

Supplementary materials “Tolerance to ceftriaxone in *Neisseria gonorrhoeae*: rapid induction in WHO P reference strain and detection in clinical isolates”.

Table S1: Tolerant colonies count of *N. gonorrhoeae* strains (n = 6) and two controls (A and B) cyclically exposed to ceftriaxone (CRO).

| Tolerant colonies count | | | | | | | |
|-------------------------|---|----|----|----|----|----|----|
| Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| A | / | / | / | / | / | / | / |
| B | / | / | / | / | / | / | / |
| 1 | / | 83 | 67 | 8 | 6 | 7 | 9 |
| 2 | / | 72 | 78 | 18 | 8 | / | / |
| 3 | / | 18 | / | 16 | 7 | 8 | 66 |
| 4 | / | / | 19 | 7 | 5 | 8 | 18 |
| 5 | / | 15 | 18 | 16 | 7 | 6 | 20 |
| 6 | / | 14 | 92 | 83 | 17 | 13 | 19 |

Table S2: No change in ceftriaxone minimum inhibitory concentration (MIC) of *N. gonorrhoeae* strains (n = 6) and two controls (A and B) cyclically exposed to ceftriaxone (CRO), determined through susceptibility testing via E-tests.

[illegible]

Table S3: The minimum inhibitory concentration (MIC) and susceptibility/resistance (S/R) of used WHO P reference and clinical *N. gonorrhoeae* strains for ceftriaxone (CRO), ciprofloxacin (CIP), and azithromycin (AZI) [46]. Plus, site of infection (anorectal – A, urogenital – U) and tolerance detection (yes – Y or no – N) of clinical isolates and gender of patients (male – M, female - F).

| Strain | MIC (µg/mL) | | | Infection site | Tolerance | Gender |
|---------|-------------|-----------|-----------|----------------|-----------|--------|
| | CRO | CIP | AZI | | | |
| WHO P | 0.004 (S) | 0.004 (S) | 4 (R) | / | / | |
| RA00002 | < 0.008 (S) | 0.5 (R) | 0.094 (S) | A | Y | M |
| RA00003 | < 0.008 (S) | 0.032 (S) | 2 (R) | A | Y | M |
| RA00007 | < 0.008 (S) | 0.023 (S) | 2 (R) | A | Y | M |
| RA00012 | < 0.008 (S) | 0.003 (S) | 0.094 (S) | U | N | M |
| RA00015 | < 0.008 (S) | 1.5 (R) | 0.19 (S) | A | Y | M |
| RA00018 | < 0.008 (S) | 3 (R) | 0.094 (S) | U | Y | M |
| RA00023 | < 0.008 (S) | 0.023 (S) | 2 (R) | U | Y | M |
| RA00024 | < 0.008 (S) | 1.5 (R) | 0.19 (S) | U | N | M |
| RA00029 | < 0.008 (S) | 0.002 (S) | 0.19 (S) | A | Y | M |
| RA00032 | < 0.008 (S) | 0.023 (S) | 1.5 (R) | A | N | M |
| RA00035 | < 0.008 (S) | 0.006 (S) | 2 (R) | A | Y | M |
| RA00045 | < 0.008 (S) | 0.006 (S) | 1.5 (R) | A | Y | M |
| RA00069 | < 0.008 (S) | 0.75 (R) | 0.19 (S) | U | N | M |
| RA00071 | < 0.008 (S) | 2 (R) | 1 (S) | A | N | M |
| 22227 | < 0.008 (S) | 0.38 (S) | 0.002 (S) | U | N | M |
| 22235 | < 0.008 (S) | 0.094 (S) | 0.002 (S) | U | N | M |
| 22249 | < 0.008 (S) | 0.125 (S) | 0.003 (S) | U | N | M |
| 22253 | < 0.008 (S) | 0.125 (S) | 0.002 (S) | U | Y | F |

Table S4: Tolerance not associated with accelerated induction of ceftriaxone resistance. Ceftriaxone minimum inhibitory concentration of *N. gonorrhoeae* tolerant colonies (n = 13) compared to controls (n = 4) under *in vitro* selective pressure via 7 consecutive crossover CRO E-test.

[illegible]