Virulence associated factors, antimicrobial resistance, and *in silico* serotyping of *Streptococcus suis*

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Background

Streptococcus suis is a common cause of disease in pigs with a significant economic impact on swine production, due to decreased performance and mortality as well as increasing antimicrobial resistance (AMR) associated with *S. suis*. Factors related to host, environment, and bacteria may play a role in the development of *S. suis* disease. Although several virulence-associated factors (VAF) including capsular polysaccharide (CPS), muramidase-released protein (MRP), extracellular protein factor (EPF), and suilysin (SLY) have been suggested to be involved in *S. suis* pathogenesis, the actual involvement of many VAFs in *S. suis* disease development remains controversial. Three key VAFs (EPF, SLY and MRP) have been characterized as important for virulence in serotype 2 isolates; however, virulent *S. suis* strains which do not produce or carry genes encoding these proteins are regularly reported in literature. The objective of this study was to describe the distribution of serotypes, VAF and AMR genes in *S. suis* isolates recovered from systemic (blood, meninges) and non-systemic (tonsil, rectum) sites of sick and healthy pigs using whole genome sequencing (WGS).

Materials and methods

In total, 273 *S. suis* isolates- recovered from 112 nursery pigs (47 isolates from systemic and 136 from non-systemic sites of 65 sick pigs; 90 isolates from non-systemic sites of 47 healthy pigs) on 17 Ontario farms between 2013 and 2018- were studied. The isolates were grouped into four different categories: Systemic-confirmed (SC), Non-systemic-confirmed (NSC), Non-systemic-probable (NSP) and Non-systemic-healthy (NSH). The SC (n=47) and NSC (n=65) isolates were recovered from systemic and non-systemic sites of 32 pigs, respectively, that were both symptomatic and confirmed to have *S. suis* recovered from at least one systemic site. The NSP (n=71) isolates were obtained from 33 non-systemic sites of symptomatic pigs, for which *S. suis* was not recovered from any systemic site. The NSH isolates (n=90) were recovered from 47 healthy pigs. All isolates were subjected to WGS and serotyped *in silico* using WGS data.

Results

Using *in silico* typing, 21 serotypes were identified with serotypes 9 (13.9%) and 2 (8.4%) as the most frequent serotypes, while 53 (19.4%) isolates remained untypable. There were significant differences in the serotyping results by PCR and in silico serotyping ($p < 0.001$). Most of the PCR-untypable isolates were re-classified to serotype 9 (9%, 8/87,) and 21 (10%, 9/87). The relative frequency of VAF genes in isolates from systemic ($p < 0.001$) and non-systemic ($p < 0.001$) sites in sick pigs was higher compared to isolates from non-systemic sites in healthy pigs. Although many VAF genes were abundant in all isolates, three genes including *dltA* ($p < 0.001$), *luxS* ($p = 0.01$) *and troA* ($p = 0.02$) were more prevalent in isolates recovered from systemic sites compared to non-systemic sites of pigs. None of the serotype 2 isolates carried *epf* or *sly* genes. Furthermore, all the serotype 9 isolates except for one mrp+ isolate belonged to the mrp-sly-epf– genotype. Overall, 62% (168/273) of the total isolates, 58% (106/183) of SC, NSC and NSP isolates collectively, and 69% (62/90) of NSH isolates- belonged to the mrp-sly-epf – genotype. Among the isolates, 98% had at least one AMR gene and 79% had genes associated with at least four drug classes. The most frequently detected AMR genes were *tetO* conferring resistance to tetracycline, and *ermB* conferring resistance to macrolide, lincosamide and streptogramin (MLS).

Conclusion

In silico typing can improve the classification of *S. suis*. Further, VAFs were determined to be more prevalent in isolates recovered from pigs with clinical signs of *S. suis* infections. However, the wide distribution of VAFs genes in *S. suis* isolates in this study suggests that other host and environmental factors may contribute to *S. suis* disease development. In fact, the VAFs may not be the direct cause of *S. suis* disease but it is likely that their presence increases the likelihood of disease development under certain circumstances. The high frequency of AMR genes within the isolates poses problems not only within the swine industry but also in public health.

Source:

M Aradanas, et al. Serotypes, Virulence-Associated Factors, and Antimicrobial Resistance of *Streptococcus suis* Isolates Recovered From Sick and Healthy Pigs Determined by Whole-Genome Sequencing. Front Vet Sci. 2021. doi: 10.3389/fvets.2021.742345.