

1 **Supplementary material**

2 ***Bacterial survival***

3 Initial investigations were undertaken to examine bacterial survival in horse blood and
4 saponin. A panel of 27 clinical bacterial isolates were collected from the Royal Free Hospital
5 Hampstead. These bacteria represented the top ten most common causes of bacteraemia in the
6 UK⁴² as well as a variety of cell wall types, morphology and growth requirements. Roughly 500
7 bacterial cells were added to 1ml PBS, 1ml defibrinated horse blood (TCS Bioscience) or 1ml 2%
8 saponin (Sigma) and vortexed, before being incubated for 2 hours at room temperature. The
9 samples were then vortexed again and four aliquots of 100µl from each sample were cultured
10 using appropriate media and incubated in appropriate conditions for 24 or 48 hours. Colony
11 counts were then performed and survival rates calculated by comparing to bacterial numbers
12 isolated from spiked PBS.

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14 Of the 28 isolates tested 26 showed better than 95% survival in 2% saponin, lower survival
15 rates were found in *Paenibacillus anaericanus* (87.2%) and *Streptococcus pneumoniae* (94%). 20
16 of the isolates showed greater than 95% survival in horse blood. Seven of the isolates had poorer
17 survival in the horse blood, *Staphylococcus epidermidis* (73%), *Streptococcus pneumoniae* (6%),
18 *Proteus vulgaris* (60.9%), *Streptococcus agalactiae* (45%), *Listeria monocytogenes* (50.3%),
19 *Propionibacterium acnes* (23.4%) and *Shigella sonnei* had a 33.3% survival rate. Nine of the
20 isolates showed altered colony morphology after incubation in horse blood, with the colonies
21 appearing smaller and with a lobate margin, marked with a * in Table X (*Escherichia coli*,
22 *Staphylococcus aureus* two isolates, *Klebsiella pneumoniae*, *Pseudomonas auriginosa* one isolate,
23 *Enterobacter cloacae*, *Salmonella Typhimurium*, *Serratia marcescens* and *Listeria*
24 *monocytogenes*).

	Isolate	% survival in HB	%Survival in 2% Saponin	% survival in HB and 2% Saponin	
1	<i>Escherichia coli</i>	100.0	102.8	101.9	*
2	<i>Staphylococcus epidermidis</i>	73.0	97.3	89.2	
3	<i>Staphylococcus aureus</i>	104.6	108.5	100.0	*
4	<i>Staphylococcus aureus</i>	101.4	101.4	103.5	*
5	<i>Enterococcus faecium</i>	101.0	100.0	97.1	
6	<i>Klebsiella pneumoniae</i>	103.1	112.5	109.4	*
7	<i>Streptococcus pneumoniae</i>	6	94.0	6.0	
8	<i>Pseudomonas aurginosa</i>	105.7	102.4	106.3	
9	<i>Pseudomonas aurginosa</i>	102.0	98.0	105.4	*
10	<i>Proteus vulgaris</i>	60.9	97.9	71.6	
11	<i>Enterobacter cloacae</i>	111.1	100.0	101.2	*
12	<i>Streptococcus agalactiae</i>	45.0	95.0	42.5	
13	<i>Bacteroides vulgatus</i>	100.0	97.5	100.8	
14	<i>Streptococcus oralis</i>	101.0	107.1	105.1	
15	<i>Streptococcus mitis</i>	95.9	104.1	116.2	
16	<i>Streptococcus anginosus</i>	104.9	107.0	105.6	
17	<i>Haemophilus influenzae</i>	96.9	103.4	96.6	
18	<i>Streptococcus pyogenes</i>	106.8	101.0	106.8	
19	<i>Salmonella sp.</i>	106.9	101.1	105.1	*
20	<i>Serratia marcescens</i>	104.7	98.1	111.8	*
21	<i>Fusobacterium necrophorum</i>	101.9	99.5	104.3	
22	<i>Listeria monocytogenes</i>	50.3	106.1	49.7	*
23	<i>Actinomyces naeslundii</i>	121.8	98.6	110.9	
24	<i>Propionibacterium acens</i>	106.5	108.6	109.7	
25	<i>Paenibacillus anaericanus</i>	23.4	87.2	25.5	
26	<i>Clostridium butyricum</i>	95.1	98.1	93.2	
27	<i>Shigella sonnei</i>	33.3	103.2	39.7	

Table 1: Survival rates of 27 bacteria in horse blood, Saponin and horse blood and Saponin combined. *
Indicates altered colony morphology.