Getting more data from bacterial inhibition assays using digital image processing

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Bacterial inhibition assays are routinely used in both the clinical and the research laboratory. The sensitivity to antibiotics and toxin production by competing bacterial strains are both determined using agar plates. Although they are widely used, these methods are semi-quantitative and rely heavily on the objectivity of the person using them. There is also a wealth of information in these agar plate based assays that cannot be detected by the human eye.

Here we present a method for defining bacterial inhibition using image processing software and custom algorithms. We used a specific yet simple process to acquire images of agar plates using a standard camera under ambient light conditions. We then processed the images using the open source software ImageJ in order to characterize the inhibition zones. Using this method, we are able to accurately quantify the zone of inhibition, even when the inhibition zone was hazy due to incomplete killing or growth reduction. We were also able to quantitatively define the strength of the inhibition by quantitively measuring how clear or hazy is the zone of inhibition. This was especially useful when studying competitive interactions between clinical bacterial strains on an agar plate. Using this method, we were able to detect and define subtle interactions that were impossible to see with the naked eye. These interactions could account for weak toxins or even resource competition due to two bacterial species using the same media components to grow.

Despite being able to accurately define and compare bacterial inhibition, we also observed that the inhibition profiles generated by the image processing are quite unique depending on which bacteria are producing the inhibition zone. In the future, it may therefore be possible identify a specific inhibition type or even a specific strain using inhibition profiles. Furthermore, this method has the potential to be completely automated and take advantage of modern data analysis pipelines.