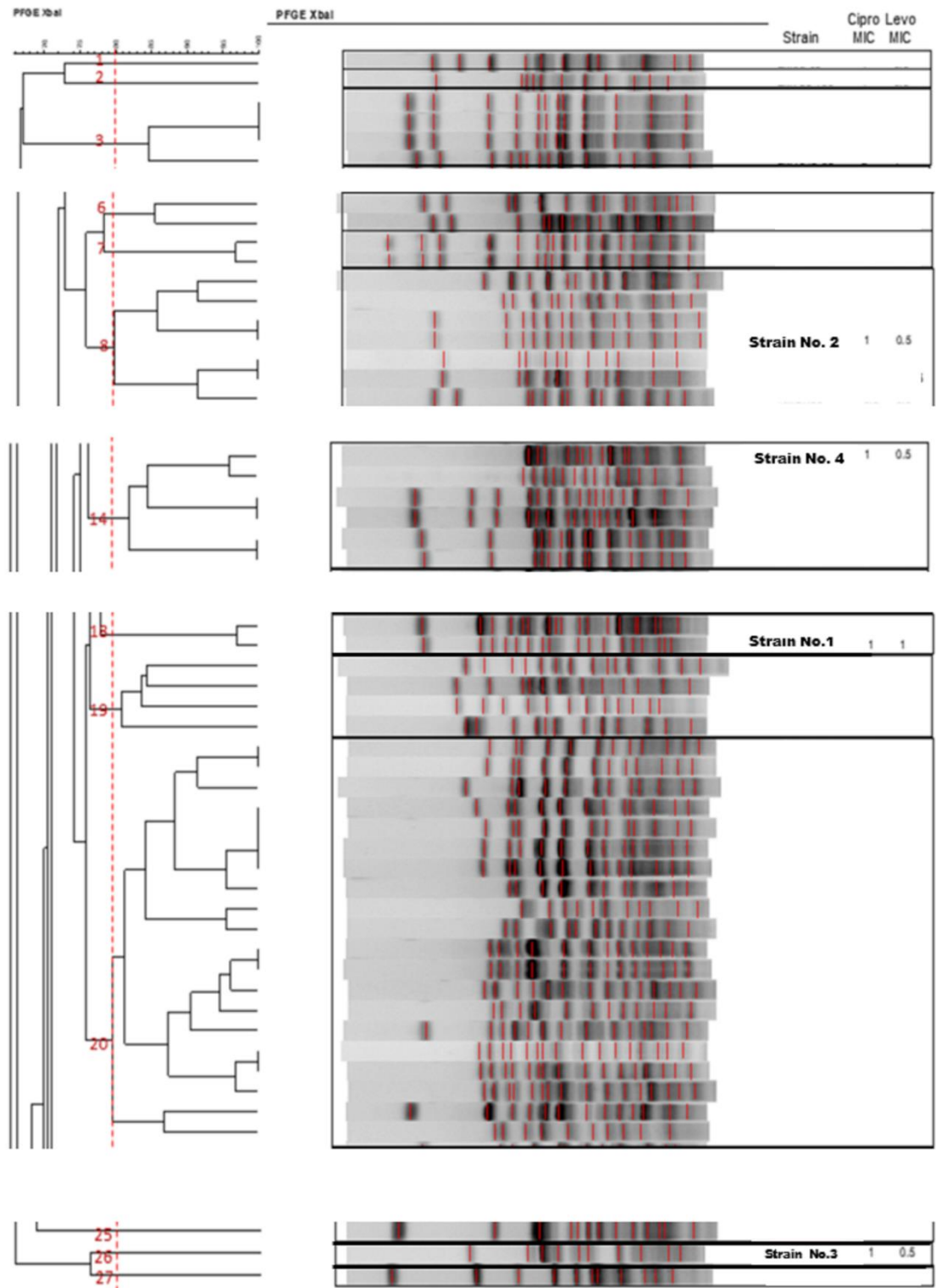


**Supplemental Figure S1.** Phylogenetic tree showing the four isolates belong to different clones, among 27 clones of *E. anophelis*



**Supplemental Figure S2.** Levofloxacin MICs for levofloxacin alone and its combination in each induction cycle

susceptible		intermediate			resistant				
Levofloxacin		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	1	4	64	512					
2	0.5	64	256	256	512				
3	0.5	32	256	512					
4	0.5	8	128	128	128	512			

Levofloxacin+0.5x MIC Mino		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	64	128	512				
2	1	2	2	2	4	4	4	4	
3	1	2	2	4	512				
4	1	1	1	4	4	32	32	32	

Levofloxacin +1x MIC Mino		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	8	8	8	64	64	64	
2	1	2	2	2	4	4	4	4	
3	1	2	2	2	2	2	2	1	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 0.5x MIC Rif		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	8	8	8	8	8	8	
2	1	2	2	2	4	4	4	4	
3	1	2	2	2	2	2	2	2	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 1x MIC Rif		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	8	8	8	8	8	8	
2	1	2	5	2	4	4	4	4	
3	1	2	5	2	2	2	2	2	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 0.5 x MIC Cef/Sul		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	8	8	8	8	8	3	
2	1	4	8	8	16	16	16	16	
3	1	2	2	2	2	2	2	2	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 1 x MIC Cef/Sul		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	4	8	8	8	8	8	8	
2	1	2	2	2	4	8	8	8	
3	1	2	2	2	2	2	2	2	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 0.5 x MIC Sul/Tri		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	2	8	8	8	8	8	8	
2	1	4	4	4	4	8	8	8	
3	1	2	2	8	128	128	512	512	
4	1	1	1	2	2	2	2	2	

Levofloxacin + 1 x MIC Sul/Tri		Levofloxacin MIC (mg/L) of mutant in steps							
Strain No.	0	1	2	3	4	5	6	7	
1	0.5	2	8	8	8	8	8	8	
2	1	4	4	4	4	8	8	8	
3	1	2	2	2	2	2	2	2	
4	1	1	1	2	2	2	2	2	

Cef/Sul = cefoperazone/sulbactam; Mino = minocycline; MIC = minimum inhibitory concentration; Rif = rifampin; Sul/Tri = sulfamethoxazole/trimethoprim.

**Supplemental Figure S3.** The MICs of antimicrobials in combination with levofloxacin in each induction cycle.

Levofloxacin + 0.5 X MIC Mino		Minocycline MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		0.25	0.5	1	1	1			
2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3		0.25	0.5	0.5	2	2	2		
4		0.25	0.25	0.5	1	1	2	2	2

Levofloxacin + 1 x MIC Mino		Minocycline MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		0.25	0.5	1	1	1	1	1	1
2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3		0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4		0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Levofloxacin + 0.5 x MIC Rif		Rifampine MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		0.25	1	1	1	1	1	1	1
2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3		0.5	0.5	0.5	1	1	1	1	1
4		0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5

Levofloxacin + 1 x MIC Rif		Rifampine MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		0.25	1	1	1	1	1	1	1
2		0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3		0.5	0.5	0.5	1	1	1	1	1
4		0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.5

Levofloxacin + 0.5 x MIC Cef/Sul		Brosym MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		16	128	128	128	128	128	128	128
2		256	256	256	256	256	256	256	256
3		256	256	256	256	256	256	256	256
4		256	256	256	256	256	256	256	256

Levofloxacin + 1 x MIC Cef/Sul		Brosym MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		16	128	128	128	128	128	128	128
2		256	256	256	256	256	256	256	256
3		256	256	256	256	256	256	256	256
4		256	256	256	256	256	256	256	256

Levofloxacin + 0.5 x MIC Sul/Tri		SXT MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		16	>32	>32	>32	>32	>32	>32	>32
2		8	>32	>32	>32	>32	>32	>32	>32
3		2	>32	>32	>32	>32	>32	>32	>32
4		4	>32	>32	>32	>32	>32	>32	>32

Levofloxacin + 1 x MIC Sul/Tri		SXT MIC (mg/L) of mutant in steps							
Strain No.		0	1	2	3	4	5	6	7
1		16	>32	>32	>32	>32	>32	>32	>32
2		8	>32	>32	>32	>32	>32	>32	>32
3		2	>32	>32	>32	>32	>32	>32	>32
4		4	>32	>32	>32	>32	>32	>32	>32

Cef/Sul = cefoperazone/sulbactam; Mino = minocycline; MIC = minimum inhibitory concentration; Rif = rifampin; Sul/Tri = sulfamethoxazole/trimethoprim.

**Supplemental Table S1.** The minimum inhibitory concentrations (MICs) of levofloxacin against *E. anophelis* and point mutation in quinolone resistance-determining regions in each step of multicycle induction and mutant selection by levofloxacin plus rifampin (0.5× and

Levofloxacin +1× MIC Rifampin					Levofloxacin +0.5× MIC Rifampin				
Strain	Cycle	Levofloxacin MIC (mg/L)	Point mutation in Gyr A ( <i>gyrA</i> )	Point mutation in GyrB ( <i>gyrB</i> )	Strain <sup>s</sup>	Cycle	levofloxacin MIC (mg/L)	Point mutation in Gyr A ( <i>gyrA</i> )	Point mutation in GyrB ( <i>gyrB</i> )
No. 1	0	0.5	N/D	N/D	No. 1	0	0.5	N/D	N/D
	1	4	N/D	N/D		1	4	N/D	N/D
	2	8	N/D	N/D		2	8	N/D	N/D
	3	8	N/D	N/D		3	8	N/D	N/D
	4	8	N/D	N/D		4	8	N/D	N/D
	5	8	N/D	N/D		5	8	N/D	N/D
	6	8	N/D	N/D		6	8	N/D	N/D
	7	8	N/D	N/D		7	8	N/D	N/D
No. 2	0	1	N/D	N/D	No. 2	0	1	N/D	N/D
	1	2	N/D	N/D		1	2	N/D	N/D
	2	2	N/D	N/D		2	2	N/D	N/D
	3	2	N/D	N/D		3	2	N/D	N/D
	4	4	N/D	N/D		4	4	N/D	N/D
	5	4	N/D	N/D		5	4	N/D	N/D
	6	4	N/D	N/D		6	4	N/D	N/D
	7	4	N/D	N/D		7	4	N/D	N/D
No. 3	0	1	N/D	N/D	No. 3	0	1	N/D	N/D
	1	2	N/D	N/D		1	2	N/D	N/D

No. 4	2	2	N/D	N/D	No. 4	2	2	N/D	N/D
	3	2	N/D	N/D		3	2	N/D	N/D
	4	2	N/D	N/D		4	2	N/D	N/D
	5	2	N/D	N/D		5	2	N/D	N/D
	6	2	N/D	N/D		6	2	N/D	N/D
	7	2	N/D	N/D		7	2	N/D	N/D
	0	1	N/D	N/D		0	1	N/D	N/D
	1	1	N/D	N/D		1	1	N/D	N/D
	2	1	N/D	N/D		2	1	N/D	N/D
	3	2	N/D	N/D		3	2	N/D	N/D
	4	2	N/D	N/D		4	2	N/D	N/D
	5	2	N/D	N/D		5	2	N/D	N/D
	6	2	N/D	N/D		6	2	N/D	N/D
	7	2	N/D	N/D		7	2	N/D	N/D

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**Supplemental Table S2.** The minimum inhibitory concentrations (MICs) of levofloxacin against *E. anophelis* and point mutation in quinolone resistance-determining regions in each step of multicycle induction and mutant selection by levofloxacin plus cefoperazone/sulbactam (0.5× and 1×MIC)

Levofloxacin + 1× MIC cefoperazone/sulbactam					Levofloxacin + 0.5× MIC cefoperazone/sulbactam				
Strain	Cycle	Levofloxacin MIC (mg/L)	Point mutation in Gyr A ( <i>gyrA</i> )	Point mutation in GyrB ( <i>gyrB</i> )	Strain	Cycle	Levofloxacin MIC (mg/L)	Point mutation in Gyr A ( <i>gyrA</i> )	Point mutation in GyrB ( <i>gyrB</i> )
No. 1	0	0.5	N/D	N/D	No. 1	0	0.5	N/D	N/D
	1	4	N/D	N/D		1	4	N/D	N/D
	2	8	N/D	N/D		2	8	N/D	N/D
	3	8	N/D	N/D		3	8	N/D	N/D
	4	8	N/D	N/D		4	8	N/D	N/D
	5	8	N/D	N/D		5	8	N/D	N/D
	6	8	N/D	N/D		6	8	N/D	N/D
No. 2	7	8	N/D	N/D	No. 2	7	8	N/D	N/D
	0	1	N/D	N/D		0	1	N/D	N/D
	1	2	N/D	N/D		1	4	N/D	N/D
	2	2	N/D	N/D		2	8	N/D	N/D
	3	2	N/D	N/D		3	8	N/D	N/D
	4	4	N/D	N/D		4	16	N/D	N/D
	5	8	N/D	N/D		5	16	N/D	N/D
No. 3	6	8	N/D	N/D	No. 3	6	16	N/D	N/D
	7	8	N/D	N/D		7	16	N/D	N/D
No. 3	0	1	N/D	N/D	No. 3	0	1	N/D	N/D

No. 4	1	2	N/D	N/D	No. 4	1	2	N/D	N/D
	2	2	N/D	N/D		2	2	N/D	N/D
	3	2	N/D	N/D		3	2	N/D	N/D
	4	2	N/D	N/D		4	2	N/D	N/D
	5	2	N/D	N/D		5	2	N/D	N/D
	6	2	N/D	N/D		6	2	N/D	N/D
	7	2	N/D	N/D		7	2	N/D	N/D
	0	1	N/D	N/D		0	1	N/D	N/D
	1	1	N/D	N/D		1	1	N/D	N/D
	2	1	N/D	N/D		2	1	N/D	N/D
	3	2	N/D	N/D		3	2	N/D	N/D
	4	2	N/D	N/D		4	2	N/D	N/D
	5	2	N/D	N/D		5	2	N/D	N/D
	6	2	N/D	N/D		6	2	N/D	N/D
	7	2	N/D	N/D		7	2	N/D	N/D

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