

Modulation of Endolysin LysECD7 Bactericidal Activity by Different Peptide Tag Fusion

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Supplementary materials

Table S2. Bacterial strains used to determine the Spectrum of activity of LysECD7-8his. Antibacterial activity* of this protein and CFU reduction* at concentration of protein upon treatment of 10^3 to 10^5 CFU/ml with 100 $\mu\text{g/ml}$.

Strain	Source	Method of Identification	Antibacterial Activity	CFU Reduction, CFU/ml	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis «Kaluga»	Animal infectious disease, chicken	Cultural, morphological and biochemical properties, serological typing	97.92%	4.95×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Infantis 4632	Animal infectious disease, chicken		100.00%	6.45×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium «Lo»	Animal infectious disease, pigeons		100.00%	2.27×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis 3B	Animal infectious disease, chicken		100.00%	6.55×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Infantis 2511	Animal infectious disease, chicken		100.00%	8.60×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium 415	Animal infectious disease, chicken		100.00%	3.50×10^3	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium 1281	Animal infectious disease, chicken		99.75%	8.08×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium Ufa	Animal infectious disease, ducks		100.00%	4.25×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium #3	Animal infectious disease, ducks		97.04%	5.58×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium #24	Animal infectious disease, chicken		92.07%	6.45×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis 25	Animal infectious disease, chicken		98.43%	3.98×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Infantis 4631	Animal infectious disease, chicken		100.00%	1.19×10^5	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Infantis 4522	Animal infectious disease, chicken		100.00%	6.30×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Dublin immobilin 2	Animal infectious disease, calf		99.88%	8.19×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Enteritidis «4B»	Animal infectious disease, chicken		99.50%	3.98×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Infantis «Kuzn»	Animal infectious disease, chicken		100.00%	9.15×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar London N2	Animal infectious disease, turkey		71.30%	7.95×10^4	
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Tiphimurium G1	Livestock wastes		Cultural, morphological and biochemical properties, MALDI-TOF	99.49%	1.55×10^5
<i>Salmonella</i>	Livestock		Cultural, morphological and	82.62%	2.52×10^4

<i>enterica</i> subsp. <i>enterica</i> serovar Enteritidis SEM4	wastes	biochemical properties		
<i>Salmonella</i> <i>enterica</i> subsp. <i>enterica</i> serovar Infantis 1271	Livestock wastes	Cultural, morphological and biochemical properties, MALDI-TOF	91.52%	1.54 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 1			100.00%	2.50 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 2			100.00%	9.30 x 10 ³
<i>Pseudomonas aeruginosa</i> 3			100.00%	1.53 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 4			100.00%	5.00 x 10 ²
<i>Pseudomonas aeruginosa</i> 5			100.00%	3.70 x 10 ³
<i>Pseudomonas aeruginosa</i> 6			100.00%	1.05 x 10 ³
<i>Pseudomonas aeruginosa</i> 7			100.00%	6.35 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 8	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	100.00%	1.66 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 9			100.00%	1.13 x 10 ⁴
<i>Pseudomonas aeruginosa</i> 10			100.00%	7.75 x 10 ³
<i>Pseudomonas aeruginosa</i> 11			81.90%	1.43 x 10 ⁵
<i>Pseudomonas aeruginosa</i> 12			0%	0
<i>Pseudomonas aeruginosa</i> 13			93.75%	3.00 x 10 ⁵
<i>Pseudomonas aeruginosa</i> 14			47.79%	3.25 x 10 ³
<i>Pseudomonas aeruginosa</i> 15			98.37%	1.48 x 10 ⁵
<i>Pseudomonas aeruginosa</i> 16			96.19%	1.24 x 10 ⁵
<i>Pseudomonas aeruginosa</i> 3086	Reference laboratory strain		99.75%	4.67 x 10 ⁵
<i>Pseudomonas aeruginosa</i> 1805	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	99.46%	5.57 x 10 ⁴
<i>Pseudomonas aeruginosa</i> PA01	Reference laboratory strain		94.48%	5.95 x 10 ⁵
<i>Pseudomonas aeruginosa</i> B-1304	Reference laboratory strain	Cultural, morphological and biochemical properties, MALDI-TOF	99.26%	3.77 x 10 ⁵
<i>Klebsiella pneumoniae</i> 1			97.85%	5.24 x 10 ⁴
<i>Klebsiella pneumoniae</i> 2			98.22%	4.42 x 10 ⁴
<i>Klebsiella pneumoniae</i> 3			95.16%	5.90 x 10 ⁴
<i>Klebsiella pneumoniae</i> 4			96.50%	5.94 x 10 ⁴
<i>Klebsiella pneumoniae</i> 5			96.52%	8.74 x 10 ⁴
<i>Klebsiella pneumoniae</i> 6			99.79%	9.63 x 10 ⁴
<i>Klebsiella pneumoniae</i> 7			99.03%	5.60 x 10 ⁴
<i>Klebsiella pneumoniae</i> 8			99.38%	9.64 x 10 ⁴
<i>Klebsiella pneumoniae</i> 9			99.18%	9.67 x 10 ⁴
<i>Klebsiella pneumoniae</i> 10	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties	87.62%	6.44 x 10 ⁴
<i>Klebsiella pneumoniae</i> 11			94.29%	3.63 x 10 ⁴
<i>Klebsiella pneumoniae</i> 12			95.03%	8.70 x 10 ⁴
<i>Klebsiella pneumoniae</i> 13			100.00%	1.96 x 10 ⁴
<i>Klebsiella pneumoniae</i> 14			99.22%	3.18E x 10 ⁴
<i>Klebsiella pneumoniae</i> 15			98.31%	9.59 x 10 ⁴
<i>Klebsiella pneumoniae</i> 16			99.00%	5.94 x 10 ⁴
<i>Klebsiella pneumoniae</i> 17			100.00%	9.75 x 10 ⁴
<i>Klebsiella pneumoniae</i> 18			96.95%	9.21 x 10 ⁴
<i>Klebsiella pneumoniae</i> 19			99.73%	9.18 x 10 ⁴
<i>Klebsiella pneumoniae</i> 20			92.16%	8.53 x 10 ⁴
<i>Klebsiella pneumoniae</i> Ts 104-14	Hospital strain, outpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	72.65%	7.61 x 10 ³
<i>Escherichia coli</i> 96			100.00%	4.60 x 10 ⁴
<i>Escherichia coli</i> 532			100.00%	1.10 x 10 ⁵
<i>Escherichia coli</i> 502			99.82%	1.41 x 10 ⁵
<i>Escherichia coli</i> 125	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	100.00%	5.70 x 10 ⁴
<i>Escherichia coli</i> 636			100.00%	1.37 x 10 ⁵
<i>Escherichia coli</i> 16			100.00%	8.45 x 10 ⁴
<i>Escherichia coli</i> 110			100.00%	4.75 x 10 ⁴
<i>Escherichia coli</i> 158			100.00%	6.30 x 10 ⁴

<i>Escherichia coli</i> 452			100.00%	6.75 x 10 ³		
<i>Escherichia coli</i> 510			100.00%	6.55 x 10 ⁴		
<i>Escherichia coli</i> 663			100.00%	3.01 x 10 ⁵		
<i>Escherichia coli</i> 185			84.47%	1.31 x 10 ⁴		
<i>Escherichia coli</i> 515			100.00%	6.85 x 10 ⁴		
<i>Escherichia coli</i> 682			99.38%	2.40 x 10 ⁴		
<i>Escherichia coli</i> 108			100.00%	7.05 x 10 ⁴		
<i>Escherichia coli</i> 503			100.00%	1.10 x 10 ⁵		
<i>Escherichia coli</i> 632			100.00%	1.05 x 10 ⁵		
<i>Escherichia coli</i> 179			100.00%	1.72 x 10 ⁴		
<i>Escherichia coli</i> 201			100.00%	5.10 x 10 ⁴		
<i>Escherichia coli</i> 533			100.00%	3.85 x 10 ⁴		
<i>Acinetobacter baumannii</i> 145			98.72%	1.93 x 10 ⁴		
<i>Acinetobacter baumannii</i> 402			99.88%	4.20 x 10 ⁴		
<i>Acinetobacter baumannii</i> MA 65			99.81%	8.04 x 10 ⁴		
<i>Acinetobacter baumannii</i> 474	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	97.49%	1.17 x 10 ⁴		
<i>Acinetobacter baumannii</i> 869			98.73%	9.33 x 10 ⁴		
<i>Acinetobacter baumannii</i> Bor			98.54%	1.15 x 10 ⁵		
<i>Acinetobacter baumannii</i> PA			99.17%	8.33 x 10 ⁴		
<i>Acinetobacter baumannii</i> 67GKB			99.85%	3.35 x 10 ⁴		
<i>Acinetobacter baumannii</i> Gar			Hospital strain, intensive care unit	Cultural, morphological and biochemical properties	99.84%	9.14 x 10 ⁴
<i>Acinetobacter baumannii</i> B-05			Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	99.42%	2.55 x 10 ⁴
<i>Acinetobacter baumannii</i> Ts 50-16	Hospital strain, intensive care unit	Cultural, morphological and biochemical properties	100.00%	1.53 x 10 ⁵		
<i>Enterobacter</i> sp. 1			99.77%	4.34 x 10 ⁴		
<i>Enterobacter</i> sp. 2			100.00%	1.41 x 10 ⁴		
<i>Enterobacter</i> sp. 3			100.00%	5.60 x 10 ³		
<i>Enterobacter</i> sp. 4			100.00%	1.59 x 10 ⁴		
<i>Enterobacter</i> sp. 5	Hospital strain, outpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	100.00%	8.10 x 10 ⁴		
<i>Enterobacter</i> sp. 6			97.08%	4.32 x 10 ⁴		
<i>Enterobacter</i> sp. 7			98.97%	1.01 x 10 ⁵		
<i>Enterobacter</i> sp. 8			97.41%	1.70 x 10 ⁴		
<i>Enterobacter</i> sp. 9			99.71%	6.78 x 10 ⁴		
<i>Enterobacter</i> sp. 10			89.48%	2.60 x 10 ⁴		

*For all experiments, antimicrobial activity is shown as the mean value from three independent experiments. The antibacterial activity was expressed as follows: Antibacterial activity (%) = 100% - (CFU_{exp}/CFU_{cont})x100%, where CFU_{exp} is the number of bacterial colonies in the experimental culture plates, and CFU_{cont} is the number of bacterial colonies in the control culture plates. . "100%" Antibacterial activity means zero colonies on CFU_{exp} when plating 10 µl from initial experimental well. Antibacterial activity was arbitrarily regarded as meaningful when it was higher than 33%. CFU reduction value was obtained using the following equation: CFU reduction = CFU_{cont} - CFU_{exp}, where CFU_{exp} is the number of bacterial colonies in the experimental culture plates, and CFU_{cont} is the number of bacterial colonies in the control culture plates.

Table S3. Bacterial strains used to compare bactericidal activity of LysECD7-8his and LysECD7-SMAP. Antibacterial activity* of these proteins and CFU reduction* at concentration of protein upon treatment of 10^3 to 10^5 CFU/ml with 50 μ g/ml.

Strain	Source	Method of Identification	Antibacterial Activity of LysECD7-8his	CFU Reduction	Antibacterial Activity of LysECD7-SMAP	CFU Reduction
<i>Klebsiella pneumoniae</i> Ts 104-14	Hospital strain, outpatient hospital		46.23%	4.84×10^3	100%	1.05×10^4
<i>Pseudomonas aeruginosa</i> 12	Hospital strain, inpatient hospital	Cultural, morphological and biochemical properties, MALDI-TOF	0%	0	100%	1.52×10^4
<i>Pseudomonas aeruginosa</i> 14			34.23%	4.45×10^3	100%	3.68×10^4
<i>Staphylococcus aureus</i> Z 73-14	Hospital strain, outpatient hospital		0%	0	99.91%	2.5×10^5
<i>Staphylococcus haemolyticus</i> G 58-0916			0%	0	92.33%	1.34×10^5

*For all experiments, antimicrobial activity is shown as the mean value from three independent experiments. The antibacterial activity was expressed as follows: Antibacterial activity (%) = $100\% - (\text{CFU}_{\text{exp}}/\text{CFU}_{\text{cont}}) \times 100\%$, where CFU_{exp} is the number of bacterial colonies in the experimental culture plates, and CFU_{cont} is the number of bacterial colonies in the control culture plates. "100%" Antibacterial activity means zero colonies on CFU_{exp} when plating 10 μ L from initial experimental well. Antibacterial activity was arbitrarily regarded as meaningful when it was higher than 33%. CFU reduction value was obtained using the following equation: $\text{CFU reduction} = \text{CFU}_{\text{cont}} - \text{CFU}_{\text{exp}}$, where CFU_{exp} is the number of bacterial colonies in the experimental culture plates, and CFU_{cont} is the number of bacterial colonies in the control culture plates.

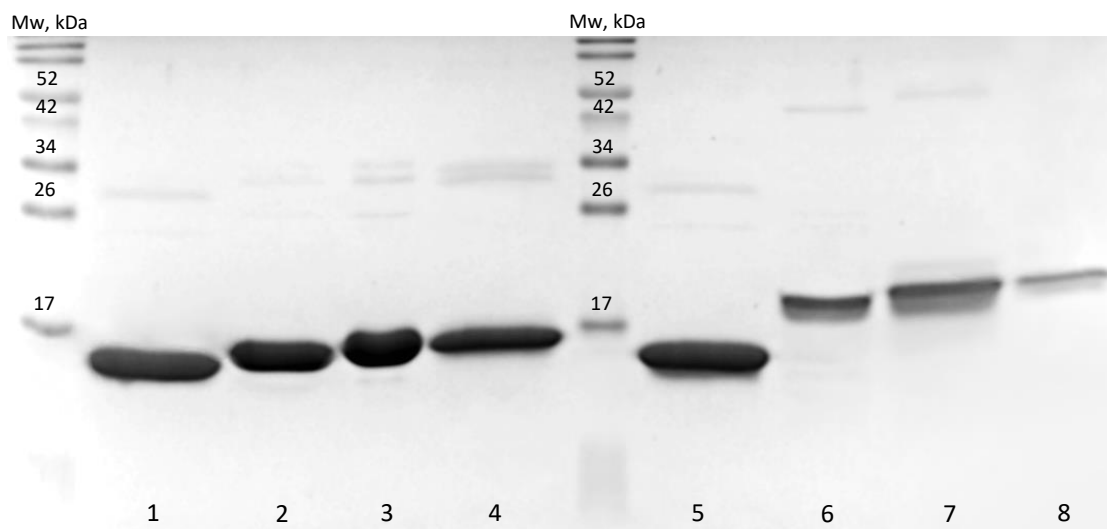
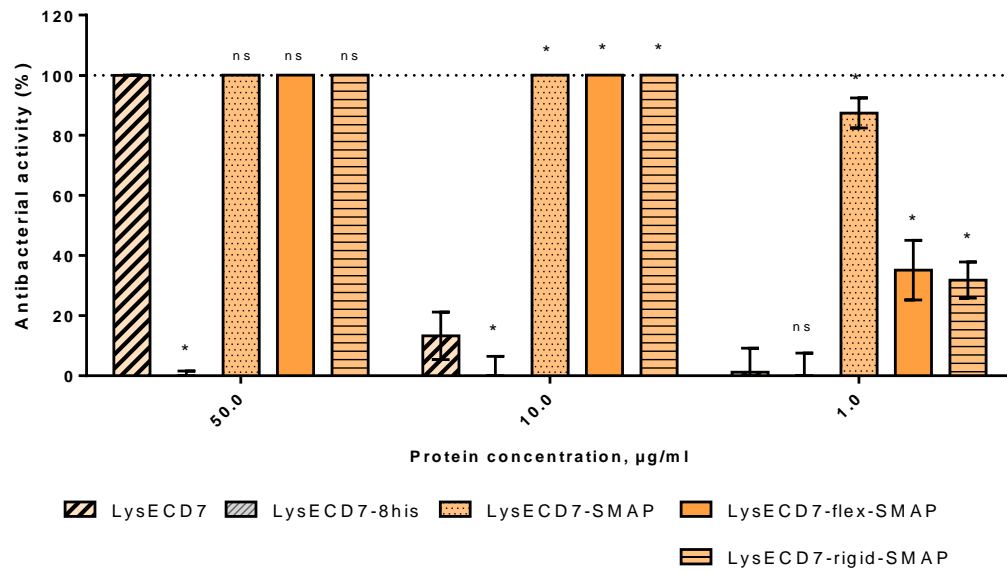


Figure S1. SDS-PAGE gel analysis of purified endolysins. 16% SDS-PAGE gel analysis of LysECD7 derivatives. Spectra™ Multicolor Broad Range Protein Ladder (Thermo Scientific, Vilnius, Lithuania) was used as a marker. 1 - LysECD7 (14.98 kDa), 2 - LysECD7-6his (15.7 kDa), 3 - LysECD7-8his (16.1 kDa), 4 - LysECD7-12his (16.5 kDa), 5 - LysECD7 (14.98 kDa), 6 - LysECD7-SMAP (17.18 kDa), 7 - LysECD7-flex-SMAP (18.16 kDa), 8 - LysECD7-rigid-SMAP (18.74 kDa).

A.



B.

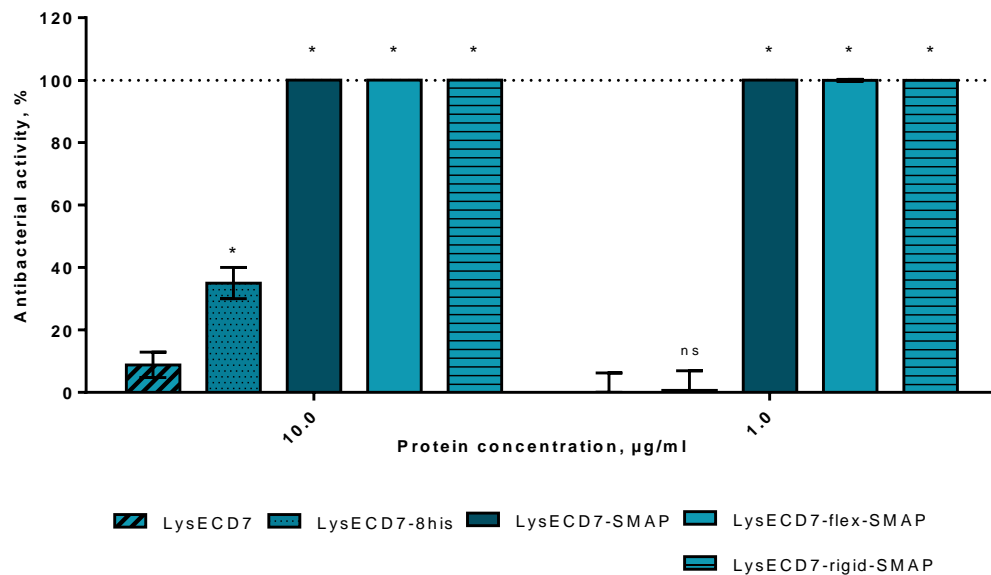


Figure S2. (a) Bactericidal activity of modified endolysins LysECD7 against *A. baumannii* Ts 50-16 in PBS buffer solution. For all experiments, the mean values are shown from three independent experiments (\pm standard deviation, SD). Asterisk (*) indicates significant effect on bactericidal activity comparing to LysECD7 enzyme ($p < 0.05$, one-way ANOVA, Dunnett's multiple comparisons test). (b) Efficiency of modified endolysins LysECD7 under different conditions. Bactericidal activity of modified endolysins LysECD7 against stationary-phase cells of *A. baumannii* Ts 50-16. For all experiments the mean values are shown from three independent experiments (\pm standard deviation, SD). Asterisk (*) indicates significant effect on bactericidal activity comparing to LysECD7 enzyme ($p < 0.05$, one-way ANOVA, Dunnett's multiple comparisons test).

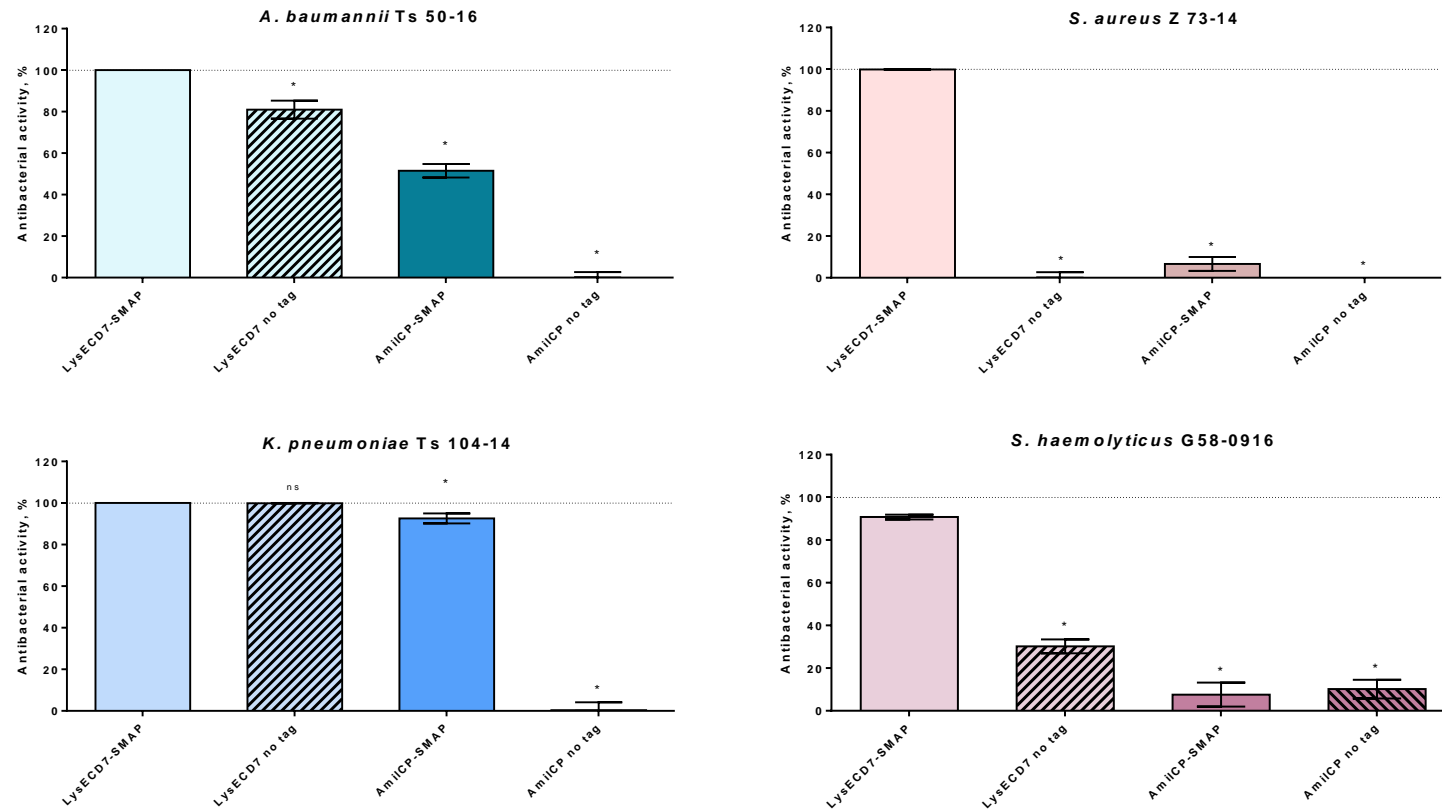


Figure S3. Influence of SMAP peptide introduction into the LysECD7 protein sequence on its antimicrobial activity. Bactericidal activity of LysECD7, its SMAP-modified version and SMAP peptide fused to a nonendolysin protein (AmiICP) against Gram-negative and Gram-positive isolates, diluted in 20 mM Tris HCl buffer pH 7.5. Proteins were assessed at following concentrations: 0.03 μM (corresponding to 0.5 $\mu\text{g/ml}$ of LysECD7-SMAP protein) for *A. baumannii* Ts 50-16 and 2.9 μM (corresponding to 50.0 $\mu\text{g/ml}$ of LysECD7-SMAP protein) for *Klebsiella* and *Staphylococcus* isolates. The culture dilution was 1 to 3×10^5 CFU/ml. For all experiments the mean values are shown from three independent experiments (\pm standard deviation, SD). Asterisk (*) indicates significant effect on bactericidal activity comparing to LysECD7-SMAP enzyme ($p < 0.05$, one-way ANOVA, Dunnett's multiple comparisons test).