

Microbiological and molecular analysis of human invasive *Streptococcus suis* isolates

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Introduction: *Streptococcus suis* is an important zoonotic agent that causes a wide variety infections in pigs (meningitis, pneumonia, septicaemia, arthritis); moreover, the most of clinically healthy pigs carry *S. suis* in their nasal cavity and tonsils, contributing to the dissemination of this pathogen. *S. suis* can be transmitted to humans by direct contact with a pig or through contaminated meat. *S. suis* infections, such as meningitis, septicaemia and endocarditis, occur mainly in the group of people occupationally exposed to pigs or pork, including veterinary practitioners, pig farm workers, abattoir workers, butchers and meat processing workers.

Purpose: The aim of this work was to perform molecular and microbiological analyses of *S. suis* humans invasive isolates from Poland.

Methods: The studied group comprised 26 *S. suis* isolates from invasive infections: 19 (73%) and 7 (27%) isolates from CSF and blood, respectively, from 21 patients (17 men, 4 women) collected during 2000-2013 in the whole area of Poland (Fig. 1). The isolates were examined by hemolysis assay on horse blood agar plates, biofilm formation in microtiter plates with human plasma fibrinogen, susceptibility testing as well as by molecular methods, such as multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) typing and analysis of the presence of potential virulence factors genes and antibiotics resistance genes by PCR and partial sequencing.

Results: All 26 *S. suis* strains represented serotype 2 and belonged to ST1. Pulsed-field gel electrophoresis typing revealed high similarity of isolates, with 23 (84.6%) isolates representing one PFGE profile (Fig. 2). All isolates were susceptible to β -lactams, aminoglycosides, glycopeptides and quinolones, and the majority of isolates (73.1%) was susceptible to macrolides and tetracycline. Seven isolates resistant to erythromycin and tetracycline contained the *ermB* and *tetO* genes. 16 (61.5%) isolates were able to form a biofilm. The majority of isolates i.e. 20 (76.9%) and 4 (15.4%) showed strong and weak hemolytic activity, respectively, and only 2 (7.7%) lacked such activity. Virulence factor genes, including *sly*, *eno*, *fbpS*, *mrp*, *sao*, *epf* were detected in the whole analyzed group. The majority of isolates possessed the variant of the *mrp* gene encoding Muramidase-released protein with a single repeat, with an exception of a single isolate, which possessed *mrp* variant with no repeats (Fig. 3). The variable region of *mrp* gene (Fig. 4) was characteristic for the European *mrp* genotype; indels in five isolates resulted in *mrp* encoding a truncated protein. In the analyzed group of isolates the *sao*-M allelic variant of *sao* gene (7 repeats) predominated; two isolates harbored atypical *sao* variant with 6 repeats. Analysis of the presence of putative four pilus gene clusters classified all the isolates into the genotype A (i.e., positive for the *srtBCD* cluster and *srtF*). None of isolates harbored either 89K pathogenicity island (89K PAI) or 105K pathogenicity island (105K PAI).

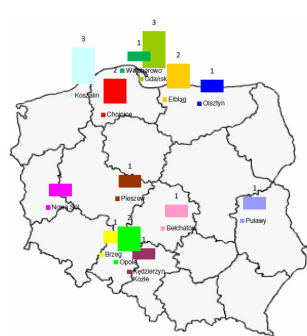


Fig. 1. *S. suis* invasive disease in Poland

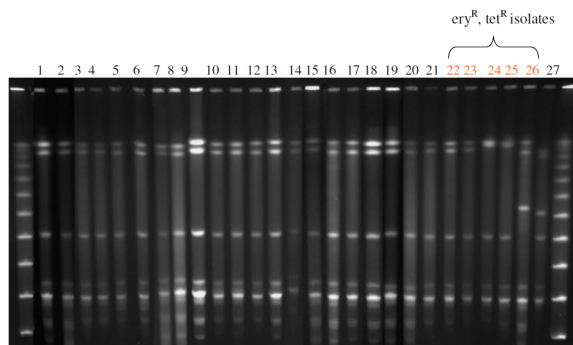


Fig. 2. PFGE-genotyping results of all isolates. Erythromycin and tetracycline resistant isolates was marked.

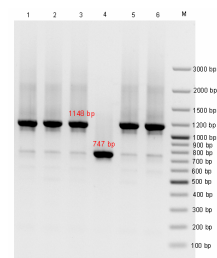


Fig. 3. PCR identification of *mrp* gene variants results

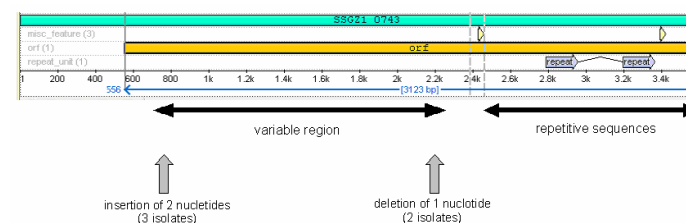


Fig. 4. Schematic presentation of gene encoding the Mrp protein.

Conclusions: *S. suis* isolates from human invasive infections in Poland are highly clonal and are associated with virulent ST1 observed worldwide. These isolates usually show good susceptibility to antimicrobials important for treatment of *S. suis* infections in humans.