

# Supplementary Materials: Therapeutic Potential of Injectable Nano-Mupirocin Liposomes for Infections Involving Multidrug-Resistant Bacteria

Ahuva Cern, Yaelle Bavli, Atara Hod, Daniel Zilbersheid, Shazad Mushtaq, Ayelet Michael-Gayego, Dinorah Barasch, Yael Feinstein Rotkopf, Allon E. Moses, David M Livermore and Yechezkel Barenholz

Table S1. Line listing of the 167 Gram-positive isolates tested

#	Organism No	Organism	Country	Year Collected	Body Location	Expected Resistance	Final Resistance
1	626678	<i>S. aureus</i> , MRSA	United States	2010	Bodily Fluids: Synovial	DAP R	VAN S / DAP NS / LNZ S
2	410696	<i>S. aureus</i> , MRSA	United States	2008	CVS: Blood	DAP R	VAN S / DAP NS / LNZ S
3	651970	<i>S. aureus</i> , MRSA	Canada	2010	INT: Wound	LNZ R	VAN S / DAP S / LNZ R
4	672232	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
5	1146992	<i>E. faecium</i>	Austria	2014	INT: Wound	LNZ R / VAN R	LNZ R / VAN R
6	1180874	<i>S. aureus</i> , MRSA	Spain	2014	GI: Liver	LNZ R	VAN S / DAP S / LNZ R
7	746091	<i>S. aureus</i> , MRSA	Spain	2011	Respiratory: Bronchials	LNZ R	VAN S / DAP S / LNZ S
8	817763	<i>S. aureus</i> , MRSA	United States	2012	Respiratory: Bronchials	LNZ R	VAN S / DAP S / LNZ R
9	651971	<i>S. aureus</i> , MRSA	Canada	2010	INT: Wound	LNZ R	VAN S / DAP S / LNZ R
10	878234	<i>S. aureus</i> , MRSA	Spain	2012	INT: Wound	DAP R	VAN S / DAP NS / LNZ S
11	649380	<i>S. aureus</i> , MRSA	United States	1997	Blood stream	VAN I	VAN I / DAP S / LNZ S
12	649390	<i>S. aureus</i> , MRSA	United States	2000	Bone/joint	VAN I	VAN I / DAP S / LNZ S
13	649391	<i>S. aureus</i> , MRSA	United States	2000	Wound	VAN I	VAN I / DAP S / LNZ S
14	1308248	<i>S. aureus</i> , MRSA	United States	2000	Cerebrospinal fluid	VAN I	VAN I / DAP NS / LNZ S
15	1308252	<i>S. aureus</i> , MRSA	United States	2000	Bile	VAN I	VAN S / DAP S / LNZ S
16	1308253	<i>S. aureus</i> , MRSA	Brazil	1998	Wound	VAN I	VAN S / DAP S / LNZ S
17	1308254	<i>S. aureus</i> , MRSA	Brazil	1999	Wound	VAN I	VAN I / DAP NS / LNZ S
18	672230	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
19	672231	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
20	672233	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
21	672234	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
22	672235	<i>S. aureus</i> , MRSA	United States	2010	Unknown	VAN R	VAN R / DAP S / LNZ S
23	1308256	<i>S. aureus</i> , MRSA	United States	2009	Wound	VAN R	VAN R / DAP S / LNZ S
24	1308258	<i>S. aureus</i> , MRSA	United States	2010	Wound	VAN R	VAN R / DAP S / LNZ S
25	649401	<i>S. aureus</i> , MRSA	United States	2001	Peritonitis	LNZ R	VAN S / DAP S / LNZ R
26	649402	<i>S. aureus</i> , MRSA	United States	2001	Peritonitis	LNZ R	VAN S / DAP S / LNZ R
27	649404	<i>S. aureus</i> , MRSA	United States	2002	Sputum	LNZ R	VAN S / DAP S / LNZ R
28	309191	<i>S. aureus</i> , MRSA	Belgium	2007	HEENT: Nose	LNZ R / VAN R	VAN S / DAP S / LNZ S
29	114489	<i>S. aureus</i> , MRSA	Italy	2001	CVS: Blood	LNZ R / VAN R	VAN S / DAP S / LNZ S
30	860769	<i>E. faecalis</i>	Colombia	2012	Bodily Fluids: Peritoneal	LNZ R / VAN R	LNZ S / VAN S / DAP NS
31	862935	<i>E. faecalis</i>	Turkey	2012	GU: Urine	LNZ R / VAN R	LNZ S / VAN S
32	925016	<i>S. aureus</i> , MRSA	Germany	2013	INT: Wound	DAP R	VAN S / DAP S / LNZ S
33	943616	<i>S. aureus</i> , MRSA	Austria	2013	INT: Skin Ulcer	DAP R	VAN S / DAP NS / LNZ S
34	1018136	<i>S. aureus</i> , MRSA	Spain	2013	INT: Wound	DAP R	VAN S / DAP NS / LNZ S

#	Organism No	Organism	Country	Year Collected	Body Location	Expected Resistance	Final Resistance
35	1018140	<i>S. aureus</i> , MRSA	Spain	2013	INT: Wound	DAP R	VAN S / DAP NS / LNZ S
36	927059	<i>S. aureus</i> , MRSA	Turkey	2013	INT: Abscess	DAP R	VAN S / DAP NS / LNZ S
37	927060	<i>S. aureus</i> , MRSA	Turkey	2013	INT: Wound	DAP R	VAN S / DAP NS / LNZ S
38	927061	<i>S. aureus</i> , MRSA	Turkey	2013	INT: Wound	DAP R	VAN S / DAP NS / LNZ S
39	928778	<i>S. aureus</i> , MRSA	Spain	2013	INT: Wound	LNZ R	VAN S / DAP S / LNZ R
40	1246376	<i>S. aureus</i> , MSSA	United States	2015	INT: Wound	-	VAN S / DAP S / LNZ S
41	1268522	<i>S. aureus</i> , MSSA	Austria	2015	INT: Wound	-	VAN S / DAP S / LNZ S
42	1268530	<i>S. aureus</i> , MRSA	Austria	2015	INT: Decubitus	-	VAN S / DAP S / LNZ S
43	1268531	<i>S. aureus</i> , MRSA	Austria	2015	INT: Skin Ulcer	-	VAN S / DAP S / LNZ S
44	1268648	<i>S. aureus</i> , MRSA	Austria	2015	INT: Wound	-	VAN S / DAP S / LNZ S
45	1272493	<i>S. aureus</i> , MSSA	United States	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
46	1282516	<i>S. aureus</i> , MSSA	Canada	2015	INT: Wound	-	VAN S / DAP S / LNZ S
47	1276929	<i>S. aureus</i> , MSSA	France	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
48	1335968	<i>S. aureus</i> , MSSA	United Kingdom	2015	INT: Wound	-	VAN S / DAP S / LNZ S
49	1285320	<i>S. aureus</i> , MRSA	United States	2015	INT: Wound	-	VAN S / DAP NS / LNZ S
50	1259571	<i>S. aureus</i> , MRSA	Denmark	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
51	1336167	<i>S. aureus</i> , MSSA	United States	2015	INT: Burn	-	VAN S / DAP S / LNZ S
52	1267763	<i>S. aureus</i> , MSSA	United States	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
53	1435278	<i>S. aureus</i> , MSSA	United States	2016	INT: Skin Ulcer	-	VAN S / DAP S / LNZ S
54	1428168	<i>S. aureus</i> , MSSA	United States	2016	INT: Wound	-	VAN S / DAP S / LNZ S
55	1475091	<i>S. aureus</i> , MSSA	France	2016	INT: Wound	-	VAN S / DAP S / LNZ S
56	1475093	<i>S. aureus</i> , MSSA	France	2016	INT: Wound	-	VAN S / DAP S / LNZ S
57	1518939	<i>S. aureus</i> , MSSA	Czech Republic	2016	INT: Wound	-	VAN S / DAP S / LNZ S
58	1518943	<i>S. aureus</i> , MSSA	Czech Republic	2016	INT: Wound	-	VAN S / DAP S / LNZ S
59	1528968	<i>S. aureus</i> , MRSA	Belgium	2016	INT: Other	-	VAN S / DAP S / LNZ S
60	1481133	<i>S. aureus</i> , MRSA	Czech Republic	2016	INT: Wound	-	VAN S / DAP S / LNZ S
61	1502818	<i>S. aureus</i> , MSSA	Italy	2016	INT: Wound	-	VAN S / DAP S / LNZ S
62	1502830	<i>S. aureus</i> , MSSA	Italy	2016	INT: Wound	-	VAN S / DAP S / LNZ S
63	1502834	<i>S. aureus</i> , MSSA	Italy	2016	INT: Wound	-	VAN S / DAP S / LNZ S
64	1540234	<i>S. aureus</i> , MSSA	Germany	2016	INT: Wound	-	VAN S / DAP S / LNZ S
65	1540236	<i>S. aureus</i> , MSSA	Germany	2016	INT: Wound	-	VAN S / DAP S / LNZ S
66	1540244	<i>S. aureus</i> , MSSA	Germany	2016	INT: Wound	-	VAN S / DAP S / LNZ S
67	1503418	<i>S. aureus</i> , MSSA	Germany	2016	INT: Wound	-	VAN S / DAP S / LNZ S
68	1503430	<i>S. aureus</i> , MSSA	Germany	2016	INT: Wound	-	VAN S / DAP S / LNZ S
69	1503437	<i>S. aureus</i> , MSSA	Germany	2016	INT: Abscess	-	VAN S / DAP S / LNZ S
70	1503428	<i>S. aureus</i> , MSSA	Germany	2016	INT: Abscess	-	VAN S / DAP S / LNZ S
71	1479809	<i>S. aureus</i> , MRSA	France	2016	INT: Abscess	-	VAN S / DAP S / LNZ S
72	1551440	<i>S. aureus</i> , MSSA	Greece	2016	Bodily Fluids: Abscess/Pus	-	VAN S / DAP S / LNZ S
73	1284277	<i>S. aureus</i> , MSSA	France	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
74	1247465	<i>S. aureus</i> , MSSA	United States	2015	INT: Wound	-	VAN S / DAP S / LNZ S
75	1303514	<i>S. aureus</i> , MRSA	Belgium	2015	INT: Wound	-	VAN S / DAP S / LNZ S
76	1260284	<i>S. aureus</i> , MSSA	Czech Republic	2015	INT: Wound	-	VAN S / DAP S / LNZ S
77	1272234	<i>S. aureus</i> , MRSA	Czech Republic	2015	INT: Wound	-	VAN S / DAP S / LNZ S
78	1479327	<i>S. aureus</i> , MRSA	Belgium	2016	INT: Abscess	-	VAN S / DAP S / LNZ S

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79	1480924	<i>S. aureus</i> , MSSA	Netherlands	2016	INT: Other	-	VAN S / DAP S / LNZ S
80	1480926	<i>S. aureus</i> , MSSA	Netherlands	2016	INT: Wound	-	VAN S / DAP S / LNZ S
81	1469091	<i>S. aureus</i> , MRSA	France	2016	Respiratory: Sputum	LNZ R	VAN S / DAP S / LNZ R
82	1319253	<i>S. aureus</i> , MSSA	Belgium	2015	INT: Wound	-	VAN S / DAP S / LNZ S
83	1311254	<i>S. aureus</i> , MSSA	Denmark	2015	INT: Wound	-	VAN S / DAP S / LNZ S
84	1332641	<i>S. aureus</i> , MSSA	United States	2015	INT: Wound	-	VAN S / DAP S / LNZ S
85	1315814	<i>S. aureus</i> , MSSA	Germany	2015	INT: Wound	-	VAN S / DAP S / LNZ S
86	1228010	<i>S. aureus</i> , MSSA	Canada	2015	Bodily Fluids: Abscess/Pus	-	VAN S / DAP S / LNZ S
87	1331930	<i>S. aureus</i> , MSSA	United Kingdom	2015	INT: Wound	-	VAN S / DAP S / LNZ S
88	1174479	<i>S. aureus</i> , MSSA	Croatia	2015	INT: Wound	-	VAN S / DAP S / LNZ S
89	1322231	<i>S. aureus</i> , MSSA	Spain	2015	INT: Wound	-	VAN S / DAP S / LNZ S
90	1258353	<i>S. aureus</i> , MRSA	Spain	2015	INT: Skin Ulcer	-	VAN S / DAP S / LNZ S
91	1262881	<i>S. aureus</i> , MSSA	Germany	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
92	1283184	<i>S. aureus</i> , MSSA	Turkey	2015	INT: Abscess	-	VAN S / DAP S / LNZ S
93	1296195	<i>S. aureus</i> , MSSA	Spain	2015	INT: Skin	-	VAN S / DAP S / LNZ S
94	1280864	<i>S. aureus</i> , MSSA	Portugal	2015	INT: Skin Ulcer	-	VAN S / DAP S / LNZ S
95	1449510	<i>S. aureus</i> , MSSA	United States	2016	INT: Wound	-	VAN S / DAP S / LNZ S
96	1464473	<i>S. aureus</i> , MSSA	Canada	2016	INT: Wound	-	VAN S / DAP S / LNZ S
97	1427879	<i>S. aureus</i> , MSSA	United States	2016	INT: Other	-	VAN S / DAP S / LNZ S
98	1412354	<i>S. aureus</i> , MSSA	Canada	2016	INT: Wound	-	VAN S / DAP S / LNZ S
99	1428822	<i>S. aureus</i> , MSSA	United States	2016	INT: Wound	-	VAN S / DAP S / LNZ S
100	1426641	<i>S. aureus</i> , MRSA	United States	2016	INT: Wound	-	VAN S / DAP S / LNZ S
101	1537751	<i>S. aureus</i> , MSSA	Canada	2016	INT: Wound	-	VAN S / DAP S / LNZ S
102	1537741	<i>S. aureus</i> , MSSA	Canada	2016	INT: Skin	-	VAN S / DAP S / LNZ S
103	1535356	<i>S. aureus</i> , MSSA	United States	2016	INT: Wound	-	VAN S / DAP S / LNZ S
104	1533772	<i>E. faecium</i>	United States	2016	Bodily Fluids: Peritoneal	LNZ R / VAN R	LNZ R / VAN R
105	1766256	<i>E. faecium</i>	United States	2017	GI: Other	VAN R	LNZ S / VAN R
106	1765156	<i>E. faecium</i>	Germany	2017	GI: Abscess	VAN R	LNZ R / VAN R
107	1569172	<i>E. faecalis</i>	Lithuania	2017	GI: Gall Bladder	-	LNZ S / VAN S
108	1602010	<i>E. faecium</i>	France	2017	GU: Urine	-	LNZ S / VAN S
109	1602013	<i>E. faecium</i>	France	2017	GU: Urine	-	LNZ S / VAN S
110	1606748	<i>E. faecalis</i>	Serbia	2017	GU: Urine	-	LNZ S / VAN S
111	1766601	<i>E. faecalis</i>	United States	2017	GU: Urine	VAN R	LNZ S/ VAN R
112	1766602	<i>E. faecalis</i>	United States	2017	GU: Urine	VAN R	LNZ S/ VAN R
113	1262561	<i>S. pyogenes</i>	Portugal	2015	INT: Cellulitis/Erysipelas	MACRO R	MACRO R
114	1317874	<i>S. pyogenes</i>	United States	2015	INT: Abscess	MACRO R	MACRO R
115	1301252	<i>S. pyogenes</i>	United States	2015	INT: Cellulitis/Erysipelas	MACRO R	MACRO S
116	1201162	<i>S. pyogenes</i>	France	2015	INT: Skin	-	MACRO S
117	1342786	<i>S. pyogenes</i>	United States	2015	INT: Wound	MACRO R	MACRO S
118	1261497	<i>S. pyogenes</i>	Italy	2015	INT: Abscess	-	MACRO S
119	1261500	<i>S. pyogenes</i>	Italy	2015	INT: Other	MACRO R	MACRO S
120	1279407	<i>S. pyogenes</i>	United Kingdom	2015	INT: Wound	-	MACRO S
121	1328069	<i>S. pyogenes</i>	Germany	2015	INT: Wound	-	MACRO S

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122	1270153	<i>S. pyogenes</i>	Russia	2015	INT: Abscess	-	MACRO S
123	1262994	<i>S. pyogenes</i>	Germany	2015	INT: Wound	MACRO R	MACRO S
124	1247617	<i>S. pyogenes</i>	United States	2015	INT: Abscess	-	MACRO S
125	1267955	<i>S. pyogenes</i>	United States	2015	INT: Wound	MACRO R	MACRO S
126	1267719	<i>S. pyogenes</i>	United States	2015	INT: Wound	-	MACRO S
127	1336219	<i>S. pyogenes</i>	United States	2015	INT: Wound	-	MACRO S
128	1279084	<i>S. pyogenes</i>	United Kingdom	2015	INT: Other	MACRO R	MACRO S
129	1424074	<i>S. pyogenes</i>	Italy	2016	INT: Abscess	-	MACRO S
130	1422650	<i>S. pyogenes</i>	Spain	2016	INT: Wound	-	MACRO S
131	1428657	<i>S. pyogenes</i>	United States	2016	INT: Abscess	-	MACRO S
132	1465140	<i>S. pyogenes</i>	Germany	2016	INT: Skin Ulcer	MACRO R	MACRO S
133	1519165	<i>S. pyogenes</i>	Spain	2016	INT: Wound	-	MACRO S
134	1447632	<i>S. pyogenes</i>	Poland	2016	INT: Wound	MACRO R	MACRO S
135	1419666	<i>S. pyogenes</i>	Russia	2016	INT: Wound	-	MACRO S
136	1413766	<i>S. pyogenes</i>	Russia	2016	INT: Wound	MACRO R	MACRO R
137	1440834	<i>S. pyogenes</i>	Czech Republic	2016	INT: Wound	MACRO R	MACRO R
138	1565572	<i>S. pneumoniae</i>	United States	2017	Respiratory: Sinuses	-	PEN I
139	1612737	<i>S. pneumoniae</i>	Germany	2017	GU: Urine	-	PEN I
140	1661575	<i>S. pneumoniae</i>	Germany	2017	GU: Urine	-	PEN S
141	1610983	<i>S. pneumoniae</i>	France	2017	GI: Liver	-	PEN S
142	1590644	<i>S. pneumoniae</i>	United Kingdom	2017	Respiratory: Sputum	PEN R	PEN R
143	1590532	<i>S. pneumoniae</i>	United Kingdom	2017	Respiratory: Sputum	PEN R	PEN R
144	1565469	<i>S. pneumoniae</i>	Switzerland	2017	Respiratory: Bronchials	-	PEN S
145	1565470	<i>S. pneumoniae</i>	Switzerland	2017	Respiratory: Bronchials	-	PEN I
146	1621647	<i>S. pneumoniae</i>	Italy	2017	Bodily Fluids: Peritoneal	-	PEN S
147	1635458	<i>S. pneumoniae</i>	Spain	2017	Bodily Fluids: Peritoneal	-	PEN S
148	1635459	<i>S. pneumoniae</i>	Spain	2017	GI: Abscess	-	PEN S
149	1591861	<i>S. pneumoniae</i>	Australia	2017	GU: Urine	-	PEN I
150	1695310	<i>S. pneumoniae</i>	Taiwan	2017	GU: Urine	PEN R	PEN R
151	1695316	<i>S. pneumoniae</i>	Taiwan	2017	Bodily Fluids: Peritoneal	PEN R	PEN R
152	1695116	<i>S. pneumoniae</i>	Philippines	2017	Respiratory: Sputum	PEN R	PEN R
153	1744977	<i>S. pneumoniae</i>	Czech Republic	2017	Bodily Fluids: Peritoneal	-	PEN I
154	1654013	<i>S. pneumoniae</i>	United States	2017	Respiratory: Bronchials	PEN R	PEN R
155	1582680	<i>S. pneumoniae</i>	Czech Republic	2017	GU: Other	-	PEN S
156	1592877	<i>S. pneumoniae</i>	Australia	2017	Respiratory: Sputum	PEN R	PEN R
157	1592882	<i>S. pneumoniae</i>	Australia	2017	Respiratory: Sputum	PEN R	PEN R
158	1616271	<i>S. pneumoniae</i>	Taiwan	2017	GU: Urine	PEN R	PEN R
159	1598607	<i>S. pneumoniae</i>	Philippines	2017	Respiratory: Sputum	-	PEN I
160	1519981	<i>S. pneumoniae</i>	France	2017	GU: Urine	-	PEN I
161	1532565	<i>S. pneumoniae</i>	Vietnam	2017	Respiratory: Sputum	PEN R	PEN R
162	1734753	<i>S. pneumoniae</i>	Japan	2017	Respiratory: Sputum	PEN R	PEN R
Spare	1765227	<i>E. faecium</i>	France	2017	GI: Gall Bladder	-	LNZ S / VAN S
Spare	1426580	<i>S. aureus</i> , MRSA	United States	2016	INT: Abscess	-	VAN S / DAP S / LNZ S

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Spare	1525771	<i>S. aureus</i> , MSSA	United States	2016	Bodily Fluids: Abscess / Pus	-	VAN S / DAP S / LNZ S
Spare	1765036	<i>E. faecalis</i>	Korea, South	2017	Bodily Fluids: Peritoneal	-	LNZ S / VAN S
Spare	1426536	<i>S. pyogenes</i>	United States	2016	INT: Abscess	MACRO R	MACRO R

DAP: daptomycin; LNZ: linezolid; MACRO: macrolide; PEN: penicillin; VAN: vancomycin; R: resistant; I: intermediate; NS: non-susceptible; S: susceptible. GU: Genital/urinary. GI: Gastrointestinal, INT: Integumentary, CVS: cardiovascular system

**Table S2.** Line listing of the MIC data for 167 the Gram-positive isolates

Organism No	Organism	MIC (µg/mL)										
		MUP	NANO	VAN	DAP	PEN	LNZ	FOX	ERY	TET	LVX	SXT
626678	<i>S. aureus</i> , MRSA	0.25	0.5	2	4	≤0.06	2	>4	>4	0.12	>4	0.12
410696	<i>S. aureus</i> , MRSA	0.25	1	2	2	>16	1	>4	>4	0.12	4	≤0.06
651970	<i>S. aureus</i> , MRSA	0.25	1	1	0.5	8	>8	>4	0.5	0.5	>4	0.5
672232	<i>S. aureus</i> , MRSA	0.12	0.5	>16	0.5	>16	2	>4	>4	16	>4	≤0.06
1146992	<i>E. faecium</i>	1	4	>16	2	>16	>8	>4	>4	4	>4	>2
1180874	<i>S. aureus</i> , MRSA	0.25	1	2	0.5	16	>8	>4	0.25	0.5	>4	≤0.06
746091	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.25	16	2	>4	0.25	0.25	>4	≤0.06
817763	<i>S. aureus</i> , MRSA	32	>64	2	1	>16	>8	>4	>4	4	>4	≤0.06
651971	<i>S. aureus</i> , MRSA	0.25	1	1	0.5	8	>8	>4	0.5	0.5	>4	0.25
878234	<i>S. aureus</i> , MRSA	0.25	0.5	2	4	8	1	>4	0.25	0.12	>4	≤0.06
649380	<i>S. aureus</i> , MRSA	0.25	0.5	4	1	16	1	>4	>4	0.25	>4	≤0.06
649390	<i>S. aureus</i> , MRSA	0.25	0.5	4	1	>16	2	>4	>4	0.25	>4	>2
649391	<i>S. aureus</i> , MRSA	16	>64	4	1	>16	2	>4	>4	0.25	>4	0.5
1308248	<i>S. aureus</i> , MRSA	>64	>64	4	2	>16	2	>4	>4	0.5	>4	>2
1308252	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.25	0.25	1	1	0.25	0.25	0.12	0.25
1308253	<i>S. aureus</i> , MRSA	>64	>64	2	1	>16	1	>4	>4	>32	>4	>2
1308254	<i>S. aureus</i> , MRSA	0.25	0.5	4	2	>16	1	>4	>4	32	>4	>2
672230	<i>S. aureus</i> , MRSA	16	32	>16	1	>16	2	>4	>4	2	>4	>2
672231	<i>S. aureus</i> , MRSA	0.12	0.25	>16	1	8	1	>4	>4	4	>4	>2
672233	<i>S. aureus</i> , MRSA	0.12	0.5	>16	0.25	>16	2	>4	>4	32	>4	≤0.06
672234	<i>S. aureus</i> , MRSA	0.25	1	>16	0.5	>16	4	>4	>4	0.5	>4	≤0.06
672235	<i>S. aureus</i> , MRSA	0.5	0.5	>16	0.5	16	4	>4	>4	0.5	>4	≤0.06
1308256	<i>S. aureus</i> , MRSA	0.25	0.5	>16	0.5	>16	2	>4	>4	0.25	>4	≤0.06
1308258	<i>S. aureus</i> , MRSA	8	16	>16	0.5	4	2	>4	>4	0.12	>4	0.25
649401	<i>S. aureus</i> , MRSA	0.12	0.25	2	1	>16	>8	>4	0.25	32	>4	>2
649402	<i>S. aureus</i> , MRSA	0.12	0.12	2	1	>16	>8	>4	0.5	32	>4	0.25
649404	<i>S. aureus</i> , MRSA	16	32	2	0.5	16	8	>4	>4	16	>4	≤0.06
309191	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	4	4	>4	>4	0.25	>4	0.12
114489	<i>S. aureus</i> , MRSA	0.25	0.5	1	1	>16	2	>4	>4	0.25	>4	0.12
860769	<i>E. faecalis</i>	32	>64	1	2	2	2	>4	>4	32	1	>2
862935	<i>E. faecalis</i>	64	>64	1	1	2	2	>4	0.25	>32	1	≤0.06
925016	<i>S. aureus</i> , MRSA	0.12	0.5	1	0.5	2	2	1	0.25	0.5	0.25	≤0.06
943616	<i>S. aureus</i> , MRSA	0.12	0.12	2	4	>16	2	>4	0.25	0.25	>4	≤0.06
1018136	<i>S. aureus</i> , MRSA	0.25	0.25	2	2	16	2	>4	0.25	0.25	4	≤0.06
1018140	<i>S. aureus</i> , MRSA	0.25	0.5	2	4	8	2	>4	0.25	0.25	>4	≤0.06
927059	<i>S. aureus</i> , MRSA	0.5	1	2	4	>16	2	>4	>4	>32	>4	0.25
927060	<i>S. aureus</i> , MRSA	0.25	1	2	2	>16	2	>4	>4	>32	>4	0.25
927061	<i>S. aureus</i> , MRSA	0.25	1	2	4	>16	2	>4	>4	>32	>4	0.25
928778	<i>S. aureus</i> , MRSA	>64	>64	1	0.25	>16	>8	>4	0.25	0.5	>4	≤0.06
1246376	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	4	2	4	0.12	0.25	0.25	≤0.06
1268522	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	1	4	0.25	0.25	0.12	≤0.06
1268530	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	>16	2	>4	>4	0.25	>4	≤0.06
1268531	<i>S. aureus</i> , MRSA	32	64	1	0.5	>16	2	>4	>4	0.25	>4	≤0.06

1268648	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	16	2	>4	>4	0.25	>4	≤0.06
1272493	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	0.5	2	4	0.25	0.25	0.25	≤0.06
1282516	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	16	2	2	0.25	0.25	0.25	≤0.06
1276929	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	≤0.06	1	2	0.25	0.25	0.25	≤0.06
1335968	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	0.5	2	2	>4	0.5	0.25	≤0.06
1285320	<i>S. aureus</i> , MRSA	0.12	0.12	0.5	2	0.12	0.5	>4	≤0.015	0.12	>4	0.25
1259571	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	16	2	>4	0.5	0.25	0.25	≤0.06
1336167	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	0.25	2	4	0.25	0.25	0.25	≤0.06
1267763	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	0.25	0.25	0.25	≤0.06
1435278	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	16	2	4	>4	0.5	0.12	≤0.06
1428168	<i>S. aureus</i> , MSSA	>64	>64	1	0.5	0.12	2	4	0.25	0.25	0.25	≤0.06
1475091	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	16	2	4	0.25	0.25	>4	≤0.06
1475093	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	>4	0.5	0.25	≤0.06
1518939	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	0.12	2	4	>4	0.12	2	≤0.06
1518943	<i>S. aureus</i> , MSSA	0.25	0.25	2	0.5	≤0.06	2	4	0.25	0.25	0.25	≤0.06
1528968	<i>S. aureus</i> , MRSA	0.12	0.5	1	0.5	>16	2	>4	0.12	>32	>4	0.5
1481133	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	>16	4	>4	0.25	0.5	0.25	≤0.06
1502818	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	0.25	0.25	0.25	≤0.06
1502830	<i>S. aureus</i> , MSSA	0.12	0.5	0.5	0.25	>16	2	4	>4	0.25	0.12	≤0.06
1502834	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	2	2	4	0.25	0.25	0.25	≤0.06
1540234	<i>S. aureus</i> , MSSA	0.12	0.25	1	0.25	2	2	2	0.25	0.12	0.12	0.12
1540236	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	0.25	0.25	0.25	≤0.06
1540244	<i>S. aureus</i> , MSSA	0.25	0.5	1	1	≤0.06	2	4	0.25	0.25	>4	≤0.06
1503418	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	>16	2	2	0.25	0.25	0.25	0.12
1503430	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	2	0.25	0.25	0.12	≤0.06
1503437	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	2	0.25	0.25	≤0.06
1503428	<i>S. aureus</i> , MSSA	0.25	0.5	0.5	0.5	≤0.06	2	2	0.25	0.25	0.25	≤0.06
1479809	<i>S. aureus</i> , MRSA	0.12	0.25	1	0.5	>16	2	>4	>4	32	4	≤0.06
1551440	<i>S. aureus</i> , MSSA	0.12	0.25	1	0.5	0.5	2	4	0.25	0.25	0.25	0.12
1284277	<i>S. aureus</i> , MSSA	0.12	0.25	1	0.5	≤0.06	2	4	0.25	0.25	0.12	≤0.06
1247465	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	16	2	4	>4	0.25	0.25	≤0.06
1303514	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	>16	2	>4	>4	0.25	>4	≤0.06
1260284	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	8	2	4	0.25	0.25	0.25	≤0.06
1272234	<i>S. aureus</i> , MRSA	0.12	0.25	1	0.5	16	2	>4	>4	0.25	>4	0.5
1479327	<i>S. aureus</i> , MRSA	0.12	0.25	2	1	>16	1	>4	>4	>32	>4	0.5
1480924	<i>S. aureus</i> , MSSA	0.25	1	1	0.5	>16	2	4	0.25	0.5	0.25	≤0.06
1480926	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	8	2	2	0.5	0.25	0.12	≤0.06
1469091	<i>S. aureus</i> , MRSA	0.12	0.5	2	1	16	>8	>4	>4	>32	>4	0.12
1319253	<i>S. aureus</i> , MSSA	0.12	0.25	1	0.5	0.12	2	4	0.25	0.25	0.25	≤0.06
1311254	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	≤0.06	2	4	0.12	0.25	0.12	≤0.06
1332641	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	≤0.06	2	2	0.25	0.5	0.25	≤0.06
1315814	<i>S. aureus</i> , MSSA	0.25	0.5	2	0.5	8	4	2	0.5	0.5	0.12	≤0.06
1228010	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	0.5	2	2	0.5	0.25	0.12	≤0.06
1331930	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	>16	2	4	0.25	0.25	0.25	≤0.06
1174479	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	16	2	2	0.25	0.25	0.25	≤0.06
1322231	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	8	2	2	0.5	0.5	0.5	≤0.06
1258353	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	>16	4	>4	>4	0.5	>4	0.12
1262881	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	≤0.06	2	2	0.5	0.25	0.25	≤0.06

1283184	<i>S. aureus</i> , MSSA	0.25	0.25	1	0.5	0.25	2	4	0.5	0.25	0.12	≤0.06
1296195	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	0.12	2	4	0.25	0.25	0.25	≤0.06
1280864	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	>4	0.25	0.25	≤0.06
1449510	<i>S. aureus</i> , MSSA	0.12	0.25	1	0.5	>16	2	4	0.25	0.25	0.12	≤0.06
1464473	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	>16	2	4	0.25	0.5	1	≤0.06
1427879	<i>S. aureus</i> , MSSA	0.12	0.25	0.5	0.25	0.25	2	4	>4	0.5	0.25	≤0.06
1412354	<i>S. aureus</i> , MSSA	0.12	0.12	0.5	0.25	8	2	2	0.25	0.5	0.12	0.12
1428822	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.25	≤0.06	1	2	0.12	0.25	0.12	≤0.06
1426641	<i>S. aureus</i> , MRSA	0.25	0.5	1	0.5	>16	2	>4	>4	0.25	4	≤0.06
1537751	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	8	2	2	0.5	0.25	0.25	≤0.06
1537741	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	0.25	0.25	0.5	≤0.06
1535356	<i>S. aureus</i> , MSSA	0.25	0.5	1	0.5	≤0.06	2	4	0.25	0.25	0.25	≤0.06
1533772	<i>E. faecium</i>	0.5	2	>16	4	>16	>8	>4	2	32	>4	>2
1766256	<i>E. faecium</i>	1	2	>16	4	>16	4	>4	>4	16	>4	>2
1765156	<i>E. faecium</i>	1	2	>16	4	>16	8	>4	>4	0.5	>4	>2
1569172	<i>E. faecalis</i>	>64	>64	1	1	2	2	>4	2	>32	1	≤0.06
1602010	<i>E. faecium</i>	1	2	1	4	>16	2	>4	2	>32	>4	>2
1602013	<i>E. faecium</i>	1	2	1	4	>16	2	>4	2	>32	>4	>2
1606748	<i>E. faecalis</i>	32	>64	1	2	2	2	>4	0.25	16	2	≤0.06
1766601	<i>E. faecalis</i>	64	>64	>16	0.5	2	2	>4	>4	>32	>4	>2
1766602	<i>E. faecalis</i>	64	>64	>16	2	8	2	>4	>4	>32	>4	>2
1262561	<i>S. pyogenes</i>	0.25	0.5	0.5	0.12	0.12	2	2	4	0.25	0.5	0.12
1317874	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	2	>4	0.25	0.5	0.25
1301252	<i>S. pyogenes</i>	0.25	0.25	0.5	0.12	≤0.06	1	≤1	0.03	0.25	0.25	0.12
1201162	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	≤1	0.03	0.25	0.5	0.12
1342786	<i>S. pyogenes</i>	0.25	0.12	0.5	0.12	≤0.06	1	≤1	0.03	0.25	0.5	≤0.06
1261497	<i>S. pyogenes</i>	0.12	0.25	0.5	0.06	≤0.06	2	≤1	0.06	0.25	0.5	≤0.06
1261500	<i>S. pyogenes</i>	0.25	0.12	0.5	0.06	≤0.06	1	≤1	0.06	0.25	0.5	≤0.06
1279407	<i>S. pyogenes</i>	0.12	0.25	0.5	0.06	0.12	2	≤1	0.03	0.25	0.5	0.5
1328069	<i>S. pyogenes</i>	0.12	0.12	0.25	0.06	≤0.06	1	2	0.03	32	0.5	0.25
1270153	<i>S. pyogenes</i>	0.25	0.25	0.5	0.06	≤0.06	4	2	0.03	0.5	0.5	0.25
1262994	<i>S. pyogenes</i>	0.25	0.12	0.5	0.12	≤0.06	2	≤1	0.06	8	0.5	0.5
1247617	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	2	0.06	0.25	1	0.25
1267955	<i>S. pyogenes</i>	0.25	0.06	0.5	0.12	≤0.06	1	≤1	0.03	0.25	0.5	≤0.06
1267719	<i>S. pyogenes</i>	0.25	0.25	0.5	0.06	0.12	2	2	0.06	0.25	0.5	0.12
1336219	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	2	0.06	0.25	2	0.12
1279084	<i>S. pyogenes</i>	0.12	0.25	0.25	0.06	≤0.06	0.5	2	0.03	8	0.25	0.25
1424074	<i>S. pyogenes</i>	1	4	0.5	0.25	0.12	2	2	0.06	0.5	1	0.12
1422650	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	≤1	0.03	0.25	0.25	≤0.06
1428657	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	2	0.03	0.25	0.5	0.5
1465140	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	2	0.03	32	0.5	≤0.06
1519165	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	≤1	0.03	>32	0.5	0.12
1447632	<i>S. pyogenes</i>	≤0.06	0.06	0.5	0.12	≤0.06	0.5	≤1	0.03	0.12	2	0.25
1419666	<i>S. pyogenes</i>	0.12	0.12	0.25	0.12	0.12	1	4	0.03	0.12	0.5	0.12
1413766	<i>S. pyogenes</i>	0.12	0.12	0.25	0.06	0.12	1	4	>4	0.12	0.5	0.12
1440834	<i>S. pyogenes</i>	0.12	0.12	0.5	0.12	0.12	1	2	>4	32	0.25	≤0.06
1565572	<i>S. pneumoniae</i>	0.5	0.5	0.25	0.12	0.12	1	4	4	0.25	1	0.12
1612737	<i>S. pneumoniae</i>	0.5	1	0.25	0.12	0.12	1	4	0.03	0.25	1	0.25



1661575	<i>S. pneumoniae</i>	0.5	1	0.5	0.5	≤0.06	2	4	0.03	0.25	1	0.25
1610983	<i>S. pneumoniae</i>	0.5	1	0.25	≤0.03	≤0.06	1	4	0.03	0.25	1	0.12
1590644	<i>S. pneumoniae</i>	0.25	1	0.25	0.25	4	1	>4	>4	16	1	>2
1590532	<i>S. pneumoniae</i>	0.5	1	0.5	0.12	4	1	>4	>4	16	1	>2
1565469	<i>S. pneumoniae</i>	0.5	1	0.25	0.12	≤0.06	1	2	0.03	0.25	1	0.25
1565470	<i>S. pneumoniae</i>	2	8	0.25	0.12	0.12	1	≤1	0.03	0.25	1	0.25
1621647	<i>S. pneumoniae</i>	4	8	0.25	0.25	≤0.06	1	2	>4	>32	0.5	0.25
1635458	<i>S. pneumoniae</i>	0.25	0.5	0.25	0.25	≤0.06	2	2	0.06	0.25	2	0.25
1635459	<i>S. pneumoniae</i>	4	8	0.25	0.25	≤0.06	1	2	0.06	0.25	1	0.25
1591861	<i>S. pneumoniae</i>	0.5	2	0.5	0.25	0.25	1	4	0.06	0.25	1	0.12
1695310	<i>S. pneumoniae</i>	0.25	0.5	0.5	0.25	4	1	>4	>4	32	1	>2
1695316	<i>S. pneumoniae</i>	0.25	0.5	1	0.25	4	2	>4	>4	>32	>4	>2
1695116	<i>S. pneumoniae</i>	2	4	0.5	0.25	8	1	>4	>4	32	0.5	0.25
1744977	<i>S. pneumoniae</i>	0.25	0.25	0.25	0.12	0.12	0.5	2	0.03	0.12	1	≤0.06
1654013	<i>S. pneumoniae</i>	0.5	1	0.5	0.5	2	1	>4	>4	>32	2	2
1582680	<i>S. pneumoniae</i>	0.25	0.25	0.25	0.25	≤0.06	1	≤1	0.03	0.25	1	0.12
1592877	<i>S. pneumoniae</i>	2	4	0.25	0.25	4	1	>4	>4	32	1	>2
1592882	<i>S. pneumoniae</i>	2	8	0.5	0.25	4	1	>4	>4	32	0.5	0.5
1616271	<i>S. pneumoniae</i>	4	8	0.25	0.12	4	1	>4	>4	32	1	>2
1598607	<i>S. pneumoniae</i>	0.25	0.25	0.25	0.25	0.12	1	4	0.03	32	1	0.25
1519981	<i>S. pneumoniae</i>	1	0.5	0.5	0.5	0.12	2	4	0.03	>32	1	0.25
1532565	<i>S. pneumoniae</i>	4	8	0.5	0.25	4	1	>4	>4	32	0.5	>2
1734753	<i>S. pneumoniae</i>	1	4	0.5	0.25	4	1	>4	>4	>32	>4	0.5
1765227	<i>E. faecium</i>	0.5	1	2	0.5	0.12	2	>4	>4	>32	2	≤0.06
1426580	<i>S. aureus</i> , MRSA	0.12	0.5	1	0.5	>16	2	>4	>4	0.25	>4	0.5
1525771	<i>S. aureus</i> , MSSA	0.12	0.5	1	0.5	≤0.06	4	2	0.5	0.5	0.25	≤0.06
1765036	<i>E. faecalis</i>	64	>64	0.5	1	2	1	>4	0.5	0.5	1	≤0.06
1426536	<i>S. pyogenes</i>	0.12	0.12	0.5	0.06	0.12	1	≤1	>4	16	0.5	0.25

MUP: mupirocin; Nano: nano-mupirocin; VAN: vancomycin; DAP: daptomycin; PEN: penicillin; LNZ: linezolid; FOX: cefoxitin; ERY: erythromycin; TER: tetracycline; LVX: levofloxacin; SXT: trimethoprim/sulfamethoxazole

**Table S3.** Mupirocin and Nano-mupirocin MICs for MRSA isolates resistant to one or more of vancomycin, daptomycin or linezolid

	MIC (µg/mL)				
Organism No	Mupirocin	Nano-mupirocin	Vancomycin	Daptomycin	Linezolid
<b>Vancomycin resistant</b>					
672232	0.12	0.5	>16	0.5	2
672230	16	32	>16	1	2
672231	0.12	0.25	>16	1	1
672233	0.12	0.5	>16	0.25	2
672234	0.25	1	>16	0.5	4
672235	0.5	0.5	>16	0.5	4
1308256	0.25	0.5	>16	0.5	2
1308258	8	16	>16	0.5	2
<b>Daptomycin non-susceptible</b>					
626678	0.25	0.5	2	4	2
410696	0.25	1	2	2	1
878234	0.25	0.5	2	4	1
943616	0.12	0.12	2	4	2
1018136	0.25	0.25	2	2	2
1018140	0.25	0.5	2	4	2
927059	0.5	1	2	4	2
927060	0.25	1	2	2	2
927061	0.25	1	2	4	2
1285320	0.12	0.12	0.5	2	0.5
1308248	>64	>64	4	2	2
1308254	0.25	0.5	4	2	1
<b>Linezolid resistant</b>					
651970	0.25	1	1	0.5	>8
1180874	0.25	1	2	0.5	>8
817763	32	>64	2	1	>8
651971	0.25	1	1	0.5	>8
649401	0.12	0.25	2	1	>8
649402	0.12	0.12	2	1	>8
649404	16	32	2	0.5	8
928778	>64	>64	1	0.25	>8
1469091	0.12	0.5	2	1	>8

**Table S4.** MICs of Nano-mupirocin, mupirocin and rifampicin during passage / multi-step selection for nine Gram-positive clinical isolates.

IHMA number	Organism	Selecting agent	MIC (µg/mL) over at day														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
649380	<i>S. aureus</i> , MRSA	Nano-mupirocin	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5
		Mupirocin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
		Rifampicin	256	256	128	256	512	256	512	256	512	512	>512	512	>512	512	512
649390	<i>S. aureus</i> , MRSA	Nano-mupirocin	1	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5
		Mupirocin	0.25	0.25	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25
		Rifampicin	0.015	0.015	0.03	0.03	0.03	0.03	0.03	0.03	0.015	0.015	0.015	0.015	0.015	0.015	0.015
1308254	<i>S. aureus</i> , MRSA	Nano-mupirocin	1	0.5	0.5	1	0.5	1	0.5	0.5	0.5	0.25	0.5	0.5	0.5	1	0.5
		Mupirocin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
		Rifampicin	2	1	2	2	2	2	2	2	2	2	4	2	2	4	2
672231	<i>S. aureus</i> , MRSA	Nano-mupirocin	0.25	0.25	0.25	0.5	0.25	0.5	0.5	0.25	0.25	0.25	0.5	0.5	0.5	1	1
		Mupirocin	0.12	0.12	0.25	1	0.5	1	0.5	0.5	1	1	1	1	0.5	1	1
		Rifampicin	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
672233	<i>S. aureus</i> , MRSA	Nano-mupirocin	0.5	0.25	0.25	0.25	0.5	0.5	0.5	0.25	0.25	0.12	0.5	0.5	0.5	0.5	0.5
		Mupirocin	0.12	0.12	0.12	0.12	0.25	0.12	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.25	0.5
		Rifampicin	0.004	0.008	0.015	0.008	0.015	0.015	0.015	0.015	0.015	0.015	0.008	0.008	0.015	0.015	0.015
672232	<i>S. aureus</i> , MRSA	Nano-mupirocin	0.25	0.25	0.5	>32	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5
		Mupirocin	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.25	0.25	0.25	0.12	0.25	0.12
		Rifampicin	0.004	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.008	0.008	0.015	0.015	0.015
1146992	<i>E. faecium</i>	Nano-mupirocin	2	4	4	4	4	4	8	8	8	4	8	8	8	8	8
		Mupirocin	1	1	2	2	2	2	2	4	2	4	4	4	4	4	4
		Rifampicin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512
1533772	<i>E. faecium</i>	Nano-mupirocin	2	2	4	8	8	16	16	16	16	8	32	16	32	32	32
		Mupirocin	0.5	0.5	0.5	1	2	2	2	4	2	4	4	8	4	8	4
		Rifampicin	4	8	8	8	16	8	16	16	16	32	16	16	32	16	16
1765156	<i>E. faecium</i>	Nano-mupirocin	1	2	1	2	4	4	8	4	4	2	8	16	8	8	8
		Mupirocin	0.5	1	2	2	2	4	2	2	4	4	8	8	8	8	8
		Rifampicin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512

Highlight in red: possible issue with MIC plate on that day

**Table S5.** MICs for potential mutants obtained in multi-step selection, as re-tested after five days of subculture on drug- free agar.

IHMA no.	Organism	Selective agent	Day	Drug	Original MIC (µg/mL)	MIC when potential mutant was isolated (µg/mL)	MIC after 5 non selective subculture (µg/mL)	Comment
649380	<i>S. aureus</i> , MRSA	Rifampicin	11	Nano-mupirocin	0.5	-	0.5	No increase of Nano-mupirocin MIC observed
				Mupirocin	0.25	-	0.25	No increase of Mupirocin MIC observed
				Rifampicin	256	>512	>512	Mutant confirmed for Rifampicin
672231	<i>S. aureus</i> , MRSA	Mupirocin	4	Nano-mupirocin	0.25	-	2	Parallel increase of Nano-Mupirocin MIC
				Mupirocin	0.12	1	0.5	Mutant confirmed for Mupirocin
				Rifampicin	0.004	-	0.004	No increase of Rifampicin MIC observed
		Nano-mupirocin	14	Nano-mupirocin	0.25	1	2	Mutant confirmed for Nano-Mupirocin
				Mupirocin	0.12	-	0.5	Parallel increase of Mupirocin MIC
				Rifampicin	0.004	-	0.004	No increase of Rifampicin MIC observed
672233	<i>S. aureus</i> , MRSA	Rifampicin	3	Nano-mupirocin	0.5	-	0.5	No increase of Nano-Mupirocin MIC observed
				Mupirocin	0.12	-	0.12	No increase of Mupirocin MIC observed
				Rifampicin	0.004	0.015	0.008	Mutant not confirmed
		Mupirocin	15	Nano-mupirocin	0.5	-	1	No increase of Nano-Mupirocin MIC observed
				Mupirocin	0.12	0.5	0.25	Mutant not confirmed
				Rifampicin	0.004	-	0.008	No increase of Rifampicin MIC observed
672232	<i>S. aureus</i> , MRSA	Rifampicin	2	Nano-mupirocin	0.25	-	0.5	No increase of Nano-Mupirocin MIC observed
				Mupirocin	0.12	-	0.12	No increase of Mupirocin MIC observed
				Rifampicin	0.004	0.015	0.008	Mutant not confirmed

IHMA number	Organism	Selecting agent	Day	Drug	Original MIC (µg/mL)	MIC when potential mutant was isolated (µg/mL)	MIC after 5 non-selective subcultures (µg/mL)	Comment
1146992	<i>E. faecium</i>	Nano-mupirocin	7	Nano-mupirocin	2	8	4	Mutant not confirmed
				Mupirocin	1	-	1	No increase of Mupirocin MIC observed
				Rifampicin	>512	-	>512	No increase of Rifampicin MIC observed
		Mupirocin	8	Nano-mupirocin	2	-	4	No increase of Nano-Mupirocin MIC observed
				Mupirocin	1	4	1	Mutant not confirmed
				Rifampicin	>512	-	>512	No increase of rifampicin MIC observed
1533772	<i>E. faecium</i>	Nano-mupirocin	4	Nano-mupirocin	2	8	8	Mutant confirmed for nano-mupirocin
				Mupirocin	0.5	-	2	Parallel increase of mupirocin MIC
				Rifampicin	4	-	4	No increase of rifampicin MIC observed
		Mupirocin	5	Nano-mupirocin	2	-	8	Parallel increase of nano-mupirocin MIC
				Mupirocin	0.5	2	2	Mutant confirmed for mupirocin
				Rifampicin	4	-	4	No increase of rifampicin MIC observed
		Rifampicin	5	Nano-mupirocin	2	-	2	No increase of nano-mupirocin MIC observed
				Mupirocin	0.5	-	0.5	No increase of mupirocin MIC observed
				Rifampicin	4	16	8	Mutant not confirmed
		Nano-mupirocin	11	Nano-mupirocin	2	32	32	Mutant confirmed for nano-mupirocin
				Mupirocin	0.5	-	8	Parallel increase of mupirocin MIC
				Rifampicin	4	-	2	No increase of rifampicin MIC observed
		Mupirocin	12	Nano-mupirocin	2	-	16	Parallel increase of nano-mupirocin MIC
				Mupirocin	0.5	8	4	Mutant confirmed for mupirocin
				Rifampicin	4	-	4	No increase of rifampicin MIC observed
1765156	<i>E. faecium</i>	Mupirocin	3	Nano-mupirocin	1	-	4	Increase of nano-mupirocin MIC
				Mupirocin	0.5	2	1	Mutant not confirmed
				Rifampicin	>512	-	>512	-
		Nano-mupirocin	5	Nano-mupirocin	1	4	4	Mutant confirmed for nano-mupirocin
				Mupirocin	0.5	-	1	No increase of mupirocin MIC observed
				Rifampicin	>512	-	>512	-
		Mupirocin	11	Nano-mupirocin	1	-	32	Parallel increase of nano-mupirocin MIC
				Mupirocin	0.5	8	8	Mutant confirmed for mupirocin
				Rifampicin	>512	-	>512	-
		Nano-mupirocin	12	Nano-mupirocin	1	16	8	Mutant confirmed for nano-mupirocin
				Mupirocin	0.5	-	2	Parallel increase of mupirocin MIC
				Rifampicin	>512	-	>512	-

**Table S6.** Line listing of agar MIC data for Nano-mupirocin, mupirocin, and rifampicin against nine Gram-positive clinical isolates, as tested at different inocula, and for two QC strains.

Organism number	Organism	Inoculum size (CFU/spot)	MIC agar (µg/mL)		
			Nano-mupirocin (diffusion) <sup>a</sup>	Mupirocin	Rifampicin
ATCC 29213	<i>S. aureus</i>	10 <sup>4</sup> (Standard)	0.12	0.12	0.004
ATCC 29213	<i>S. aureus</i>	10 <sup>4</sup> (Standard)	0.12	0.12	0.004
ATCC 29213	<i>S. aureus</i>	10 <sup>4</sup> (Standard)	0.12	0.06	0.004
ATCC 29212	<i>E. faecalis</i>	10 <sup>4</sup> (Standard)	32	16	1
ATCC 29212	<i>E. faecalis</i>	10 <sup>4</sup> (Standard)	32	32	1
ATCC 29212	<i>E. faecalis</i>	10 <sup>4</sup> (Standard)	32	32	1
649380	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.12	0.12	>512
649380	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>0.25</b>	<b>0.25</b>	<b>&gt;512</b>
649380	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.25	0.25	>512
649380	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.12	0.25	>512
649390	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.25	0.25	0.015
649390	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>2</b>	<b>1</b>	<b>0.015</b>
649390	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.5	0.25	0.015
649390	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.25	0.25	0.015
1308254	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.25	0.25	1
1308254	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>0.5</b>	<b>1</b>	<b>2</b>
1308254	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.25	0.25	1
1308254	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.25	0.25	1
672231	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.12	0.12	≤0.002
672231	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>0.5</b>	<b>0.25</b>	<b>≤0.002</b>
672231	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.12	0.12	≤0.002
672231	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.12	0.06	≤0.002
672233	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.12	0.06	0.004
672233	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>0.5</b>	<b>1</b>	<b>0.015</b>
672233	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.12	0.25	0.008
672233	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.12	0.12	0.008
672232	<i>S. aureus</i> , MRSA	10 <sup>4</sup> (Standard)	0.06	≤0.015	0.004
672232	<i>S. aureus</i> , MRSA	10 <sup>7</sup>	<b>0.5</b>	<b>1</b>	<b>0.015</b>
672232	<i>S. aureus</i> , MRSA	10 <sup>6</sup>	0.06	0.12	0.008
672232	<i>S. aureus</i> , MRSA	10 <sup>5</sup>	0.06	0.06	0.004
1146992	<i>E. faecium</i>	10 <sup>4</sup> (Standard)	no growth	no growth	>512
1146992	<i>E. faecium</i>	10 <sup>7</sup>	<b>2</b>	<b>4</b>	<b>&gt;512</b>
1146992	<i>E. faecium</i>	10 <sup>6</sup>	2	2	>512
1146992	<i>E. faecium</i>	10 <sup>5</sup>	1	2	>512
1533772	<i>E. faecium</i>	10 <sup>4</sup> (Standard)	1	1	4
1533772	<i>E. faecium</i>	10 <sup>7</sup>	<b>2</b>	<b>2</b>	<b>16</b>
1533772	<i>E. faecium</i>	10 <sup>6</sup>	1	1	8
1533772	<i>E. faecium</i>	10 <sup>5</sup>	1	1	8
1765156	<i>E. faecium</i>	10 <sup>4</sup> (Standard)	1	0.5	>512
1765156	<i>E. faecium</i>	10 <sup>7</sup>	<b>2</b>	<b>4</b>	<b>&gt;512</b>
1765156	<i>E. faecium</i>	10 <sup>6</sup>	2	2	>512
1765156	<i>E. faecium</i>	10 <sup>5</sup>	1	1	>512

In bold: MIC used to calculate the concentrations for the Spontaneous mutation frequency (SMF) experiment.

- a- Nano-mupirocin was spread on petri dishes containing solidified supplemented QC agar. The plates were allowed to dry at room temperature before bacteria were added. This method was used to prevent nano-mupirocin liposome degradation at temperature higher than 40°C that are usually required to prepare agar panels

**Table S7.** Line listing of single-step spontaneous mutation frequency data for nine Gram-positive isolates tested with Nano-mupirocin, mupirocin, and rifampicin

IHMA number	Organism	Inoculum	Selective agent [µg/mL]	Selective agent (× MIC)	Potential mutants at 24 h incubation	Confirmed mutants at 24 h	SMF at 24h
649380	<i>S. aureus</i> , MRSA	2.52E+09	Nano-mupirocin [1]	4 ×	2	1/2	3.97E-10
649380	<i>S. aureus</i> , MRSA	2.52E+09	Nano-mupirocin [2]	8 ×	5	2/2	1.98E-09
649380	<i>S. aureus</i> , MRSA	2.52E+09	Nano-mupirocin [4]	16 ×	6	2/2	2.38E-09
649380	<i>S. aureus</i> , MRSA	2.52E+09	Mupirocin [1]	4 ×	0		<3.97E-10
649380	<i>S. aureus</i> , MRSA	2.52E+09	Mupirocin [2]	8 ×	0		<3.97E-10
649380	<i>S. aureus</i> , MRSA	2.52E+09	Mupirocin [4]	16 ×	0		<3.97E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Nano-mupirocin [8]	4 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Nano-mupirocin [16]	8 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Nano-mupirocin [32]	16 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Mupirocin [4]	4 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Mupirocin [8]	8 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Mupirocin [16]	16 ×	0		<5.21E-10
649390	<i>S. aureus</i> , MRSA	1.92E+09	Rifampicin [0.06]	4 ×	50	2/2	2.60E-08
649390	<i>S. aureus</i> , MRSA	1.92E+09	Rifampicin [0.12]	8 ×	32	2/2	1.67E-08
649390	<i>S. aureus</i> , MRSA	1.92E+09	Rifampicin [0.25]	16 ×	26	2/2	1.35E-08
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Nano-mupirocin [2]	4 ×	29	2/2	3.63E-08
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Nano-mupirocin [4]	8 ×	27	2/2	3.38E-08
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Nano-mupirocin [8]	16 ×	73	2/2	9.13E-08
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Mupirocin [4]	4 ×	0		<1.25E-09
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Mupirocin [8]	8 ×	0		<1.25E-09
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Mupirocin [16]	16 ×	0		<1.25E-09
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Rifampicin [8]	4 ×	>600	2/2	>7.5E-07
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Rifampicin [16]	8 ×	>600	2/2	>7.5E-07
1308254	<i>S. aureus</i> , MRSA	8.00E+08	Rifampicin [32]	16 ×	>600	2/2	>7.5E-07
672231	<i>S. aureus</i> , MRSA	2.06E+09	Nano-mupirocin [2]	4 ×	3	1/2	7.28E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Nano-mupirocin [4]	8 ×	0		<4.85E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Nano-mupirocin [8]	16 ×	1	1/1	4.85E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Mupirocin [1]	4 ×	0		<4.85E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Mupirocin [2]	8 ×	0		<4.85E-10



IHMA number	Organism	Inoculum	Selective agent [µg/mL]	Selective agent (× MIC)	Potential mutants at 24 h incubation	Confirmed mutants at 24 h	SMF at 24h
672231	<i>S. aureus</i> , MRSA	2.06E+09	Mupirocin [4]	16 ×	0		<4.85E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Rifampicin [0.008]	4 ×	Lawn	0/2	<4.85E-10
672231	<i>S. aureus</i> , MRSA	2.06E+09	Rifampicin [0.015]	8 ×	41	2/2	1.99E-08
672231	<i>S. aureus</i> , MRSA	2.06E+09	Rifampicin [0.03]	16 ×	42	2/2	2.04E-08
672233	<i>S. aureus</i> , MRSA	1.48E+09	Nano-mupirocin [2]	4 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Nano-mupirocin [4]	8 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Nano-mupirocin [8]	16 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Mupirocin [4]	4 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Mupirocin [8]	8 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Mupirocin [16]	16 ×	0		<6.76E-10
672233	<i>S. aureus</i> , MRSA	1.48E+09	Rifampicin [0.06]	4 ×	160	2/2	1.08E-07
672233	<i>S. aureus</i> , MRSA	1.48E+09	Rifampicin [0.12]	8 ×	>349	2/2	>2.36E-07
672233	<i>S. aureus</i> , MRSA	1.48E+09	Rifampicin [0.25]	16 ×	>600	2/2	>4.05E-07
672232	<i>S. aureus</i> , MRSA	2.22E+09	Nano-mupirocin [2]	4 ×	1	1/1	4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Nano-mupirocin [4]	8 ×	1	1/1	4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Nano-mupirocin [8]	16 ×	0		<4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Mupirocin [4]	4 ×	0		<4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Mupirocin [8]	8 ×	0		<4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Mupirocin [16]	16 ×	1	0/1	<4.50E-10
672232	<i>S. aureus</i> , MRSA	2.22E+09	Rifampicin [0.06]	4 ×	128	2/2	5.77E-08
672232	<i>S. aureus</i> , MRSA	2.22E+09	Rifampicin [0.12]	8 ×	79	2/2	3.56E-08
672232	<i>S. aureus</i> , MRSA	2.22E+09	Rifampicin [0.25]	16 ×	18	2/2	8.11E-09
1146992	<i>E. faecium</i>	5.55E+08	Nano-mupirocin [8]	4 ×	3	0/2	<1.80E-09
1146992	<i>E. faecium</i>	5.55E+08	Nano-mupirocin [16]	8 ×	12	0/2	<1.80E-09
1146992	<i>E. faecium</i>	5.55E+08	Nano-mupirocin [32]	16 ×	4	1/2	3.60E-09
1146992	<i>E. faecium</i>	5.55E+08	Mupirocin [16]	4 ×	0		<1.80E-09
1146992	<i>E. faecium</i>	5.55E+08	Mupirocin [32]	8 ×	0		<1.80E-09
1146992	<i>E. faecium</i>	5.55E+08	Mupirocin [64]	16 ×	0		<1.80E-09
1533772	<i>E. faecium</i>	1.36E+09	Nano-mupirocin [8]	4 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Nano-mupirocin [16]	8 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Nano-mupirocin [32]	16 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Mupirocin [8]	4 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Mupirocin [16]	8 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Mupirocin [32]	16 ×	0		<7.35E-10
1533772	<i>E. faecium</i>	1.36E+09	Rifampicin [64]	4 ×	63	2/2	4.63E-08

IHMA number	Organism	Inoculum	Selective agent [µg/mL]	Selective agent (× MIC)	Potential mutants at 24 h incubation	Confirmed mutants at 24 h	SMF at 24h
1533772	<i>E. faecium</i>	1.36E+09	Rifampicin [128]	8 ×	82	2/2	6.03E-08
1533772	<i>E. faecium</i>	1.36E+09	Rifampicin [256]	16 ×	60	2/2	4.41E-08
1765156	<i>E. faecium</i>	1.46E+09	Nano-mupirocin [8]	4 ×	2	1/2	6.85E-10
1765156	<i>E. faecium</i>	1.46E+09	Nano-mupirocin [16]	8 ×	5	2/2	3.42E-09
1765156	<i>E. faecium</i>	1.46E+09	Nano-mupirocin [32]	16 ×	1	0/1	<6.85E-10
1765156	<i>E. faecium</i>	1.46E+09	Mupirocin [16]	4 ×	0		<6.85E-10
1765156	<i>E. faecium</i>	1.46E+09	Mupirocin [32]	8 ×	0		<6.85E-10
1765156	<i>E. faecium</i>	1.46E+09	Mupirocin [64]	16 ×	1	0/1	<6.85E-10

**Table S8.** Line listing of MICs for variants selected in spontaneous mutation frequency experiments.

Label	MIC (µg/mL)			Comment
	Nano-mupirocin	Mupirocin	Rifampicin	
<i>S. aureus</i> , MRSA. 649380 parent isolate	0.5	0.25	>512	-
11- 649380- NANO-MUP- 1 µg/mL- 24H.1	0.5	0.25	>512	Not confirmed
11- 649380- NANO-MUP- 1 µg/mL- 24H.2	32	16	>512	Confirmed, increase in MUP MIC
11- 649380- NANO-MUP- 2 µg/mL- 24H.1	32	16	>512	Confirmed, increase in MUP MIC
11- 649380- NANO-MUP- 2 µg/mL- 24H.2	64	16	>512	Confirmed, increase in MUP MIC
11- 649380- NANO-MUP- 4 µg/mL- 24H.1	64	16	>512	Confirmed, increase in MUP MIC
11- 649380- NANO-MUP- 4 µg/mL- 24H.2	64	16	>512	Confirmed, increase in MUP MIC
<i>S. aureus</i> , MRSA. 649390 parent isolate	1	0.25	0.015	-
12- 649390- RIF- 0.06 µg/mL- 24H.1	1	0.25	16	Mutant confirmed
12- 649390- RIF- 0.06 µg/mL- 24H.2	1	0.25	128	Mutant confirmed
12- 649390- RIF- 0.12 µg/mL- 24H.1	2	0.25	16	Mutant confirmed
12- 649390- RIF- 0.12 µg/mL- 24H.2	2	0.25	256	Mutant confirmed
12- 649390- RIF- 0.25 µg/mL- 24H.1	2	0.5	256	Mutant confirmed
12- 649390- RIF- 0.25 µg/mL- 24H.2	2	0.5	>512	Mutant confirmed
<i>S. aureus</i> , MRSA. 1308254	1	0.25	2	-
17- 1308254- NANO-MUP- 2 µg/mL- 24H.1	64	16	2	Confirmed, increase in MUP MIC
17- 1308254- NANO-MUP- 2 µg/mL- 24H.2	64	16	2	Confirmed, increase in MUP MIC
17- 1308254- NANO-MUP- 4 µg/mL- 24H.1	32	8	2	Confirmed, increase in MUP MIC
17- 1308254- NANO-MUP- 4 µg/mL- 24H.2	32	16	2	Confirmed, increase in MUP MIC
17- 1308254- NANO-MUP- 8 µg/mL- 24H.1	32	8	2	Confirmed, increase in MUP MIC
17- 1308254- NANO-MUP- 8 µg/mL- 24H.2	32	16	2	Confirmed, increase in MUP MIC
17- 1308254- RIF- 8 µg/mL- 24H.1	2	0.25	>512	Mutant confirmed
17- 1308254- RIF- 8 µg/mL- 24H.2	0.5	0.25	>512	Mutant confirmed
17- 1308254- RIF- 16 µg/mL- 24H.1	1	0.25	>512	Mutant confirmed
17- 1308254- RIF- 16 µg/mL- 24H.2	0.5	0.25	>512	Mutant confirmed
17- 1308254- RIF- 32 µg/mL- 24H.1	0.5	0.25	>512	Mutant confirmed
17- 1308254- RIF- 32 µg/mL- 24H.2	0.5	0.25	>512	Mutant confirmed
<i>S. aureus</i> , MRSA. 672231	0.25	0.06	0.004	-
19- 672231- NANO-MUP- 2 µg/mL- 24H.1	32	8	0.004	Confirmed, increase in MUP MIC
19- 672231- NANO-MUP- 2 µg/mL- 24H.2	0.25	0.12	0.004	Not confirmed
19- 672231- NANO-MUP- 8 µg/mL- 24H.1	32	8	0.004	Confirmed, increase in MUP MIC
19- 672231- RIF- Lawn- 24H.1	0.25	0.12	0.004	Not confirmed
19- 672231- RIF- Lawn- 24H.2	0.25	0.12	0.004	Not confirmed
19- 672231- RIF- 0.015 µg/mL- 24H.1	0.5	0.12	0.06	Mutant confirmed
19- 672231- RIF- 0.015 µg/mL- 24H.2	0.25	0.06	1	Mutant confirmed
19- 672231- RIF- 0.03 µg/mL- 24H.1	0.25	0.12	32	Mutant confirmed
19- 672231- RIF- 0.03 µg/mL- 24H.2	0.25	0.12	512	Mutant confirmed
<i>S. aureus</i> , MRSA. 672233	0.5	0.06	0.008	-
20- 672233- RIF- 0.06 µg/mL- 24H.1	0.5	0.12	512	Mutant confirmed
20- 672233- RIF- 0.06 µg/mL- 24H.2	0.5	0.12	256	Mutant confirmed
20- 672233- RIF- 0.12 µg/mL- 24H.1	1	0.25	1	Mutant confirmed
20- 672233- RIF- 0.12 µg/mL- 24H.2	0.5	0.12	512	Mutant confirmed
20- 672233- RIF- 0.25 µg/mL- 24H.1	1	0.25	2	Mutant confirmed

Label	MIC (µg/mL)			Comment
	Nano-mupirocin	Mupirocin	Rifampicin	
20- 672233- RIF- 0.25 µg/mL- 24H.2	0.5	0.12	512	Mutant confirmed
<i>S. aureus</i> , MRSA. 672232	0.5	0.06	0.004	-
4- 672232- NANO-MUP- 2 µg/mL- 24H.1	64	16	0.008	Confirmed, increase in MUP MIC
4- 672232- NANO-MUP- 4 µg/mL- 24H.1	4	1	0.008	Confirmed, increase in MUP MIC
4- 672232- MUP- 16 µg/mL- 24H.1	0.5	0.12	0.008	Not confirmed
4- 672232- RIF- 0.06 µg/mL- 24H.1	0.5	0.12	0.5	Mutant confirmed
4- 672232- RIF- 0.06 µg/mL- 24H.2	0.5	0.12	0.5	Mutant confirmed
4- 672232- RIF- 0.12 µg/mL- 24H.1	0.5	0.12	2	Mutant confirmed
4- 672232- RIF- 0.12 µg/mL- 24H.2	0.5	0.12	4	Mutant confirmed
4- 672232- RIF- 0.25 µg/mL- 24H.1	0.5	0.12	>512	Mutant confirmed
4- 672232- RIF- 0.25 µg/mL- 24H.2	0.5	0.12	>512	Mutant confirmed
<i>E. faecium</i> . 1146992	4	1	>512	-
5- 1146992- NANO-MUP- 8 µg/mL- 24H.1	4	1	>512	Not confirmed
5- 1146992- NANO-MUP- 8 µg/mL- 24H.2	4	1	>512	Not confirmed
5- 1146992- NANO-MUP- 16 µg/mL- 24H.1	4	1	>512	Not confirmed
5- 1146992- NANO-MUP- 16 µg/mL- 24H.2	4	1	>512	Not confirmed
5- 1146992- NANO-MUP- 32 µg/mL- 24H.1	32	8	>512	Confirmed, increase in MUP MIC
5- 1146992- NANO-MUP- 32 µg/mL- 24H.2	8	1	>512	Not confirmed
<i>E. faecium</i> . 1533772	2	0.5	4	-
104- 1533772- RIF- 64 µg/mL- 24H.1	2	0.5	>512	Mutant confirmed
104- 1533772- RIF- 64 µg/mL- 24H.2	2	0.5	>512	Mutant confirmed
104- 1533772- RIF- 128 µg/mL- 24H.1	1	0.5	512	Mutant confirmed
104- 1533772- RIF- 128 µg/mL- 24H.2	2	0.5	512	Mutant confirmed
104- 1533772- RIF- 256 µg/mL- 24H.1	2	0.5	512	Mutant confirmed
104- 1533772- RIF- 256 µg/mL- 24H.2	2	0.5	512	Mutant confirmed
<i>E. faecium</i> . 1765156	2	0.5	>512	-
106- 1765156- NANO-MUP- 8 µg/mL- 24H.1	16	4	>512	Confirmed, increase in MUP MIC
106- 1765156- NANO-MUP- 8 µg/mL- 24H.2	2	1	>512	Not confirmed
106- 1765156- NANO-MUP- 16 µg/mL- 24H.1	32	8	>512	Confirmed, increase in MUP MIC
106- 1765156- NANO-MUP- 16 µg/mL- 24H.2	16	8	>512	Confirmed, increase in MUP MIC
106- 1765156- NANO-MUP- 32 µg/mL- 24H.1	2	0.5	>512	Not confirmed
106- 1765156- MUP- 64 µg/mL- 24H.1	2	0.5	>512	Not confirmed

NANO-MUP: Nano-mupirocin; MUP: Mupirocin; RIF: Rifampicin.

**Table S9.** MICs of Nano-mupirocin, mupirocin and tetracycline for three *N. gonorrhoea* clinical isolates during multi-step passaging study.

IHMA No	Organism	Selective agent	MIC (µg/mL) at day :														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1073248	<i>N. gonorrhoea</i>	Nano-mupirocin	0.03	0.06	0.03	0.03	0.03	0.03	0.06	0.06	0.06	0.06	0.06	0.12	0.12	0.12	0.25
		Mupirocin	0.03	0.03	0.03	0.03	0.03	0.015	0.06	0.06	0.06	0.12	0.12	0.12	0.06	0.12	0.12
		Tetracycline	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5
1075014	<i>N. gonorrhoea</i>	Nano-mupirocin	0.03	0.03	0.03	0.03	0.03	0.015	0.015	0.06	0.015	0.03	0.06	0.06	0.06	0.12	0.12
		Mupirocin	0.015	0.015	0.03	0.015	0.015	0.008	0.03	0.03	0.03	0.06	0.06	0.06	0.03	0.06	0.06
		Tetracycline	0.5	0.5	1	0.25	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5
1123849	<i>N. gonorrhoea</i>	Nano-mupirocin	0.03	0.06	0.03	0.06	0.015	0.03	0.06	0.06	0.06	0.06	0.06	0.12	0.06	0.25	0.12
		Mupirocin	0.015	0.03	0.03	0.03	0.008	0.008	0.03	0.03	0.03	0.06	0.06	0.12	0.06	0.12	0.12
		Tetracycline	1	1	2	0.5	1	0.5	1	2	1	2	2	2	2	2	2

**Table S10.** MIC data for potential mutants of *N. gonorrhoea* as re-tested after five days subculture on drug-free agar

IHMA No	Organism	Selective agent	Day	Drug	Original MIC (µg/mL)	MIC when potential mutant was isolated (µg/mL)	MIC after 5 non-selective subcultures (µg/mL)	Comment
1073248	<i>N. gonorrhoea</i>	Mupirocin	10	Nano-mupirocin	0.03	-	0.03	No increase of nano-mupirocin MIC observed
				Mupirocin	0.03	0.12	0.03	Mutant not confirmed
				Tetracycline	0.5	-	0.5	No increase of tetracycline MIC observed
		Nano-mupirocin	12	Nano-mupirocin	0.03	0.12	0.06	Mutant not confirmed
				Mupirocin	0.03	-	0.03	No increase of mupirocin MIC observed
				Tetracycline	0.5	-	0.5	No increase of tetracycline MIC observed
		Nano-mupirocin	15	Nano-mupirocin	0.03	0.25	0.12	Mutant confirmed for nano-mupirocin
				Mupirocin	0.03	-	0.06	Small increase of mupirocin MIC
				Tetracycline	0.5	-	0.5	No increase of tetracycline MIC observed
1075014	<i>N. gonorrhoea</i>	Mupirocin	10	Nano-mupirocin	0.03	-	0.015	No increase of nano-mupirocin MIC observed
				Mupirocin	0.015	0.06	0.015	Mutant not confirmed
				Tetracycline	0.5	-	1	No increase of tetracycline MIC observed
		Nano-mupirocin	14	Nano-mupirocin	0.03	0.12	0.015	Mutant not confirmed
				Mupirocin	0.015	-	0.008	No increase of mupirocin MIC observed
				Tetracycline	0.5	-	0.5	No increase of tetracycline MIC observed
1123849	<i>N. gonorrhoea</i>	Mupirocin	10	Nano-mupirocin	0.03	-	0.03	No increase of nano-mupirocin MIC observed
				Mupirocin	0.015	0.06	0.015	Mutant not confirmed
				Tetracycline	1	-	2	No increase of tetracycline MIC observed
		Nano-mupirocin	12	Nano-mupirocin	0.03	0.12	0.03	Mutant not confirmed for nano-mupirocin
				Mupirocin	0.015	-	0.03	Small increase of mupirocin MIC observed
				Tetracycline	1	-	4	No increase of tetracycline MIC observed
		Mupirocin	15	Nano-mupirocin	0.03	-	0.06	No increase of nano-mupirocin MIC observed
				Mupirocin	0.015	0.12	0.03	Mutant not confirmed
				Tetracycline	1	-	2	No increase of tetracycline MIC observed

**Table S11.** Line listing of MIC data for three *N. gonorrhoea* clinical isolates tested at different inocula and for one QC strain.

Organism No	Organism	Target inoculum size (CFU/spot)	MIC agar (µg/mL)			Comment
			Nano-mupirocin diffusion	Mupiricin	Tetracycline	
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	0.002	0.5	Test performed with supplement B
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	0.002	0.5	Test performed with supplement B
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	0.001	0.5	Test performed with supplement B
1073248	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.03	0.03	0.5	Test performed with supplement B
1073248	<i>N. gonorrhoea</i>	10 <sup>7</sup>	0.06	0.06	1	Test performed with supplement B
1073248	<i>N. gonorrhoea</i>	10 <sup>6</sup>	0.03	0.06	0.5	Test performed with supplement B
1073248	<i>N. gonorrhoea</i>	10 <sup>5</sup>	0.03	0.03	0.5	Test performed with supplement B
1075014	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.015	0.03	0.5	Test performed with supplement B
1075014	<i>N. gonorrhoea</i>	10 <sup>7</sup>	0.06	0.06	1	Test performed with supplement B
1075014	<i>N. gonorrhoea</i>	10 <sup>6</sup>	0.03	0.06	1	Test performed with supplement B
1075014	<i>N. gonorrhoea</i>	10 <sup>5</sup>	0.015	0.015	0.5	Test performed with supplement B
1123849	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.03	0.03	2	Test performed with supplement B
1123849	<i>N. gonorrhoea</i>	10 <sup>7</sup>	0.06	0.06	4	Test performed with supplement B
1123849	<i>N. gonorrhoea</i>	10 <sup>6</sup>	0.03	0.06	4	Test performed with supplement B
1123849	<i>N. gonorrhoea</i>	10 <sup>5</sup>	0.03	0.03	2	Test performed with supplement B
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	≤0.0005	0.5	Test performed with supplement VX
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	≤0.0005	0.5	Test performed with supplement VX
ATCC 49226	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	≤0.0005	≤0.0005	0.5	Test performed with supplement VX
1073248	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.03	0.015	0.5	Test performed with supplement VX
1073248	<i>N. gonorrhoea</i>	10 <sup>7</sup>	<b>0.03</b>	<b>0.03</b>	<b>4</b>	Test performed with supplement VX
1075014	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.015	0.015	1	Test performed with supplement VX
1075014	<i>N. gonorrhoea</i>	10 <sup>7</sup>	<b>0.03</b>	<b>0.03</b>	<b>2</b>	Test performed with supplement VX
1123849	<i>N. gonorrhoea</i>	10 <sup>4</sup> (Standard)	0.03	0.015	2	Test performed with supplement VX
1123849	<i>N. gonorrhoea</i>	10 <sup>7</sup>	<b>0.03</b>	<b>0.03</b>	<b>4</b>	Test performed with supplement VX

Supplement B: Becton Dickinson; Product code: 227610; Batch: 6201840; Expiry date: 07.07.2018

Supplement VX: Becton Dickinson; Product code: 233541; Batch: 7082782; Expiry date: 19.01.2020

**Table S12.** Line listing of spontaneous mutation frequency (SMF) data for *N. gonorrhoea*

IHMA No	Organism	Inoculum	Selective agent [µg/mL]	Selective agent (× MIC)	Potential mutant at 24 h incubation	Mutants confirmed at 24 h	SMF at 24h
1073248	<i>N. gonorrhoea</i>	2.12E+08	Nano-mupirocin [0.12]	4 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Nano-mupirocin [0.25]	8 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Nano-mupirocin [0.5]	16 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Mupirocin [0.12]	4 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Mupirocin [0.25]	8 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Mupirocin [0.5]	16 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Tetracycline [16]	4 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Tetracycline [32]	8 ×	0	NA	<4.72E-09
1073248	<i>N. gonorrhoea</i>	2.12E+08	Tetracycline [64]	16 ×	0	NA	<4.72E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Nano-mupirocin [0.12]	4 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Nano-mupirocin [0.25]	8 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Nano-mupirocin [0.5]	16 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Mupirocin [0.12]	4 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Mupirocin [0.25]	8 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Mupirocin [0.5]	16 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Tetracycline [8]	4 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Tetracycline [16]	8 ×	0	NA	<5.15E-09
1075014	<i>N. gonorrhoea</i>	1.94E+08	Tetracycline [32]	16 ×	0	NA	<5.15E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Nano-mupirocin [0.12]	4 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Nano-mupirocin [0.25]	8 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Nano-mupirocin [0.5]	16 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Mupirocin [0.12]	4 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Mupirocin [0.25]	8 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Mupirocin [0.5]	16 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Tetracycline [16]	4 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Tetracycline [32]	8 ×	0	NA	<1.06E-09
1123849	<i>N. gonorrhoea</i>	9.40E+08	Tetracycline [64]	16 ×	0	NA	<1.06E-09



**Table S13.** Exposure parameters ( $C_{\max}$  and  $AUC_{\text{INF}}$ ) for Nano-mupirocin in rates, according to dose and as compared with mean values

	$C_{\max}/\text{Dose}$ ( $\mu\text{g/mL}$ )/( $\text{mg/kg}$ )	$AUC_{\text{INF}}/\text{Dose}$ ( $\text{hr}\cdot\mu\text{g/mL}$ )/( $\text{mg/kg}$ )	% of average $C_{\max}/\text{Dose}$	% of average $AUC_{\text{INF}}/\text{Dose}$
<b>Day 1</b>				
<b>Male</b>				
10 mg/kg	16.1	82.0	87	88
30 mg/kg	18.4	91.5	100	98
100 mg/kg	20.9	106.0	113	114
Mean	18.4	93.2		
<b>Female</b>				
10 mg/kg	21.6	80.8	100	85
30 mg/kg	19.4	97.2	90	102
100 mg/kg	24.0	108.5	111	114
Mean	21.7	95.5		
<b>Day 14</b>				
<b>Male</b>				
10 mg/kg	24.8	123.4	103	94
30 mg/kg	24.9	128.8	104	98
100 mg/kg	22.3	142.1	93	108
Mean	24.0	131.4		
<b>Female</b>				
10 mg/kg	26.3	105.3	113	95
30 mg/kg	21.2	104.8	91	94
100 mg/kg	22.3	122.7	96	111
Mean	23.3	110.9		

## 1. Methods

### *Nano-mupirocin preparation*

Nano-mupirocin was prepared as described elsewhere [1]. Briefly, the 3 lipids that form the liposomes (HSPC: cholesterol: mPEG DSPE(2000 Da)), were dissolved in ethanol at a weight ratio of 3:1:1, and were mechanically hydrated by stirring at 65 °C with 200 mM calcium acetate pH 5.5 solution containing 15% (*w/w*) HPCD. The liposomal dispersions were downsized by stepwise extrusion using polycarbonate filter membranes of defined pore size and subjected to diafiltration using a Quixstand Benchtop System (GE Healthcare, Uppsala, Sweden) against isotonic 10% sucrose. These ‘nano-liposomes.’ exhibiting a trans membrane acetate gradient and high intraliposomal concentration of calcium ions and of HPCD were remotely loaded by 10 min incubation at 65 °C with a 15 mg/mL mupirocin solution in 200 mM phosphate buffer, pH 6.3.

The fluorescently labeled lissamine-rhodamine B phosphatidylethanolamine (LRPE)-Nano-mupirocin liposomes (LRPE-Nano-mupirocin) were prepared by incubating Nano-mupirocin with

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (LR-PE) (Avanti Polar Lipids, AL, USA) dissolved in ethanol at a final molar ratio of 0.2 mole % of the liposomal lipids for 5 min at 37 °C and then overnight at 4 °C. Note that LRPE is labeled in the polar head group of the phospholipid and therefore is retained within the nanoliposomes.

Nanoliposomes size was measured by the dynamic light scattering method, performed with a Zetasizer Nano Series ZEN3600F instrument (Malvern Instruments, Malvern, UK).

## **2. Bioanalytical methods**

Bioanalytical testing was performed at two sites for the PK and PK/BD studies. Slightly different assays were used at these, as described below.

### *2.1. Pharmacokinetic study*

#### **Sample preparation**

Plasma samples (20 µL) were extracted in 1 mL of 0.1% formic acid in acetonitrile containing 100 ng/mL mycophenolic acid (internal standard, IS). Following vortexing and centrifugation, the upper phase was diluted × 5 in water. Mupirocin stock standards were prepared in methanol. Further dilutions of the standards were prepared in a 50/50 acetonitrile/water solution.

#### **LC-MS/MS method**

A HPLC-tandem mass spectrometry (LC-MS/MS) assay was used to quantify mupirocin in the plasma samples, with acceptable accuracy and precision, over the concentration range of 0.500–500 µg/mL. Chromatography was performed under reverse phase conditions using a Shimadzu (Kyoto, Japan) HPLC System. The analytical column used was Synergi, Polar-RP, 2.5 µm, 100Å, 50 × 2.0 mm (Phenomenex, Torrance, CA, USA). The injection volume was 5 µL, the oven temperature was maintained at 40 °C and the autosampler tray temperature was maintained at 5 °C. A mixture of water:MeOH:formic acid (80:20:1) was used for washing the needle prior to each injection cycle.

The chromatographic separation was achieved using a linear gradient program at a constant flow rate of 0.5 mL/min over a total run time of 4 min. An outline of the mobile phase gradient program is summarized as below:

Time (min)	Solvent A (%)	Solvent B (%)
	0.1% Formic Acid in Water	0.1% Formic Acid in Acetonitrile
0.0	90	10
1.0	80	20
2.5	20	80
3.0	20	80
3.1	90	10
4.0	90	10

Mupirocin and mycophenolic acid (IS) were detected in positive ion mode using an AB Sciex API 4000 mass spectrometer (Framingham, MA, USA) with electrospray ionization (ESI) employing the 'multiple reaction monitoring' (MRM) mode of acquisition. The TurboIonspray® probe temperature was set at 500 °C with the ion spray voltage at 2500 V. The curtain gas was set at 30 psi. The nebulizer gas (Gas 1) was set to 50 psi, the turbo heater gas (Gas 2) at 50 psi and the collision gas (CAD) at 8 psi. The entrance potentials (EP), collision energy potentials (CE), collision cell exit potentials (CXP) and declustering potentials (DP) for the monitored transitions are given below. The dwell time was 100 ms. Data acquisition and analysis were performed with Analyst 1.6.2 software distributed by AB Sciex.

Multiple reaction monitoring (MRM) transitions and parameters for Mupirocin and the IS in positive ion mode

	Precursor (m/z)	Product (m/z)	DP (V)	EP (V)	CE (eV)	CXP (V)	Rt (min)
Mupirocin	501.5	309.2	63.0	6.20	21.0	7.9	2.71
IS	321.3	207.2	61.9	9.24	31.4	12.0	2.83

m/z: mass to charge ratio; DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential; V: volts; eV: electronvolts; Rt: retention time. .

## 2.2. In-house PK and BD studies

### Preparation of plasma samples

Plasma samples were spiked with mycophenolic acid (IS) to a concentration of 10 µg/mL and diluted either 5-fold or 50-fold with acetonitrile. Samples were then vortexed, centrifuged, and the upper phase was diluted 1:1 with double distilled water. Samples were analyzed by the LCMS/MS method. Mupirocin concentrations were calculated based on a calibration curve of mupirocin in plasma from untreated mice.

### Preparation of swab samples

Following sampling, swabs were weighed and immediately placed in a 15 mL tube containing 2 mL of acetonitrile. To each tube, 25 µL of 3 µg/mL mycophenolic acid (IS) in methanol were added. The tubes were vortexed vigorously for 1 min, then the swab was removed and the tube was placed in an evaporator until complete dryness was achieved. Next, 75 µL of MeOH was added followed by vortex (1 min), sonication (0.5 min), and centrifugation (10 min, 4,000 rpm). The upper phase was collected and analyzed by the LCMS/MS method. The mupirocin concentration in the swab was calculated based on a calibration curve of mupirocin spiked into swabs obtained from untreated mice. The absolute amount of mupirocin recovered from each swab was related to the average weight of vaginal secretion 3.45 mg (SD = 2.53) collected per swab, to calculate the local mupirocin concentration as µg/g. This average was based on 18 measurements, including swabs from both treated and untreated mice.

### LC-MS/MS method

Chromatography was performed under reverse phase conditions using a Thermo Scientific system (San Jose, CA, USA), which includes a Dionex pump with degasser module and an Accela Autosampler. The chromatographic separations were performed on a Kinetex™ (Phenomenex, Torrance, CA, USA) column (C18, 2.6 µm particle size, 100 Å pore size, 50 × 2.1 mm), protected by a SecurityGuard™ (Phenomenex, Torrance, CA, USA) cartridge (C18, 4 × 2.1 mm). The injection volume was 5 µL, the oven temperature was maintained at 40 °C, and the autosampler tray temperature at 4 °C. The chromatographic separation was achieved using a linear gradient program at a constant flow rate of 0.35 mL/min over a total run time of 8 min. An outline of the mobile phase gradient program is summarized in the table below.

Time (min)	Solvent A (%)	Solvent B (%)
	0.1% formic acid in water	0.1% formic acid in acetonitrile
0.0	90	10
2.3	55	45
4.0	55	45
4.3	10	90
5.0	10	90
5.5	90	10
8.0	90	10

The column effluent was diverted away from the mass spectrometer during the first 1.3 min of the run. A mixture of water:MeOH (1:1) was used for washing the needle prior to each injection cycle. Mupirocin and mycophenolic acid (IS) were detected by a TSQ Quantum Access Max mass spectrometer in positive ion mode using electron spray ionization (ESI) and the multiple reaction monitoring (MRM) mode of acquisition. The spray voltage, sheath and auxiliary gas were set at 4500 V, 30 and 35 (arbitrary units), respectively. The capillary transfer tube temperature was set at 250 °C and the tube lens was set at 85 V for mupirocin and 88V for mycophenolic acid. The vaporizer temperature within the H-ESI source was 400 °C. The scan time was 40 ms.

The following transitions were monitored:

Mupirocin: m/z 501 → 309 (quantifier), collision energy (CE) 15 V and m/z 501 → 327 (qualifier), CE 12 V, retention time (RT) 4.1 min.

Mycophenolic acid: m/z 321 → 207 (quantifier), CE 21 V and m/z 321 → 303 (qualifier), CE 10 V, retention time (RT) 4.0 min.

Data acquisition and processing were carried out using the Xcalibur program (Thermo Scientific, San Jose, CA, USA). Quantitative calibration (1–5000 ng/mL) was performed before every batch of samples. The calibration curves were created using peak-area ratios (analyte versus internal standard). The calibration curve ( $y = a + bx$ ) was obtained by weighted (1/y) linear least-squares regression of the measured peak-area ratios ( $y$ ) of mupirocin/mycophenolic acid versus the concentration of mupirocin added to the plasma ( $x$ ). The limit of quantification (LOQ) was 1 ng/mL for mupirocin.