Reconstructing plasmids of multi-resistant *E. coli* isolated from retail poultry meat

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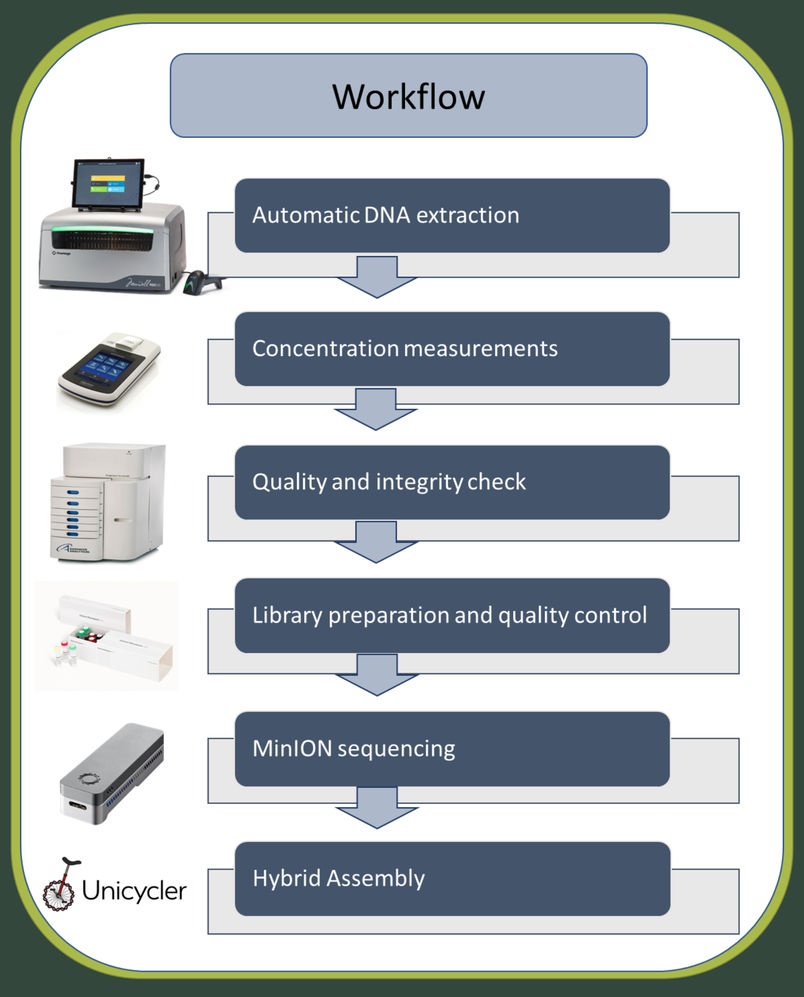
Introduction

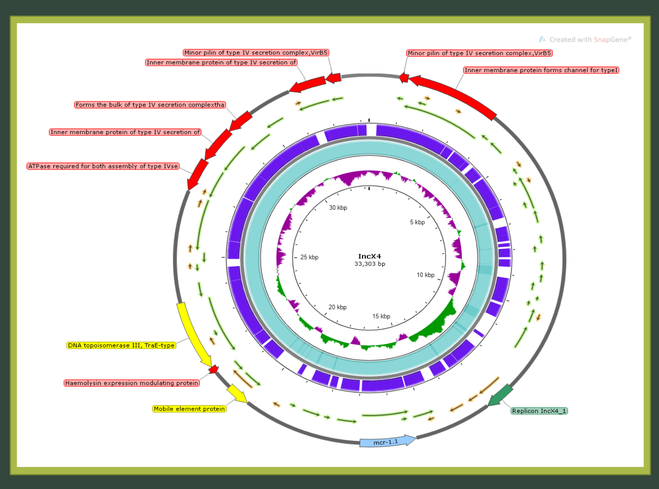
Escherichia coli is often used as an indicator organism in monitoring of food chain production for presence of AMR. Antimicrobial resistant bacteria have been associated with limited therapeutic options, prolonged duration of infections and high rate of treatment failures. Monitoring of plasmid- associated genes is especially crucial due to increased possibility of horizontal transfer. Our aim was to reconstruct and characterise plasmids harbouring resistance determinants in *E. coli* from poultry meat.

Pic. 1 Isolate Escherichia coli - MacConkey agar



Materials and methods

DNA was extracted from seven multi-resistant (MDR) *E. coli* originating from broiler meat. Quantity, quality and integrity of genomic material was evaluated by Qubit, Nanodrop and Fragment Analyzer, respectively. Sequencing of short fragments was done with use of Illumina platform (Nextera XT Library Prep and MiSeq). Sequencing of long reads was done by Oxford Nanopore platform (Native barcoding genomic kit and MinION). After hybrid assembly (Unicycler), analysis was done by Abricate with PlasmidFinder, ResFinder, VirulenceFinder 2.0, databases.



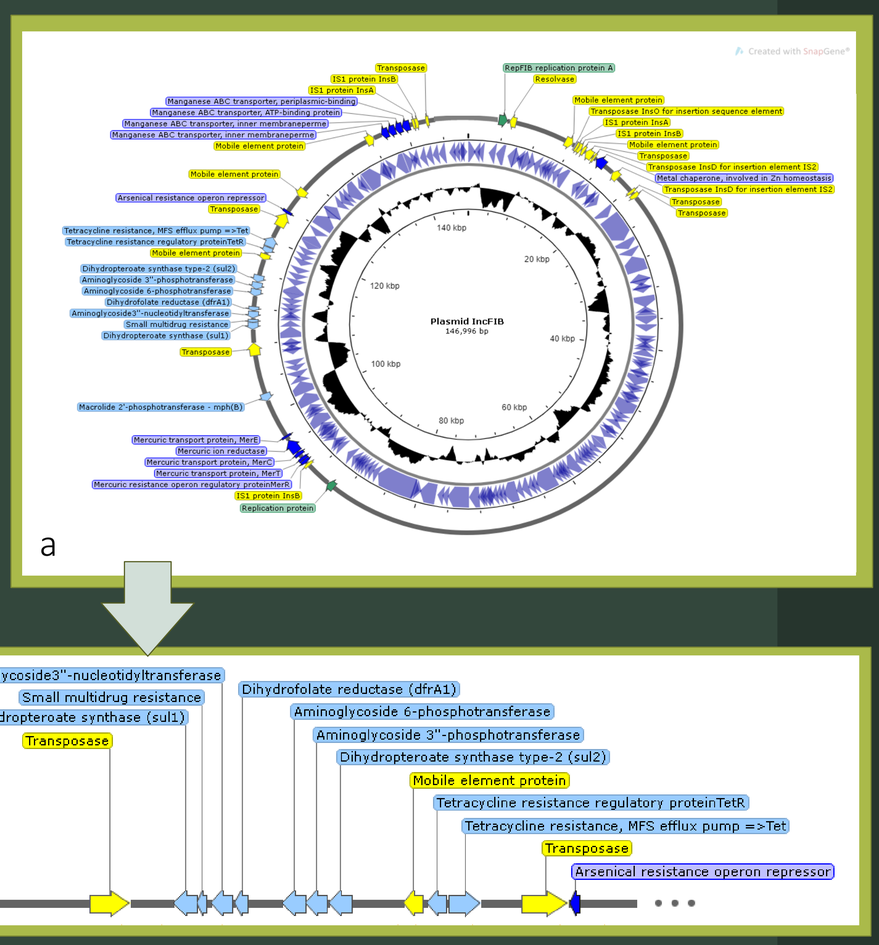
Pic. 2 Plasmid IncX4 harbouring mcr1

Table 1 Plasmids harbouring resistance genes

Isolate	Plasmids	Resistance genes
no. 29	IncX4 (33.3kb)	*mcr-1.1*
	IncX1 (29.6kb)	*tet(A), sul3, ant(3'')-Ia, cmlA1, aadA2, dfrA12*
no. 30	IncI1 (114.6kb)	*tet(A), aadA2, cmlA1, ant(3'')-Ia, sul3, blaSHV-12*
no. 33	IncFIB (149.4kb)	bla_{TEM-1B}, *tet(B), aph(3'')-Ib, aph(6)-Id*
	IncI1 (114.7kb)	*tet(A), aadA2, cmlA1, ant(3'')-Ia, sul3,* bla_{SHV-12}
	IncX1 (33.7kb)	bla_{TEM-1B}
no. 42	IncFIB (147kb)	*dfrA17, aadA5, sul2,* $bla_{CTX-M-1}$
	IncI1 (114.7kb)	*tet(A),* bla_{TEM-1B}
no. 44	IncFIB (146.9kb)	*mph(B), sul1, ant(3'')-Ia, dfrA1, aph(6)-Id, aph(3'')-Ib, sul2, tet(A)*
	IncB/O/K/Z (97kb)	bla_{SHV-12}, *sul2*
no. 47	ColE10/IncI1 (116.9kb)	*floR,* bla_{TEM-1B}
	IncB/O/K/Z (87.9kb)	bla_{CMY-2}
	IncX1 (49.3kb)	*tet(A),* bla_{TEM-1B}
no. 48	IncI1 (30.2kb)	*tet(A), sul2*

Results

Due to reconstruction, a total of thirty-one complete plasmid structures were obtained. Isolates harboured from two to seven plasmid types, with IncFIB being most common. Four multi-replicon plasmids were detected - IncFIB/FIC (n=2; 105-116 kb), ColE10/IncI1 (117 kb) and Col/ColRNAI (5,7 kb). Plasmids harbouring AMR determinants ranged from 30 kb to 116 kb. Presence of forty-nine genes encoding resistance to various antimicrobial classes was confirmed. Most of which was associated with plasmids (n=47), only two genes ($bla_{CTX-M-1}$) were allocated on chromosome. All isolates showed resistance to cephalosporins - ESBL via bla_{SHV-12} (n= 3), $bla_{CTX-M-1}$ (n=3) and AmpC via bla_{CMY-2} (n=1), mostly linked with various plasmids (IncFIB, IncI1, IncB/O/K/Z). One *E. coli* harboured colistin-resistant gene *mcr1* localised on IncX4. In addition we characterised six plasmids (IncI1, IncFIB, IncX1) harbouring MDR cassettes.



Pic. 3 (a, b) Plasmid IncFIB harbouring multi-resistance cassette

Conclusion

Study showed high mobilisation of various resistance genes, including multi-resistance cassettes in *E. coli* from poultry meat.

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