Supporting Information for

Antimicrobial Susceptibility Testing by Using Virulent Phages to Evaluate Bacterial Viability

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Isolation of virulent phages

Hospital sewage collected from Southwest Hospital of Third Military Medical University was used to isolate virulent phages according to a lambda phage isolation protocol with minor modification (Calendar 2006). Briefly, $CaCl_2$ was added into the hospital sewage with a final concentration of 1.0 M, followed by centrifugation at 10000 *g* for 10 min. Afterward, the collected supernatant was filtered through a 0.22-μm filtration membrane. Subsequently, the host *P. aeruginosa* was mixed with the filtrate and cultured at 37 °C overnight. The host bacterial cells in the culture were removed by filtering through the same filtration membrane. Then 0.3 mL of *P. aeruginosa* culture and 0.3 mL of filtrate were mixed and incubated for 15 min at room temperature, followed by adding with 2.0 mL of semisolid LB broth. After mixing well, the semisolid LB broth was poured into solid LB broth plate, followed by incubation at 37 °C overnight. Isolation of the virulent phages was identified through the forming of single plaque.

Amplification and purification of virulent phages

When the ratio of phages to host bacteria was above 10, the host bacteria can be completely captured and lysed by the phages (Kutter and Sulakvelidze 2005). Therefore, virulent phages titration above 1.0×10^7 PFU mL^{-1} was required in this protocol. The isolated single plaque of virulent phages was added into *P. aeruginosa* solution at early logarithmic phase. Then the *P. aeruginosa* solution was continuously cultured till it turned to transparent. To release the virulent phages from the cell debris, the lysate was added with NaCl (0.058 g mL^{-1}), followed by centrifugation at 10000 *g* and 4 °C. Subsequently, 10% (w/v) PEG 8000 was added into the supernatant to precipitate virulent phages at 0 °C overnight. After centrifugal separation at 12000 *g* and 4 °C, the virulent phages solution was mixed with equal volume of chloroform, and centrifuged at 5000 *g* for 10 min. Finally, the virulent phages solution was stored in 0.05 M Tris-HCl buffer (pH 7.5) containing 0.2% $MgSO_4.7H_2O$ at 4 °C. The purified phage was used to conduct the following detection of antimicrobial susceptibility testing (AST) of *P. aeruginosa*. Standard plaque assay procedure was

used to determine the titration of the obtained virulent phages. The titration of virulent phages remained stable for half year at 4 °C.

Measurement of lysis time of virulent phages

One hundred microliters of *P. aeruginosa* solution was diluted with 0.9% NaCl solution to reach a final concentration of 5.0×10^5 CFU mL^{-1}, followed by adding with 50 μL of virulent phages solution at 2.0×10^9 PFU mL^{-1}. The mixture was cultured at 37 °C for various duration. For BL detection, 50 μL of the obtained lysate and the same volume of ATP BL detection reagent were mixed in the microplate in turn to trigger the BL signal at room temperature.

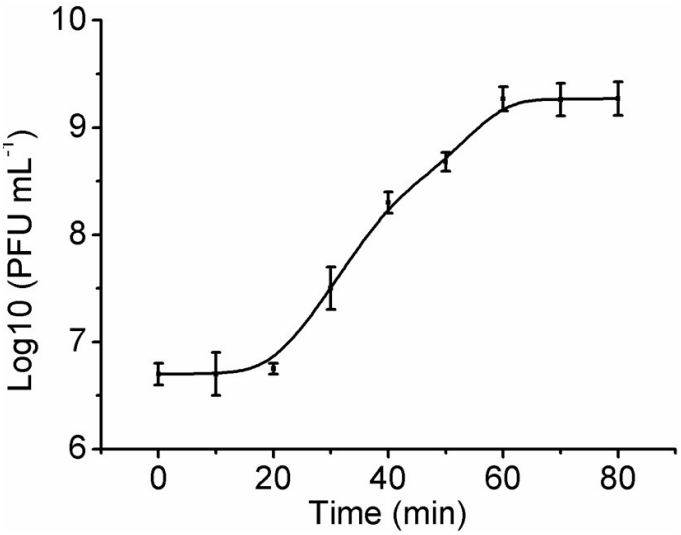

Figure S1. Single-step growth curve of the isolated virulent phage.

Table S1. The principle of AST by using virulent phages to evaluating bacterial viability. S: susceptible, I: intermediate, R: resistant.

Group	Sample	Lysis property of antibiotics	Susceptibility	BL signal	Origin
Control (Solvent group, SG)	Bacteria	Lytic antibiotics	R	-	
			S	+	Antibiotics
		Non-lytic antibiotics	R	-	
			S	-	
Testing (Phage group, PG)	Bacteria + phages	Lytic antibiotics	R	+	Phages
			S	+	Antibiotics
		Non-lytic antibiotics	R	+	Phages
			S	-	

Table S2. MIC interpretive standards for *P. aeruginosa*. S: susceptible, I: intermediate, R: resistant.

Report Group	Antibiotic	MIC Interpretive Criteria(μg mL^{-1})		
		S	I	R
A	PIP	≤16	32-64	≥128
A	CAZ	≤8	16	≥32
A	GEN	≤4	8	≥16
A	TOB	≤4	8	≥16
B	LVX	≤2	4	≥8

Table S3. MIC and MBC results of *P. aeruginosa* to different antibiotics performed by microbroth dilution method. S: susceptible, I: intermediate, R: resistant.

Antibiotic	MIC (μg mL^{-1})		MBC (μg mL^{-1})
	Value	Susceptibility	
PIP	256	R	512
CAZ	64	R	128
GEN	8	I	8
TOB	<4	S	<4
LVX	16	R	16

SI references

R. Calendar, The Bacteriobacteriophages, 2nd ed., Oxford University Press, UK, 2006.

E.Kutter, A. Sulakvelidze,Bacteriophages: Biology and Applications, 1st ed., CRC Press, Boca Raton, 2005.