

1 **Supplemental Information**

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3 **Effects of Microplate Type and Broth Additives on Microdilution MIC Susceptibility**

4 **Assays**

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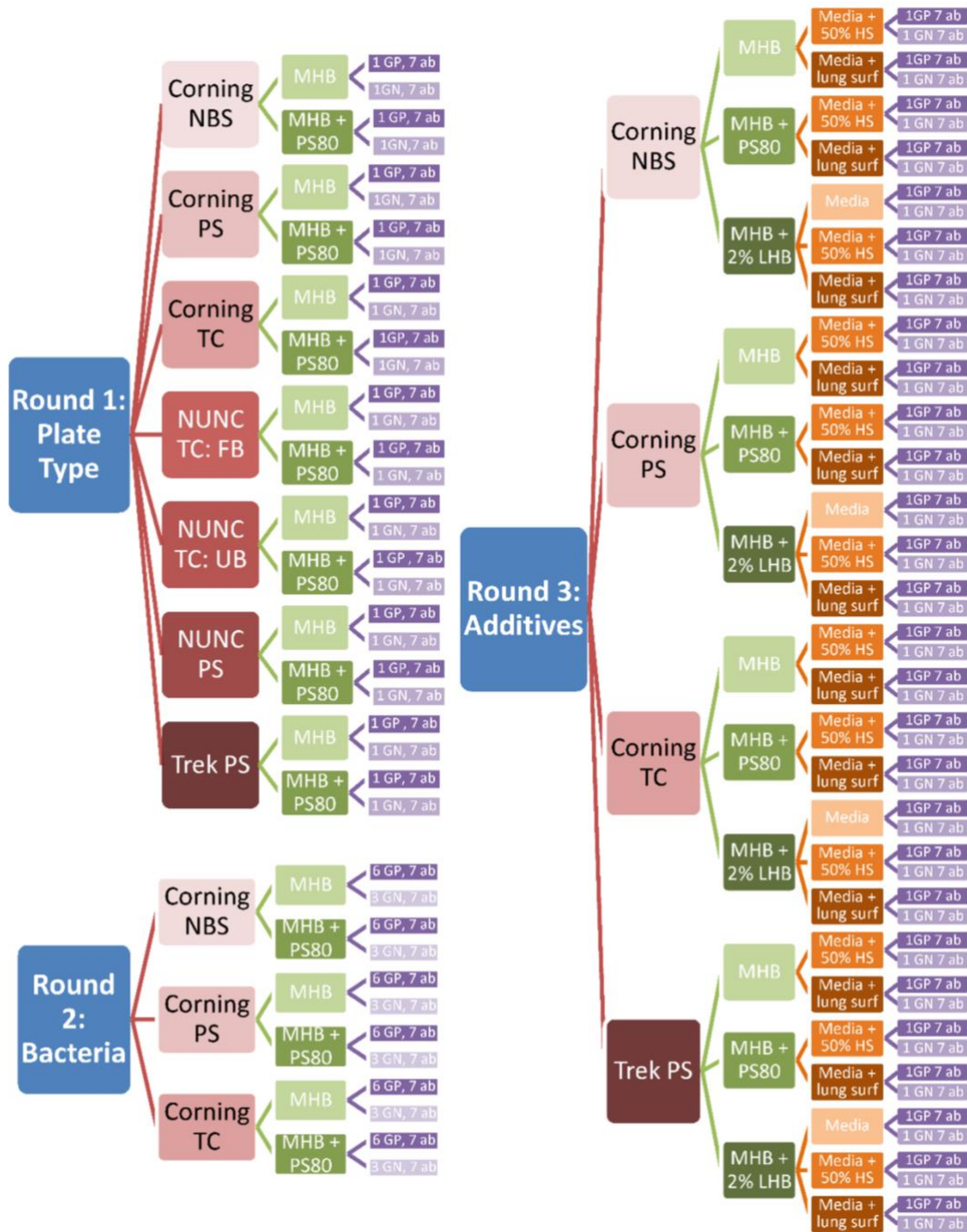
13 Running Head: Plate and Additive Effects on Broth MIC Determinations

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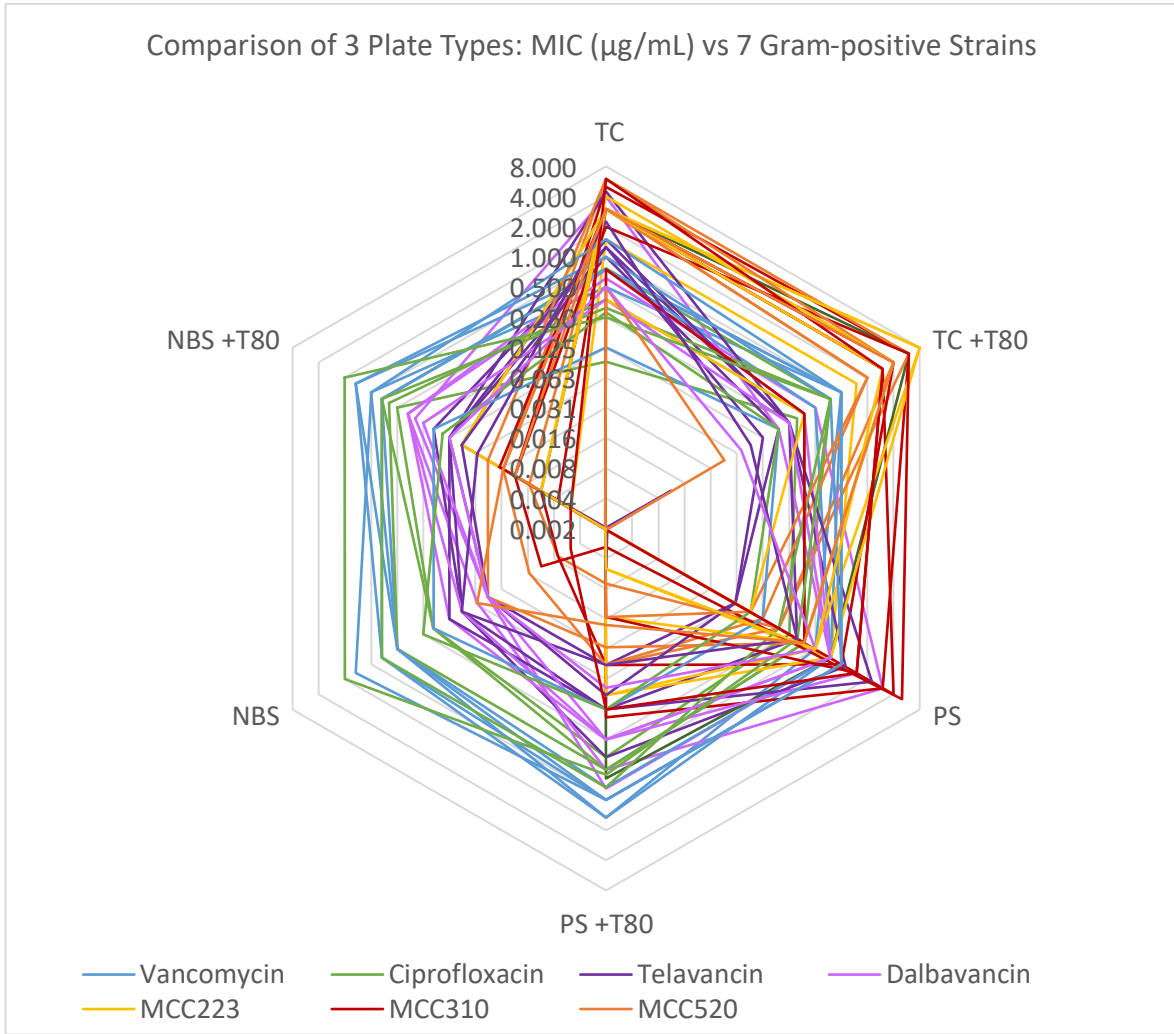
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20 **Figure S1.** Experimental Design

21 (Ab = antibiotic, GP = Gram-positive bacteria, GN = Gram-negative bacteria,

22 HS = human serum, LHB = lysed horse blood, MHB = Mueller-Hinton Broth, PS =

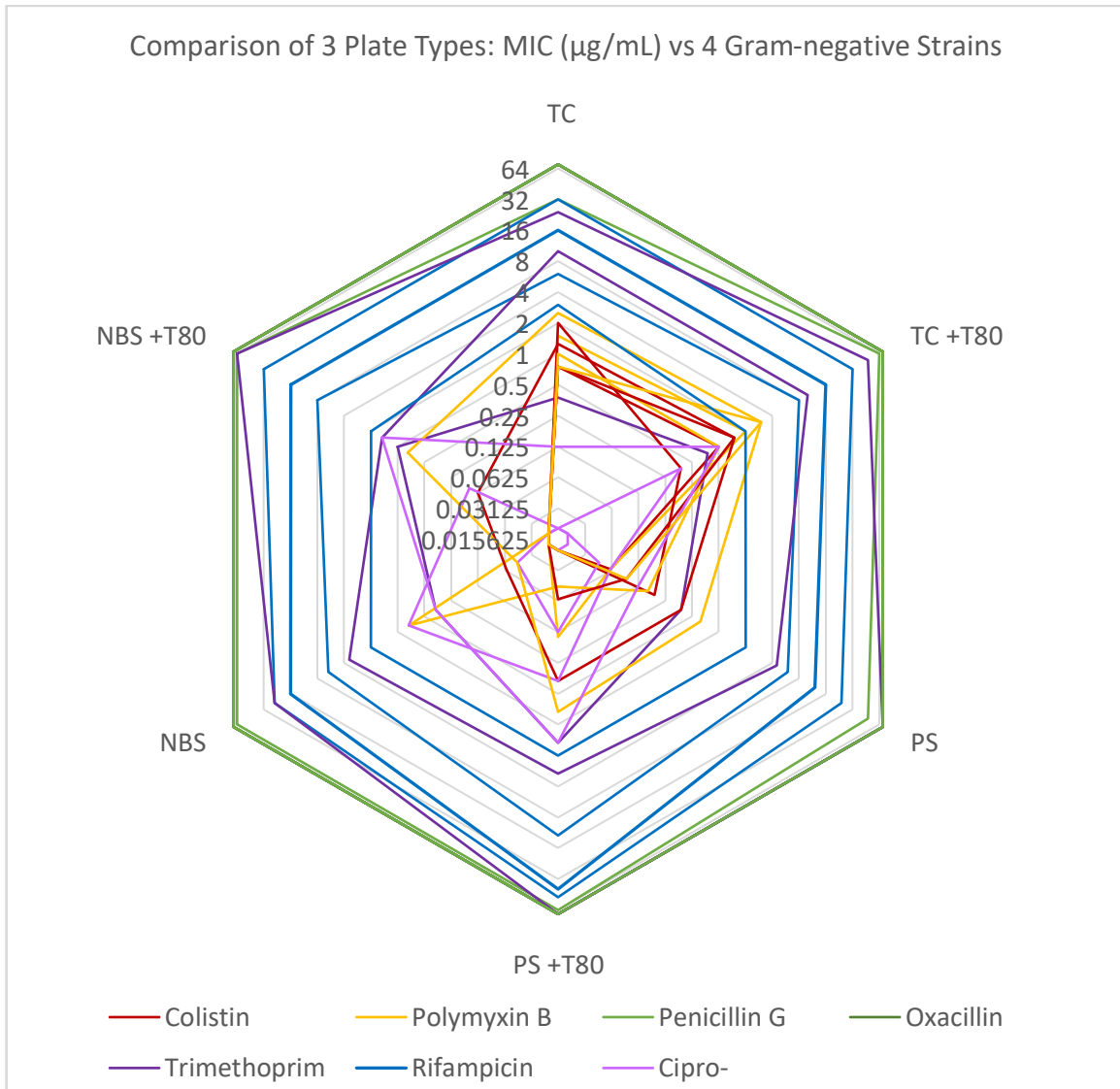
23 polysorbate 80)



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27 **Figure S2.** Comparison of antibiotic MICs determined in three plate types with and
28 without the addition of 0.002% Tween-80, against 7 strains of Gram-positive bacteria
29 (MRSA, MSSA, GISA, *S. pneumoniae*, MDR *S. pneumoniae*, *S. pyogenes*, *E. faecalis*).
30 Note: each hexagon represents one antibiotic-strain pair, with the same colour used for
31 one antibiotic against all 7 strains. If the MIC remains constant across plate
32 types/conditions, the plot should be symmetrical, as for vancomycin (blue).

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Figure S3. Comparison of antibiotic MICs determined in three plate types with and without the addition of 0.002% Tween-80, against 4 strains of Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*). Note: each hexagon represents one antibiotic-strain pair, with the same colour used for one antibiotic against all 7 strains. If the MIC remains constant across plate types/conditions, the plot should be symmetrical, as for rifampicin (blue).

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45 **Table S1.** Comparison of 3 Plate Types vs Seven Gram Positive Bacteria in Mueller
 46 Hinton Broth in the presence and absence of Tween-80 (polysorbate 80) (MIC, µg/mL)

Bacteria	Plate	Media	Antibiotic						
			Vanco- mycin	Cipro- floxacin	Tela- vancin	Dalba- vancin	MCC223	MCC310	MCC520
MRSA	Corning	-T80	0.5	0.125-0.25	0.03-0.06	0.03-0.06	≤0.003	≤0.003	0.007
	NBS	+T80	1	0.5	0.125	0.125-0.5	0.003-0.007	0.007	0.015
<i>S. aureus</i> ATCC 43300	Corning	-T80	0.5	0.06-0.125	1	4	1-2	2-4	2-4
	TC	+T80	1	0.125-0.5	0.125-0.25	0.125-0.25	1-2	2-4	4
	Corning	-T80	0.5	0.25	0.06	0.5-1	0.5-1	4	0.125-0.25
	PS	+T80	1-2	0.25-0.5	0.03-0.06	0.03-0.125	0.003-0.007	≤0.003	0.007
MSSA	Corning	-T80	0.5-1	0.125-0.25	0.06-0.125	0.06	≤0.003	≤0.003	≤0.003
	NBS	+T80	1-2	0.5-1	0.125-0.25	0.25-0.5	0.06-0.125	0.015-0.03	0.03
<i>S. aureus</i> ATCC 25923	Corning	-T80	0.5-1	0.03-0.125	0.5-2	0.25-1	2-4	2-8	4-8
	TC	+T80	1	0.5-1	0.25	0.5	4-8	4-8	4-8
	Corning	-T80	0.5-1	0.25-0.5	0.25-2	2-4	0.5-1	2-4	0.125-0.25
	PS	+T80	1-2	0.5	0.25-0.5	0.5	0.25-1	0.06-0.25	0.03-0.06
<i>S. pneum- oniae</i>	Corning	-T80	0.5-1	0.5	0.125	0.06-0.125	≤0.003	0.007-0.015	0.03-0.06
	NBS	+T80	0.5-1	0.25-1	0.125	0.25-0.5	0.06-0.125	0.015-0.03	0.03-0.06
ATCC 33440	Corning	-T80	0.5-1	0.25-0.5	1-2	0.5-1	2-4	2	4-8
	TC	+T80	1	0.25-0.5	0.25	0.25-0.5	8	4-8	4
	Corning	-T80	1	0.25-0.5	0.5-4	1-2	0.5-1	2-8	0.125-0.25
	PS	+T80	1	0.5	0.125	0.25	0.06-0.125	0.003-0.06	0.03
<i>S. pyogenes</i>	Corning	-T80	0.125-0.25	0.25	≤0.003	≤0.003	≤0.003	≤0.003	≤0.003
	NBS	+T80	0.125-0.25	0.06-0.25	0.003-0.015	≤0.003	≤0.003	≤0.003	≤0.003
ATCC 12344	Corning	-T80	0.125	0.06-0.5	≤0.003	≤0.003	0.25-0.5	0.5-1	0.5
	TC	+T80	0.125-0.25	0.125-0.25	0.007-0.015	≤0.003	0.25-0.5	0.25-0.5	0.03-0.06
	Corning	-T80	0.125	0.06-0.125	≤0.003	≤0.003	0.06-0.125	0.25-0.5	≤0.003
	PS	+T80	0.125	0.125	0.003-0.007	≤0.003	≤0.003	≤0.003	≤0.003
<i>S. pneum- oniae</i>	Corning	-T80	0.5	0.5-1	0.03-0.06	0.03-0.06	≤0.003	≤0.003	≤0.003
	NBS	+T80	1-2	0.5-1	0.06-0.125	0.25	0.007-0.015	0.007-0.06	0.015-0.03
ATCC 700677	Corning	-T80	1	0.125-0.5	0.5-2	0.25-0.5	4	2-4	2-4
	TC	+T80	0.5-1	0.5-1	0.125	0.25	4	2-4	2
	Corning	-T80	1	0.125-0.25	0.06	0.5-1	0.25-0.5	1-2	0.06-0.125
	PS	+T80	1	0.5-1	0.06-0.125	0.25	0.015	0.015	0.015
<i>E. faecalis</i>	Corning	-T80	1-2	2	0.06-0.125	0.03-0.06	≤0.003	0.003-0.007	0.06
	NBS	+T80	1	2	0.06	0.125	0.007-0.015	0.003-0.007	0.03
ATCC 29212	Corning	-T80	1-2	0.25	0.5-4	0.5	2-4	4-8	2-4
	TC	+T80	1	0.5-1	0.06-0.125	0.015-0.125	2-4	2-4	4
	Corning	-T80	1	0.125-0.5	0.125-0.5	0.5	0.5	1-2	0.25-0.5
	PS	+T80	1	0.125-1	0.03-0.06	0.03-0.125	0.003-0.007	0.125	0.007-0.03
<i>S. aureus</i> GISA NRS 2;	Corning	-T80	0.5	64	0.06-0.125	0.125	≤0.003	0.007	0.015
	NBS	+T80	0.5-1	64	0.125	0.25-0.5	0.06-0.125	0.007-0.015	0.03
ATCC 700698	Corning	-T80	0.5-1	32-64	1-8	0.5-1	2-4	2-4	2-4
	TC	+T80	0.5	64	0.25	0.25-0.5	8	4	2
	Corning	-T80	0.5-1	32->64	0.125-0.5	0.5-1	0.5	1	0.125
	PS	+T80	0.5-1	64	0.125	0.25	0.06-0.125	0.03-0.06	0.03-0.06

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50 **Table S2.** Comparison of 3 Plate Types vs Four Gram Negative Bacteria in Mueller
 51 Hinton Broth in the presence and absence of Tween-80 (polysorbate 80) (MIC, $\mu\text{g/mL}$)

Bacteria	Plate	Media	Antibiotic						
			Colistin	Ciprofloxacin	Oxacillin	Trimethoprim	Polymyxin B	Penicillin G	Rifampicin
<i>E. coli</i> ATCC 25922	Corning	-T80	≤ 0.03	≤ 0.03	>64	0.25-0.5	≤ 0.03	64	4-8
	NBS	+T80	≤ 0.03	≤ 0.03	>64	1	≤ 0.03	64	8
	Corning	-T80	0.5-1	≤ 0.03	>64	0.25-0.5	1-2	32	4-8
	TC	+T80	1	≤ 0.03	>64	0.5-1	2	64	8
	Corning	-T80	0.06	≤ 0.03	>64	0.25-0.5	0.06	32-64	4-8
	PS	+T80	≤ 0.03	≤ 0.03	>64	1-2	0.03-0.25	64	8-16
<i>K. pneumoniae</i> ATCC 13883	Corning	-T80	≤ 0.03	0.03-0.06	>64	4-8	0.03-0.125	>64	16
	NBS	+T80	≤ 0.03	≤ 0.03	>64	1-2	≤ 0.03	>64	16
	Corning	-T80	2	≤ 0.03	>64	4-16	1	>64	16
	TC	+T80	0.25-0.5	≤ 0.03	>64	4-16	1	>64	16
	Corning	-T80	0.125-0.25	0.03-0.06	>64	1-8	0.06-0.25	>64	8-16
	PS	+T80	≤ 0.03	0.125	>64	2-4	0.03-0.06	>64	16-64
<i>P. aeruginosa</i> ATCC 10145	Corning	-T80	0.06	0.03-0.125	>64	>64	0.03-0.06	>64	16-32
	NBS	+T80	0.125	0.06-0.25	>64	>64	0.03-0.125	>64	32
	Corning	-T80	0.5-2	≤ 0.03	>64	>64	1-4	>64	32
	TC	+T80	1-2	0.25-0.5	>64	>64	2-4	>64	32
	Corning	-T80	0.25-0.5	0.06	>64	>64	0.25-1	>64	16-32
	PS	+T80	0.25-0.5	0.25-0.5	>64	>64	0.5-1	>64	32-64
<i>A. baumannii</i> ATCC 19606	Corning	-T80	≤ 0.03	0.25-0.5	>64	16-32	≤ 0.03	>64	2
	NBS	+T80	≤ 0.03	1-2	>64	64	≤ 0.03	>64	2
	Corning	-T80	0.5-1	0.125	>64	16-32	0.5-1	>64	2-4
	TC	+T80	1-2	1	>64	32-64	2-4	>64	2
	Corning	-T80	0.06-0.125	0.125	>64	>64	0.06-0.125	>64	2
	PS	+T80	0.06	1-2	>64	>64	≤ 0.03	>64	2