

Host soluble factors cause changes in *Staphylococcus epidermidis* antibiotic susceptibility and biofilm formation ability

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Supplementary figures

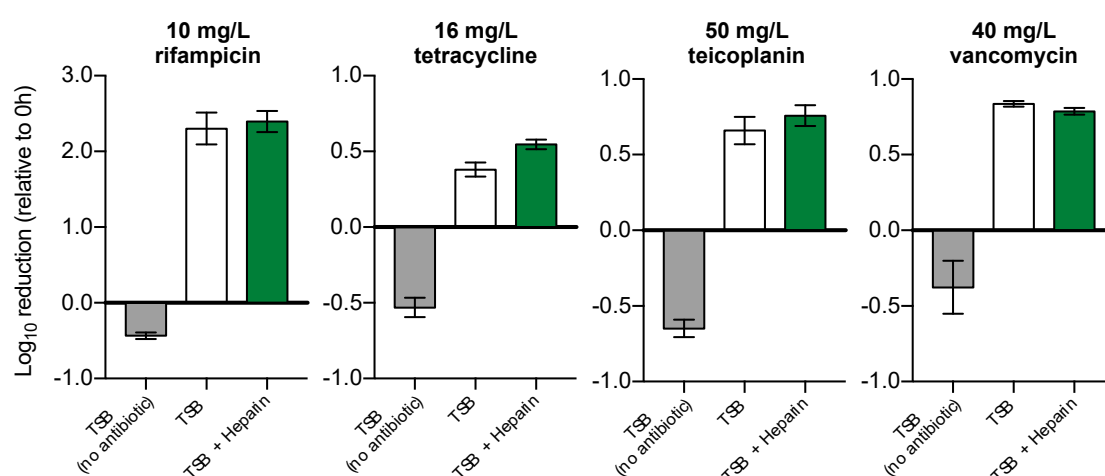


Figure S1. Effect of heparin on the activity of the antibiotics tested. Reduction in the CFU counts of *S. epidermidis* RP62A grown in the presence of heparin upon 3 hours of incubation with rifampicin, tetracycline, teicoplanin, and vancomycin at their respective peak serum concentration (PSC). Data are represented as mean \pm SEM (n = 3-4). One-way ANOVA with Dunnett's multiple comparisons test found no differences between TSB and TSB plus heparin.

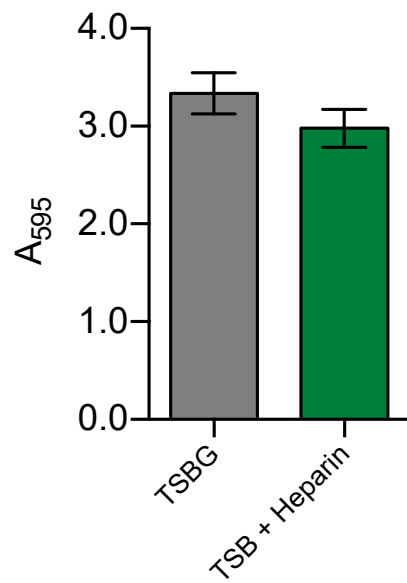


Figure S2. Effect of heparin on *S. epidermidis* biofilm formation. Data are represented as mean \pm SEM (n = 5). One-way ANOVA with Dunnett's multiple comparisons test found no significant differences between TSB and TSB plus heparin.

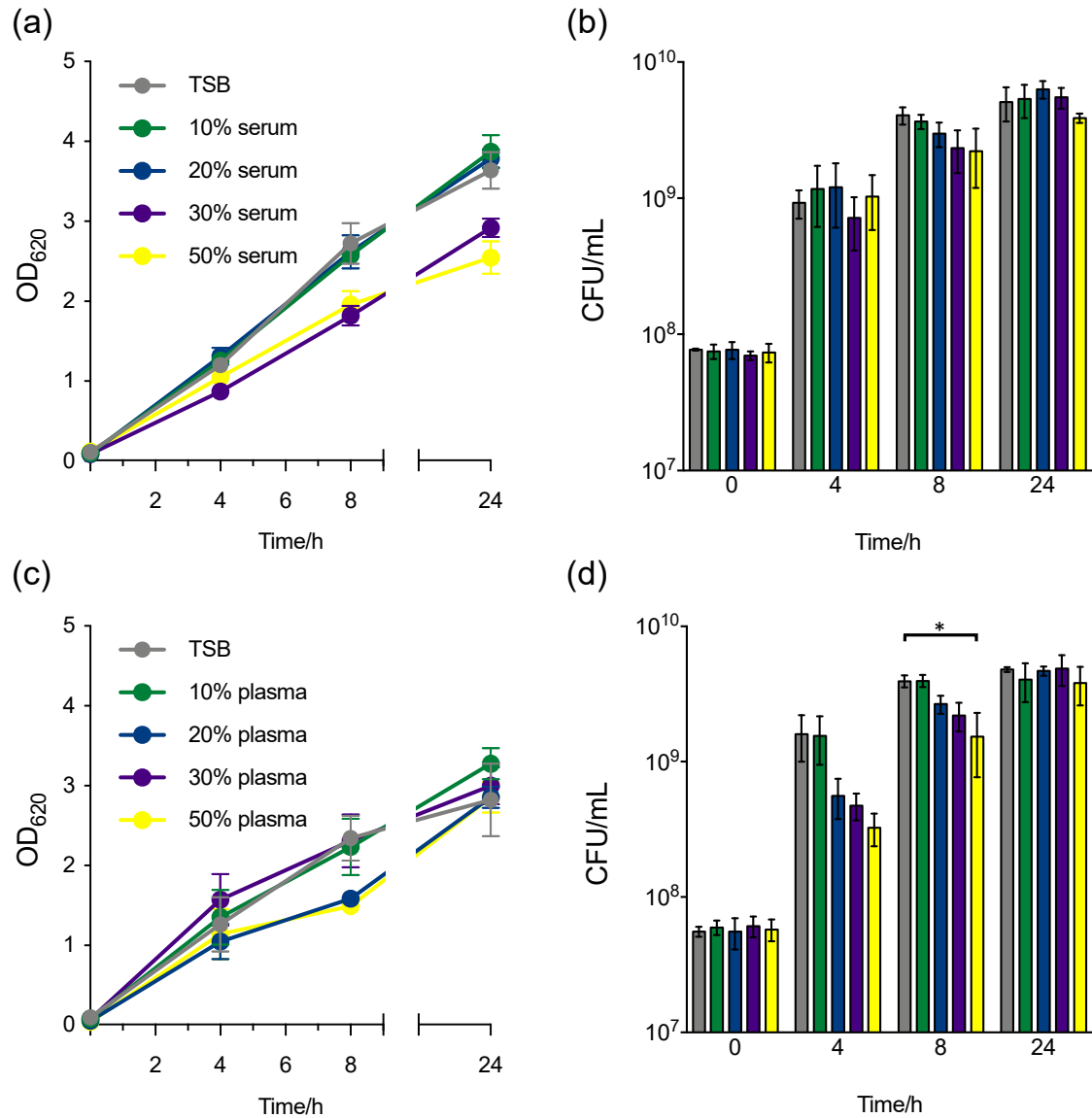


Figure S3. Effect of human serum and plasma on *S. epidermidis* growth. Bacteria were grown in conical flasks for 24 h at 37°C, 120 rpm in serum (a and b)- or plasma (c and d)- enriched TSB and growth was monitored regarding OD₆₂₀ (a and c) and CFU counts (b and d). Data are represented as mean \pm SEM (n = 3). Significant differences were determined by two-way ANOVA with Dunnett's multiple comparisons tests. *P < 0.05 vs TSB.

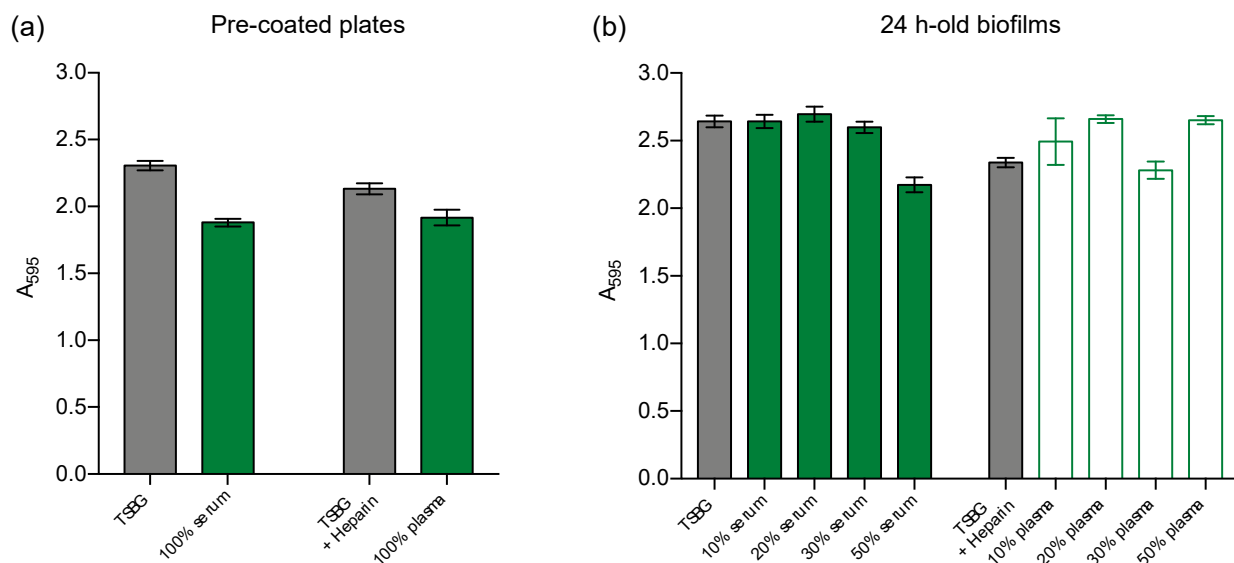


Figure S4. Effect of human serum and plasma at different stages of biofilm formation. (a) Biofilm formation on microplates pre-coated with human serum/ plasma; (b) Effect of human serum/plasma on 24 h-old biofilms. Data represent the mean \pm SEM of 6 technical replicates from a single experiment.

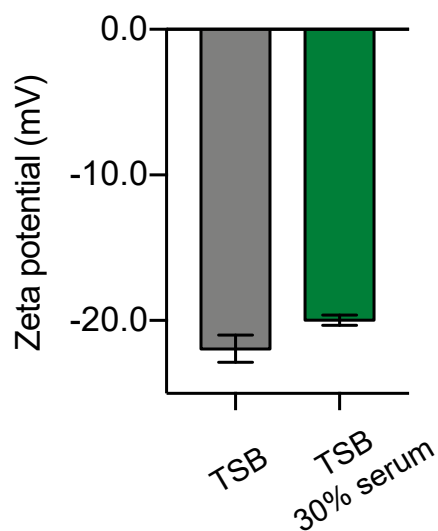


Figure S5. Effect of human serum on the bacterial surface charge. Data represent the mean \pm SEM of 10 zeta potential measurements from a single experiment.

Supplementary tables

Table S1. List of primers used in this study.

Target gene	Forward sequence (5' - 3')	Reverse sequence (5' - 3')	Product size (bp)	Efficiency (%)
<i>16S</i>	GGGCTACACACGTGCTACAA	GTACAAGACCCGGAACGTA	176	91.0
<i>gyrB</i>	GCATTGTTGACGGGTATTGG	CATCAACATCGGCATCAGTC	88	101.5
<i>atlE</i>	GTAGATGTTGTGCCCCAAGG	ACCTGATAGCGCTGAAAACG	201	98.5
<i>embp</i>	ATCGCAGCAAGTACATCCACCA	ATATTCTGTTGCATCCGCACGA	112	102.9
<i>gehD</i>	AATCGAGCGCTTTAGTTCC	TGGTGGTACGGTATCCGATT	176	103.1
<i>sdrG</i>	GGACGATTACACGACGATACAGCA	GAGGCAAGTCACCTTGTCCTTGA	140	98.0
<i>sepA</i>	TCTTAAGGCATCTCCGCCTA	GTCTGGTGCGAATGATGTTG	196	89.0
<i>sspA</i>	GTGGCTAATGGTTTGTACCC	TCCTAATGGCAAATTTGTGG	201	105.1
<i>dltA</i>	ACACATATGGACCAACTGAAGCTA	CCTCAATGACAAGTTCTCCTTCTT	150	94.0
<i>fntC</i>	CGCCCTCATCATAGCATTG	CCAATTGGATCACCCAAAAC	182	103.8

Table S2. List of antibiotics used in this study

Antimicrobial agent	Class	Mechanism of action
Dicloxacillin	β -lactams	Inhibition of cell wall synthesis
Rifampicin	Rifamycins	Inhibition of RNA synthesis
Teicoplanin	Glycopeptides	Inhibition of cell wall synthesis
Tetracycline	Tetracyclines	Inhibition of protein synthesis
Vancomycin	Glycopeptides	Inhibition of cell wall synthesis

Table S3. MIC ranges (in mg/L) of five antimicrobial agents for *S. epidermidis* RP62A in the presence of human serum and plasma. TSBH, TSB supplemented with heparin ($n = 3-4$)

Antimicrobial agent	TSB	Human serum			TSBH	Human plasma		
		10%	20%	30%		10%	20%	30%
Dicloxacillin	4-8	4	2-4	2-4	2	2	2	2-4
Rifampicin	0.004	0.008	0.008-0.016	0.008-0.031	0.002-0.004	0.008	0.008	0.008-0.016
Teicoplanin	1-4	2-4	2-4	2-4	1-2	2-4	2-4	4
Tetracycline	0.25	0.25	0.25	0.125-0.25	0.25	0.25	0.25	0.25-0.5
Vancomycin	2-4	2	2-4	2	2	2	2	2

Table S4. MBC ranges (in mg/L) of five antimicrobial agents for *S. epidermidis* RP62A in the presence of human serum and plasma. TSBH, TSB supplemented with heparin ($n = 3-4$)

Antimicrobial agent	TSB	Human serum			TSBH	Human plasma		
		10%	20%	30%		10%	20%	30%
Dicloxacillin	≥ 64	≥ 64	> 64	> 64	> 2	> 64	> 64	> 64
Rifampicin	0.16	0.031- 0.063	0.031	0.031- 0.063	0.008	0.031- 0.063	0.031- 0.063	0.031 - \geq 0.063
Teicoplanin	4	≥ 8	≥ 8	≥ 8	4 - > 8	> 8	≥ 8	≥ 8
Tetracycline	0.5- 4	> 4	> 4	> 4	> 4	> 4	> 4	> 4
Vancomycin	> 8	≥ 8	> 8	≥ 8	8	4 - > 8	4 - > 8	4 - ≥ 8