

Supplementary information file

Table S1. Quality control growth ranges. *ATCC = American Type Culture Collection

Bacterial type	Quality control	Fluoroquinolone	Range (µg/mL)	Source
<i>S. aureus</i> <i>S. pseudintermedius</i>	<i>S. aureus</i> ATCC 29213	Enrofloxacin	0.03-0.12	CLSI VET01A4 (2013)
		Marbofloxacin	0.12-0.5	CLSI VET01A4 (2013)
		Pradofloxacin	0.03-0.12	CLSI VET01A4 (2013)
		Ciprofloxacin	0.125-0.5	EUCAST 2022
		Moxifloxacin	0.016-0.125	EUCAST 2022
<i>E. coli</i>	<i>E. coli</i> ATCC 25922	Enrofloxacin	0.008-0.03	CLSI VET01A4 (2013)
		Marbofloxacin	0.008-0.03	CLSI VET01A4 (2013)
		Pradofloxacin	0.008-0.03	CLSI VET01A4 (2013)
		Ciprofloxacin	0.004-0.016	EUCAST 2022
		Moxifloxacin	0.008-0.06	EUCAST 2022

Table S2. Fluoroquinolones (veterinary and human) clinical breakpoints (CBP)

Bacterial type	Drug	Susceptibility CBP (µg/mL)	Resistance CBP (µg/mL)	Source of CBP	ECOFF (µg/mL)
<i>S. aureus</i>	ENR	<4	≥4	CLSI VET01S ED5:2020	
	MAR	<4	≥4	CLSI VET01S ED5:2020	
	PRA	<2	≥2	CLSI VET01S ED5:2020	
	CIP	<4	≥4	CLSI M100- D32:2022	1 (EUCAST 2022)
	MOX	<2	≥2	CLSI M100- D32:2022	0.25 (EUCAST 2022)
<i>S. pseudintermedius</i>	ENR	<4	≥4	CLSI VET01S ED5:2020	0.5 (EUCAST 2022)
	MAR	<4	≥4	CLSI VET01S ED5:2020	
	PRA	<2	≥2	CLSI VET01S ED5:2020	
	CIP	<4	≥4	CLSI M100- D32:2022	
	MOX	<2	≥2	CLSI M100- D32:2022	
<i>E. coli</i>	ENR	<4	≥4	CLSI VET01S ED5:2020	0.125 (EUCAST 2022)
	MAR	<4	≥4	CLSI VET01S ED5:2020	
	PRA	<2	≥2	CLSI VET01S ED5:2020	
	CIP	<1	≥1	CLSI M100- D32:2022	0.06 (EUCAST 2022)
	MOX	≤ 0.25	>0.25	EUCAST 2022	0.25 (EUCAST 2022)

Figure S1. MBC distributions in *S. aureus* (first row), *S. pseudintermedius* (second row) and *E. coli* (third row) of 5 fluoroquinolones. Blue dotted lines indicate the clinical breakpoint, the highest MIC value considered susceptible, and the epidemiological cut-off (ECOFF), the MIC value that separates wild-type from non wild-type bacteria.

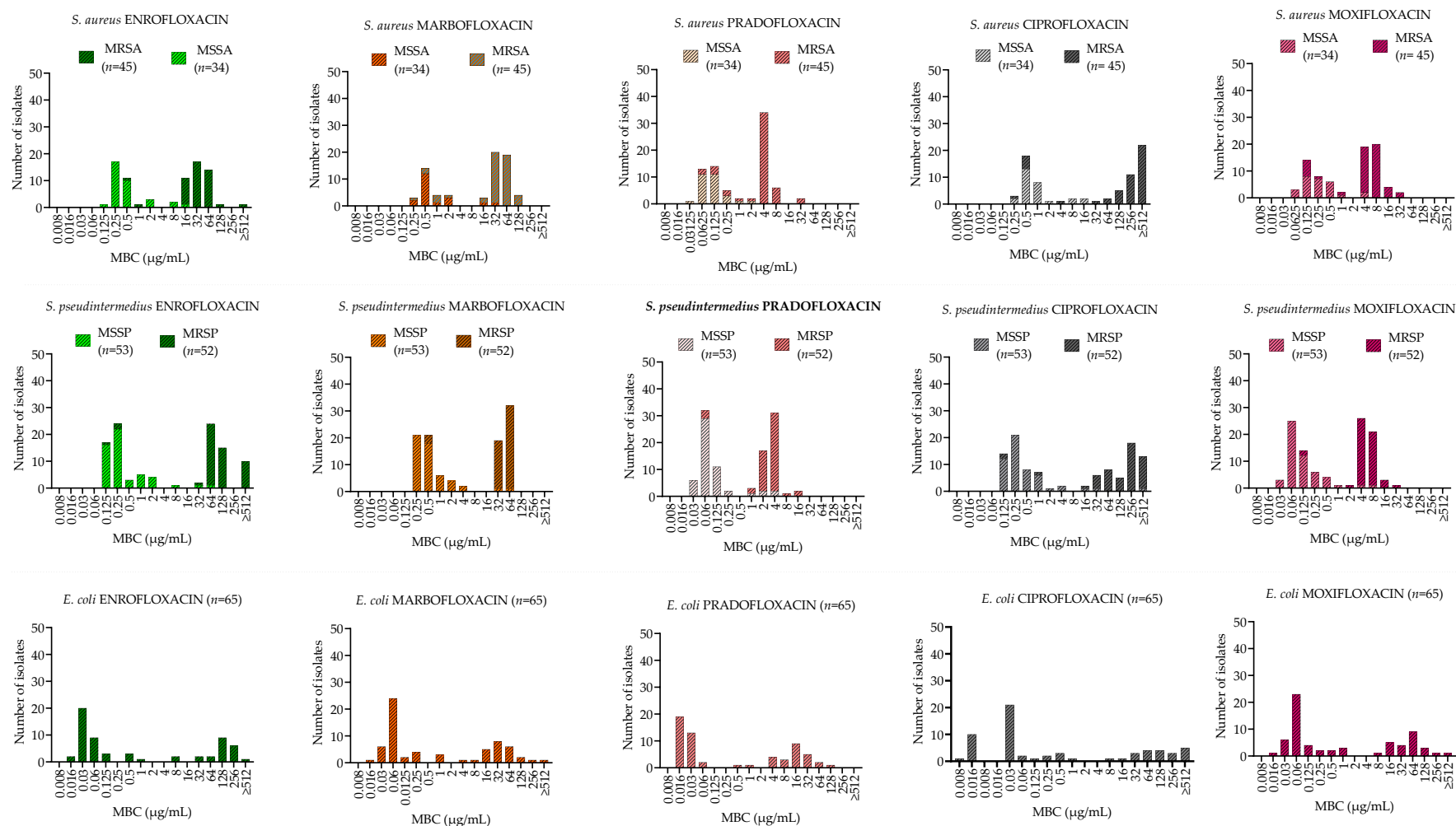


Table S3. Statistical comparison between MICs of veterinary and human FQs in *S. aureus*, *S. pseudintermedius* and *E. coli*. Anderson Darling test compared the areas of the distributions between MS, MR staphylococci and *E. coli* among the FQs. Friedman's test compared the medians (MIC₅₀) of the aforementioned distributions. Dunn's post hoc analysis was carried out for pairwise comparison between FQs.

Bacterial species	Anderson Darling test + Friedman's test		Dunn's post hoc analysis (Pairwise comparison)				
			ENR	MAR	PRA	CIP	MOX
MSSA (n=34)	MSSA ***	ENR	-	MSSA *	MSSA ***	MSSA *	MSSA (NS)
				MRSA (NS)	MRSA ***	MRSA ***	MRSA **
	MRSA ***	MAR	-	-	MSSA ***	MSSA (NS)	MSSA ***
					MRSA ***	MRSA *	MRSA ***
		PRA	-	-	-	MSSA ***	MSSA (NS)
					MRSA ***	MRSA (NS)	
MRSA (n=45)	MSSA ***	CIP	-	-	-	-	MSSA ***
							MRSA ***
	MRSA ***	MOX	-	-	-	-	-
		ENR	-	MSSP ***	MSSP ***	MSSP (NS)	MSSP ***
				MRSP (NS)	MRSP ***	MRSP (NS)	MRSP ***
MSSP (n=53)	MSSP ***	MAR	-	-	MSSP ***	MSSP (NS)	MSSP ***
					MRSP ***	MRSP (NS)	MRSP ***
	MRSP ***	PRA	-	-	-	MSSP ***	MSSP (NS)
						MRSP ***	MRSP (NS)
		CIP	-	-	-	-	MSSP ***
							MRSP ***
MRSP (n=52)	MSSP ***	MOX	-	-	-	-	-
	MRSP ***	ENR	-	(NS)	***	(NS)	(NS)
		MAR	-	-	***	(NS)	(NS)
		PRA	-	-	-	***	***
		CIP	-	-	-	-	(NS)
<i>E. coli</i> (n=65)	***	MOX	-	-	-	-	-
	***	ENR	-	(NS)	***	(NS)	(NS)
		MAR	-	-	***	(NS)	(NS)
		PRA	-	-	-	***	***
		CIP	-	-	-	-	(NS)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = not statistically significant ($p > 0.05$).

Table S4. Statistical comparison between MBCs of veterinary and human FQs in *S. aureus*, *S. pseudintermedius* and *E. coli*. Anderson Darling test compared the areas of the distribution between MS and MR staphylococci and in *E. coli* among the FQs. Friedman's test compared the medians (MBC₅₀) of the beforementioned distributions. Dunn's post hoc analysis was carried out for pairwise comparison between FQs.

Bacterial species	Anderson Darling test + Friedman's test	Dunn's post hoc analysis (Pairwise comparison)				
MSSA (n=34)	MSSA ***	ENR	MAR	PRA	CIP	MOX
		ENR	MSSA * MRSA (NS)	MSSA *** MRSA ***	MSSA * MRSA ***	MSSA (NS) MRSA ***
	MRSA ***	MAR	-	MSSA *** MRSA ***	MSSA (NS) MRSA ***	MSSA *** MRSA ***
		PRA	-	-	MSSA *** MRSA ***	MSSA (NS) MRSA (NS)
		CIP	-	-	-	MSSA *** MRSA ***
		MOX	-	-	-	-
MSSP (n=53)	MSSP ***	ENR	MAR	PRA	CIP	MOX
		ENR	MSSP * MRSP *	MSSP *** MRSP ***	MSSP (NS) MRSP (NS)	MSSP *** MRSP ***
	MRSP ***	MAR	-	MSSP *** MRSP ***	MSSP (NS) MRSP **	MSSP *** MRSP ***
		PRA	-	-	MSSP *** MRSP ***	MSSP (NS) MRSP (NS)
		CIP	-	-	-	MSSP *** MRSP ***
		MOX	-	-	-	-
<i>E. coli</i> (n=65)	***	ENR	MAR	PRA	CIP	MOX
		ENR	(NS)	***	(NS)	(NS)
		MAR	-	***	*	(NS)
		PRA	-	-	***	***
		CIP	-	-	-	*
		MOX	-	-	-	-

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS = not statistically significant ($p > 0.05$)