAAA GCAACATCAA AGACCAGTAC AAACCGGAGA TCGCGAAAGT TGCGGAGAAA
661   CTGAGCGAAT ATCCGAACGC AACCGCACGT ATTGAAGGTC ATACCGATAA CACCGGTCCG
721   CGTAAACTGA ACGAACGTCT GTCTCTGGCA CGTGCAAATA GCGTTAAAAG CGCGCTGGTT
781   AACGAATATA ACGTCGACGC AAGCCGTCTG TCTACCCAAG GTTTTGCTTG GGATCAGCCG
841   ATTGCGGACA ACAAAACCAA AGAAGGCCGC GCAATGAACC GTCGCGTTTT TGCGACCATT
901   ACCGGTTCTC GTACCGTTGT TGTTCAACCG GGTCAAGAAG CAGCAGCACC GGCAGCAGCA
961   CAA
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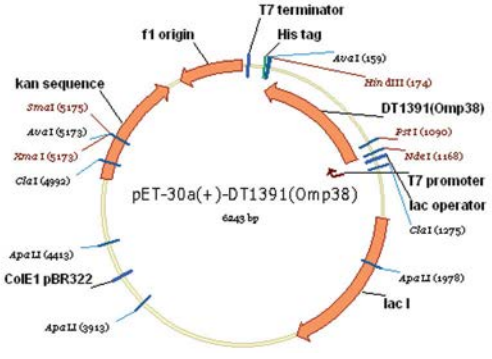
CAATGA AAGCTT

HindIII

B



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