

Fig. S1

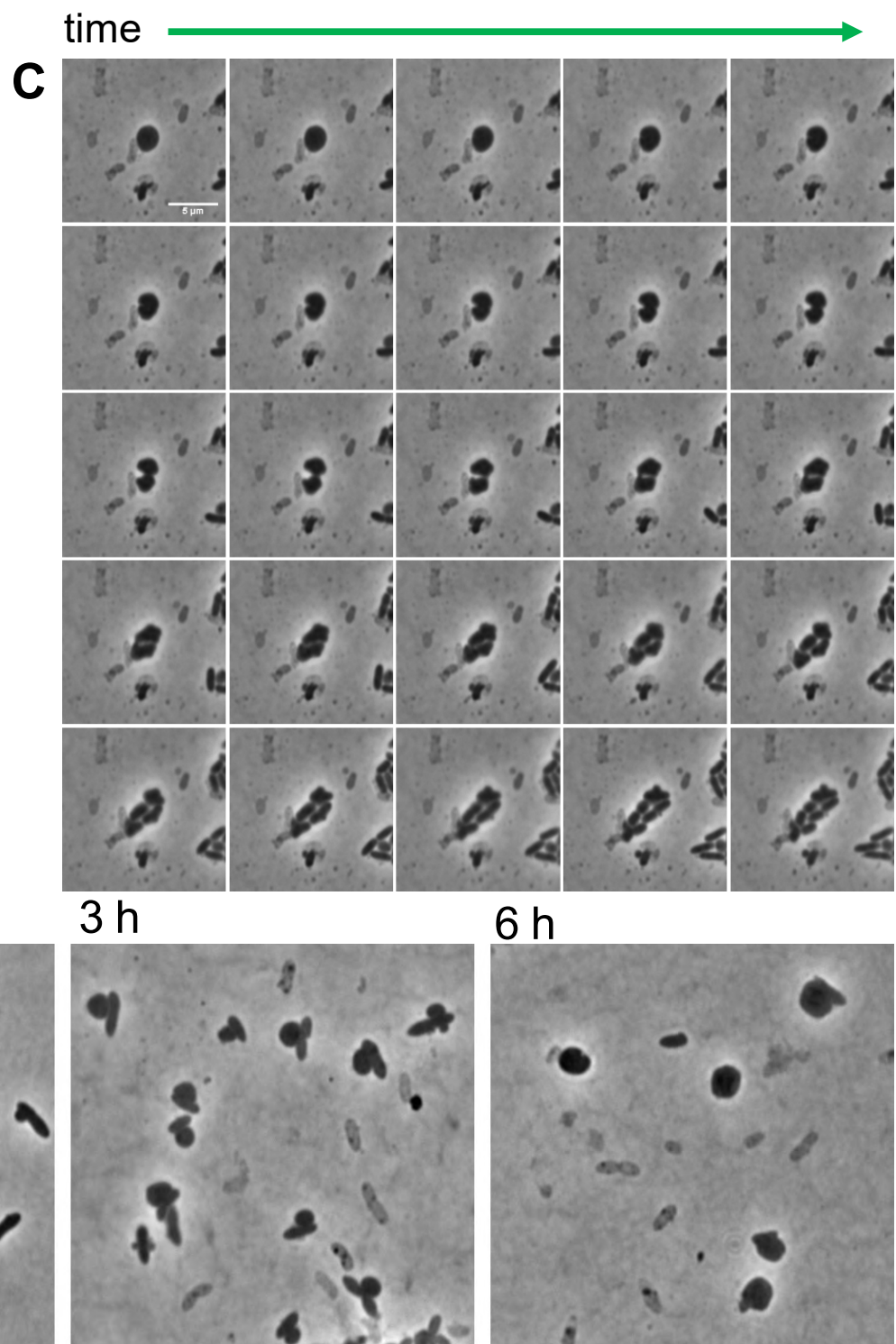
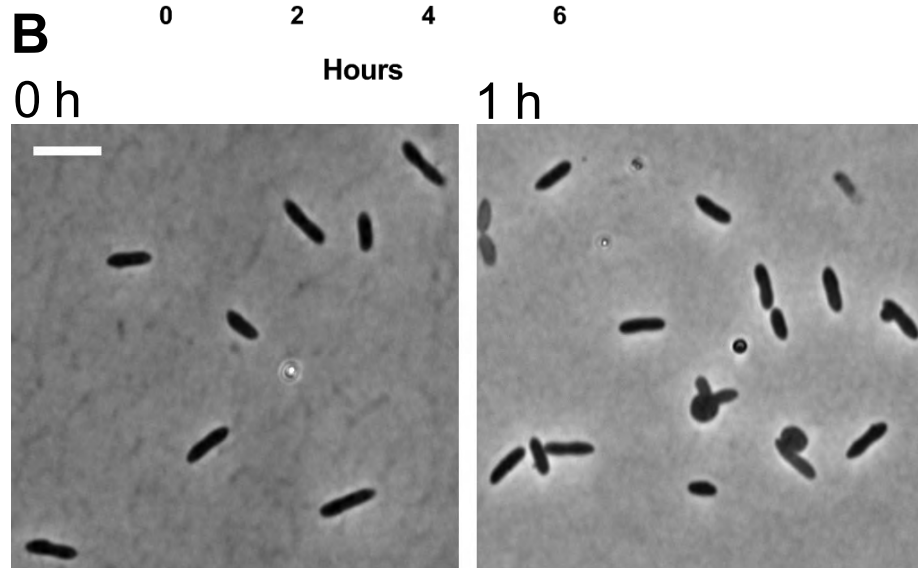
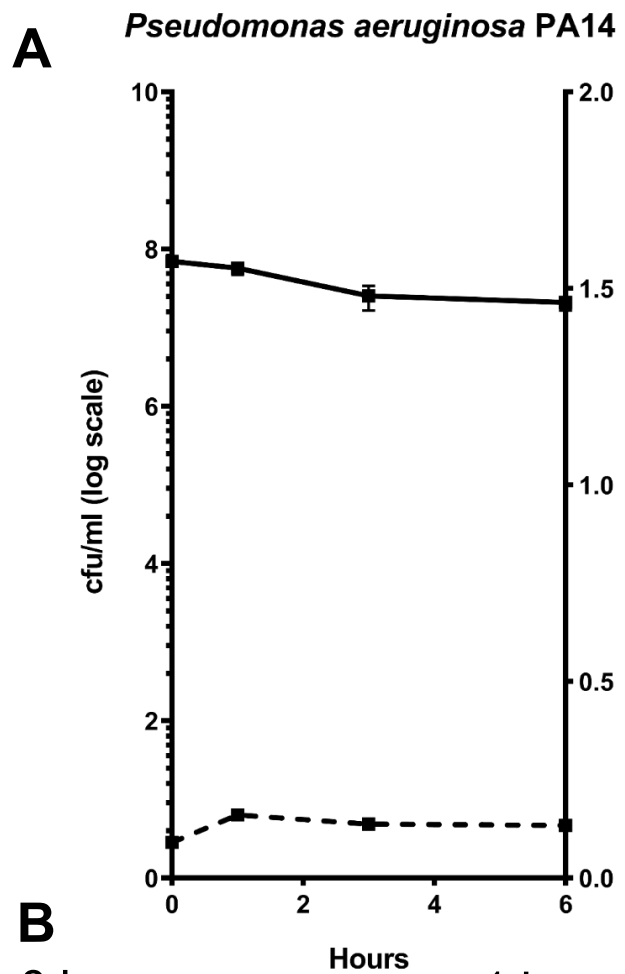


Fig. S2

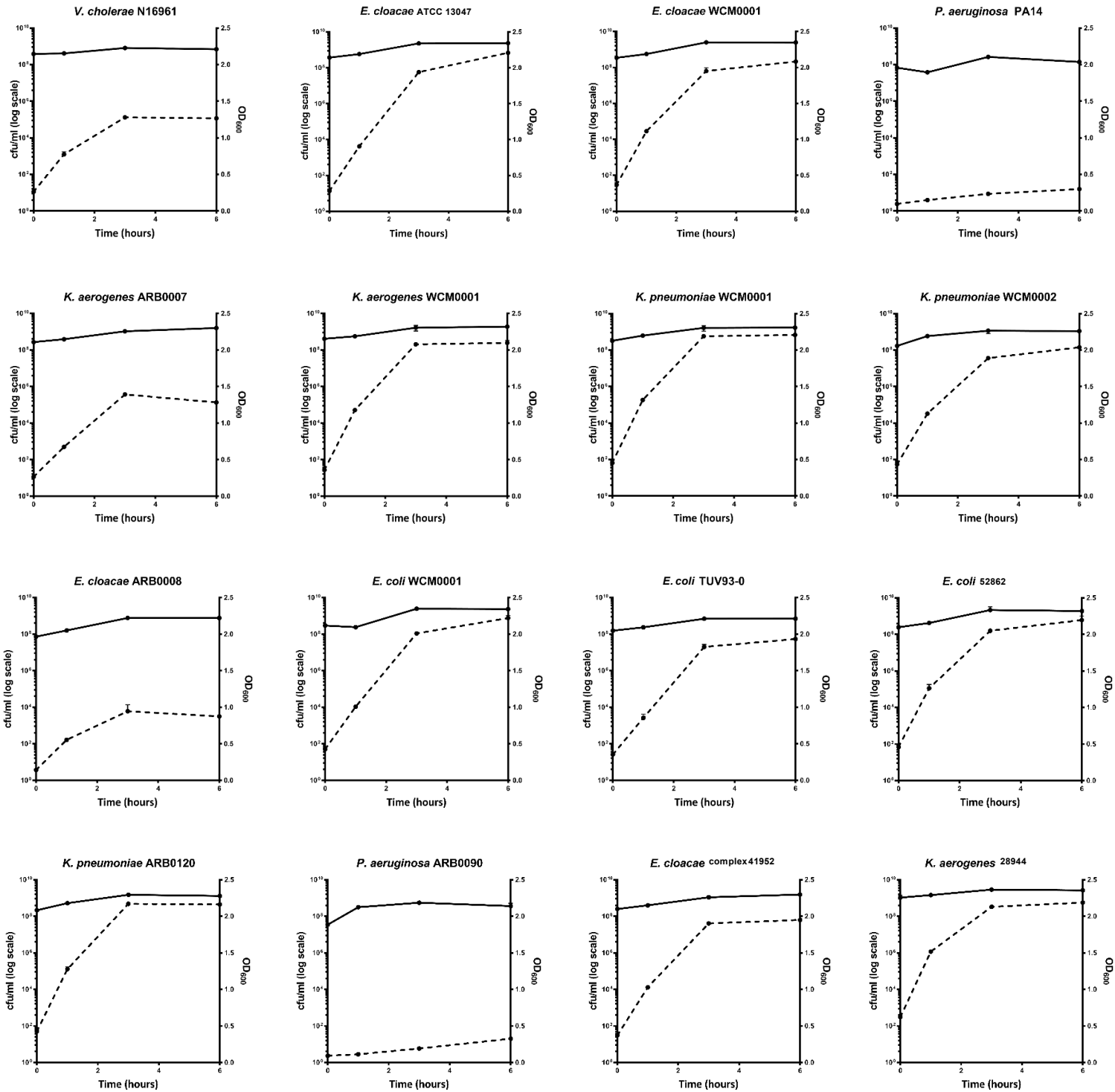


Fig. S3

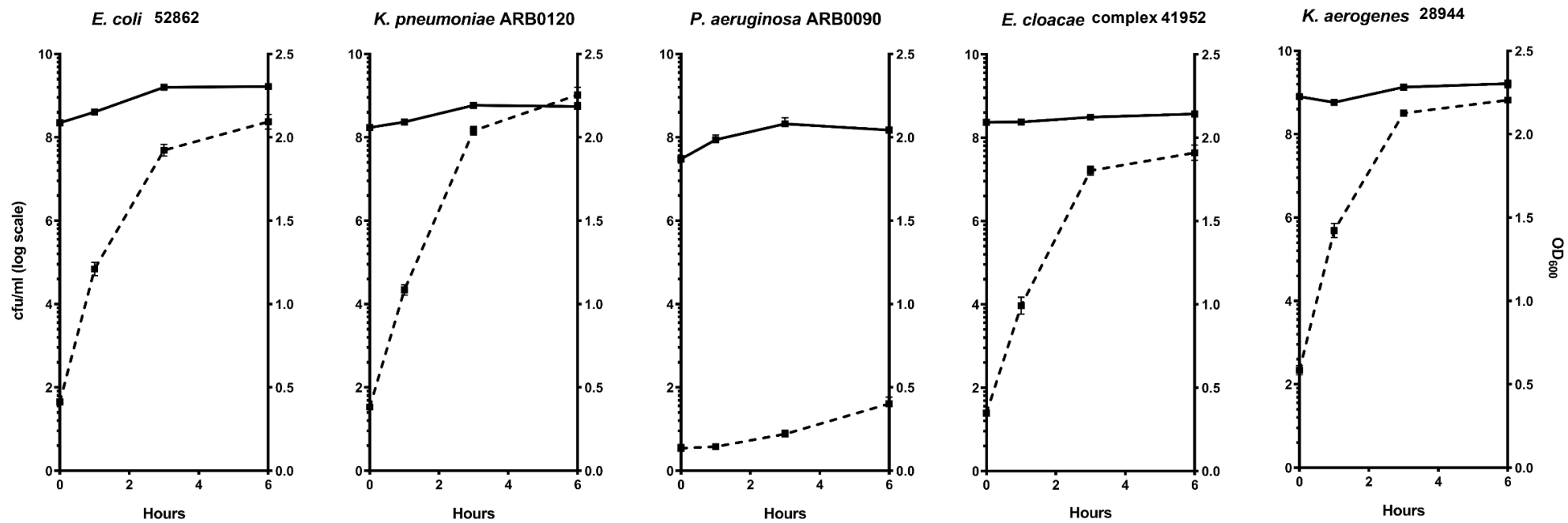
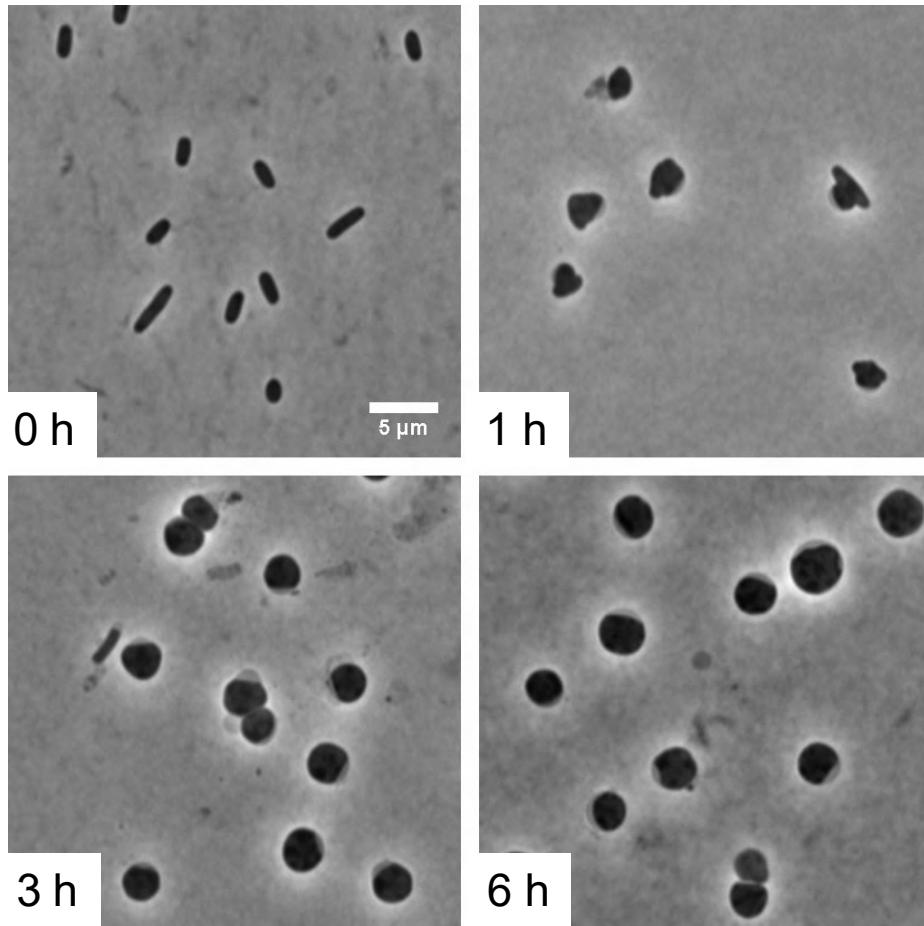


Fig. S4

E. cloacae ATCC13047

A



B

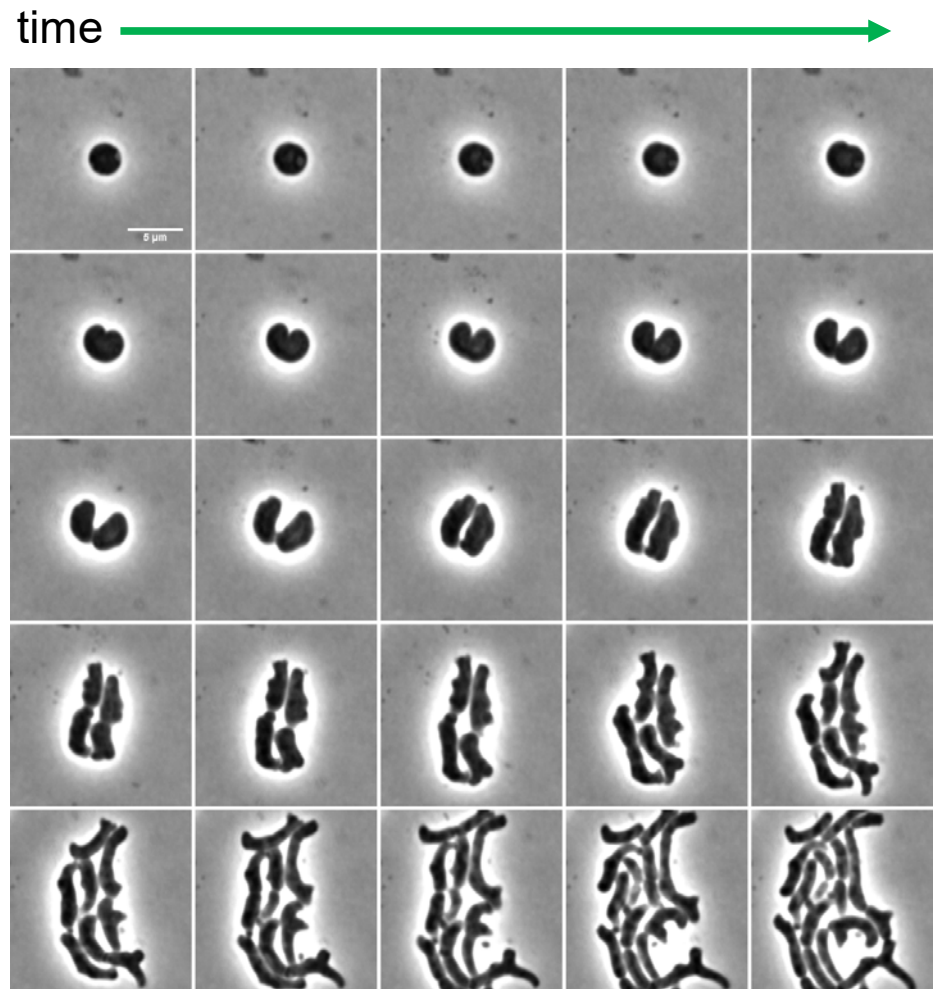
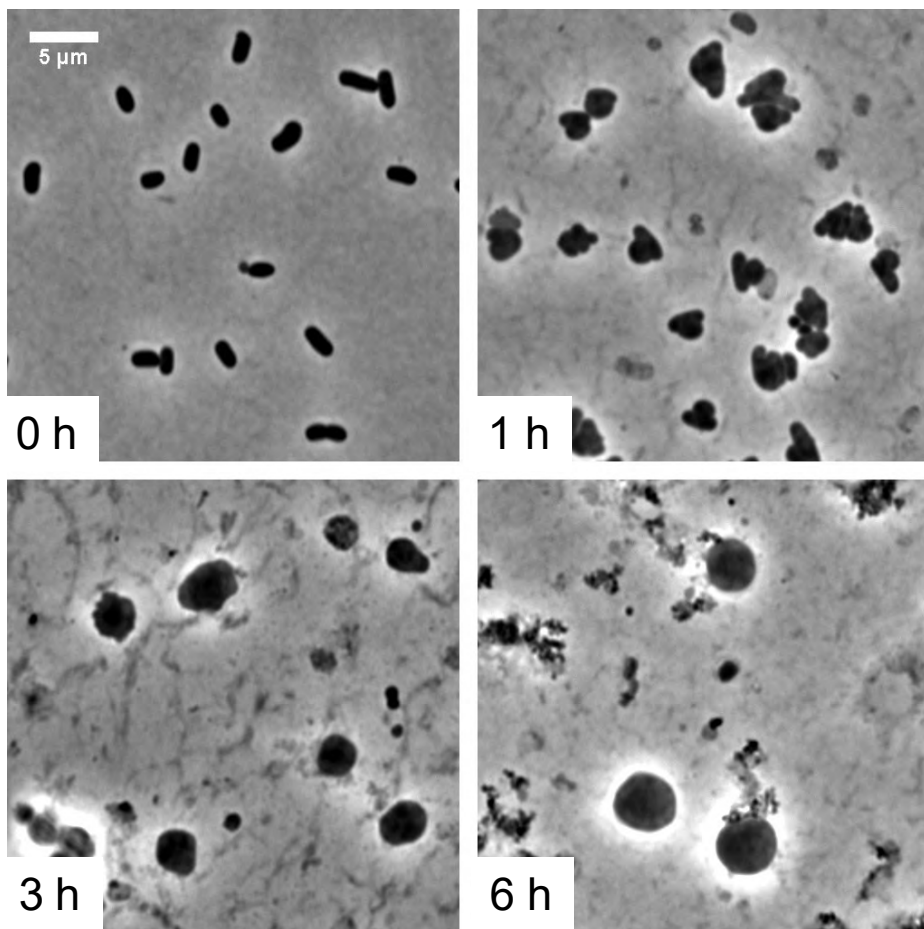


Fig. S5

K. aerogenes ARB0007

A



B

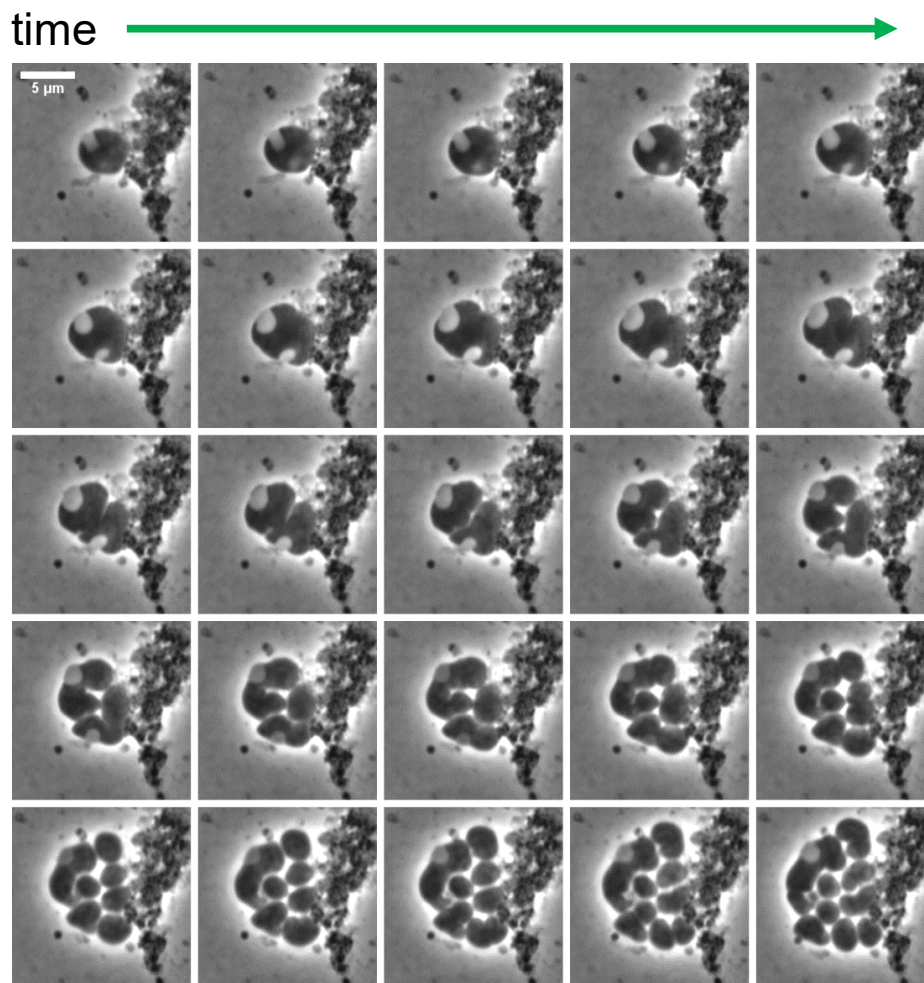
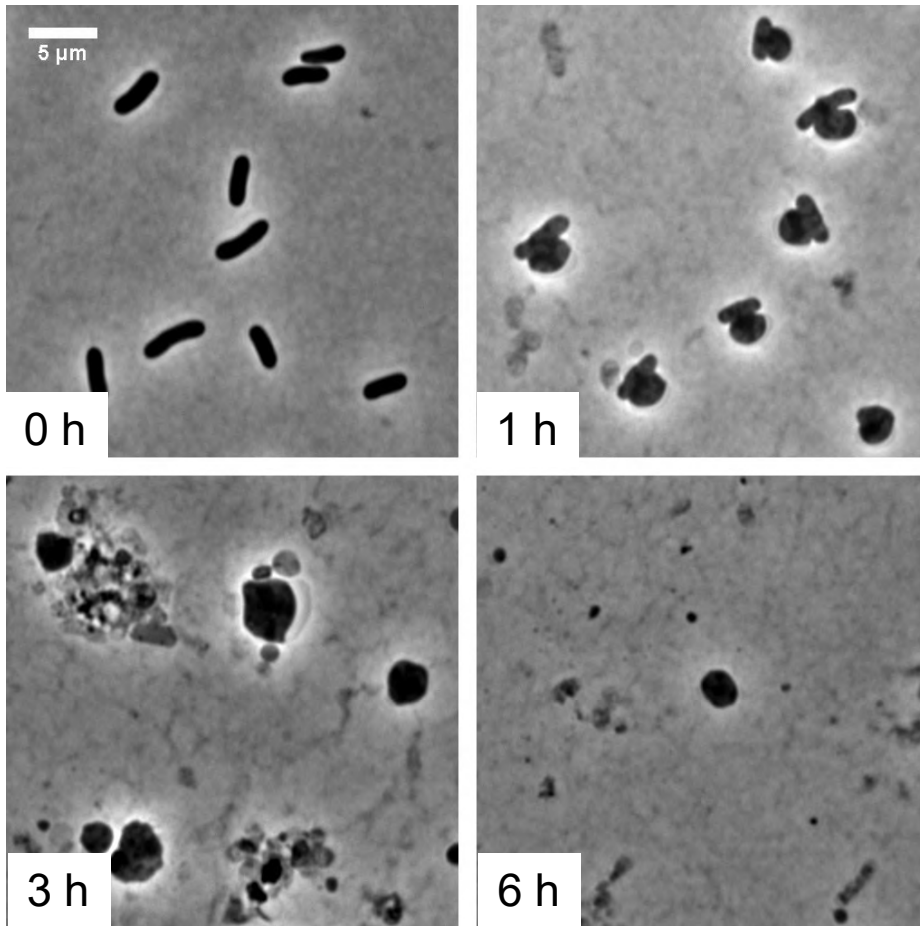


Fig. S6

K. pneumoniae WCM0002

A



B

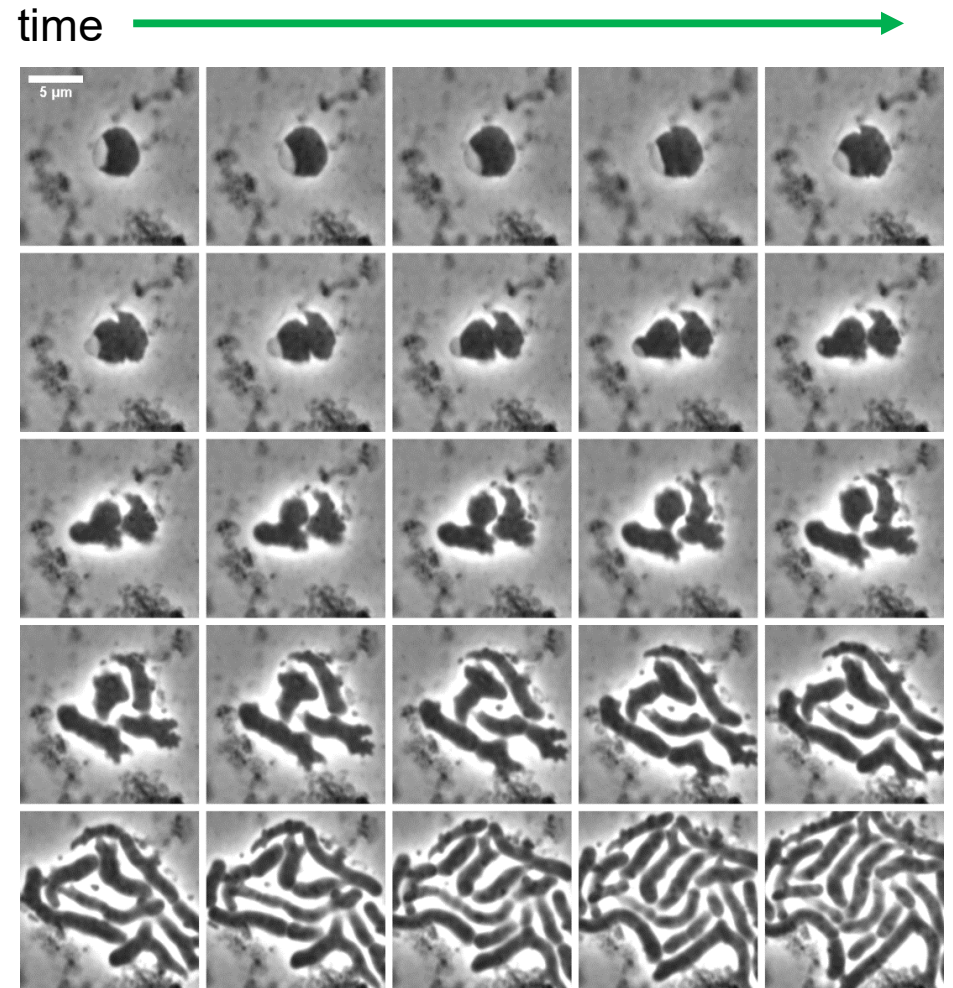


Fig. S7

E. cloacae ARB0008

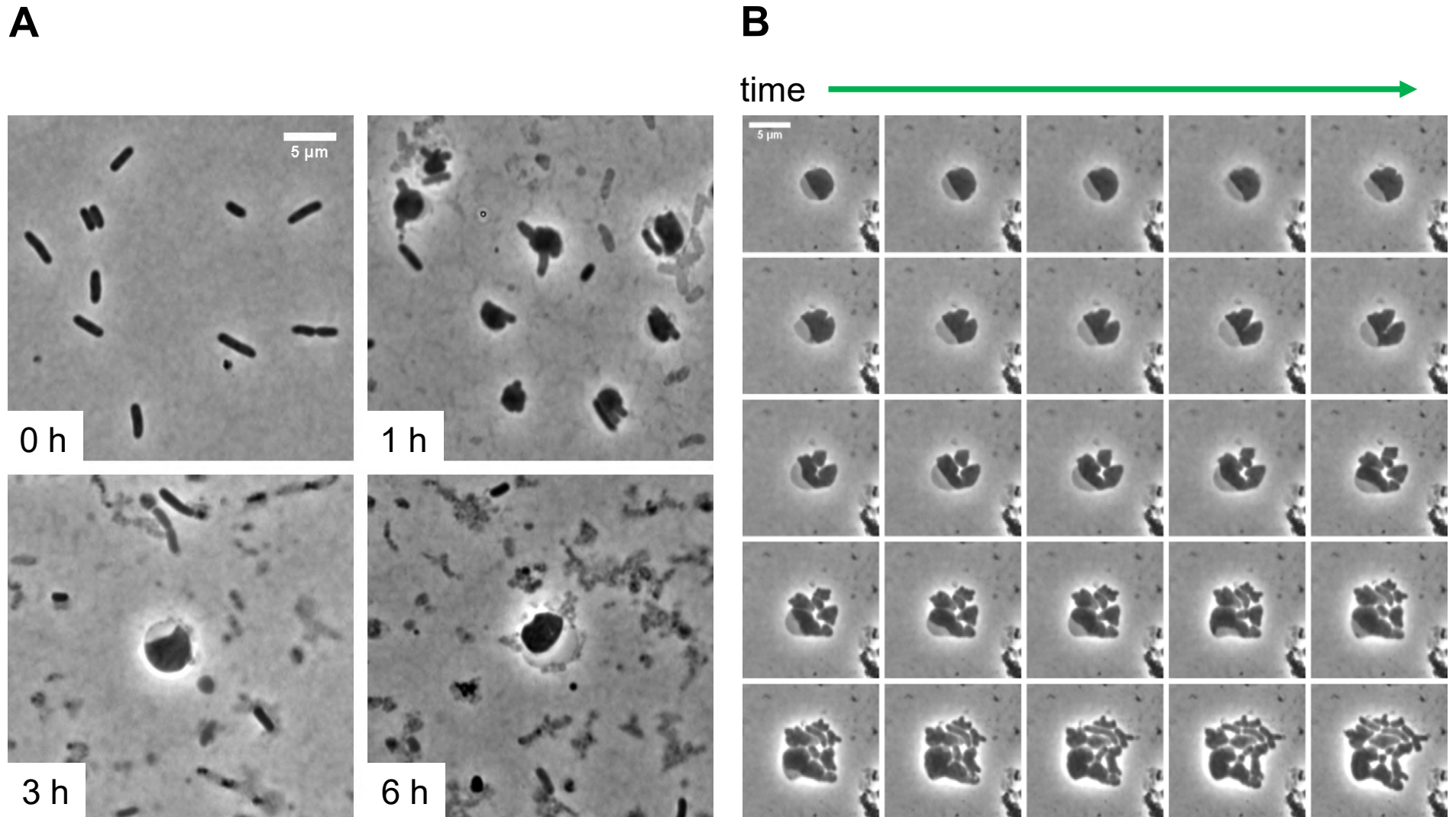


Fig. S8

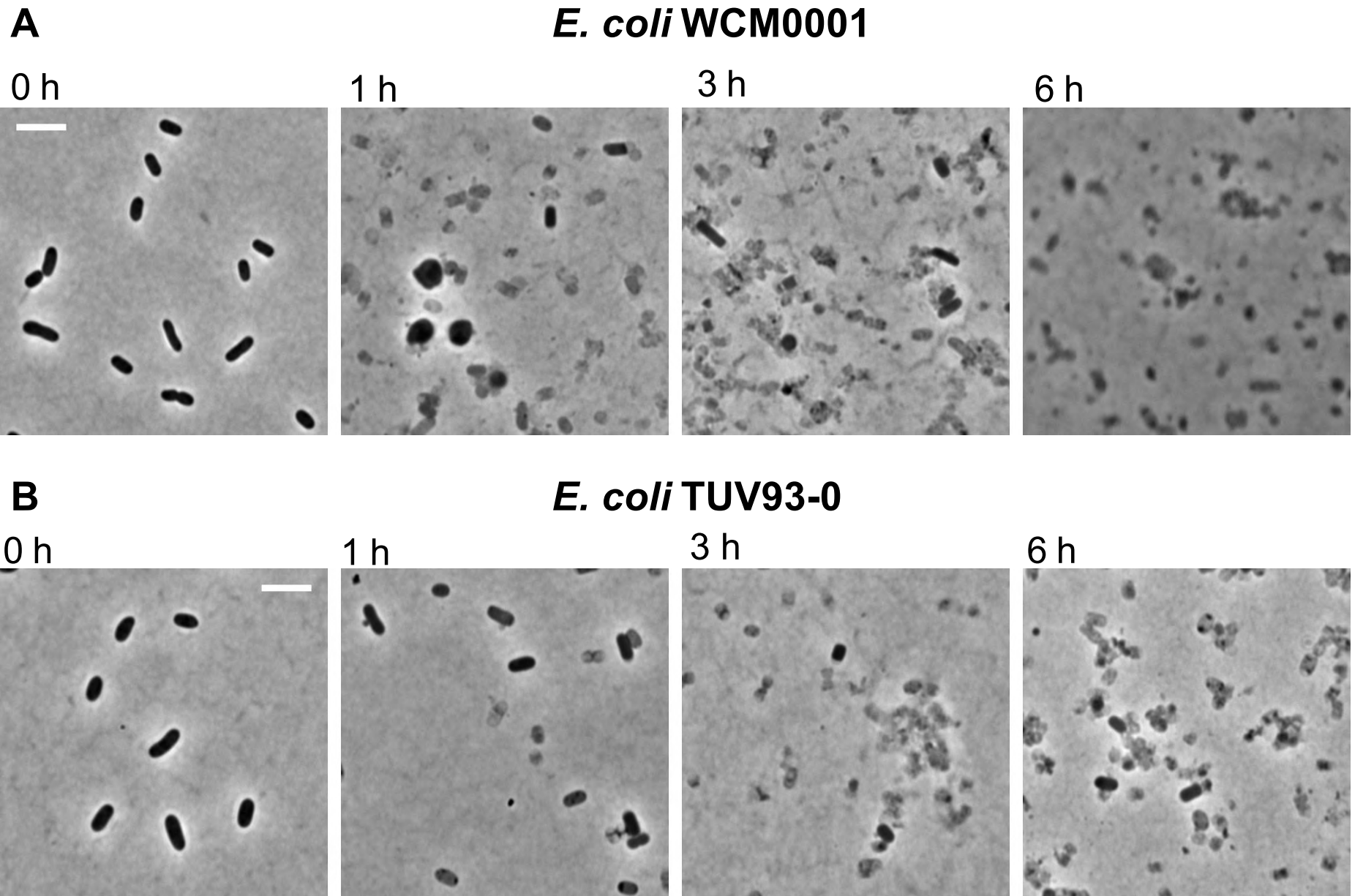


Fig. S9

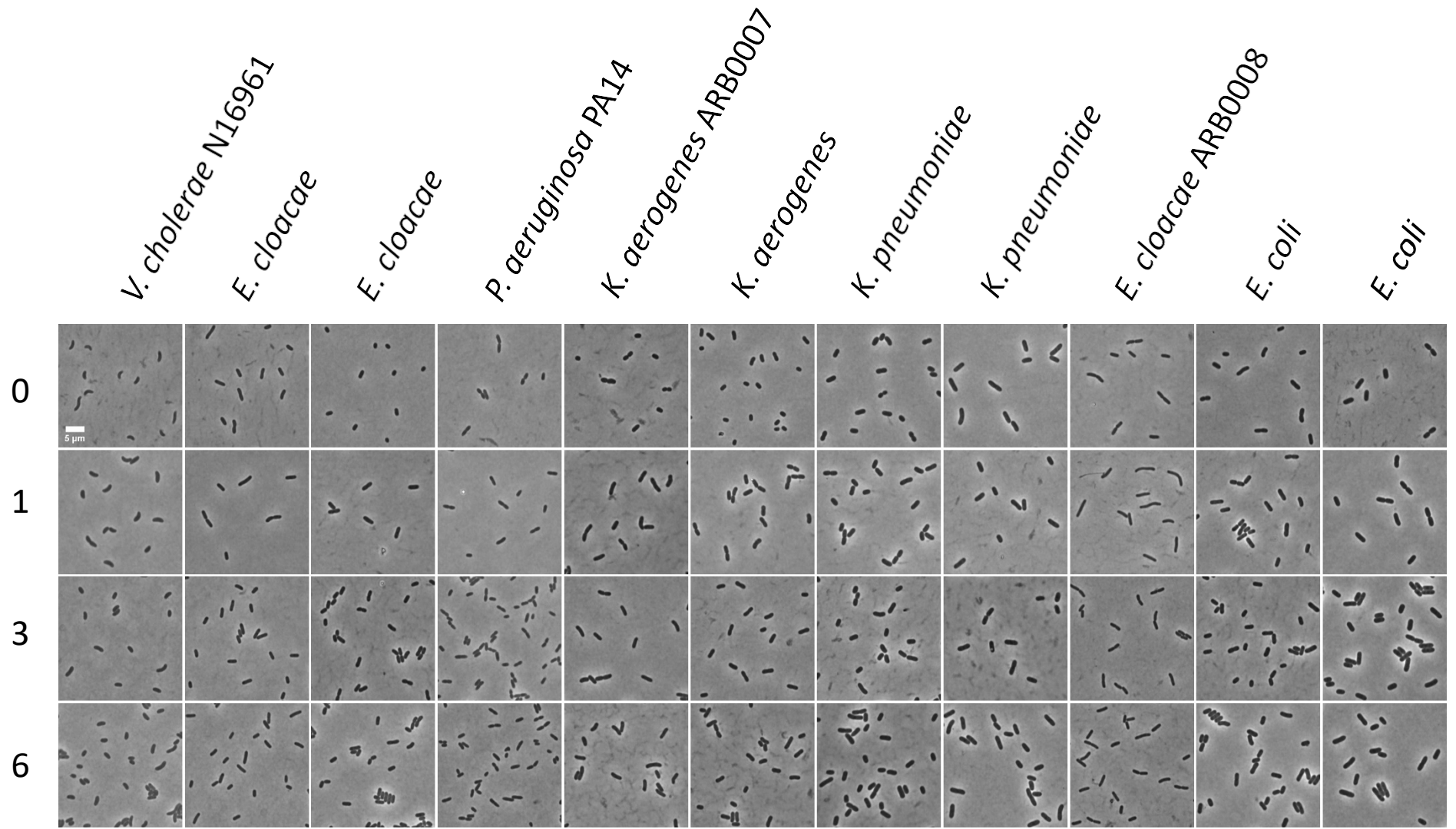


Fig. S10

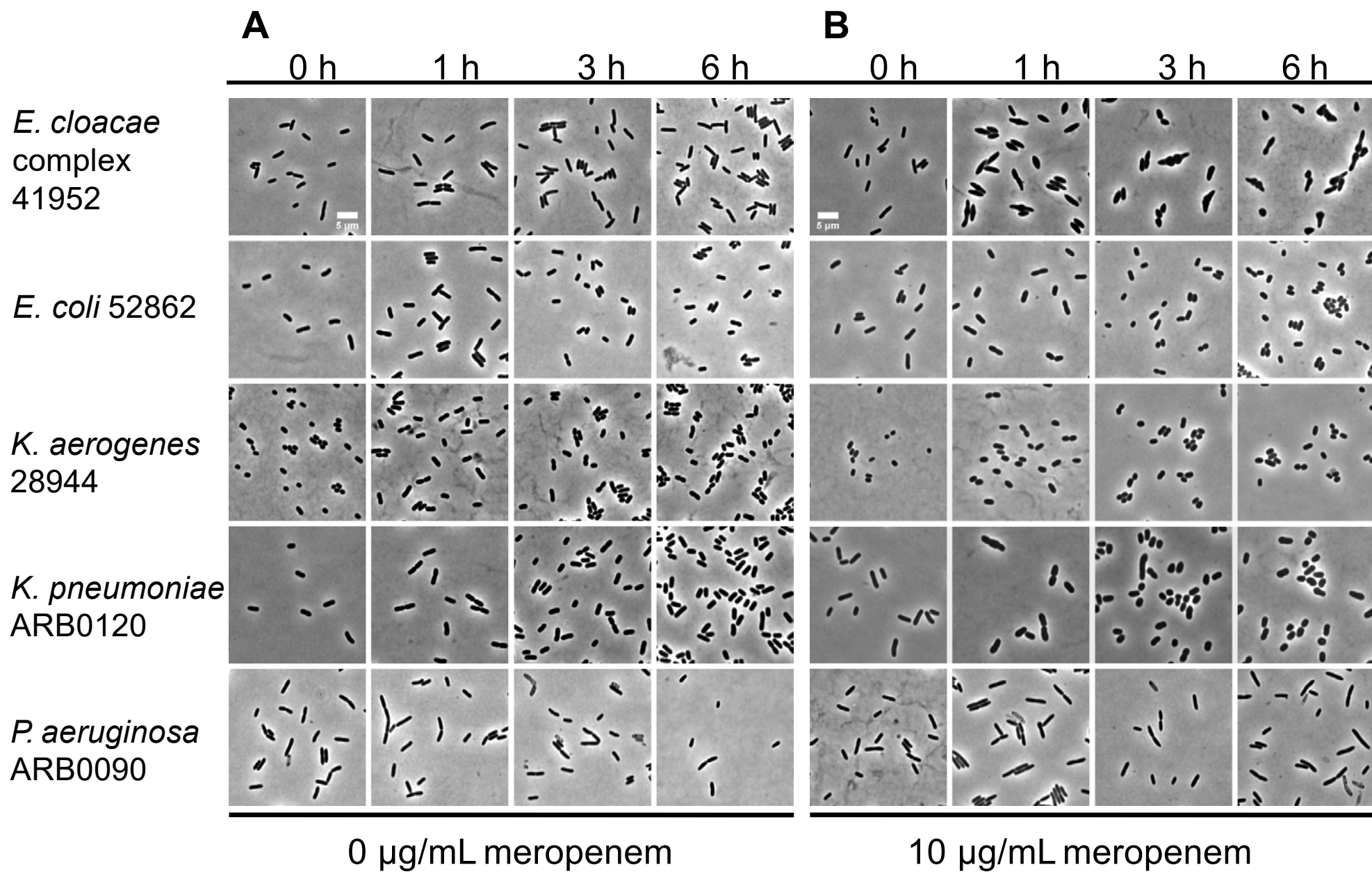


Fig. S11

Supplemental Figure Legends

Figure S1. Spheroplast formation and recovery in *V. cholerae* N16961

(A) Survival (cfu/mL, blue; OD₆₀₀, red) in the presence of 10 µg/mL meropenem; error bars show the standard error of the mean of three biological replicates. (B) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem. (C) Time-lapse images showing recovery upon removal of meropenem with purified NDM-1 carbapenemase (frames were acquired 5 min apart). Scale bars, 5 µm.

Figure S2. Spheroplast formation and recovery in *P. aeruginosa* PA14

(A) Survival (cfu/mL, solid lines; OD₆₀₀, dotted lines) in the presence of 10 µg/mL meropenem, error bars show the standard error of the mean of three biological replicates. (B) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem. (C) Time-lapse montage of spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours. Both *E. coli* isolates were omitted since no spheroplasts were observed after 6 hours of meropenem treatment. Scale bars, 5 µm.

Figure S3. Growth of susceptible/non-resistant and carbapenemases-producing isolates without antibiotic treatment.

Growth of the indicated isolates in BHI+ (cfu/mL, solid lines; OD₆₀₀, dotted lines), error bars show standard error of the mean of two biological replicates.

Figure S4. Growth of KPC-producing *Enterobacteriaceae* in the presence of meropenem. Growth of the indicated isolates in BHI+ (cfu/mL, solid lines; OD₆₀₀, dotted lines) supplemented with 10 µg/mL meropenem, error bars show standard error of the mean of two biological replicates.

Figure S5. Spheroplast formation and recovery in *E. cloacae* ATCC 13047

(A) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem in BHI+. **(B)** Time-lapse montage of spheroplast recovery upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were acquired 5 min apart for another 2 hours. Scale bars, 5 µm.

Figure S6. Spheroplast formation and recovery in *K. aerogenes* ARB0007

(A) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem in BHI+. **(B)** Time-lapse montage of spheroplast recovery upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+/0.8% (w/v) agarose. Images were acquired 5 min apart for another 2 hours. Scale bars, 5 µm.

Figure S7. Spheroplast formation and recovery in *K. pneumoniae* WCM0002

(A) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem.

(B) Time-lapse montage of spheroplast recovery upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were acquired 5 min apart for another 2 hours. Scale bars, 5 µm.

Figure S8. Spheroplast formation and recovery in *E. cloacae* ARB0008

(A) Spheroplast formation at 0, 1, 3, and 6 hours after exposure to 10 µg/mL meropenem.

(B) Time-lapse montage of spheroplast recovery upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were acquired 5 min apart for another 2 hours. Scale bars, 5 µm.

Figure S9. Absence of spheroplasts in *E. coli* isolates. Overnight cultures of *E. coli* WCM0001 **(A)**, and TUV93-0 **(B)** were subcultured 1:10 (final volume 5 mL) into pre-warmed BHI+ liquid medium supplemented with 10 µg/mL meropenem and imaged at the indicated time points. Scale bar, 5 µm.

Figure S10. Cell Morphology without antibiotic treatment. Overnight cultures of the indicated isolates were diluted 10-fold into BHI+ liquid media, and imaged at the indicated time points. Scale bar, 5 µm.

Figure S11. Cell Morphology of KPC-producing *Enterobacteriaceae*.

Overnight cultures of the indicated isolates were diluted 10-fold into fresh BHI+ liquid media containing vehicle **(A)**, or meropenem (10 µg/mL) **(B)**, and imaged at the indicated time points. Scale bar, 5 µm.

Supplemental Movie 1. *E. cloacae* WCM0001 post-meropenem recovery.

Time-lapse movie of *E. cloacae* WCM0001 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 2. *K. aerogenes* WCM0001 post-meropenem recovery.

Time-lapse movie of *K. aerogenes* WCM0001 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 3. *K. pneumoniae* WCM0001 post-meropenem recovery.

Time-lapse movie of *K. pneumoniae* WCM0001 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 4. *V. cholerae* N16961 post-meropenem recovery.

Time-lapse movie of *V. cholerae* N16961 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 5. *P. aeruginosa* PA14 post-meropenem recovery.

Time-lapse movie of *P. aeruginosa* PA14 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 6. *E. cloacae* ATCC 13047 post-meropenem recovery.

Time-lapse movie of *E. cloacae* ATCC 13047 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 7. *K. aerogenes* ARB0007 post-meropenem recovery.

Time-lapse movie of *K. aerogenes* ARB0007 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 8. *K. pneumoniae* WCM0002 post-meropenem recovery.

Time-lapse movie of *K. pneumoniae* WCM0002 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.

Supplemental Movie 9. *E. cloacae* ARB0008 post-meropenem recovery.

Time-lapse movie of *E. cloacae* ARB0008 spheroplasts upon removal of meropenem after 6 hours of treatment. The antibiotic was removed by addition of purified NDM-1 carbapenemase, followed by time-lapse microscopy on BHI+ agarose pads (0.8 % [w/v] agarose). Images were then acquired 5 min apart for another 2 hours.