# C-032. 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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## Abstract *(revised)*

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

**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 1 heteroresistance. Bacteria were pregrown for 2 hours (to assure log phase) and 10 µl of a 5E6 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 µg/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 1 MRSA strains were 0.6 to 2.0 $hr^{-1}$ throughout the test. Heteroresistance effects initially slowed one Class 1 MRSA strain's mean population 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 possible direct extraction from clinical specimens.

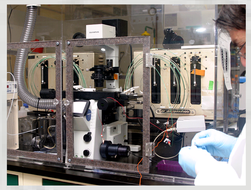
## Introduction

- Broad-spectrum resistance mechanisms to β-lactam antibiotics present challenges to the clinician.
- The production of a modified penicillin binding protein (PBP2a) encoded by the *mecA* gene is the well-known mechanism of the MRSA phenotype.
- MRSA is a clinically important phenotype requiring rapid and accurate identification.
- CLSI recommends a disk diffusion method for detection of MRSA, requiring overnight culturing.
- A method requiring a relatively small number of starting bacteria, compatible with direct-from-specimen analysis, would greatly reduce the total time-to-result for MRSA identification.

**The purpose of this investigation was to determine whether a new automated analytical method based on small numbers of bacteria could identify MRSA with accuracy comparable to that of the FOX disk diffusion test, but in less time.**

## Assay Methods

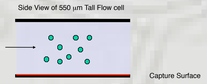
Direct observation of bacterial response to oxacillin was performed on a custom benchtop instrument that combines a disposable fluidic cartridge with automated digital microscopy, motion control, and image analysis software.



BACcel System

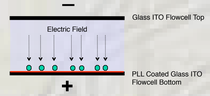
### Sample Introduction

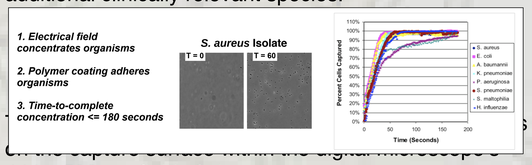
- *S. aureus* isolates were resuspended in electrokinetic capture buffer at 1 x $10^6$ CFU/mL.
- The samples were introduced into flowcells and the cartridge was placed on the reader.
- Flowcells were constructed of transparent top and bottom surfaces allowing microscope imaging.




### Capture and Concentration

- Cells were electrokinetically concentrated to the flowcells' capture surface coated with poly-L-lysine.
- Time-to-capture was less than 60 seconds for *S. aureus* isolates and less than 180 seconds for a panel of additional clinically relevant species.

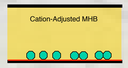



- [illegible] on the capture surface within the digital microscope's 444 x 592 µm field of view.

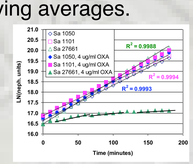
### Oxacillin Challenge

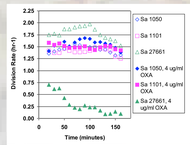
- The flowcells were then rinsed with cation-adjusted Mueller-Hinton broth (MHB), removing electrokinetic concentration buffer.
- The system performed 2 parallel assays:
  - Growth of immobilized cells in MHB for 3 hours.
  - OXA susceptibility test consisting of exposure to OXA at 4 µg/mL in MHB for 3 hours.
- Time sequence images were acquired by the system for each of the flowcells.
- Division rates in the OXA medium were used to call each strain as OXA resistant or susceptible.
- Each test required less than 3 hours of run time.
- 32 MRSA and 27 MSSA strains and isolates were run in duplicate.
- Total time-to-result was 5 hours including pre-growth.
- FOX disk diffusion tests were performed on the same strains, following CLSI procedures.




## Population Growth

- Population growth was assessed using nephelometry measurements integrating across the microscope's field of view.
- Nonlogarithmic growth rates were estimated using moving averages.

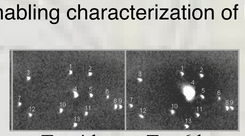


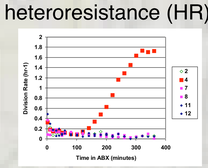




**Log phase growth was accurately quantified as evidenced by high $R^2$ linear fits. Susceptible strains are characterized by nonlinear growth. Moving averages were used to calculate instantaneous division rates.**

## Individual Clone Growth

- Image processing algorithms were developed to extract individual clone nephelometry intensity and calculate clonal growth rates.
- Automated image analysis enables tracking of individual cells and progeny on a clone-by-clone basis.
- Clone analysis detects low frequency clonal resistance, enabling characterization of heteroresistance (HR).

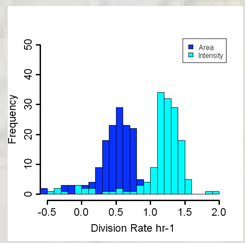



**Growth rate measurements for 1 growing and 10 non-growing clones, enabling heteroresistance characterization.**

## 3D Growth Characterization

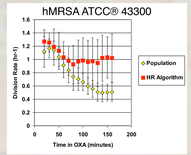
- 3-dimensional clustering of *Staphylococci* during growth presents unique challenges to accurate growth rate determination.
- Intensity-based nephelometry algorithms were developed to account for 3D clonal growth.

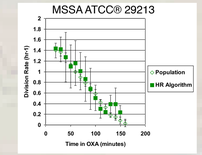



**Comparison of clonal area (2D) and nephelometry intensity based division rates for clones growing in the presence of OXA (MRSA ATCC® 35548). Clonal nephelometry intensity and population nephelometry division rates (1.06 $hr^{-1}$) agree. Area measurements significantly underestimate growth, potentially resulting in very major errors (VME, false susceptibility).**

## Heteroresistance Characterization

- Population growth measurements underestimate minority HR subpopulation growth rates, potentially masking hMRSA strains.
- HR characterization algorithms were developed to detect resistant subpopulations and eliminate VMEs (false susceptibility).



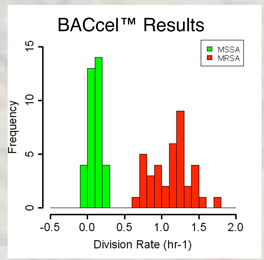

**Detection and growth quantification of a heteroresistant sub-population in MRSA ATCC® 43300 (left). The unbiased algorithm does not significantly alter MSSA ATCC® 29213 susceptible strain division rates (right). Error bars represent one standard deviation of replicated runs.**

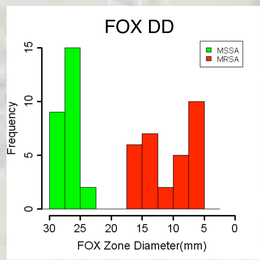
## Analysis Methods

- 5 point moving averages were calculated for all strains.
- HR detection performed on strains with division rates < 0.6 $hr^{-1}$ at the end of the assay.
- Endpoint division rates were averaged across strains.
- Strains were called:
  - **MRSA** when endpoint division rate ≥ 0.6 $hr^{-1}$
  - **MSSA** when endpoint division rate < 0.6 $hr^{-1}$
- Results were compared to FOX DD.

## Results

- The BACcel system used a 3-hour endpoint division rate to correctly characterize 32 of 32 MRSA and 27 of 27 MSSA strains and isolates when compared to FOX DD.




## Conclusions

- **The system accurately characterized growth of *S. aureus* in control and antibiotic conditions.**
- **Heteroresistant subpopulations were detected and their growth rates were quantified.**
- **The system required small numbers of bacteria, rapidly characterizing them as MRSA or MSSA.**
- **The method shows promise for use with direct-from-specimen samples.**