

Supplementary Material

Heterogeneous colistin-resistance phenotypes coexisting in *Stenotrophomonas maltophilia* isolates influence colistin susceptibility testing

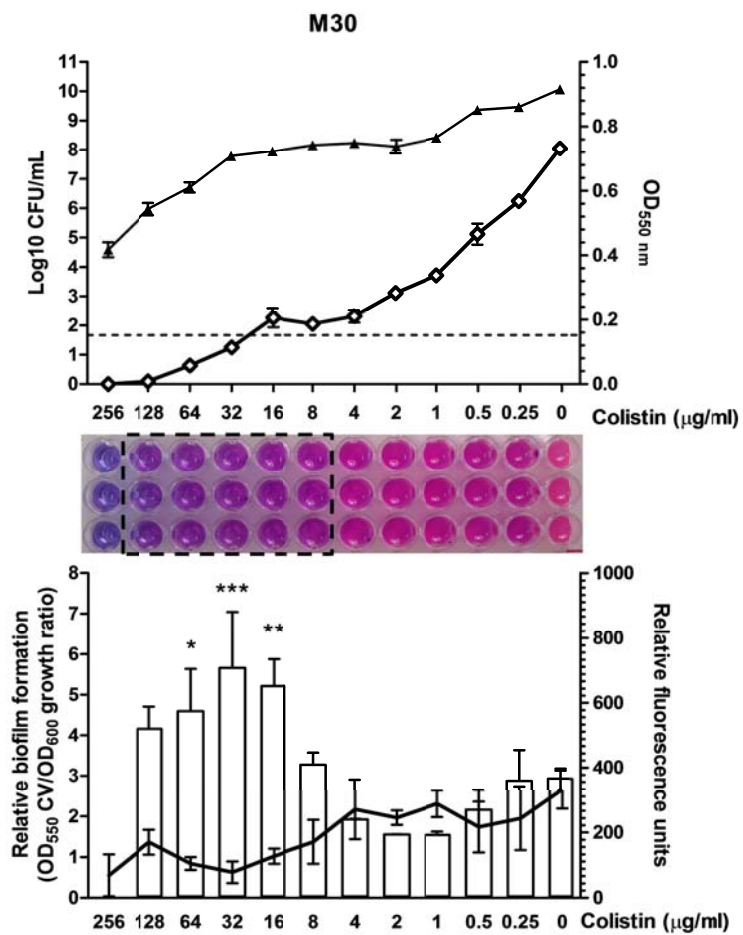
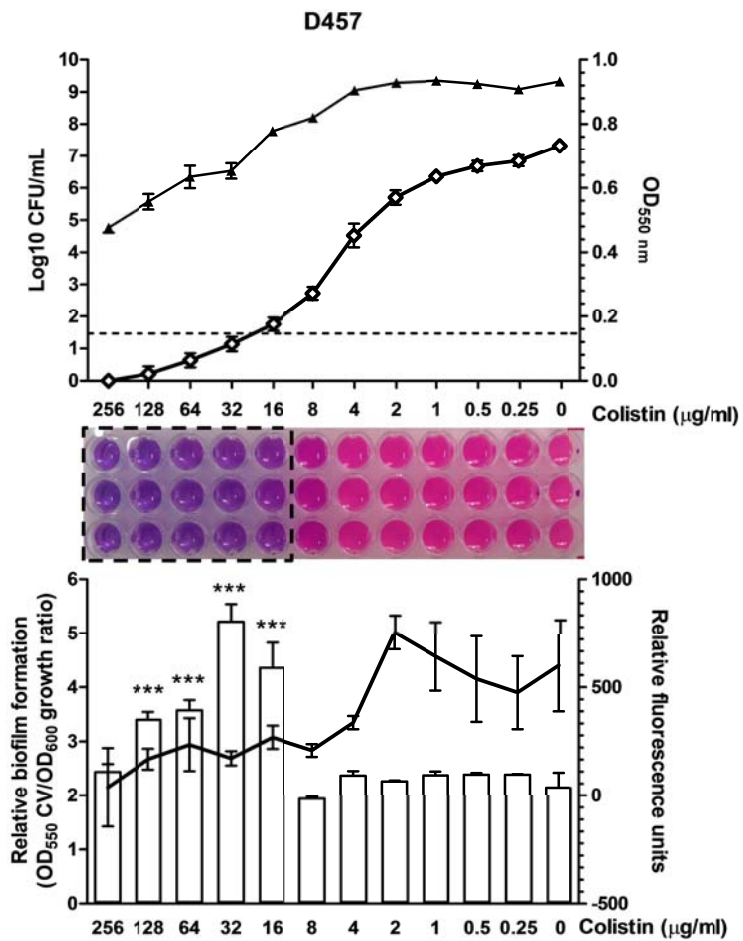
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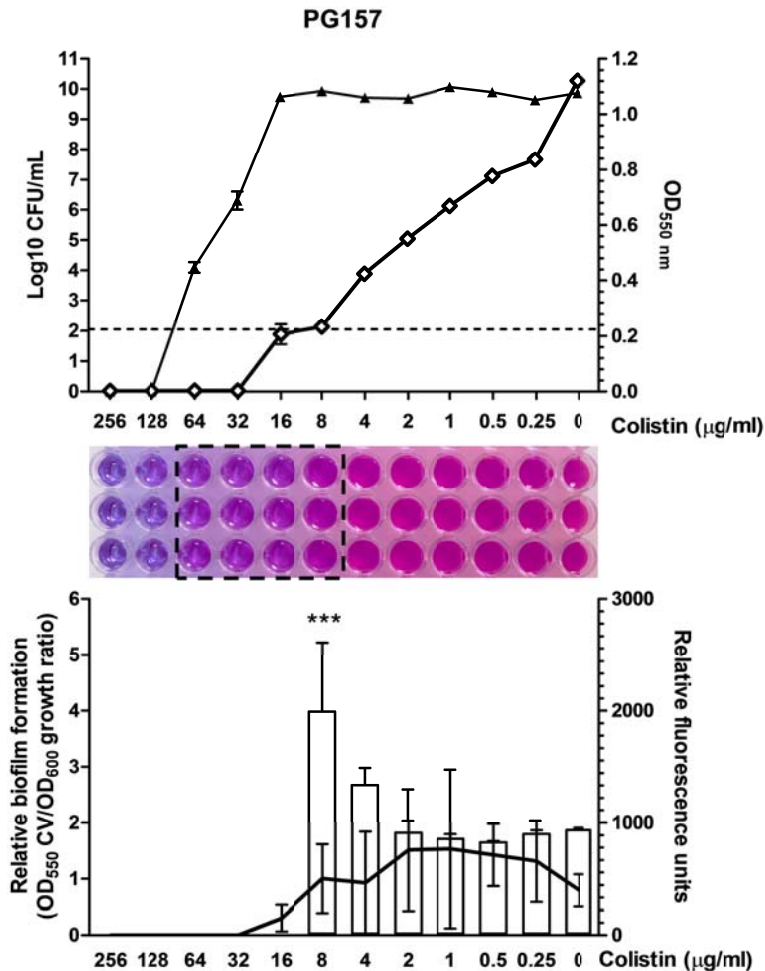
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Supplementary figures

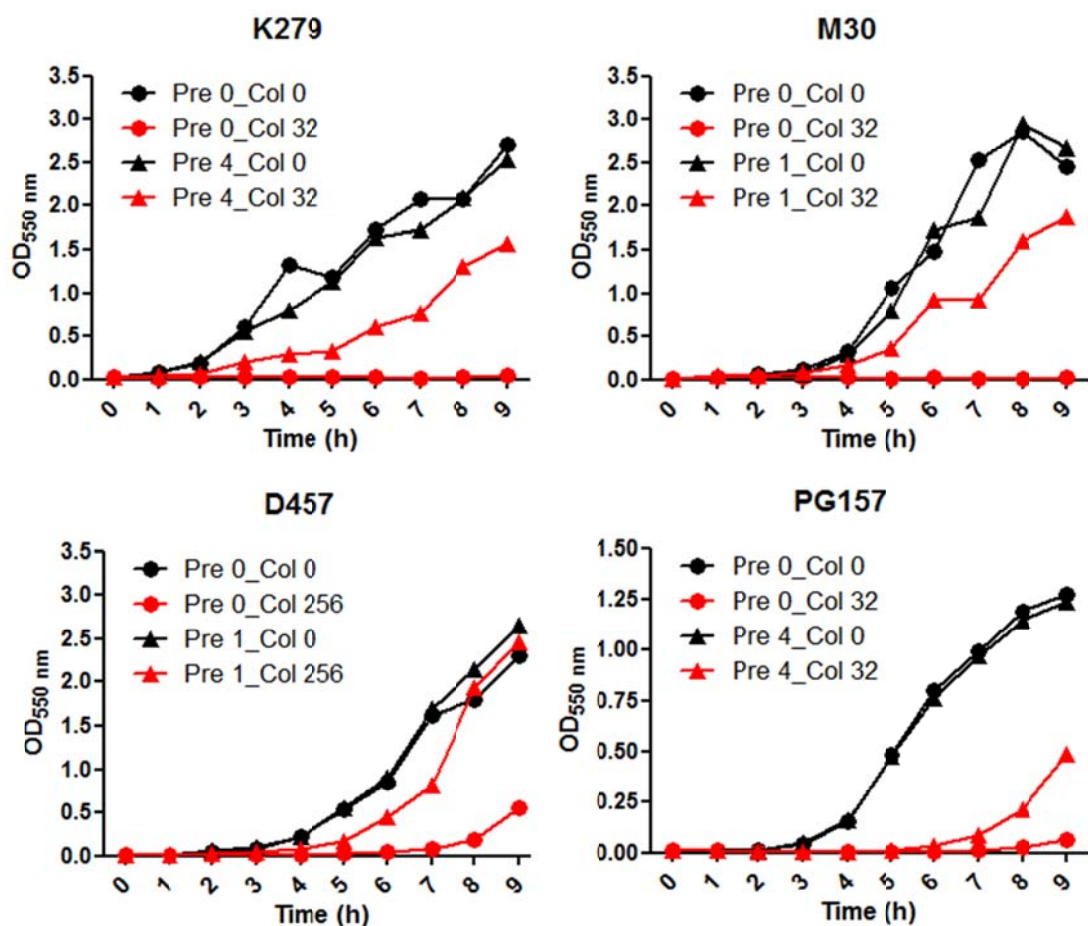
	Colistin (mg/L)											
	Resistant							Susceptible				
Strains	256	128	64	32	16	8	4	2	1	0.5	0.25	
Strains showing heteroresistant response to colistin												
PU140	←MIC											
OUI41	←MIC											
PC187	MIC											
PR45		MIC										
EV5520			MIC									
PC257			MIC									
PC255			MIC									
P626				MIC								
M30				MIC								
PL20				MIC								
D457				MIC								
PG157				MIC								
B5565				MIC								
PR59				MIC								
PC232				MIC								
K279a					MIC							
E50					MIC							
PC314					MIC							
B285						MIC						
UV74						MIC						
PC312						MIC						
OL13						MIC						
PC274						MIC						
S5720						MIC						
OS87						MIC						
PU101						MIC						
OUI48						MIC						
PC186						MIC						
OCI94						MIC						
PC313						MIC						
PC240							MIC					
OS91							MIC					
P815							MIC					
ATCC13637							MIC					
PC245								MIC				
OR41								MIC				
PU126								MIC				
4834-R									MIC			
OCI97									MIC			
OL16									MIC			
PU109									MIC			
OUI353									MIC			
PC226									MIC			
OUI110										MIC		
PU153										MIC		
OCI323										MIC		
OUI343										MIC		
H5726											MIC→	
Strains showing homogeneous response to colistin												
S3149			MIC									
OR79				MIC								
OL11					MIC							
PU100					MIC							
S289						MIC						
B5742							MIC					
PU102							MIC					
OG156								MIC				
PL12										MIC		
OUI111											MIC	
OUI52											MIC	
E77											MIC→	
L9-5R5											MIC→	

Supplementary Figure S1. Graphical representation of the resazurin-based 96-well plate BMD method for the determination of the minimum inhibitory concentration (MIC) of colistin in a panel of 61 *S. maltophilia* strains. The color changes were observed 30 min after addition of resazurin (pink color indicates growth and blue means inhibition of growth). The purple color in the graph indicates the purple/pink “transition zone”. The MIC was taken as the first well in the microdilution plate exhibiting 80% growth inhibition compared to the absorbance (OD550) of the well without antibiotic (arrows indicate MIC values out of the detection limits). The clinical breakpoint for resistance (2 mg/L) is indicated with a thicker black line.





Supplementary Figure S2: Uninterpretable results during colistin MIC determination by the BMD method in CAMHB broth for the colistin-resistant clinical strains D457, M30 and PG157 (Upper graphic in each panel). The optical density at 550 nm (white diamond) and CFU/ml (black triangle) of each well in the microtiter plate was measured after 24 hours of incubation at 37°C. Error bars are generated from three replicates. Colistin concentrations are in serial two-fold dilutions starting at 256 mg/L. The colistin MIC was taken as the lowest concentration that inhibited 80% of growth (OD₅₅₀) as compared to the control well without antibiotic (dashed line in the graph). A picture of three replicas of the microdilution wells with the calorimetric indicator resazurin is shown below each graph with the purple/pink transition zone boxed. The lower graphic in each panel shows total biofilm formation relative to bacterial growth in each well of the BMD plate as a bar graph (Y axis on the left) and the viability of the biofilm determined by resazurin staining as a line graph (Y axis on the right). Fluorescence units are expressed as relative to the biofilm formed in each well of the microtiter plate. Error bars are generated from four replicates in each experiment. Statistically significant increases in biofilm formation with respect to untreated control are indicated: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (one-way ANOVA with Dunnett's multiple comparison test).



Supplementary Figure S3: Adaptive response to colistin in four isolates of *S. maltophilia* showing resistance to colistin by the broth microdilution method. Effect of preincubation with sub-inhibitory concentrations of colistin (Pre in mg/L) on the growth of selected strains in CAMHB supplemented with an inhibitory concentration of colistin (Col in mg/L).