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Direct Identification of MRSA and MLS_B Phenotypes in *Staphylococcus aureus* Using Small Numbers of Immobilized Cells.

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Abstract:

Background: Conventional phenotyping methods require growth of large numbers of bacteria, which lengthens the total time-to-result. We report results for a new method that uses approximately 100-500 bacterial cells and enables 4-hour MRSA and MLS_B phenotype detection.

Methods: A multi-channel fluidic device used computerized microscopy of immobilized bacteria to measure bacterial growth rates. Borderline oxacillin resistant isolates (CDC) were exposed to cefoxitin (FOX) in one fluidic channel to predict MRSA and, in another channel, erythromycin (ERY) followed by clindamycin (CLI) to identify MLS_B phenotypes. 78 strains were *mecA*-positive and 56 were *mecA*-negative. 44 of the 78 *mecA*-positive isolates and 14 of the 56 *mecA*-negative isolates exhibited inducible or constitutive CLI resistance as determined by D-test. Colonies were resuspended from agar plates, pre-grown for 2 hours, and then 10 µL of a 7E5 CFU/mL inoculum was delivered to each flowcell. Bacteria were concentrated onto a poly-L-lysine-coated glass surface, capturing approximately 100-500 cells in the microscope's field of view. MRSA detection used 1 µg/mL of FOX followed by 6 µg/mL FOX. MLS_B detection used 0.1 µg/mL ERY followed by 0.5 µg/mL CLI. The system acquired images every 10 minutes and computed growth rates of the cell population throughout the test. The system classified strains according to differences in growth rates between the antibiotic exposed organisms and control organisms. Results were compared to *mecA* PCR results and D-tests.

Results: The FOX assay correctly classified 78 of 78 *mecA*-positive and 56 of 56 *mecA*-negative isolates in a total test time of 4 hours. 43 of 44 of the *mecA*-positive isolates and 14 of 14 of the *mecA*-negative isolates expressing CLI resistance were correctly identified.

Conclusions: Direct measurement of growth rates of small numbers of immobilized bacteria enabled rapid identification of the MRSA and MLS_B phenotypes in *S. aureus*. The method shows promise for rapid testing of a bacterial sample compatible with direct extraction from clinical specimens.

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Topic (Complete): C02 Antimicrobial Susceptibility Testing Methods - Gram-Positive Non-Molecular

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ASM Member (or who has submitted an application) : Steven Metzger