Reduced Sensitivity of Oxacillin-Screening Agar for Detection of MRSA ST398 from Colonized Pigs

Methicillin-resistant Staphylococcus aureus (MRSA) bacteria are opportunistic, zoonotic pathogens presenting major challenges for health care providers. The pig-adapted lineage, MRSA ST398, is now recognized worldwide (8), and pigs are increasingly considered an important source of community-acquired MRSA (3). Early detection of ST398 on farms is critical in order to prevent spread and human infection. MRSA routine detection methods are typically based on those described for humans, often involving selective enrichment broth and oxacillin-supplemented agar (2 to 6 μg/ml) (2, 6, 7). Direct inoculation of swabs from carriage sites onto selective agar is less sensitive than inoculation after enrichment (2), but enrichment also hinders bacterial quantitation.

Seven gnotobiotic piglets wereatraumatically inoculated with MRSA ST398 spa t0111, as part of a wider study on bacterial antagonism. Groups of piglets were housed in three isolators (Bell, Inc., United Kingdom); two groups were also inoculated with potential bacterial antagonists, while two control animals received ST398 only. Swabs from both nostrils, skin behind one ear, and skin over the sacrum (both 9 cm²) were vortexed in normal saline and serially diluted suspensions plated onto mannitol-salt agar (MSA) supplemented with 0, 2, 4, or 6 μg/ml oxacillin (Sigma-Aldrich); colonies were counted after 48 hours (37°C). mecA presence was confirmed for selected isolates (1), and mecA transcription was measured quantitatively (4) from swabs collected 4 days after inoculation with ST398 only, using the laboratory inoculation strain as a control. Relative gene expression was expressed as the ratio of target (pta and 16S RNA) gene concentrations. Wilcoxon’s rank sum test was used to compare MRSA and mecA transcriptions from different media and sites.

Cytological examination confirmed colonization of piglets with coccoid bacteria. From all sites, CFU numbers were significantly higher on MSA and MSA with 2 μg/ml than on MSA with 4 (P < 0.01) and 6 (P < 0.01) μg/ml oxacillin (Table 1). Small colonies (<0.5-mm diameter), confirmed as ST398 by phenotypic and mecA tests, became detectable on MSA with higher oxacillin concentrations after prolonged incubation (56 to 72 h). Subculture of these small colonies onto selective agar is less sensitive than inoculation after enrichment (2), but enrichment also hinders bacterial quantitation.

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The reduced and delayed growth of ST398 suggests a reversible attenuation of antibiotic resistance on animals as corroborated by reduced mecA expression. A similar observation has been described previously and was attributed to bacterial strain competition (5). In contrast, attenuation seen in our gnotobiotic piglets was competition independent. Possible cell envelope perturbation, translational attenuation of resistance genes, or defective metabolism due to growth arrest in vivo could also have had an impact on resistance expression. These findings suggest that ST398 has distinctive ecology compared to human-adapted MRSA.

This study demonstrates that MRSA ST398 may be missed using conventional screening methods and indicates that screening studies using direct plating onto oxacillin agar may underestimate the prevalence of this lineage and should be interpreted with care.

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REFERENCES

TABLE 1. Average MRSA ST398 populations

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Mean ± SE log no. of CFU/swab or cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSA</td>
</tr>
<tr>
<td>Nostrils</td>
<td>6.33 ± 0.14</td>
</tr>
<tr>
<td>Ear</td>
<td>5.17 ± 0.11</td>
</tr>
<tr>
<td>Sacrum</td>
<td>5.87 ± 0.13</td>
</tr>
</tbody>
</table>

*Values shown are average numbers of CFU/swab (for nostrils) or average numbers of CFU/cm² (for ear and sacrum) from gnotobiotic animals at day 12 postinoculation, after 48 h of incubation at 37°C. All values for MSA plus 6 μg/ml oxacillin were 0. MSA, mannitol-salt agar.


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