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1 **Assessment of Phenotype Microarray plates for rapid and high-throughput**
2 **analysis of collateral sensitivity networks**
3
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13
14 **ABSTRACT**
15
16 The crisis of antimicrobial resistance is driving research into the phenomenon of collateral
17 sensitivity. Sometimes, when a bacterium evolves resistance to one antimicrobial, it becomes
18 sensitive to others. In this study, we have investigated the utility of Phenotype Microarray
19 (PM) plates for identifying collateral sensitivities with unprecedented throughput. We
20 assessed the relative resistance/sensitivity phenotypes of nine strains of *Staphylococcus*
21 *aureus* (two laboratory strains and seven clinical isolates) towards the 72 antimicrobials
22 contained in three PM plates. In general, the PM plates reported on resistance and sensitivity
23 with a high degree of reproducibility. However, a rigorous comparison of PM growth
24 phenotypes with minimum inhibitory concentration (MIC) measurements revealed a trade-off
25 between throughput and accuracy. Small differences in PM growth phenotype did not
26 necessarily correlate with changes in MIC. Thus, we conclude that PM plates are useful for
27 the rapid and high-throughput assessment of large changes in collateral sensitivity
28 phenotypes during the evolution of antimicrobial resistance, but more subtle examples of
29 cross-resistance or collateral sensitivity cannot be reliably identified using this approach.

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30 **INTRODUCTION**

31

32 Collateral sensitivity is when bacteria develop resistance to one antibiotic, and in doing so
33 increase their susceptibility to one or more others. The phenomenon was first observed in
34 1952 [1] but it was largely ignored in the subsequent decades. With the emergence of the
35 antimicrobial resistance crisis, collateral sensitivity has garnered new attention because it
36 offers the potential to preserve the utility of our diminishing supply of antibiotics [2-4]. Were
37 collateral sensitivity to be understood in a systematic and predictable manner, a clinician
38 could treat a persistent infection by cycling through antibiotics in such a way that the second
39 antibiotic was chosen for its enhanced efficacy against resistant microorganisms that develop
40 during treatment with the first.

41

42 Many studies into the potential of collateral sensitivity have focused on strains of *Escherichia*
43 *coli* [5-8], *Pseudomonas aeruginosa* [9] or *Staphylococcus aureus* [10, 11] that were
44 subjected to evolution *in vitro*. In each case, a strain was exposed to one antibiotic, resistant
45 mutants were isolated, and their sensitivities towards up to 25 other antibiotics were tested.
46 The clinical relevance of this *in vitro* approach has been questioned because of the
47 stochasticity of evolution [12] and because it emphasises mutationally acquired resistance,
48 rather than the more common scenario of horizontally transferred resistance [13]. In turn, this
49 has led to studies seeking to identify collateral sensitivities that are more directly relevant in
50 clinical settings. For example, 10 genetically-diverse clinical urinary tract isolates of *E. coli*
51 showed broadly conserved patterns of collateral sensitivity to a panel of 16 antimicrobials
52 [14]. With evidence accumulating that collateral sensitivities may indeed be predictable [15,
53 16] – but with outstanding questions about how to apply this knowledge – the field is primed
54 to advance rapidly.

55

56 In this work we aimed to develop, implement and validate a high-throughput screen for
57 collateral sensitivities. Agar dilution and broth dilution methods [17] to determine differences
58 in minimum inhibitory concentration (MIC) are highly accurate but labour intensive. As a
59 result, it is unusual for any given study to test for collateral sensitivities towards >20
60 antimicrobials. We hypothesised that higher throughput experiments might reveal previously-
61 overlooked sensitivities, which in turn could help to accelerate the field.

62

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63 Phenotype Microarray (PM) plates have become a widely used tool for phenotypic
64 characterization of microorganisms [18, 19]. Each 96-well PM plate contains different
65 nutrient sources, growth additives or, in the case of PM plates 11-20, antimicrobial
66 compounds. Each of PM plates 11 to 20 contains 24 antimicrobials, present at different
67 concentrations in four wells. A redox dye is added to each well, which changes from
68 colourless to purple in response to microbial metabolic activity. The rate of colour formation
69 can be monitored automatically using an OmniLog instrument; however, we and others have
70 successfully scored colour development by eye [18, 20-22]. Persistent, well-specific
71 variability has also been observed in OmniLog data collected for plates PM 1-10 [23],
72 suggesting that data collection by eye or by OmniLog is equally valid. We set out to assess
73 whether PM plates could provide sensitive and reproducible enough data to be useful in
74 building collateral sensitivity networks. We concentrated on scoring growth data by eye, in
75 order to develop a protocol with the broadest possible applicability.
76
77 In our proof-of-principle experiments, we have focused on the Gram-positive bacterium, *S.*
78 *aureus*. We have compared resistance and sensitivity to 72 antimicrobials (three PM plates)
79 between seven clinical isolates of methicillin-resistant *S. aureus* (MRSA), one laboratory
80 strain, and one descendent of this strain that was evolved *in vitro* towards oxacillin resistance.
81 Overall we found PM plates to provide reproducible data, although their correlation with
82 broth microdilution was more variable.
83
84
85 **MATERIALS AND METHODS**
86
87 **Materials**
88
89 Antibiotics and other specialty chemicals were from Sigma-Aldrich (St Louis, MO, USA)
90 unless noted otherwise. Cefazolin, demeclocycline and oxacillin were from Melford
91 Laboratories (Ipswich, Suffolk, UK).
92
93 Phenotype Microarray plates (Biolog, Hayward, CA, USA) were used for chemical
94 sensitivity testing. PM plates 11-13 were used as these cover a range of common and
95 clinically relevant antibiotics, as well as other antimicrobial compounds.
96

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97 **General microbiological methods**

98

99 *S. aureus* was cultured in Mueller-Hinton (MH) medium (ForMedium, Hunstanton, UK) at
100 37°C. Seven isolates of community-acquired MRSA were obtained from the collection held
101 at Christchurch Hospital, New Zealand. These were identified as MRSA-BE, -BK, -BR, -SO,
102 -SY, -TT and -UB. The laboratory strain *S. aureus* ATCC 25923 was tested in parallel.
103 Further, *S. aureus* ATCC 25923 was evolved to be more oxacillin resistant by serial
104 passaging. An MH broth culture (1 mL) was incubated at 37°C until the optical density
105 (OD_{600}) exceeded 1.0. Then a 10 µL aliquot was used to inoculate a new 1 mL culture with an
106 increased oxacillin concentration. Serial passaging continued until no further bacterial growth
107 was observed and the resulting isolate was termed 25923evo.

108

109 **Phenotype microarrays**

110

111 Cell lines to be assayed were cultured overnight in 3 mL of MH broth. Each saturated culture
112 was diluted to an OD_{600} of 0.5 ± 0.05, for use as the PM plate inoculum as described below.

113

114 Each batch of three PM plates was prepared based upon the manufacturer's protocol for
115 Gram-positive bacteria. Inoculum solution A (6 ml, total volume) was assembled by
116 combining 1 ml of diluted overnight culture with inoculating fluid IF-0 (sold by Biolog at
117 1.2× concentration) and redox Dye H (sold by Biolog at 100× concentration).

118

119 A Gram-positive additive solution (12× concentration) was made, which comprised 24 mM
120 $MgCl_2$, 12 mM $CaCl_2$, 0.06% (w/v) yeast extract, 0.06% (v/v) Tween 80, 30 mM D-glucose
121 and 60 mM sodium pyruvate.

122

123 Inoculum solution B (35 ml, total volume) was prepared by combining the following
124 components: 29.2 ml of inoculating fluid IF-10b (Biolog; sold as a 1.2× stock); 2.92 ml of the
125 Gram-positive additive solution; 350 µl of Dye H (100× stock); and 2.53 ml of inoculum
126 solution A (described above). Aliquots of inoculum solution B (100 µl) were dispensed into
127 each well of PM plates 11-13. Plates were incubated for 24 h at 37°C without shaking.

128

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129 After incubation, growth in each well was scored by eye. Each well was given a score out of
130 2: 0 for colourless (no growth); 1 for light purple (intermediate growth); and 2 for dark purple
131 (full growth). Each antimicrobial was present in four wells at increasing concentrations.
132 Growth scores across the four wells were summed. Maximal resistance therefore
133 corresponded to a growth score of 8, whereas a growth score of 0 corresponded to complete
134 sensitivity.
135
136 **Determination of minimum inhibitory concentrations**
137
138 Minimum inhibitory concentrations (MICs) for six antimicrobial compounds were
139 determined by the broth microdilution method, as described previously [17, 20]. The first
140 column of wells in a 96-well plate was filled with 200 µl of MH broth plus the antimicrobial
141 at the maximum concentration to be tested. The remaining wells contained 100 µl MH broth.
142 This was used to begin a two-fold dilution series, which covered 12 concentrations. A 100 µl
143 aliquot from column 1 was transferred into the wells in column 2, pipetted up and down to
144 mix, and repeated for the remaining rows. A 3-µl aliquot of saturated cell culture, diluted to
145 OD_{600} = 0.5, was used to inoculate each well. Plates were sealed with a breathable membrane
146 (Aeraseal) and incubated in an Incumix plate shaker (Select Bioproducts) at 37°C and 600
147 rpm for 16 h. The percentage of growth inhibition in each well was calculated by measuring
148 OD_{600} in a plate reader (PerkinElmer) and comparing it to a blank and a well with no
149 antibiotic, according to the following formula (Clinical and Laboratory Standards Institute,
150 https://clsi.org/):
151
152 *Percentage inhibition = 100 × (OD_{600} of untreated well – OD_{600} of well at known*
153 *concentration plus cells) / (OD_{600} of untreated well – OD_{600} of blank).*
154
155 The first well which showed ≥95% inhibition was deemed to be the MIC cut-off for that
156 isolate.

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157 **RESULTS**

158

159 We assessed the accuracy of PM plates for the discovery of collateral sensitivity networks by
160 assaying seven clinical isolates and two laboratory strains of *S. aureus*. We set out to test two
161 aspects of the accuracy of these assays: reproducibility and reliability. Reproducibility was
162 tested by comparing independent biological replicates in duplicate microarray assays.
163 Reliability was assessed by correlating the relative levels of resistance observed in PM assays
164 with MICs determined using the broth microdilution method [17], which is a particularly
165 common test used in clinical settings [24].

166

167 **Reproducibility**

168

169 All nine strains of *S. aureus* were assayed in duplicate using PM plates 11-13. In total, this
170 experiment therefore probed 648 combinations (9 strains × 72 antimicrobials). The
171 antimicrobials in PM plates 11-13 were grouped according to their mode of action: cell wall-
172 acting antibiotics (16 compounds); protein synthesis inhibitors (22 compounds); inhibitors of
173 nucleic acid synthesis (11 compounds); repurposed cancer and anti-psychotic drugs (7
174 compounds); and others including antiseptics, disinfectants and metal ions (16 compounds).
175 The full list of compounds is provided in the Supporting Information (S1 Table). As
176 described in the Materials and Methods, relative resistance to each antimicrobial was
177 assessed using a growth score that ranged from 0 (completely sensitive) to 8 (maximally
178 resistant). Independent duplicates were performed several weeks apart. Examples of the PM
179 assays are depicted in Fig 1.

180

181

182 **Fig. 1. Examples of growth in phenotype microarray plates.** The growth of *S. aureus* ATCC 25923
183 in four wells of increasing enoxacin, erythromycin and doxycycline concentrations is shown. The
184 intensity of colour development (due to the presence of a redox dye) was scored by eye, 24 h after
185 inoculation. It was scored as either 0 (no growth), 1 (intermediate growth) or 2 (full growth). Summing
186 the growth in all four wells yielded a score out of 8 for each antimicrobial. In the examples shown,
187 growths scores of 8, 2 and 3 were recorded for enoxacin, erythromycin and doxycycline, respectively.
188

189

190 Overall, the PM data revealed high levels of resistance in all *S. aureus* strains to many of the
191 compounds tested (Fig 2 and S1-S5 Fig, Supporting Information). As expected, for example,
192 the aminoglycosides were not effective against this Gram-positive bacterium (S2 Fig).

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193 Comparing the MRSA isolates with *S. aureus* ATCC 25923 revealed hints of collateral
194 sensitivity; for example, the MRSA isolates all appeared more sensitive to doxycycline (S2
195 Fig) and 5-fluorouracil (S4 Fig). As expected, *S. aureus* ATCC 25923 showed a low level of
196 oxacillin resistance (average growth score = 1.5), whereas the strain evolved from it in the
197 presence of oxacillin (25923evo) showed increased resistance (growth score = 3).
198
199
200 **Fig. 2. Phenotype microarray plates yield reproducible resistance/sensitivity data.** The plot
201 summarizes 1,296 PM growth assays: 72 antimicrobials × 9 *S. aureus* strains, each done in biological
202 duplicate. Each symbol represents the median PM growth score, with the error bars showing the
203 range of the two scores. In 544 of 648 antimicrobial/strain combinations, scores were identical in
204 duplicate assays. In only 29 cases do the error bars span more than one point on the 8-point scale.
205
206
207 Comparing the replicated sets of PM data revealed that the growth scores for each
208 antimicrobial were identical between duplicates 84% of the time (Fig 2). When the growth
209 scores were different, it was most commonly only by one point on the 8-point scale. The
210 scores differed by one point 11.5% of the time, and only differed by more than one point in
211 4.5% of the duplicated assays (29 of 648 combinations). Most of the variability in duplicates
212 was observed for the β-lactams, the tetracyclines and the metal chlorides. In one extreme
213 case, 25923evo returned growth scores of 0 and 8 in duplicate vancomycin tests. The other
214 eight *S. aureus* strains showed reproducible growth scores of 0 for vancomycin, suggesting to
215 us that the score of 8 was the result of a technical or manufacturing error. The overarching
216 conclusion of this experiment was that PM plates yield reproducible data on relative
217 resistance and sensitivity, across both clinical and laboratory strains of *S. aureus*.
218

219 Correlation with broth microdilution

220
221 PM plates have been compared favourably against disc diffusion, broth microdilution or
222 molecular methods in terms of throughput, chemical use and time cost [25]. However, the
223 same authors also pointed out that PM plates are best used for preliminary estimates of
224 inhibitory concentrations. We wanted to gauge whether PM assays are sensitive and accurate
225 enough to discover novel collateral sensitivities and build large-scale resistance/sensitivity
226 networks. Therefore, we compared the output from our PM assays with classical MIC
227 determination by broth microdilution.
228

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229 Within our resistance/sensitivity network (S1-S5 Fig) we noted that the nine *S. aureus* strains
230 responded very similarly to some compounds, but for others the response was highly
231 variable. For example, the clinical isolates MRSA-BR and MRSA-TT were highly resistant to
232 erythromycin (average growth scores of 8 and 7.5 respectively) while most of the other
233 strains we tested were relatively sensitive (growth scores of 2 to 4). On the other hand, all
234 nine strains showed moderate-to-high levels of resistance to cefazolin (growth scores of 5.5
235 to 8). This reproducible inter-strain variability in the PM data provided us with a way to
236 rigorously assess the degree of correlation with broth microdilution.
237
238 We selected six antimicrobial compounds to analyze in more detail. Erythromycin and
239 oxacillin had highly variable efficacies against the nine *S. aureus* strains. Nickel chloride and
240 2,2′-dipyridyl elicited moderate variability in growth scores between strains. Cefazolin and
241 demeclocycline had relatively uniform efficacies against all strains. The growth scores for
242 each strain against each of these six antimicrobials are summarized in Fig 3. In order to
243 appraise the sensitivity and accuracy of our PM assays, we also determined the MICs for
244 these six compounds. Our goal was to assess whether the large and small growth differences
245 we observed in the wells of PM plates truly reflected differences in antimicrobial
246 resistance/sensitivity.
247
248
249 **Fig 3. Phenotype microarray growth scores for nine *S. aureus* strains in the presence of six**
250 **different antimicrobials.** The radial axis depicts the average growth score from independent
251 duplicates. The growth scores of the seven MRSA clinical isolates are plotted in shades of blue and
252 green. Data for the laboratory strain *S. aureus* ATCC 25923 and its evolved descendent, 25923evo,
253 are plotted in orange and red.
254
255
256 The comparison of PM growth scores and broth microdilution MICs is shown in Fig 4. In
257 general, large differences in PM growth score faithfully reflected large differences in MIC.
258 For example, a high growth score for erythromycin (8 or 7.5) corresponded to an MIC of 128
259 μg/ml, whereas a low growth score (2) corresponded to MICs of 8 μg/ml or less. At the same
260 time, the correlation between PM growth score and MIC was far from perfect. The five
261 strains that showed a growth score of 2 in erythromycin-containing PM wells varied in their
262 MICs from 2 μg/ml (MRSA-SO) to 8 μg/ml (MRSA-BE and MRSA-UB). Another strain
263 with an MIC of 8 μg/ml (MRSA-SY) had a growth score of 4, not 2.
264

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265

266 **Fig 4. Heat map to correlate PM growth scores and MICs for nine *S. aureus* strains and six**
267 **antimicrobials.** PM growth scores are shaded from dark red (score = 8) to white (score = 0). MICs
268 are also colour-coded, to highlight their level of agreement or disagreement with the corresponding
269 growth score. Compounds are grouped according to the level of variability the nine strains showed in
270 their PM growth scores.

271

272

273 This pattern was consistent across clinical and laboratory strains, and across all of the
274 antimicrobials tested. Results on the polar ends of each scale were more consistent than
275 intermediary scores. For 5 out of the 6 antimicrobials, the highest MICs were directly
276 correlated with the highest PM growth scores (darkest shading in Fig 4). The outlier was
277 MRSA-SY, which reproducibly failed to grow in any oxacillin-containing PM well, but
278 which was highly resistant to oxacillin (MIC = 64 μg/ml) in replicated broth microdilution
279 assays. Similarly, for 5 of the antimicrobials, the lowest MICs were reflected in the lowest
280 PM growth scores (lightest shading in Fig 4). The exception was 2,2′-dipyridyl, for which the
281 most sensitive strain (*S. aureus* ATCC 25923, MIC = 32 μg/ml) grew unusually well in the
282 PM assay (growth score = 7).

283

284 On the other hand, differences in PM growth score of less than 2 units could not reliably
285 predict differences in MIC. For example, growth scores of 4, 5 or 6 could all correspond to a
286 demeclocycline MIC of 0.0156 μg/ml (Fig 4). Similarly, growth scores of 5, 7 or even 7.5
287 could correspond to a nickel chloride MIC of 64 μg/ml.

288

289 The manufacturer of PM plates (Biolog Inc.) does not disclose the concentrations of
290 compounds in their plates. The sizes of the incremental increases in concentration across the
291 four PM wells are also unknown for each compound. Not knowing the working range of
292 concentrations complicates the use of PM plates to assess sensitivity/resistance networks. For
293 example, six of the nine *S. aureus* strains showed full growth in all four cefazolin-containing
294 PM wells; that is, they had growth scores of 8. However, the cefazolin MICs of these strains
295 ranged from 1 μg/ml to 16 μg/ml (Fig 4). The most likely explanation is that the four PM
296 wells containing cefazolin range from a very low concentration up to, perhaps, 0.5 μg/ml. If
297 this is the case, any strain with an MIC > 0.5 μg/ml will show full growth in all four wells;
298 however, it becomes impossible to assess the relative resistance or sensitivity of any strain
299 that fulfils this criterion.

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300 **DISCUSSION**

301

302 This study has emphasized the power – and potential pitfalls – of PM plates for the large-
303 scale assessment of cross resistance and collateral sensitivity. We were able to rapidly obtain
304 resistance and sensitivity data for 72 antimicrobials, which is many more than have been
305 tested in previous studies [8, 12, 14, 15]. It would be straightforward to expand our approach
306 to more of the antimicrobial-containing PM plates. In total, plates 11-20 contain 237
307 antimicrobials; testing the entire set would represent an order of magnitude increase in
308 screening breadth compared to current approaches. Moreover, scoring PM growth by eye (Fig
309 1) proved to be a fast, technically straightforward, cost effective and reproducible way to
310 collect resistance and sensitivity data. In independent duplicates, carried out several weeks
311 apart, we obtained identical growth scores in 544 of 648 antimicrobial/strain combinations.
312 Of the remaining combinations, 75 differed by a single point on our 8-point growth scale (Fig
313 2). This corresponded to the difference between no growth and intermediate growth, or
314 intermediate growth and full growth, in one of the four wells containing a given
315 antimicrobial.

316

317 When PM growth scores were carefully compared with MIC data obtained by broth
318 microdilution, a trade-off between throughput and accuracy became apparent. Small
319 differences in PM growth score did not reliably correlate with MIC. Our data suggest that a
320 difference in growth score of at least 2 points is required to indicate a genuine difference in
321 MIC between two *S. aureus* strains. For example, the PM assays suggested that many of the
322 MRSA strains were more sensitive to demeclocyline, nickel chloride or 2,2′-dipyridyl than
323 the laboratory strain *S. aureus* ATCC 25923 (Fig 3). However, the differences in PM growth
324 score were small and the evidence for increased sensitivity was not borne out by MIC testing
325 (Fig 4). The level of agreement (or disagreement) between PM scores and MICs was
326 comparable for the clinical isolates of MRSA and the laboratory strains *S. aureus* 25923 and
327 25923evo. For the purpose of discovering novel collateral sensitivities, the power of these
328 assays appears to be limited to detecting large reductions in resistance. With one exception
329 (MRSA-SY in oxacillin), differences of ≥16-fold in MIC between any two strains were
330 always correlated with differences in PM growth.

331

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332 Our results build on previous findings that laboratory strains are not necessarily good models
333 for exploring collateral sensitivity in the clinical setting [12, 26]. Our laboratory-evolved,
334 oxacillin-resistant strain 25923evo frequently behaved closer to its parent, *S. aureus* ATCC
335 25923, than to the clinical MRSA isolates. Collateral sensitivities could not be extrapolated
336 from 25923evo to the MRSA isolates, either from PM results or from MIC data.
337
338 There remains a chasm between the expanding body of laboratory research into collateral
339 sensitivity and the implementation of this research as a therapeutic strategy. A high-
340 throughput assay using PM plates could go some way towards bridging this gap. The assay
341 we have implemented here offers speed and breadth, and is capable of reproducibly
342 identifying large differences in cross-resistance or collateral sensitivity between otherwise
343 closely-related strains.
344
345
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347
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353
354
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422

423

424 ## SUPPORTING INFORMATION

425

426 **S1 Table. Full list of antimicrobial compounds tested in PM plates 11, 12 and 13,**
427 **grouped according to mode of action.**

428

429 **S1 Fig. Phenotype microarray growth scores for nine *S. aureus* strains in the presence of**
430 **cell wall-acting antibiotics.** Scores are an average of two biological replicates, with 8
431 representing maximum relative resistance around the exterior of the radar and 0 representing
432 complete sensitivity at the centre. MRSA isolates are in shades of blue and green. Laboratory
433 strains *S. aureus* ATCC 25923 and 25923evo are orange and red respectively.

434

435 **S2 Fig. Phenotype microarray scores for nine *S. aureus* strains in the presence of**
436 **protein synthesis-inhibiting antibiotics.** Scores are an average of two biological replicates,
437 with 8 representing maximum relative resistance around the exterior of the radar and 0
438 representing complete sensitivity at the centre. MRSA isolates are in shades of blue and
439 green. Laboratory strains *S. aureus* ATCC 25923 and 25923evo are orange and red
440 respectively.

441

442 **S3 Fig. Phenotype microarray scores for nine *S. aureus* strains in the presence of**
443 **antibiotics that inhibit nucleic acid synthesis.** Scores are an average of two biological
444 replicates, with 8 representing maximum relative resistance around the exterior of the radar
445 and 0 representing complete sensitivity at the centre. MRSA isolates are in shades of blue and
446 green. Laboratory strains *S. aureus* ATCC 25923 and 25923evo are orange and red
447 respectively.

448

449 **S4 Fig. Phenotype microarray scores for nine *S. aureus* strains in the presence of**
450 **repurposed cancer and antipsychotic drugs.** Scores are an average of two biological
451 replicates, with 8 representing maximum relative resistance around the exterior of the radar

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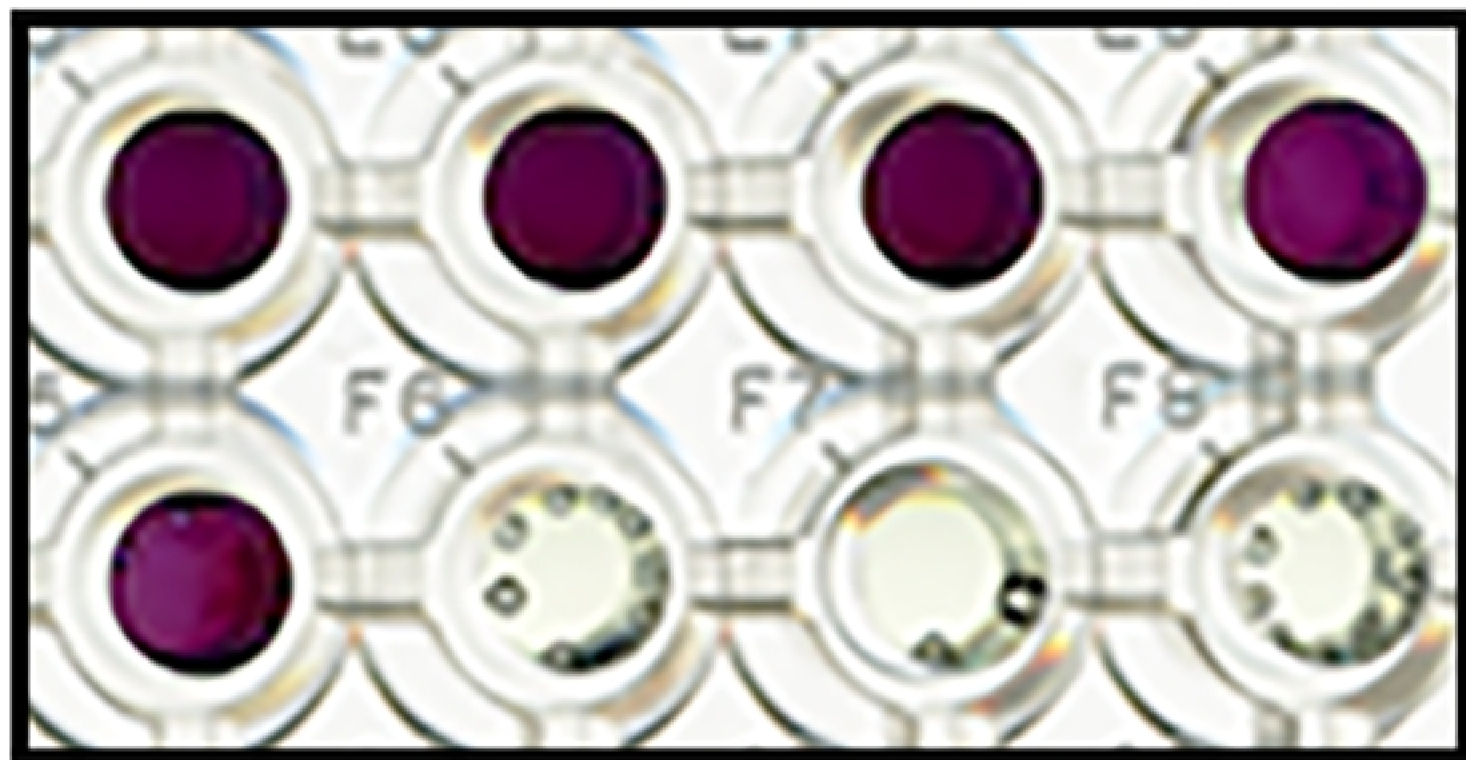
452 and 0 representing complete sensitivity at the centre. MRSA isolates are in shades of blue and
453 green. Laboratory strains *S. aureus* ATCC 25923 and 25923evo are orange and red
454 respectively.
455
456 **S5 Fig. Phenotype microarray scores for nine *S. aureus* strains in the presence of**
457 **antiseptics, disinfectants, metal ions, etc.** Scores are an average of two biological replicates,
458 with 8 representing maximum relative resistance around the exterior of the radar and 0
459 representing complete sensitivity at the centre. MRSA isolates are in shades of blue and
460 green. Laboratory strains *S. aureus* ATCC 25923 and 25923evo are orange and red
461 respectively.



Enoxacin

Erythromycin

Doxycycline

Fig 1

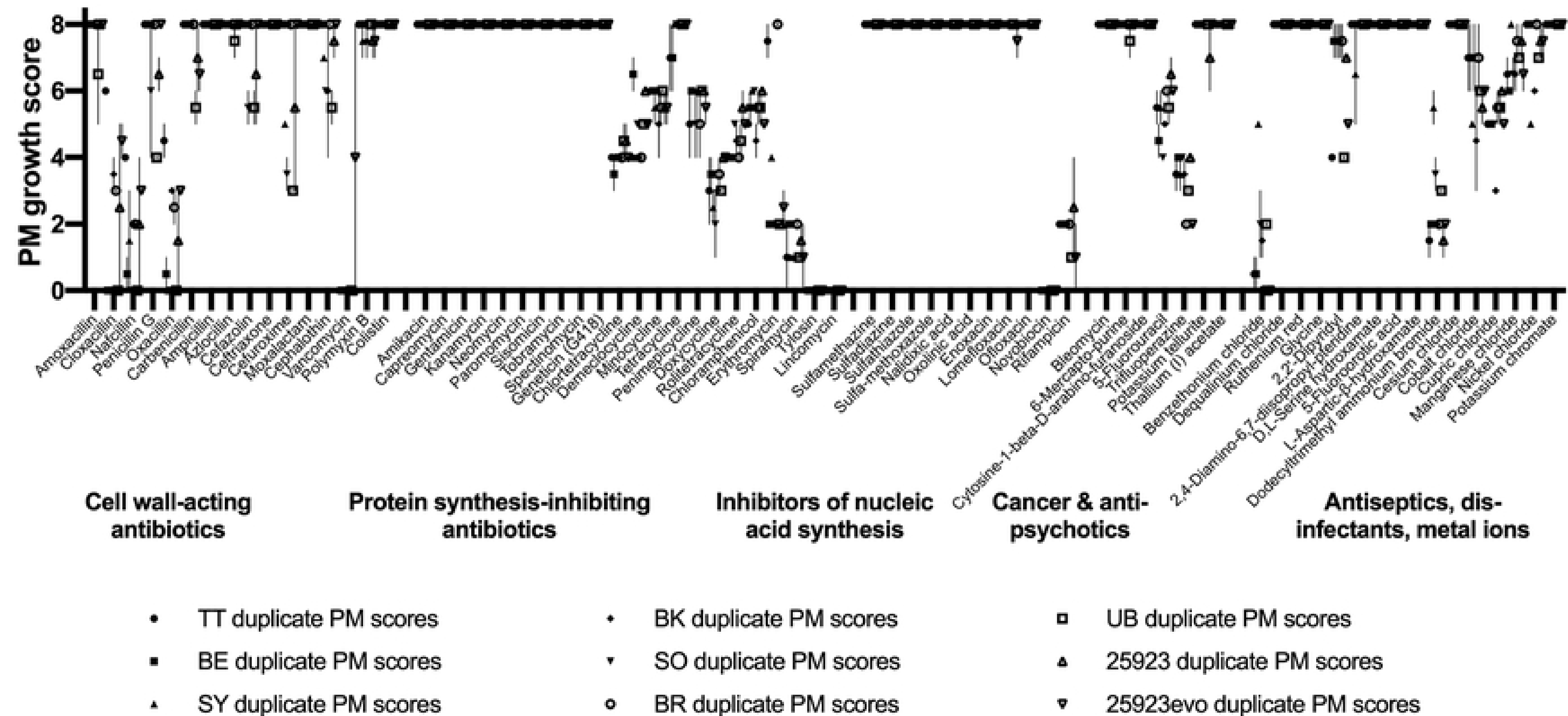


Fig 2

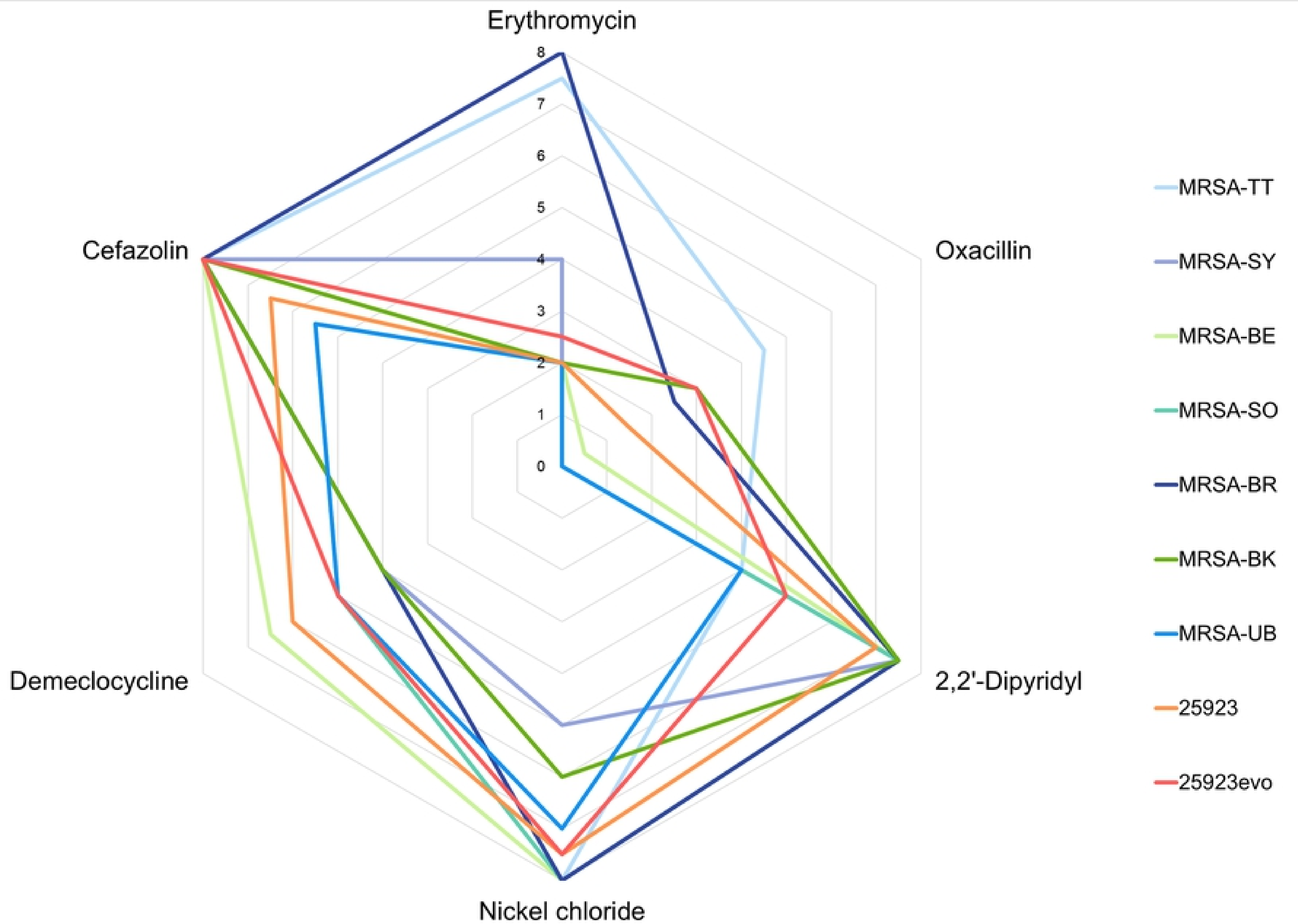


Fig 3

CLINICAL ISOLATES

MRSA isolate	Erythromycin PM score	Erythromycin MIC µg/ml	Oxacillin PM score	Oxacillin MIC µg/ml	2,2'-Dipyridyl PM score	2,2'-Dipyridyl MIC µg/ml	Nickel chloride PM score	Nickel chloride MIC µg/ml	Demeclocycline PM score	Demeclocycline MIC µg/ml	Cefazolin PM score	Cefazolin MIC µg/ml
BR	8	128	2.5	0.25	7.5	512	8	128	4	0.0313	8	2
TT	7.5	128	4.5	8	4	128	8	128	4	0.0156	8	16
SY	4	8	0	64	7.5	64	5	64	4	0.0156	8	4
SO	2	2	0	< 0.125	7.5	512	8	128	5	0.0313	5.5	< 0.125
BE	2	8	0.5	0.25	7.5	128	8	128	6.5	0.0625	8	8
UB	2	8	0	< 0.125	4	64	7	64	5	0.0156	5.5	< 0.125
BK	2	4	3	0.25	7.5	64	6	32	4	0.0313	8	2

LABORATORY STRAINS

	Erythromycin PM score	Erythromycin MIC µg/ml	Oxacillin PM score	Oxacillin MIC µg/ml	2,2'-Dipyridyl PM score	2,2'-Dipyridyl MIC µg/ml	Nickel chloride PM score	Nickel chloride MIC µg/ml	Demeclocycline PM score	Demeclocycline MIC µg/ml	Cefazolin PM score	Cefazolin MIC µg/ml
25923evo	2.5	4	3	1	5	256	7.5	128	5	0.0313	8	1
25923	2	4	1.5	< 0.125	7	32	7.5	64	6	0.0156	6.5	< 0.125

High variability compounds (Erythromycin, Oxacillin) — Intermediate variability compounds (2,2'-Dipyridyl, Nickel chloride) — Low variability compounds (Demeclocycline, Cefazolin)

Fig 4