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A new mass spectrometry based bioassay for the direct assessment of hyaluronidase activity and inhibition.

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Abstract

The development of drug resistance by bacterial pathogens is a growing threat. Drug resistant infections have high morbidity and mortality rates, and treatment of these infections is a major burden on the health care system. One potential strategy to prevent the development of drug resistance would be the application of therapeutic strategies that target bacterial virulence. Hyaluronidase is virulence factor that plays a role in the ability of Gram-positive bacteria such as *Staphyloccus aureus* and *Streptococcus agalactiae* to spread in tissue. As such, this enzyme could be a target for the development of future anti-virulence therapies. To facilitate the identification of hyaluronidase inhibitors, quantitative and reproducible assays of hyaluronidase activity are required. In the present study, we developed a new mass spectrometry based bioassay for this purpose. This assay directly measures the quantity of a degradation product (3-(4-deoxy-β-D-gluc-4-enuronosyl)-N-acetyl-D-glucosamine) produced by the hyaluronidase enzyme. Validation parameters for the new assay are as follows: repeatability, <7%; intermediate precision, <10%; range, 0.78-50 μM; limit of detection, 0.29 μM ; and limit of quantification, 0.78 μM. Using the new assay, the IC_{50} value for a published inhibitor of *S. agalactiae* hyaluronidase, ascorbic acyl 6-palmitate, was 8.0 ± 1.0 μM. We also identified a new