

Direct Identification of Methicillin Resistant *Staphylococcus aureus* (MRSA) Using Small Numbers of Immobilized Cells and Response to Oxacillin (OXA) by Automated Growth Analysis

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REVISED ABSTRACT

Background: Conventional MRSA phenotyping methods rely on large numbers of bacteria, increasing the total time-to-result. We report initial results for a new method requiring 500 bacterial cells, enabling rapid MRSA identification.

Methods: A microfluidic device, using computerized microscopy of immobilized bacteria, was used to measure bacterial growth rates. Tests were performed on 14 MRSA and 19 Methicillin Susceptible *Staphylococcus aureus* (MSSA) ATCC® strains, and on 18 MRSA and 8 MSSA clinical isolates. 2 MRSA strains exhibited Class I heteroresistance. Bacteria were pre-grown for 2 hours (to assure log phase) and 10 μ L of a 5×10^6 cfu/mL inoculum was delivered to the flowcell. Bacteria were concentrated onto a poly-L-lysine glass surface, capturing approximately 500 cells in the microscope's field of view. MHB with 4 μ g/mL oxacillin (OXA) and 2% NaCl was introduced into the flowcell. An adjacent flowcell contained a growth control of the same strain (no OXA). The system acquired images every 10 minutes, and computed growth rates of the bacterial cell population throughout the test. The system classified strains according to differences in growth rates between the OXA-exposed organisms and control organisms. Results were compared to those for cefoxitin (FOX) disk diffusion (DD).

Results: Growth rate differences, used to assess phenotypes, correctly classified 32 of 32 MRSA and 27 of 27 MSSA strains consistent with FOX DD results. Following OXA challenge, growth arrest (defined as mean population division rate < 0.6 hr⁻¹) of MSSA strains occurred in less than 4 hours. Population mean division rates for non-Class I MRSA strains were 0.6 to 2.0 hr⁻¹ throughout the test. Heteroresistance effects initially slowed one Class I MRSA strain's mean division rate to 0.5 hr⁻¹; though growth acceleration was detected 4 hours post OXA exposure.

Conclusions: Direct measurement of growth rates of small numbers of immobilized bacteria enabled rapid identification of the MRSA phenotype in *S. aureus*. The method shows promise for rapid MRSA and MSSA differentiation using an inoculum size compatible with direct extraction from clinical specimens.
