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P0779 Characterization of an IncA/C mega-plasmid encoding multidrug resistance and pathogenicity among Salmonella enterica serotype Heidelberg strains

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Background: *Salmonella enterica* serotype Heidelberg (*S*. Heidelberg) ranks among the most prevalent causes of human invasive non-typhoidal salmonellosis for which antimicrobial therapy is required. Extended spectrum cephalosporins (ESCs) are considered the drugs of choice in children or when fluoroquinolones are contraindicated in adults. ESC-resistant *S*. Heidelberg strains, exhibiting predominantly the AmpC phenotype and harbouring bla_{CMY-2} gene, have been extensively reported in Canada and the USA, whereas their introduction to Europe has only recently been documented. The aim of this study was to characterize IncA/C mega-plasmids recovered from ESC-resistant *S*. Heidelberg strains recently introduced into the Netherlands.

Materials/methods: Six IncA/C plasmids were included in the study due to their association with the bla_{CMY-2} gene. The genetic relatedness of their *S*. Heidelberg host was assessed by *Xba*I-PFGE. Plasmids were characterized by PCR-based replicon typing, S1-PFGE and filter-mating assays. Plasmid DNA was extracted from *E. coli* DH10b transformants and sequenced using MinION and 150 bp paired-end Illumina MiSeq libraries. High quality filtered reads were used for *de novo* MinION/Illumina hybrid assemblies and annotation. The pathogenic potential of a representative plasmid was assessed in the *Galleria mellonella* infection model.

Results: *S*. Heidelberg hosts were assigned to three different PFGE-types, namely *XbaI*.1965 (n=1), *XbaI*.1973 (n=4) and *XbaI*.X (n=1). The bla_{CMY-2}-encoding plasmids (approximately 218 Kb) were assigned to the IncA/C family and found to be transferable with frequencies of between $1.0*10^{-9}$ and $7.0*10^{-9}$ transconjugants per donor cell.
Plasmid sequences shared 99% identity and a typical IncA/C backbone. Annotation predicted the presence of 211 coding sequences, including resistance [bla_{CMY-2}, *sul*2, *tet*(A)] and virulence (*Yersinia* high-pathogenicity island) genes. Data from the *G. mellonella* infection model suggests that the presence of the IncA/C mega-plasmid results in increased virulence, based on larval mortality and morbidity over 96 h, as well as histopathological observations in *G. mellonella* larvae, six hours post infection.

Conclusions: The occurrence of a conjugative mega-plasmid encoding for decreased susceptibility to first-line antimicrobial agents and increased virulence among this invasive *Salmonella enterica* serotype, as well as its potential transmission to other Enterobacteriaceae warrants further investigation and continued surveillance.