Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information: Electrochemically Monitoring the Antibiotic Susceptibility of Pseudomonas aeruginosa Biofilms

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CALIBRATION CURVE FOR PA14 GROWN IN TRYPTICASE SOY BROTH (TSB):

Square wave voltammograms (SWVs) for concentrations of pyocyanin from 0 to 50 µM were obtained using three different disposable Zensor electrodes. The dilution series was repeated twice and each concentration was scanned three times per disposable electrode. The average maximum current for both runs through the dilution series was averaged and plotted versus current.

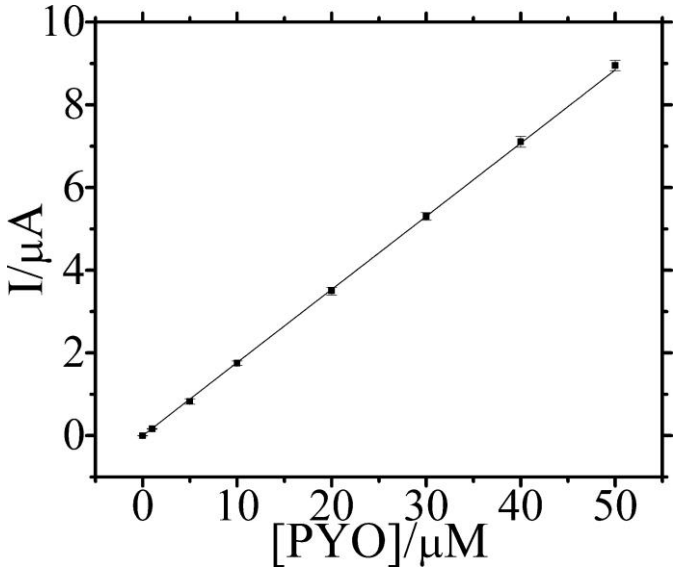

Fig. S1: Maximum current from SWVs of pyocyanin in 30 g/L TSB from 0 to 50 µM. SWVs were performed from -0.5 to 0 V at a frequency of 15 Hz and an amplitude voltage of 50 mV. Linear fit: I/µA=0.18[PYO]/µM ; I = current and [PYO] = concentration of pyocyanin in TSB.

SCANNING ELECTRON MICROGRAPHS OF PA14 GROWN IN CHAMBERS:

P. aeruginosa samples were prepared for SEM analysis to determine where in the growth chamber the bacteria were collecting. Samples were prepared by peeling the PDMS from the electrode and fixing the bacteria using a 2.5% glutaraldehyde in 0.1 M cacodyalate buffer (pH 7.2) at 4°C for 2 hours. Samples were dehydrated in an increasing dilution series of ethanol and then critical point dried using liquid CO_2.

Resulting SEMs are reported in Fig. S2 and S3 and show the clear presence of biofilms growing in the chamber.

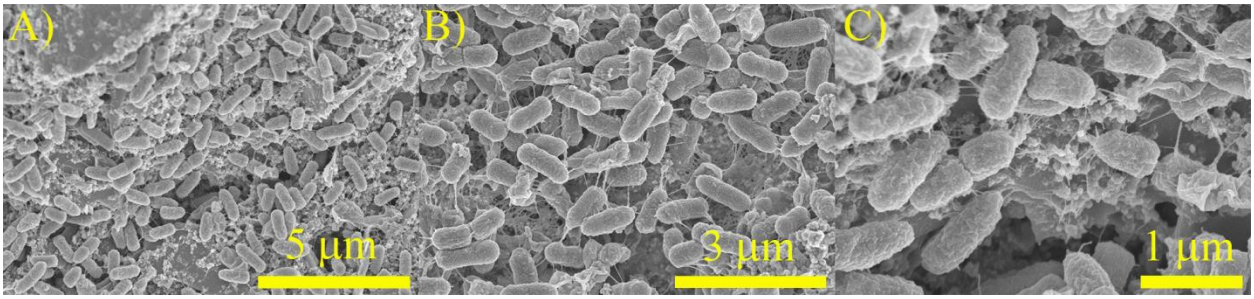

Fig. S2: SEM Images of *P. aeruginosa* grown on the working electrode of a three electrode cell. From left to right, SEM images were taken at a magnification of 9,000 X, 15,000 X, and 30,000 X at 3 kV. Note the presence of a large number of cells in all three images interlaced embedded in extracellular matrix.

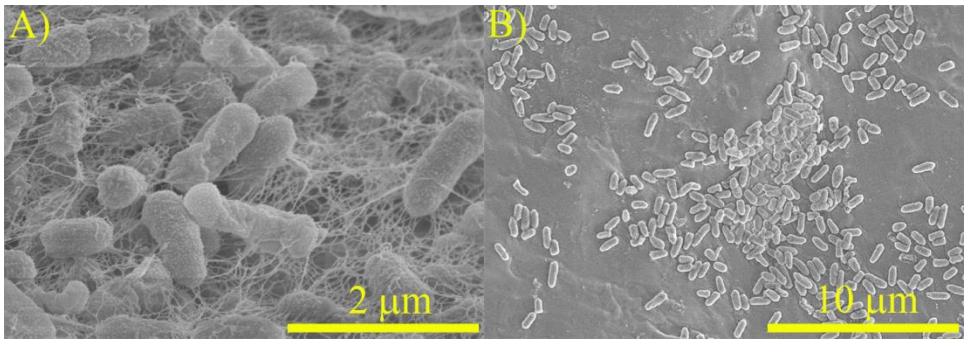

Fig. S3: SEM Images of A) *P. aeruginosa* cells in the access hole of a PDMS device leading to the chamber area containing the sensor, and B) cells attached to the surface of the PDMS that forms the top of the sensing chamber.

SQUARE WAVE VOLTAMMOGRAMS OVER TIME:

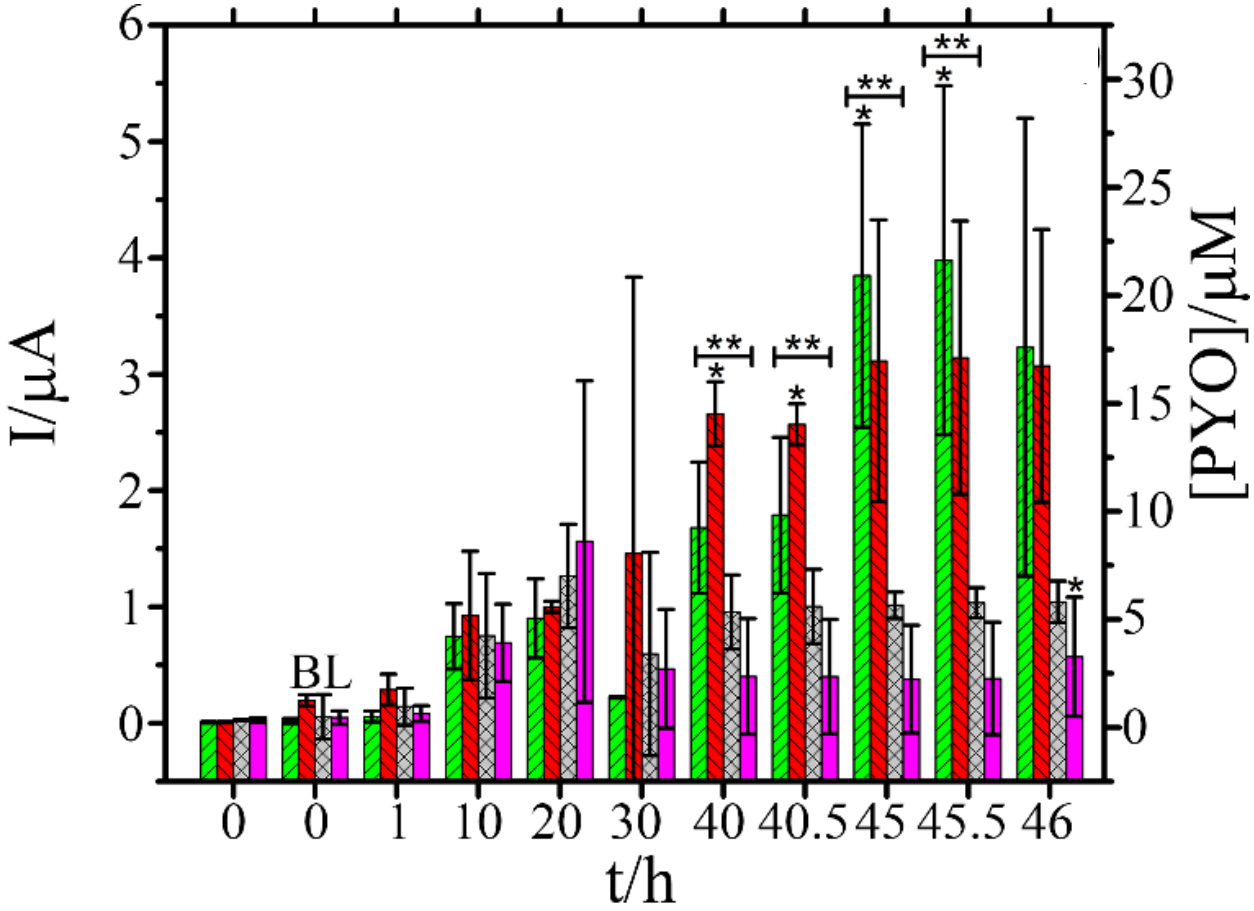

Fig. S4. Response of PA14 biofilms at selected time points during the 48 hour experiments. (BL=Bacteria loaded into the chamber). Left axis: average peak current (blank subtracted) measured over time in PA14 cultures exposed to colistin sulfate at 0 (green right slash), 4 (red left slash, low MIC), 16 (blue crosses, High MIC), and 100 mg/L (pink no slash lines). Right axis: Approximate pyocyanin concentration based on calibration curve. * indicates time points where only two replicates were used. ** indicates P<0.05 from ANOVA analysis of 16 and 100 mg/L antibiotic concentrations against the control.

SWVs were taken every 30 minutes during exposure of cells to colistin sulfate. *Pseudomonas aeruginosa* and *Escherichia coli* were exposed separately to colistin sulfate at 4, 16, and 100 mg/L at a flow rate of 100 nL/min. Of interest is the complete lack of discernible peaks from SWVs for *E. coli* cells exposed to colistin sulfate compared to *P. aeruginosa* (Fig. S5-S7). Scans are from one replicate but are representative all acquired scans.

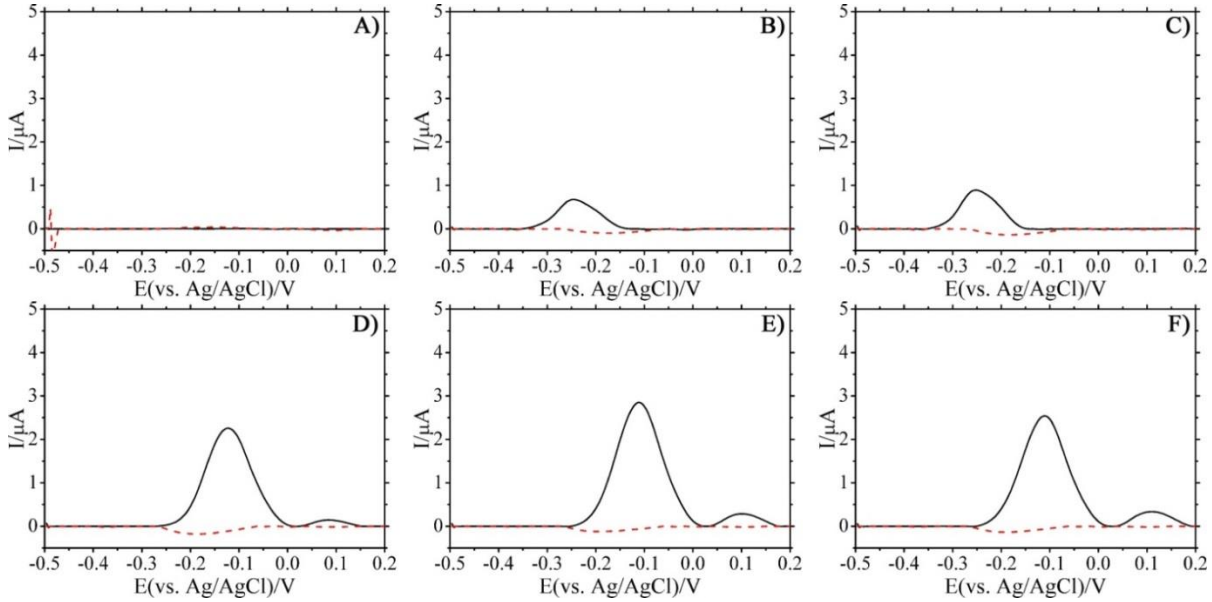

Fig. S5: SWVs of PA14 (solid black lines) and *E. coli* (red dashed liens) cultured in trypticase soy broth after loading 24 μL of overnight culture after A) 0 h, B) 12 h, C) 22 h, D) 35 h, E) 40 h, and F) 45 h. Flow of 4 mg/L colistin sulfate in fresh TSB at 100 nL/min was initiated at 22 h. SWVs performed from -0.5 to 0.2 V at a frequency of 15 Hz and an amplitude voltage of 50 mV.

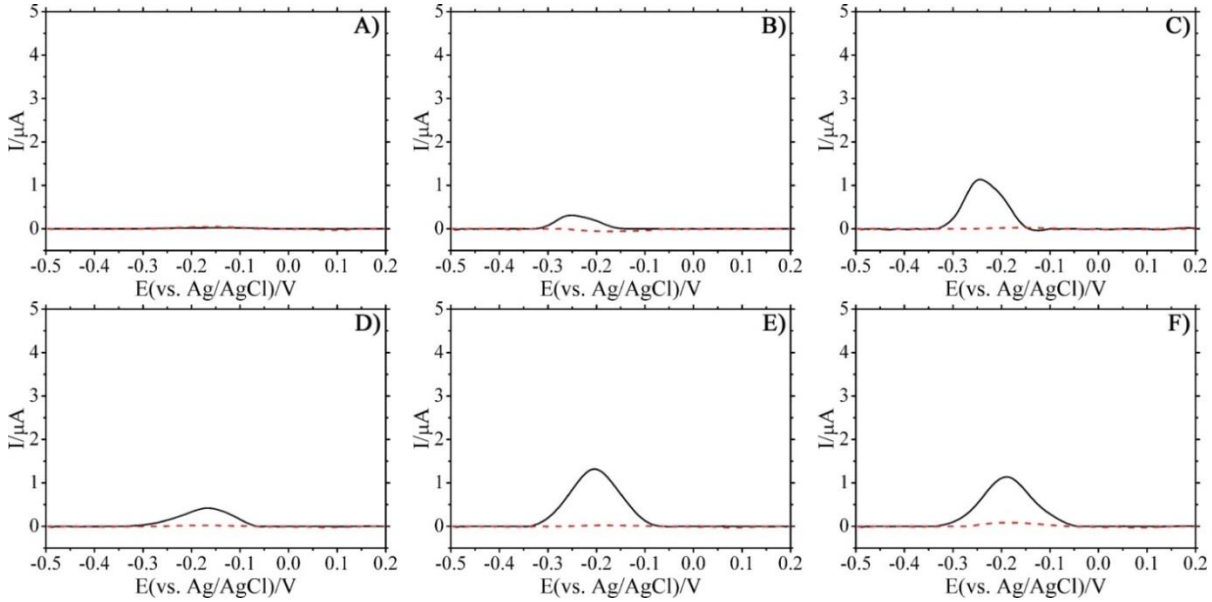

Fig. S6: SWVs of PA14 (solid black lines) and *E. coli* (red dashed lines) cultured in trypticase soy broth after loading 24 μL of overnight culture after A) 0 h, B) 12 h, C) 22 h, D) 35 h, E) 40 h, and F) 45 h. Flow of 16 mg/L colistin sulfate in fresh TSB at 100 nL/min was initiated at 22 h. SWVs performed from -0.5 to 0.2 V at a frequency of 15 Hz and an amplitude voltage of 50 mV.

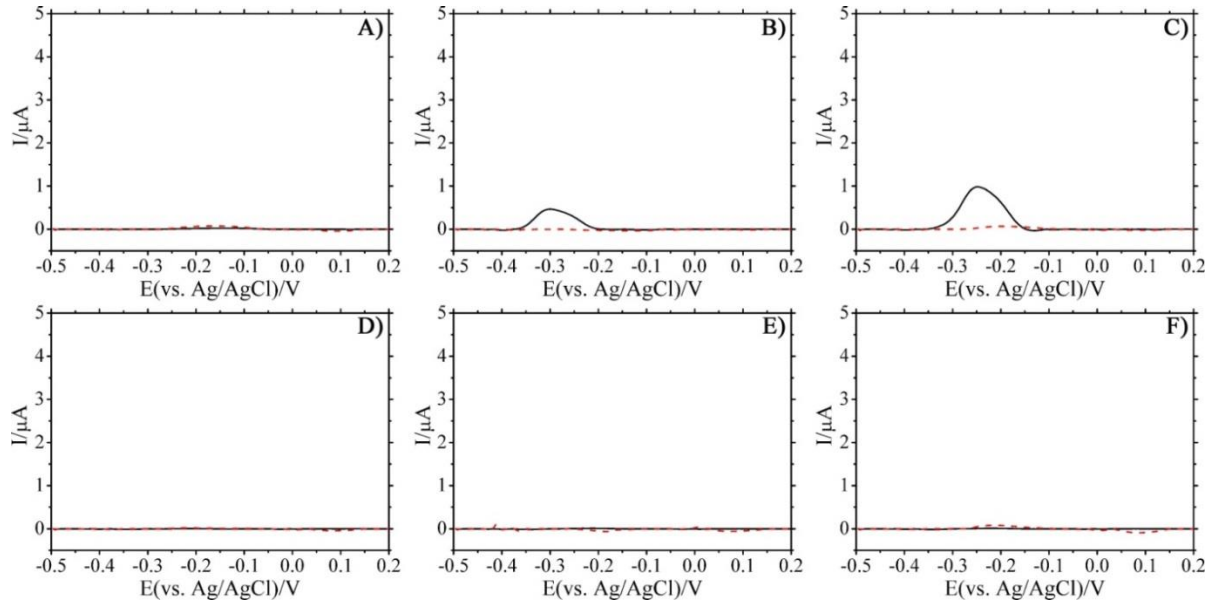

Fig. S7: SWVs of PA14 (solid black lines) and *E. coli* (red dashed lines) cultured in trypticase soy broth after loading 24 μL of overnight culture after A) 0 h, B) 12 h, C) 22 h, D) 35 h, E) 40 h, and F) 45 h. Flow of 100 mg/L colistin sulfate in fresh TSB at 100 nL/min was initiated at 22 h. SWVs performed from -0.5 to 0.2 V at a frequency of 15 Hz and an amplitude voltage of 50 mV.

EXPOSING PA14 TO AMPICILLIN:

PA14 cells were exposed to 100 mg/L of ampicillin under the same conditions as those subjected to 100 mg/L colistin sulfate exposure. A comparison of SWVs with the two antibiotics is shown in Fig. S7. Of note is that cells exposed to ampicillin continued to show an electrochemical response compared to those exposed to colistin sulfate. This was expected since ampicillin is known to be ineffective at killing *P. aeruginosa*.

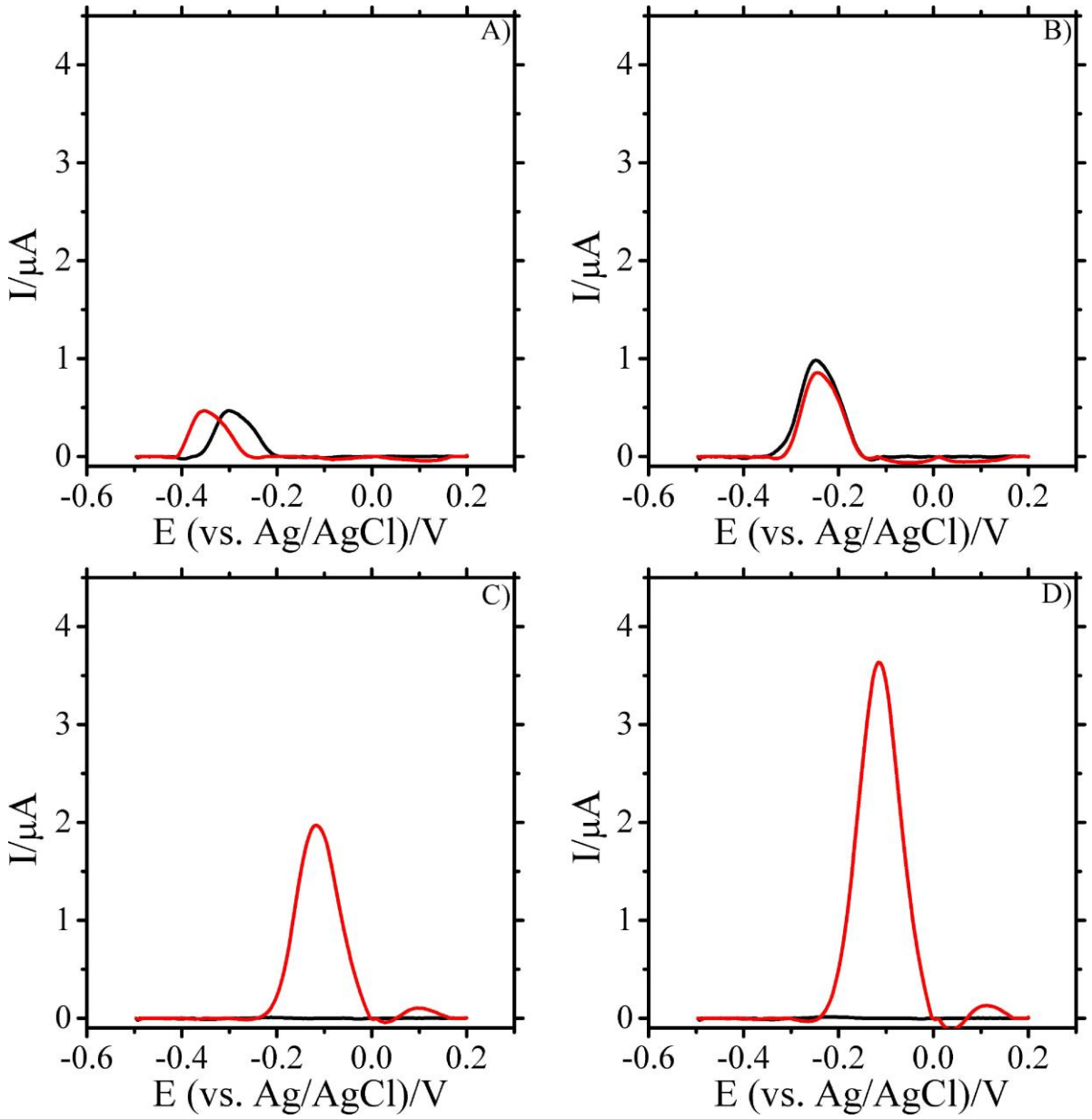

Fig. S8 SWVs of *P. aeruginosa* grown in TSB without flow for A) 12 h. The cells were then exposed to flowing 100 mg/L ampicillin (red lines) or colistin sulfate (black lines) in TSB starting at B) 22 h. SWVs C) 35 h and D) 40 h after the start of the experiment. Baseline signal was subtracted from resulting scans. SWV performed from -0.5 to 0.2 volts at a frequency of 15 Hz and an amplitude voltage of 50 mV.

EXPOSING PA14 LIQUID CULTURES TO COLISTIN PLATES

Liquid cultures of PA14, grown over night in 3 mL of TSB, were plated directly onto cetrimide agar plates containing 0, 4, and 100 mg/L of colistin sulfate. These plates were incubated overnight at 37 °C. The plates were then inspected for bacterial growth (Fig. S8). Plates containing 0 and 4 mg/L colistin sulfate showed growth, whereas plates containing 100 mg/L colistin sulfate showed no colony formation. Colony formation at the lower MIC value of 4 mg/L colistin sulfate agrees with the results reported in Fig. 3 and Fig. 4.

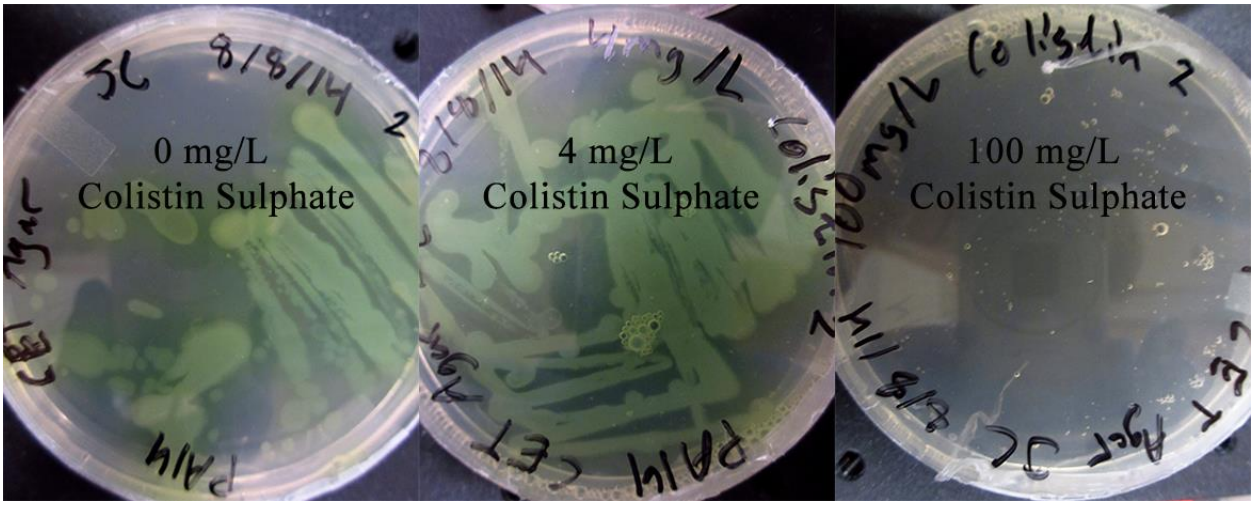

Fig. S9 Images of PA14 colonies after 20 hours of growth at 37 °C on cetrimide agar plates mixed with different concentrations of colistin sulfate.

VIABILITY OF CELLS AFTER EXPOSURE TO COLISTIN SULFATE:

After exposure to colistin sulpahte at 4 and 100 mg/L flowing at 100 nL/min, the PDMS chambers were peeled from the disposable electrodes. A sterile loop was placed onto the electrodes, then streaked onto fresh TSB agar plates. Samples were incubated at 37 °C, and monitored for growth. Cells exposed to 4 mg/L colistin sulfate began producing visible colonies after only 4 h of incubation. Over 24 h of incubation was required for colonies to form from cells exposed to 100 mg/L colistin sulfate. This indicates that cells were affected by the presence of colistin sulfate in solution at higher concentrations.

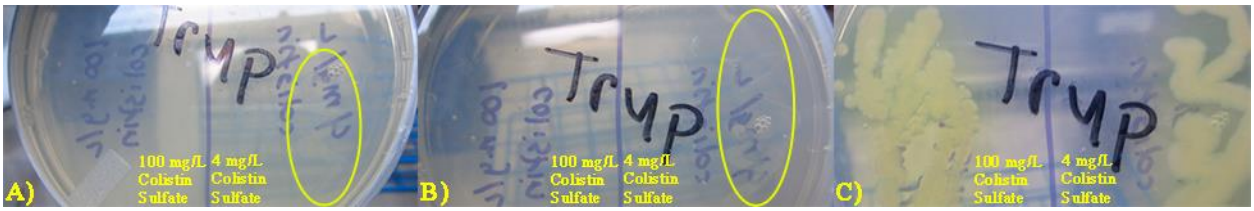

Fig. S10: PA14 streaked onto TSB after exposure to 100 mg/L and 4 mg/L colistin for 20 hours within PDMS flow chambers. Photographs show the plate after A) 4.33 h, B) 6.5 h, and C) 74.3 h of incubation. The plate was incubated at 37 °C for the first 24 hours, then grown at room temperature for the remaining time to prevent the agar from drying out.