FULL-LENGTH PLASMID SEQUENCING FOR IMPROVED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE: THE CASE OF SALMONELLA INFANTIS (OHEJP FULL FORCE STUDY).

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INTRODUCTION

Current European monitoring systems for antimicrobial resistance (AMR) fall short in identifying the drivers of the horizontal transmission of AMR genes. Although an increasing emphasis is put on genomic-based surveillance, mobile genetic elements (MGEs) remain challenging to resolve from short-read sequence data due to their chimeric, modular and repetitive nature. The goal of the FULL_FORCE consortium (One Health EJP) is to broadly introduce long-read sequencing into EU veterinary and public health institutions by supplying 17 EU partners with a technological toolbox and hands-on training in Oxford Nanopore Technologies (ONT) MinION™ sequencing. The OHEJP Full Force consortiumconducted a relevant study on Salmonella Infantis, in which the pESI megaplasmid (300 kb) carrying antimicrobial resistance genes, including extended spectrum beta-lactamase (ESBL), and conferring multidrug resistance is increasingly circulating throughout Europe in both humans and animals.

METHODOLOGY

Oxford Nanopore Technology will be applied to a selection of S. Infantis strains from seven countries, which areisolated from three different sources (human, animal and food/feed). This will, in turn, allow us to study the phylogeny of full-length pESI plasmids across sectors and borders, and lead to insights into its divergence, evolution and spread among Member States. Moreover, we plan to perform conjugation studies to assess the risk of plasmid spread towards other Salmonella serotypes.

RESULTS

Nine EU partner institutions from seven countries mined their existing short-read sequence collections for the presence of genetic markers of the pESI plasmid in S. Infantis strains, i.e. an IncFIB backbone containing the gene conferring increased bacterial tolerance to environmental mercury (mer operon), yersiniabactin, K88-like fimbria fim, tet(A), sul1, and in some cases, the dfrA14 resistance gene. Raw Illumina™ reads of 232 resulting strains were mapped against the S. Infantis reference strain (LN649235.1), followed by SNP based phylogeny. We identified eight clusters of S. Infantis strains, four of which encoded blaCTX-

M-1 and one of which encoded blaCTX-M-65. Long-read data will be analysed using the Full Force Plasmid Assembler (FFPA v1.0), a python script which joins best-in-field tools to trim and QC short and long sequence reads (qcat, Trimmomatric), enables species identification through Kraken and performs either Nanopore or hybrid assemblies through Unicycler. At the One Conference 2022, we will present results of these hybrid assemblies, and insights into the spread and evolution of the pESI megaplasmid in Europe.

DISCUSSION

The effective implementation of long-read sequencing across public health and veterinary labs can provide a paradigm shift in EU AMR surveillance, delivering insights into dominant MGEs, which are driving resistance among commensal and pathogenic Enterobacterales in Europe.