

Supplementary Materials

Article Title: Antibiotics against *Pseudomonas aeruginosa* on Human Skin Cell Lines: Determination of the Highest Non-Cytotoxic Concentrations with Antibiofilm Capacity for Wound Healing Strategies.

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Preliminary Evaluation.

High Concentration of Antibiotics Cause a Reduction in Cell Proliferation in Skin Cell Lines.

For HFs, 1.25 mg/mL AMK, 0.02 mg/mL CIP, 0.034 mg/mL COL and 1 mg/mL MER were the concentrations maintaining a cell proliferation level comparable to the control (Figure S1). For hKTs, 1.25 mg/mL AMK, 0.02 mg/mL CIP, 0.017 mg/mL COL and 1 mg/mL MER (Figure S2) were chosen for the following evaluation. It should be note that cell proliferation after AMK and COL treatments were notably reduced when compared to the control group.

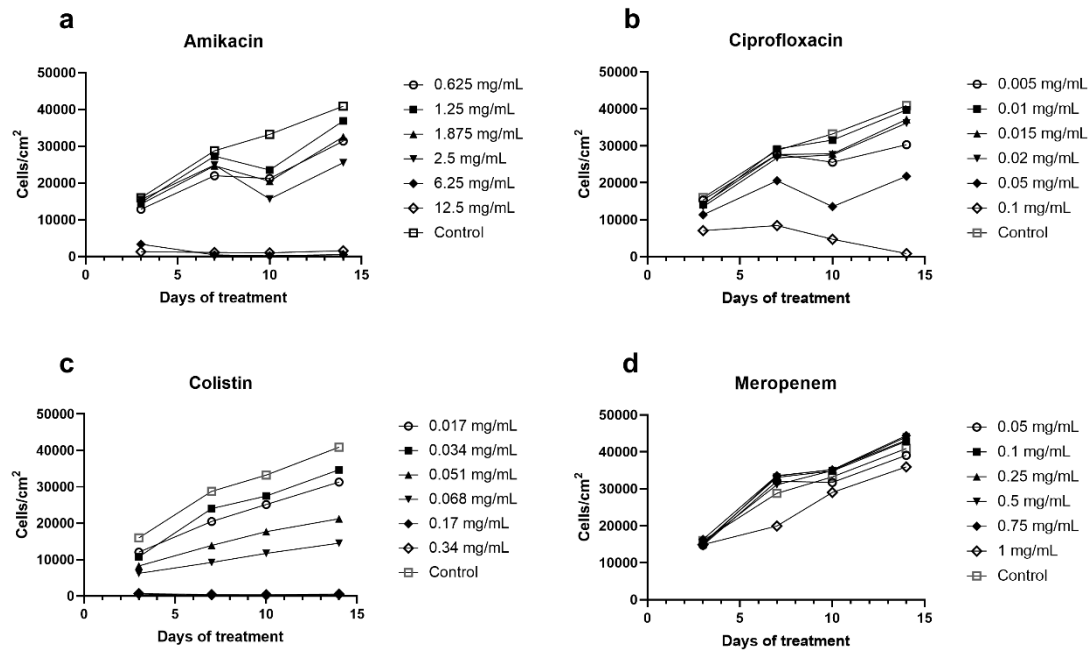


Figure S1. HF proliferation after (a) AMK (0.625 - 12.5 mg/mL), (b) CIP (0.005 - 0.1 mg/mL), (c) COL (0.017 - 0.34 mg/mL) and (d) MER (0.05 - 1 mg/mL) treatments in a 14-day follow-up.

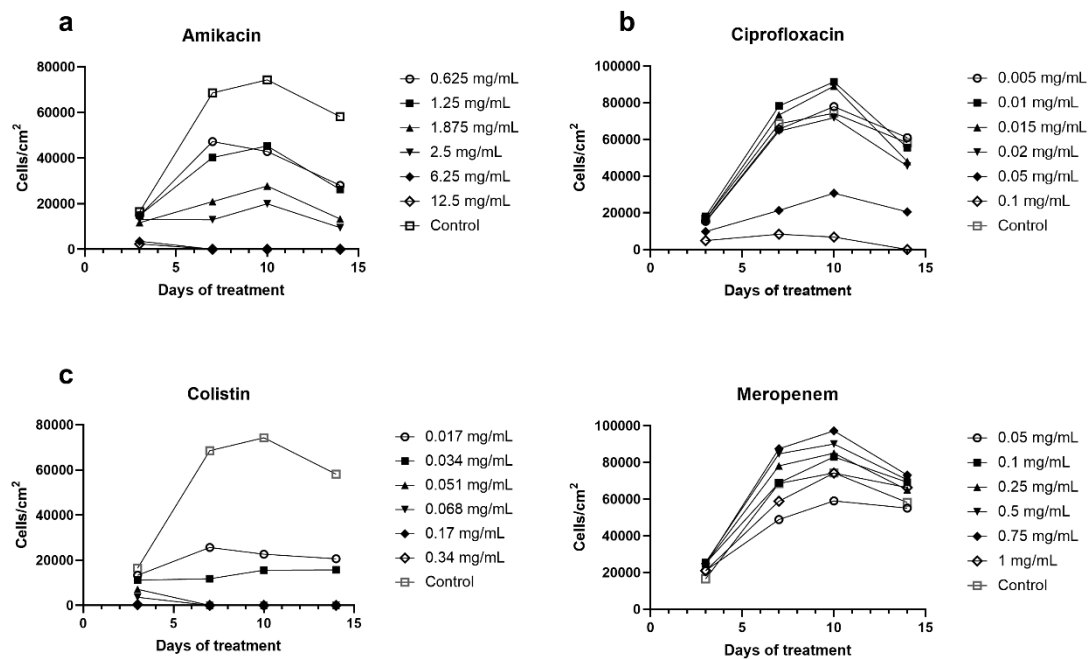


Figure S2. hKT proliferation after (a) AMK (0.625 - 12.5 mg/mL), (b) CIP (0.005 - 0.1 mg/mL), (c) COL (0.017 - 0.34 mg/mL) and (d) MER (0.05 - 1 mg/mL) treatments in a 14-day follow-up.

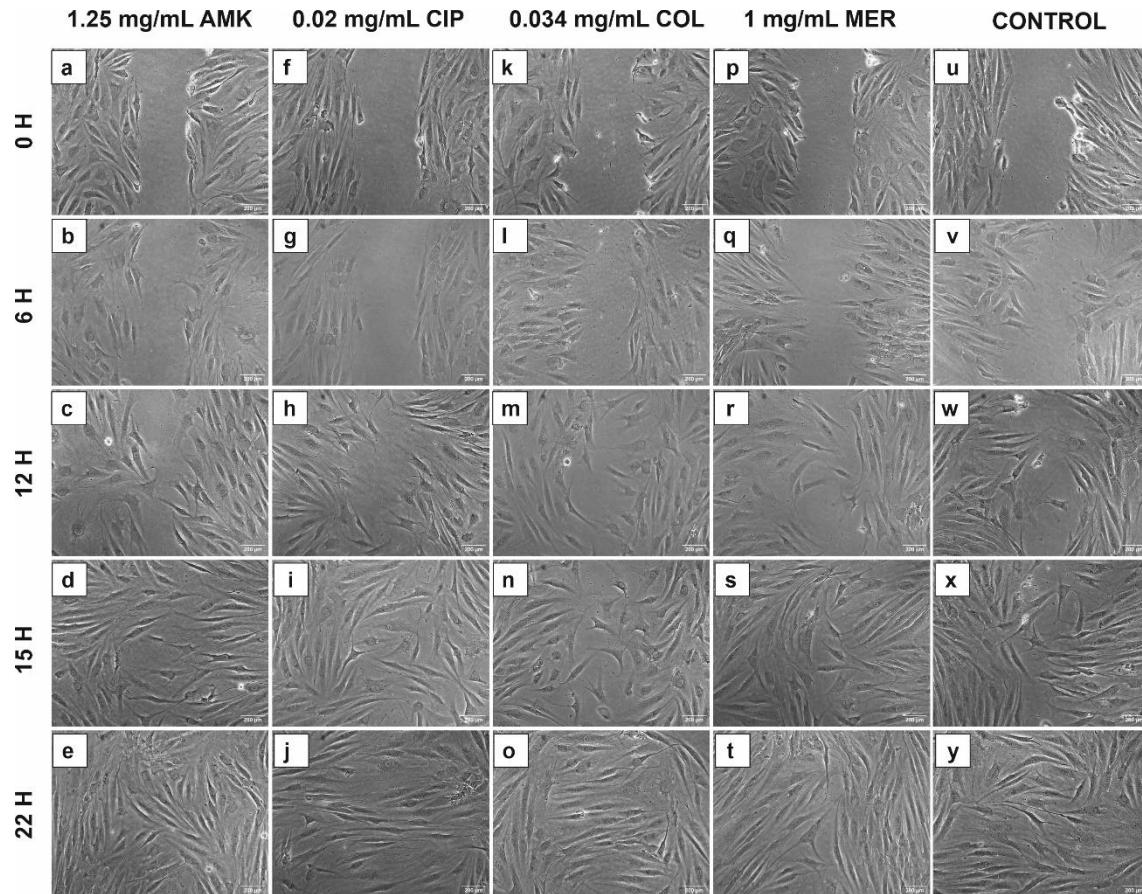


Figure S3. Wound closure in HFs after antibiotic treatments. I. (a-e) Wound closure assay was used on HFs after 1.25 mg/mL AMK treatment at 0,6,12, 15 and 22 h after scratching, respectively. (f-j) HFs after 0.02 mg/mL CIP treatment at 0,6,12, 15 and 22 h after scratching, respectively. (k-o) HFs after 0.034 mg/mL COL treatment at 0,6,12, 15 and 22 h after scratching, respectively. (p-t) HFs after 1 mg/mL MER treatment at 0,6,12, 15 and 22 h after scratching, respectively. (u-y) Control at 0, 6, 12, 15 and 22 h after scratching, respectively. n = 3. Magnification 10x.

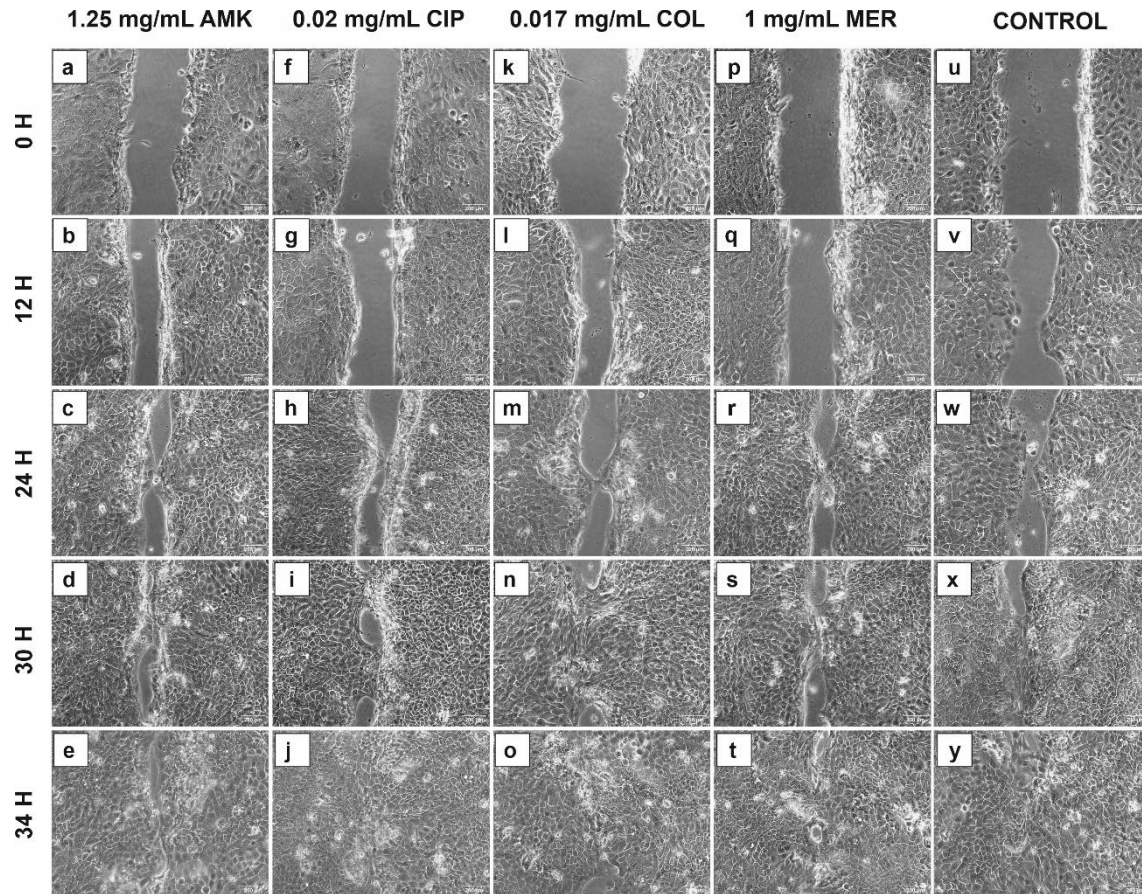


Figure S4. Wound closure in HF after antibiotic treatments. (a-e) Wound closure assay on hKTs after 1.25 mg/mL AMK treatment at 0, 12, 24, 30 and 34 h after scratching, respectively. (f-j) hKTs after 0.02 mg/mL CIP treatment at 0, 12, 24, 30 and 34 h after scratching, respectively. (k-o) hKTs after 0.017 mg/mL COL treatment at 0, 12, 24, 30 and 34 h after scratching, respectively. (p-t) hKTs after 1 mg/mL MER treatment at 0, 12, 24, 30 and 34 h after scratching, respectively. (u-y) Control at 0, 12, 24, 30 and 34 h after scratching, respectively. n = 3. Magnification 10x.

Table S1. IL-10 concentration levels in HF supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

Treatment	D3	D7	D10	D14
Amikacin 1.25 mg/mL	12.76 ± 1.99	14.97 ± 0.28	17.70 ± 0.22	15.94 ± 3.86
Ciprofloxacin 0.02 mg/mL	11.31 ± 0.76	15.97 ± 1.56	17.08 ± 0.57	18.19 ± 0.03
Colistin 0.034 mg/mL	11.03 ± 0.54	12.47 ± 0.11	12.28 ± 1.05	13.27 ± 0.45
Meropenem 1 mg/mL	10.69 ± 0.37	10.34 ± 1.73	12.33 ± 0.36	13.95 ± 1.19
Control	14.43 ± 0.08	17.53 ± 0.11	20.34 ± 0.82	20.20 ± 0.79

Table S2. bFGF concentration levels in HF supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	181 ± 6.67	224.33 ± 23.33	229.33 ± 21.66	274.33 ± 50
<i>Ciprofloxacin 0.02 mg/mL</i>	206 ± 48.33	294.33 ± 43.33	236 ± 41.67	294.33 ± 3.33
<i>Colistin 0.034 mg/mL</i>	197.67 ± 30	204.33 ± 33.33	212.66 ± 28.33	214.33 ± 16.66
<i>Meropenem 1 mg/mL</i>	192.67 ± 5	211 ± 30	236 ± 48.33	237.66 ± 13.33
<i>Control</i>	279.33 ± 25	292.66 ± 31.66	366 ± 38.33	414.33 ± 20

Table S3. TNF-α concentration levels in HF supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	692.18 ± 38.75	760.93 ± 7.5	888.43 ± 70	643.43 ± 172.5
<i>Ciprofloxacin 0.02 mg/mL</i>	617.18 ± 13.75	617.18 ± 13.75	800.93 ± 107.5	697.18 ± 3.75
<i>Colistin 0.034 mg/mL</i>	505.93 ± 7.5	485.93 ± 85	466.43 ± 23	558.43 ± 62.5
<i>Meropenem 1 mg/mL</i>	484.68 ± 23.75	617.18 ± 46.25	643.43 ± 77.5	688.43 ± 45
<i>Control</i>	673.43 ± 2.5	697.18 ± 36.25	963.43 ± 97.5	742.18 ± 1.25

Table S4. IL-6 concentration levels in HF supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	4348.89 ± 2233.33	1607.22 ± 380.55	1654.44 ± 133.33	3293.33 ± 1983.33
<i>Ciprofloxacin 0.02 mg/mL</i>	2871.11 ± 1433.33	1782.22 ± 455.55	2185 ± 102.78	2773.89 ± 1002.78
<i>Colistin 0.034 mg/mL</i>	3276.67 ± 1300	1679.44 ± 575	1754.44 ± 372.25	2385 ± 1163.89
<i>Meropenem 1 mg/mL</i>	2346.11 ± 902.78	1665.55 ± 516.65	1271.11 ± 77.78	1482.22 ± 361.11
<i>Control</i>	3371.11 ± 1783.33	1685 ± 191.67	1679.44 ± 202.77	2193.33 ± 672.25

Table S5. IL-10 concentration levels in hKTs cells supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	5.18 ± 0.15	4.67 ± 0.03	4.78 ± 0.26	5.07 ± 0.66
<i>Ciprofloxacin 0.02 mg/mL</i>	4.78 ± 0.37	7.22 ± 0.54	8.82 ± 0.77	10.49 ± 0.68
<i>Colistin 0.017 mg/mL</i>	3.10 ± 0.17	3.70 ± 0.03	3.33 ± 0.06	2.74 ± 0.15
<i>Meropenem 1 mg/mL</i>	4.21 ± 1.45	5.07 ± 0.09	7.45 ± 1.39	7.71 ± 0.29
<i>Control</i>	4.49 ± 0.54	8.76 ± 1.96	9.81 ± 1.65	15.09 ± 1.42

Table S6. bFGF concentration levels in hKTs cells supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	126 ± 8.33	116 ± 5	124.33 ± 0	129.33 ± 1.66
<i>Ciprofloxacin 0.02 mg/mL</i>	122.66 ± 1.66	134.33 ± 13.33	219.33 ± 5	251 ± 30
<i>Colistin 0.017 mg/mL</i>	142.66 ± 21.66	129.33 ± 1.66	127.66 ± 3.33	122.67 ± 5
<i>Meropenem 1 mg/mL</i>	134.33 ± 10	151 ± 6.67	207.66 ± 3.33	234.33 ± 23.33
<i>Control</i>	136 ± 8.33	202.67 ± 15	309.33 ± 15	556 ± 1.67

Table S7. TNF- α concentration levels in hKTs cells supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	279.68 ± 26.25	293.43 ± 10	350.93 ± 7.5	299.68 ± 26.25
<i>Ciprofloxacin 0.02 mg/mL</i>	267.18 ± 6.25	395.93 ± 15	570.93 ± 62.5	519.68 ± 56.25
<i>Colistin 0.017 mg/mL</i>	240.93 ± 15	284.68 ± 18.75	382.18 ± 28.75	268.43 ± 15
<i>Meropenem 1 mg/mL</i>	242.18 ± 6.25	374.68 ± 23.75	454.68 ± 28.75	514.68 ± 36.25
<i>Control</i>	299.68 ± 18.75	507.18 ± 68.75	629.68 ± 8.75	678.43 ± 15

Table S8. IL-6 concentration levels in hKTs supernatants for each treatment and control at days 3, 7, 10 and 14; n = 3, values expressed as mean (pg/mL).

<i>Treatment</i>	<i>D3</i>	<i>D7</i>	<i>D10</i>	<i>D14</i>
<i>Amikacin 1.25 mg/mL</i>	876.7 ± 11.1	862.8 ± 2.8	960 ± 138.9	823.9 ± 2.8
<i>Ciprofloxacin 0.02 mg/mL</i>	843.35 ± 22.25	1023.85 ± 30.55	1154.45 ± 33.35	1040.55 ± 47.25
<i>Colistin 0.017 mg/mL</i>	829.45 ± 2.75	996.1 ± 13.9	1010 ± 22.2	1018.35 ± 8.35
<i>Meropenem 1 mg/mL</i>	782.25 ± 5.55	1162.75 ± 108.35	1312.75 ± 91.65	757.2 ± 15.9
<i>Control</i>	854.4 ± 0	1271.1 ± 27.8	1110 ± 72.2	1032.2 ± 0