ISWAVLD 2023

International Symposium of the World Association of Veterinary Laboratory Diagnosticians

29 JUNE-1 JULY 2023 Congress Centre Lyon

Towards the veterinary diagnostics of the future

Main topic : Animal Health

New methods to characterize bacterial biofilm, the example of Streptococcus equi.

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Introduction: The biofilm allows multiple bacteria to survive in a hostile environment because it provides greater resistance to the host's immune response, antibiotics, and disinfectants than bacteria in their free form. The biofilm is a complex heterogeneous structure consisting of one or more bacterial populations, embedded in a self-produced extracellular matrix, which could bind to a biotic or abiotic surface. Biofilms were first described in human medicine and found to be implicated in animal bacterial infections. *Streptococcus equi* subsp *equi* (SEE) is the causative agent of strangles and its persistence in guttural pouches of asymptomatic carriers plays an important role in the spread of the disease. SEE is a ?-hemolytic and Lancefield's group C *Streptococcus* found only in Equidae and believed to have evolved from an ancestral strain of *Streptococcus equi* subsp *zooepidemicus* (SEZ). This last is associated with a wide variety of diseases in horses and other animals including humans. While SEZ is able to produce biofilm, SEE's ability to form it is still undescribed.
Methods: Six SEE strains (isolated from carrier [10-146, 15-001, 58-450] and acute [8-550, 12-313, 39-275] strangle horses) and three SEZ [6-884, 10-150, 39-477] strains were used to analyze and compare their biofilm formation ability. All these strains have been isolated from guttural pouch washes of horses included in the first longitudinal field study on strangles in France, performed from 2016 to 2019. In vitro biofilm production by SEE et SEZ was characterized by four methodologies: 1) Crystal violet staining (CV), 2) Single-frequency impedance measurement (iCELLigence Real-Time Cell Analysis /RTCA instrument), 3) 3D inverted microscopy using live/dead staining, 4) the Bioflux 200 microfluidic system associated with microscopy. A strain of Pseudomonas aeruginosa known to produce biofilm was used as positive control and sterile media as a negative control. Three independent experiments with three replicates per strain were carried out for each protocol.
Results: The ability of SEZ strains to produce biofilm was confirmed by all four methods. According to the results obtained, two groups could be distinguished among the SEE strains analyzed. The strains of group A, including strains 8-550, 12-313, and 15-001, presented small colonies and average biofilm with mean OD590 comprised between 0.205 and 0.218 by CV. The strains of group B, containing strains 10-146, 39275, and 58-450, presented the largest mucosal colonies and showed weak biofilm with a mean OD590 comprised between 0.0015 and 0.035. These observations were confirmed by impedance measurement, 3D microscopy, and Bioflux visualizations.
Conclusion: Our study revealed that biofilm production in SEE was mainly associated with morphotypes. More investigations are necessary to understand the relationship between the biofilm production ability of isolated strains and the asymptomatic carrier/acute status of strangles horses.
These same experiments were performed on other bacterial species such as Pseudomonas aeruginosa isolated from human and animal samples. The obtained results thus allow a better understanding of the biofilm formation mechanism and composition to consider new therapeutic strategies.