Supplemental material for

Title

High-resolution bacterial cytological profiling reveals intrapopulation morphological variations upon antibiotic exposure

Authors

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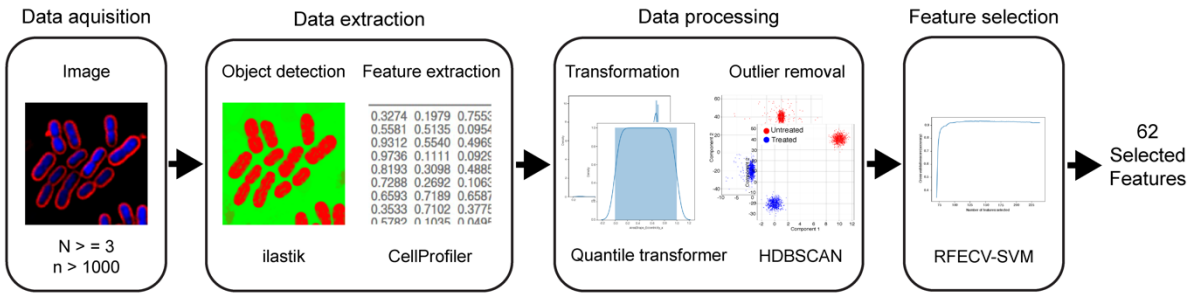
A



B

			Accuracy = 0.9297	
	MOA profile by BCP	Precision	Recall	F1-score
Minocycline (MIN)	P1	0.97	0.91	0.94
Amikacin (AMI)	P2	0.88	0.96	0.92
Piperacillin (PIP)	C1	1.00	0.97	0.99
Meropenem (MER)	C2	0.99	0.99	0.99
Ciprofloxacin (CIP)	D1	0.95	1.00	0.98
Rifampicin (RIF)	R1	0.93	0.91	0.92
Triclosan (TCS)	L1	0.84	0.85	0.84
Colistin (CST)	M1	0.86	0.91	0.89
Untreated		0.95	0.86	0.90

Figure S1. Feature selection pipeline for single antibiotic treatment and cell profile classification accuracy

(A) Data flow of cell feature selection starting from image acquisition, data extraction via ilastik and CellProfiler, data transformation and outlier removal, and feature selction via RFECE-SVM machine learning model. (B) Precision table of single cell profile classification using 62 features selected by RFECE-SVM.

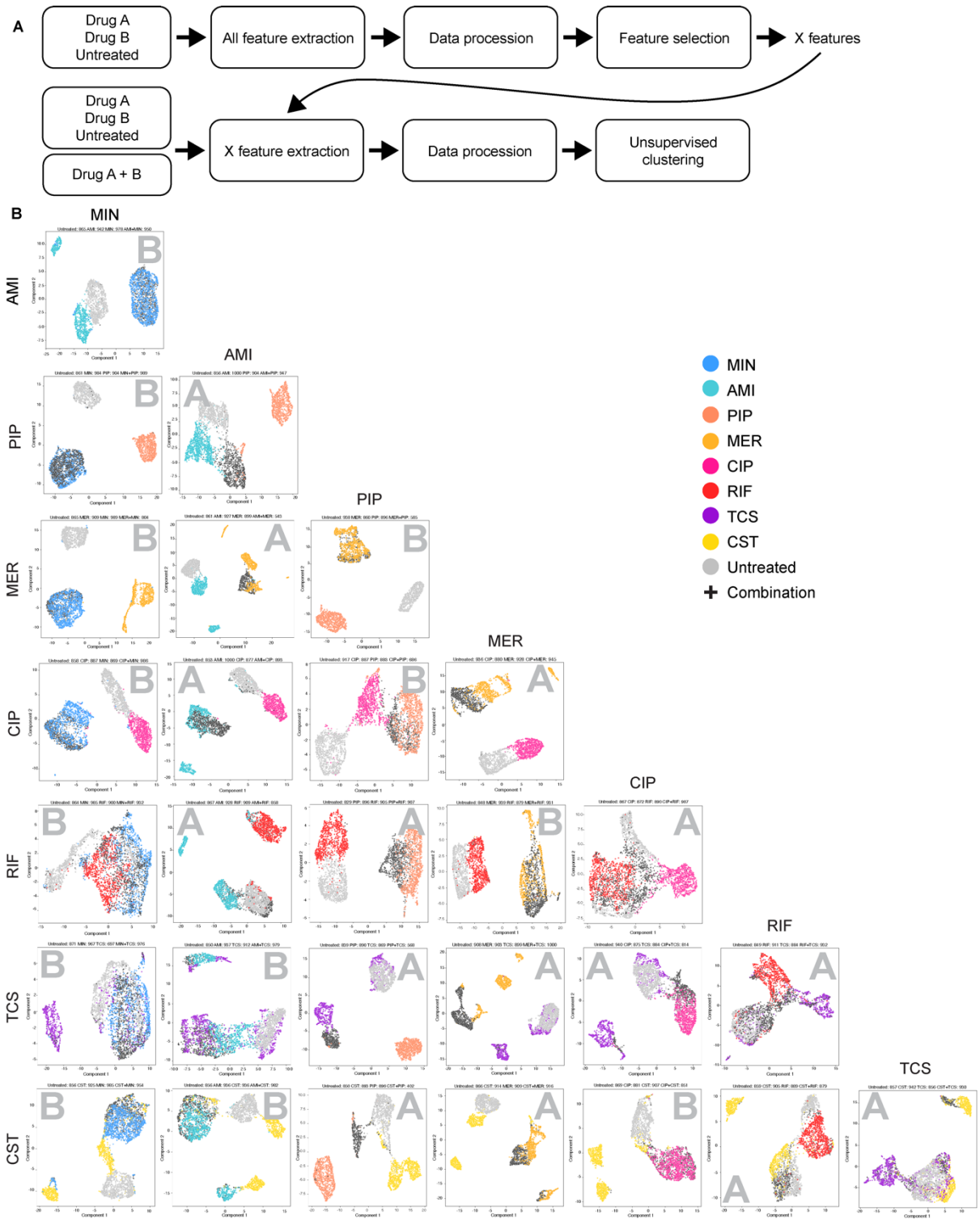


Figure S2. Feature selection pipeline for antibiotic combination treatment and the profiles of all combinations

(A) Data flow of cell feature selection from two antibiotics and unsupervised data analysis of antibiotic combination profiles. (B) PaCMAP plots of all 28 combination with designated combination type (Type A and B) indicated.

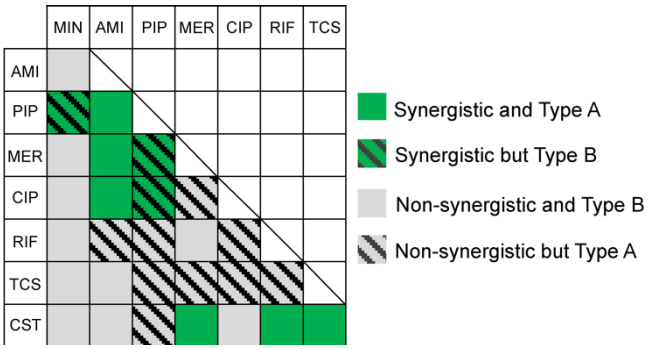


Figure S3. Combinatorial profile types and synergism of antibiotic pairs
Drug interaction is synergistic when the Σ FICs is ≤0.5, otherwise it is non-synergistic.

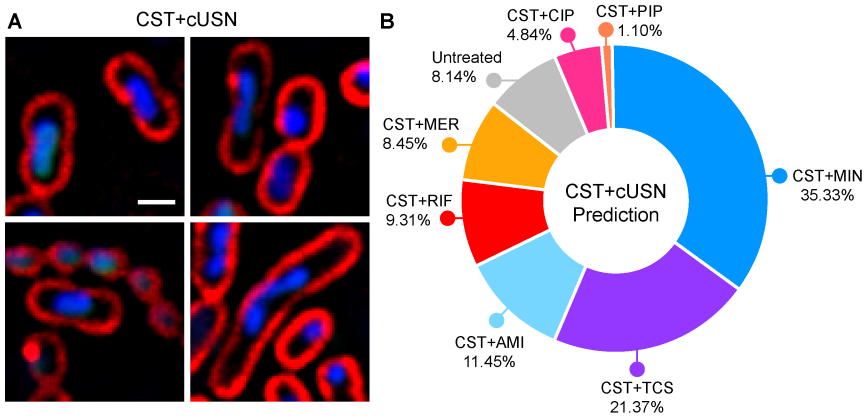


Figure S4. Cells treated with commercial usnic acid (cUSN) in combination with colistin (CST) resulted in multiple morphological changes and were classified into multiple antibiotic combinations

(A**)** Representative cell images of CST+cUSN-treated cells showing multiple morphological changes. *A. baumannii* cells treated with 0.25 x MIC of CST + 250 μM cUSN for 1 hour. Scale bar represents 1 μm. (B) CST+cUSN-treated cells were classified into different profiles by the predictive mode.

Table S1. Table showing the antibiotics used in this study, their MIC, their MOA profiles and cellular pathways inhibited.

Antibiotic name	MIC (μg/mL)	MOA profile by BCP	Cellular pathway
Minocycline (MIN)	0.50	P1	Protein translation
Amikacin (AMI)	4.00	P2	
Piperacillin (PIP)	40.00	C1	Cell wall synthesis
Meropenem (MER)	0.45	C2	
Ciprofloxacin (CIP)	0.45	D1	DNA replication
Rifampicin (RIF)	1.00	R1	RNA transcription
Triclosan (TCS)	0.05	L1	Lipid synthesis
Colistin (CST)	0.62	M1	Membrane integrity

Table S2. Table showing the selected features for each experiment.

Object features (_x = membrane, _y = DNA)	Single antibiotic (Figure 1)	Antibiotic combination (Figure 2)																												CST + USN classification (Figure 3)
		AMI+RIF	AMI+CIP	CIP+RIF	AMI+CST	CIP+CST	CST+MER	CST+MIN	CST+PIP	CST+RIF	CST+TCS	AMI+MER	CIP+MER	AMI+MIN	CIP+MIN	MER+MIN	MIN+PIP	MIN+RIF	MIN+TCS	AMI+PIP	CIP+PIP	MER+PIP	PIP+RIF	MER+RIF	RIF+TCS	AMI+TCS	CIP+TCS	MER+TCS	PIP+TCS	
AreaShape_Area_x	X		X		X		X	X		X	X				X	X	X	X		X	X	X		X	X	X	X	X	X	X
AreaShape_BoundingBoxArea_x															X	X										X				
AreaShape_Compactness_x																	X									X				
AreaShape_Eccentricity_x	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X		X	X	X	X	X	X	X	X
AreaShape_EquivalentDiameter_x	X						X				X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
AreaShape_EulerNumber_x																															
AreaShape_Extent_x															X						X						X			
AreaShape_FormFactor_x																										X				
AreaShape_MajorAxisLength_x	X		X					X		X	X			X	X	X	X	X	X	X	X					X		X	X	X
AreaShape_MaxFeretDiameter_x	X		X				X	X		X	X	X			X	X	X			X	X					X	X	X	X	X
AreaShape_MaximumRadius_x					X	X						X									X	X	X			X			X	X
AreaShape_MeanRadius_x	X	X						X		X	X	X		X	X	X	X	X			X	X				X		X	X	X
AreaShape_MedianRadius_x							X	X							X	X	X	X	X				X	X						X
AreaShape_MinFeretDiameter_x	X							X					X	X	X	X	X	X	X											X
AreaShape_MinorAxisLength_x	X						X		X	X	X				X		X				X					X				X
AreaShape_Orientation_x				X						X								X					X							
AreaShape_Perimeter_x		X										X			X	X				X	X			X		X			X	
AreaShape_Solidity_x	X		X										X	X	X	X	X						X			X	X			X
AreaShape_Zernike_0_0_x	X	X			X	X	X	X	X	X	X		X	X	X	X	X	X	X			X	X	X		X	X	X			X
AreaShape_Zernike_1_1_x																		X								X					
AreaShape_Zernike_2_0_x	X			X						X			X		X			X	X				X	X	X	X	X	X	X	X
AreaShape_Zernike_2_2_x	X					X							X		X		X							X		X	X			X
AreaShape_Zernike_3_1_x																															
AreaShape_Zernike_3_3_x															X																
AreaShape_Zernike_4_0_x	X			X						X					X						X		X	X		X		X	X	X
AreaShape_Zernike_4_2_x	X			X						X	X				X				X						X	X	X	X	X	X
AreaShape_Zernike_4_4_x	X			X						X					X						X	X	X	X					X	X
AreaShape_Zernike_5_1_x															X											X					
AreaShape_Zernike_5_3_x															X																
AreaShape_Zernike_5_5_x																										X					
AreaShape_Zernike_6_0_x										X					X						X	X	X			X					
AreaShape_Zernike_6_2_x															X						X		X								
AreaShape_Zernike_6_4_x							X			X					X								X	X							
AreaShape_Zernike_6_6_x															X																
AreaShape_Zernike_7_1_x										X					X								X			X					
AreaShape_Zernike_7_3_x															X																
AreaShape_Zernike_7_5_x															X																
AreaShape_Zernike_7_7_x																							X								
AreaShape_Zernike_8_0_x															X						X										
AreaShape_Zernike_8_2_x																										X					
AreaShape_Zernike_8_4_x																							X	X							
AreaShape_Zernike_8_6_x															X		X					X								X	
AreaShape_Zernike_8_8_x															X											X					
AreaShape_Zernike_9_1_x															X																
AreaShape_Zernike_9_3_x																															
AreaShape_Zernike_9_5_x															X																
AreaShape_Zernike_9_7_x															X											X					
AreaShape_Zernike_9_9_x																								X							
Intensity_IntegratedIntensityEdge_OriBlue_x	X										X	X		X			X		X	X					X	X	X	X	X	X	X
Intensity_IntegratedIntensityEdge_OriGreen_x	X				X		X	X		X	X	X									X	X	X			X		X			
Intensity_IntegratedIntensity_OriBlue_x	X	X						X		X			X					X				X	X	X	X	X		X	X	X	X
Intensity_IntegratedIntensity_OriGreen_x	X						X	X			X		X					X				X	X			X	X	X	X	X	X
Intensity_LowerQuartileIntensity_OriBlue_x					X	X	X			X	X				X						X					X		X	X	X
Intensity_LowerQuartileIntensity_OriGreen_x	X	X						X	X	X	X				X			X	X		X	X	X	X	X	X	X	X		X
Intensity_MADIntensity_OriBlue_x	X	X	X	X		X				X	X		X		X	X		X	X				X	X	X	X	X		X	X
Intensity_MADIntensity_OriGreen_x											X											X		X	X	X		X			
Intensity_MassDisplacement_OriBlue_x															X								X								
Intensity_MassDisplacement_OriGreen_x							X	X			X				X				X		X				X	X		X	X	X
Intensity_MaxIntensityEdge_OriBlue_x	X	X	X	X						X	X	X		X	X			X	X		X		X	X	X	X				X
Intensity_MaxIntensityEdge_OriGreen_x															X											X					
Intensity_MaxIntensity_OriBlue_x		X		X				X		X					X	X	X		X		X				X	X				X
Intensity_MaxIntensity_OriGreen_x															X								X			X					
Intensity_MeanIntensityEdge_OriBlue_x	X	X		X	X		X			X	X							X	X				X	X	X	X		X		X
Intensity_MeanIntensityEdge_OriGreen_x	X				X		X	X	X	X	X				X			X		X	X	X	X	X	X	X		X	X	X
Intensity_MeanIntensity_OriBlue_x							X				X				X			X								X		X	X	
Intensity_MeanIntensity_OriGreen_x							X																			X				X
Intensity_MedianIntensity_OriBlue_x							X				X				X									X		X		X	X	
Intensity_MedianIntensity_OriGreen_x	X			X			X			X					X			X			X		X		X	X			X	
Intensity_MinIntensityEdge_OriBlue_x	X	X	X		X					X	X	X		X	X		X		X				X	X	X	X	X	X	X	X
Intensity_MinIntensityEdge_OriGreen_x		X								X					X		X				X	X	X							
Intensity_MinIntensity_OriBlue_x	X	X	X		X			X			X	X		X	X			X	X					X	X	X	X	X	X	X
Intensity_MinIntensity_OriGreen_x															X									X		X				X
Intensity_StdIntensityEdge_OriBlue_x	X	X	X		X	X				X	X	X		X	X		X	X	X	X	X		X	X	X	X	X		X	X
Intensity_StdIntensityEdge_OriGreen_x															X																
Intensity_StdIntensity_OriBlue_x	X	X		X				X		X	X	X	X		X			X	X		X		X	X	X	X	X	X		X	X
Intensity_StdIntensity_OriGreen_x											X						X									X					X
Intensity_UpperQuartileIntensity_OriBlue_x															X											X		X			
Intensity_UpperQuartileIntensity_OriGreen_x								X							X						X										

Table S2 cont.

Object features (_x = membrane, _y = DNA)	Single antibiotic (Figure 1)	Antibiotic combination (Figure 2)																												CST + USN classification (Figure 3)
		AMI+RIF	AMI+CIP	CIP+RIF	AMI+CST	CIP+CST	CST+MER	CST+MIN	CST+PIP	CST+RIF	CST+TCS	AMI+MER	CIP+MER	AMI+MIN	CIP+MIN	MER+MIN	MIN+PIP	MIN+RIF	MIN+TCS	AMI+PIP	CIP+PIP	MER+PIP	PIP+RIF	MER+RIF	RIF+TCS	AMI+TCS	CIP+TCS	MER+TCS	PIP+TCS	
AreaShape_Area_y	x						x								x				x							x	x	x	x	
AreaShape_BoundingBoxArea_y			x	x							x	x		x								x				x		x		
AreaShape_Compactness_y	x							x		x					x			x	x				x			x				x
AreaShape_Eccentricity_y	x			x			x	x		x	x	x	x	x	x			x	x				x	x		x		x		x
AreaShape_EquivalentDiameter_y							x								x				x					x				x	x	
AreaShape_EulerNumber_y																														
AreaShape_Extent_y	x	x													x		x	x		x	x		x			x			x	
AreaShape_FormFactor_y			x					x		x					x				x					x		x				x
AreaShape_MajorAxisLength_y								x			x		x		x												x		x	x
AreaShape_MaxFeretDiameter_y						x									x												x	x	x	x
AreaShape_MaximumRadius_y	x						x	x			x	x			x	x	x	x	x		x	x	x	x	x	x	x	x	x	x
AreaShape_MeanRadius_y	x		x					x			x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x		x
AreaShape_MedianRadius_y	x		x	x		x	x				x		x		x				x		x	x		x	x	x	x	x		x
AreaShape_MinFeretDiameter_y	x	x		x		x	x			x		x	x		x	x	x	x		x	x		x	x	x	x		x		x
AreaShape_MinorAxisLength_y	x	x		x				x		x	x	x	x		x	x	x	x	x		x		x	x	x	x		x		x
AreaShape_Orientation_y										x														x						
AreaShape_Perimeter_y															x			x						x			x			
AreaShape_Solidity_y	x			x			x	x					x	x	x	x	x	x	x					x	x		x			x
AreaShape_Zernike_0_0_y	x			x			x	x		x			x	x	x			x	x					x	x		x		x	x
AreaShape_Zernike_1_1_y																														
AreaShape_Zernike_2_0_y											x		x		x		x		x					x	x	x				x
AreaShape_Zernike_2_2_y	x			x											x		x	x	x					x						x
AreaShape_Zernike_3_1_y															x											x				
AreaShape_Zernike_3_3_y																														
AreaShape_Zernike_4_0_y				x		x				x			x		x						x			x		x				
AreaShape_Zernike_4_2_y		x		x						x					x											x		x		x
AreaShape_Zernike_4_4_y				x						x					x			x					x	x						x
AreaShape_Zernike_5_1_y															x											x				
AreaShape_Zernike_5_3_y															x															
AreaShape_Zernike_5_5_y															x											x				
AreaShape_Zernike_6_0_y								x							x						x		x							x
AreaShape_Zernike_6_2_y				x				x							x			x			x			x	x	x				x
AreaShape_Zernike_6_4_y															x						x			x		x				x
AreaShape_Zernike_6_6_y	x									x					x	x	x	x					x	x		x				
AreaShape_Zernike_7_1_y																														
AreaShape_Zernike_7_3_y															x															
AreaShape_Zernike_7_5_y																					x		x	x						
AreaShape_Zernike_7_7_y										x																				
AreaShape_Zernike_8_0_y															x		x													
AreaShape_Zernike_8_2_y											x				x					x						x				
AreaShape_Zernike_8_4_y											x				x		x		x											x
AreaShape_Zernike_8_6_y						x									x						x					x				
AreaShape_Zernike_8_8_y				x											x						x	x	x	x	x	x				x
AreaShape_Zernike_9_1_y															x															
AreaShape_Zernike_9_3_y															x															
AreaShape_Zernike_9_5_y				x											x											x				
AreaShape_Zernike_9_7_y																								x		x				
AreaShape_Zernike_9_9_y																							x	x						
Intensity_IntegratedIntensityEdge_OriBlue_y	x		x	x		x		x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
Intensity_IntegratedIntensityEdge_OriGreen_y	x		x			x	x	x	x	x	x	x		x	x	x	x	x			x		x	x	x	x				x
Intensity_IntegratedIntensity_OriBlue_y	x	x			x					x	x			x					x					x	x	x	x	x	x	x
Intensity_IntegratedIntensity_OriGreen_y	x	x	x	x	x		x	x		x	x	x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x
Intensity_LowerQuartileIntensity_OriBlue_y	x	x	x	x	x		x	x		x	x	x	x	x	x	x		x	x	x	x		x	x		x	x	x		x
Intensity_LowerQuartileIntensity_OriGreen_y	x	x		x	x	x	x	x		x	x				x			x					x	x	x	x				x
Intensity_MADIntensity_OriBlue_y	x			x	x	x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
Intensity_MADIntensity_OriGreen_y	x	x			x						x	x			x		x	x	x		x				x	x	x	x		x
Intensity_MassDisplacement_OriBlue_y											x				x			x	x					x	x	x		x	x	x
Intensity_MassDisplacement_OriGreen_y	x	x		x	x	x	x	x	x	x	x	x	x		x			x	x				x	x	x	x	x	x	x	
Intensity_MaxIntensityEdge_OriBlue_y															x											x				
Intensity_MaxIntensityEdge_OriGreen_y	x										x				x				x							x		x	x	x
Intensity_MaxIntensity_OriBlue_y	x							x		x	x			x	x		x	x	x		x				x	x				x
Intensity_MaxIntensity_OriGreen_y																														
Intensity_MeanIntensityEdge_OriBlue_y	x			x		x	x			x	x				x				x		x			x	x	x	x		x	x
Intensity_MeanIntensityEdge_OriGreen_y	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x
Intensity_MeanIntensity_OriBlue_y											x				x				x							x	x		x	
Intensity_MeanIntensity_OriGreen_y	x	x		x						x					x								x	x	x	x	x	x		
Intensity_MedianIntensity_OriBlue_y	x			x		x		x					x	x	x		x	x	x		x			x		x	x			x
Intensity_MedianIntensity_OriGreen_y	x	x		x			x			x	x							x			x		x	x	x	x	x	x		
Intensity_MinIntensityEdge_OriBlue_y	x	x		x				x		x	x				x	x	x	x	x		x		x	x	x	x	x	x	x	x
Intensity_MinIntensityEdge_OriGreen_y												x			x								x	x		x				
Intensity_MinIntensity_OriBlue_y	x	x		x	x		x			x	x			x	x	x		x			x		x	x	x	x		x	x	x
Intensity_MinIntensity_OriGreen_y										x													x	x						
Intensity_StdIntensityEdge_OriBlue_y		x			x					x	x							x					x	x	x		x	x	x
Intensity_StdIntensityEdge_OriGreen_y												x			x										x					
Intensity_StdIntensity_OriBlue_y	x				x	x	x	x	x	x	x							x		x					x		x		x	x
Intensity_StdIntensity_OriGreen_y	x	x	x	x	x	x				x	x	x		x	x			x	x	x					x	x			x	x
Intensity_UpperQuartileIntensity_OriBlue_y											x				x			x						x	x					
Intensity_UpperQuartileIntensity_OriGreen_y		x								x		x											x	x	x	x				

Table S3. Table showing the antibacterial activity of natural product-derived compounds against *B. subtilis* and *A. baumannii*.

Natural product-derived compounds	**MIC (μM)**		**FIC (μM) (+ 0.25xMIC CST)**
	B. subtilis	***A. baumannii***	***A. baumannii***
Anacardic acid	**31.5**	> 500	> 500
Chrysin	> 500	> 500	> 500
α-Mangostin	**7.8**	> 500	> 500
Mansonone G	**15.6**	> 500	> 500
Melodorinone B	> 500	> 500	> 500
Methyl 4-hydroxybenzoate	> 500	> 500	> 500
Phenoxyethanol	> 500	> 500	> 500
Pinostrobin	> 500	> 500	> 500
Piperic acid	> 500	> 500	> 500
Piperine	> 500	> 500	> 500
Usnic acid (USN)	**1.5**	> 500	**250**
Commercial usnic acid (cUSN)	**1.5**	> 500	**250**