Characterisation of three NDM-5 *E. coli* isolates from meat, Hungary

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Areas of concern – emergence of resistance - CPE

Increase in reported *E. coli* carbapenemase producers

Country	Sample	MLST	Carbapenem resistance gene	Plasmid replicon gene	
Czechia	21_P_BA_4003_4_K	898	blaNDM-5	IncY, IncR, IncX3, IncX3, IncFIB(K), IncFIB	chromosome (IS30 and IS5)
Czechia	21_P_BA_225_3_K	10	blaNDM-5	IncX3, **IncR**	
Hungary	M2021_10044802_2_E	405	blaNDM-5	p0111, **IncFIB**, IncX4	
Hungary	M2021_10043982_E	405	blaNDM-5	p0111, **IncFIB**, IncX4	
Hungary	M2021_10044824_1_E	405	blaNDM-5	p0111, **IncFIB**, IncX4	
Italy	21112463I110X6	5229	blaOXA-181	IncFIB, IncX4, IncI1, IncFIA	rep_cluster_1195
Italy	21094270C326X6	5229	blaOXA-181	IncX3, IncFIB, **IncFIC**	
Italy	21098725F502X6	617	blaNDM-5	**IncFIA, IncFIB**, IncX4, IncI-alpha	
Italy	21102457L202X6	38	blaOXA-48	-	chromosome (IS10A)

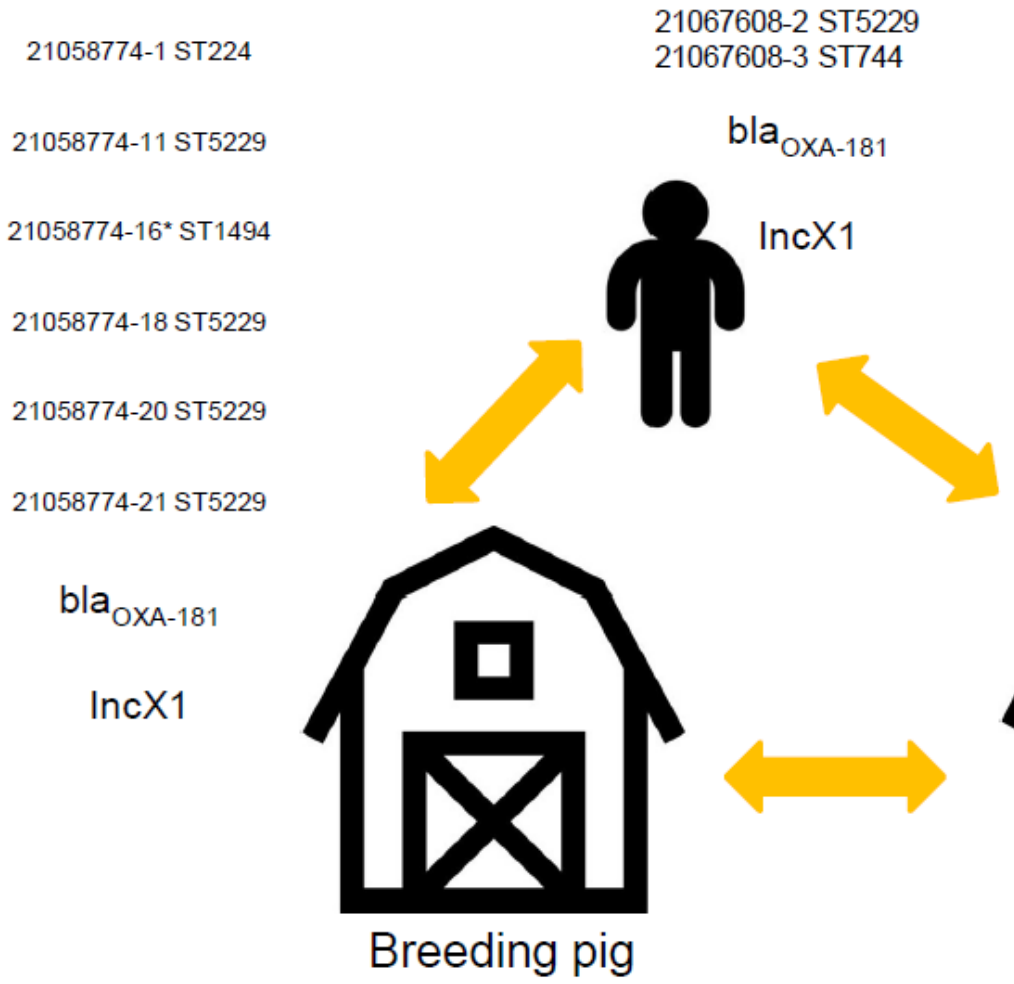
PlasmidFinder & Mob-suite: Tool for clustering and reconstruction of plasmids from draft assemblies.

EFSA reported CPE

Year	Country	Matrix	Gene Family	Gene	Number of isolates
2015	Germany	Fatenning pigs	VIM	*bla* VIM-1	1
2016	Romania	Broilers	OXA	*bla* OXA -162	2
		Broiler meat	OXA	*bla* OXA -162	1
2017	Germany	Fatenning pigs	VIM	*bla* VIM-1	1
2018	-	-	-	-	No isolates detected
2019	Germany	Pig meat	VIM	*bla* VIM-1	1
		Fatenning pigs	OXA	*bla* OXA -48	1
			GES	*bla* GES-5	1
2020	Austria	Broilers	VIM	*bla* VIM-1	1
2021	Hungary	Bovine meat	NDM	*bla* NDM-5	1
		Pig meat	NDM	*bla* NDM-5	2
	Spain	Fatenning pigs	OXA	*bla* OXA -48	2
	Czechia	Fatenning pigs	NDM	*bla* NDM-5	3
	Italy	Fatenning pigs	OXA	*bla* OXA -48	1
			OXA	*bla* OXA -181	20
		bovine animals < 1 year of age	NDM	*bla* NDM-5	1
			OXA	*bla* OXA -181	4
2022	Austria	Broilers	VIM	*bla* VIM-1	2
	Italy	Broilers	VIM	*bla* VIM-1	1
		Fattening turkeys	OXA	*bla* OXA -181	2

Epidemiological follow up and trace-back

Epidemiological Investigation: Case1



The hazard of carbapenemase (OXA-181)-producing *Escherichia coli* spreading in pig and veal calf holdings in Italy in the genomics era: Risk of spill over and spill back between humans and animals

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Emergence of carbapenemase producing *E. coli* Three isolates from Hungary

- Three multidrug resistant *bla*$_{NDM-5}$-harbouring *Escherichia coli* ST405 isolates were recovered from **beef and pork** collected **at retail** in Hungary, 2021
- Same ST 405 – know from human clinical cases
- Identical AMR and plasmid profile
- Initial analysis showed clonality, but no relation to NDM-5 producers from Czechia or Italy
- Clonality confirmed by both SNP analysis and cgMLST
  - Comparative analysis confirmed that isolates were clonally related (up to 2 SNPs)
  - Identical hierarchical clustering of cgMLST (HierCC) within Enterobase
    - belonging to the same HC2-172694 group

E. coli ST 405

- ST405 is a recognised human uropathogenic ST
  - Increasingly associated with multidrug resistance and virulence

- Comparison showed the most similar genomes available in Enterobase (same HC2)
  - isolate of human origin from Australia
  - two other isolates of unknown origin from the UK

- AMR genotypes were concordant with their phenotypes and included:
  - $bla_{\text{NDM-5}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$
  - *sul1*, *dfrA12*, *qepA4*, *tet*(B), *mph*(A)
  - point mutations in *gyrA* and *parCE* genes,

Emergence of carbapenemase producing *E. coli* -Three isolates from Hungary

- MinION sequencing in addition to Illumina for hybrid assembly of plasmid

- *bla*$_{NDM-5}$ was located on a IncFIB-IncFII hybrid plasmid
  - closely related to a plasmids from clinical *E. coli* isolated in USA (among others)
    - only bla$_{CTX-M-15}$-ISEcp1 transposition unit was absent

- Additional promoters or mutations indicate increased expression of both *bla*$_{CTX-M-15}$ and *bla*$_{NDM-5}$

- IncFII-IncFIB plasmids contained the *tra* and *trb* gene clusters required for conjugal transfer
  - conjugation experiments showed transfer of *bla*$_{NDM-5}$ was transferred along with the IncX4 plasmid to the recipient *E. coli* K12
  - transconjugants exhibited antimicrobial susceptibility profiles in concordance with the genetic content

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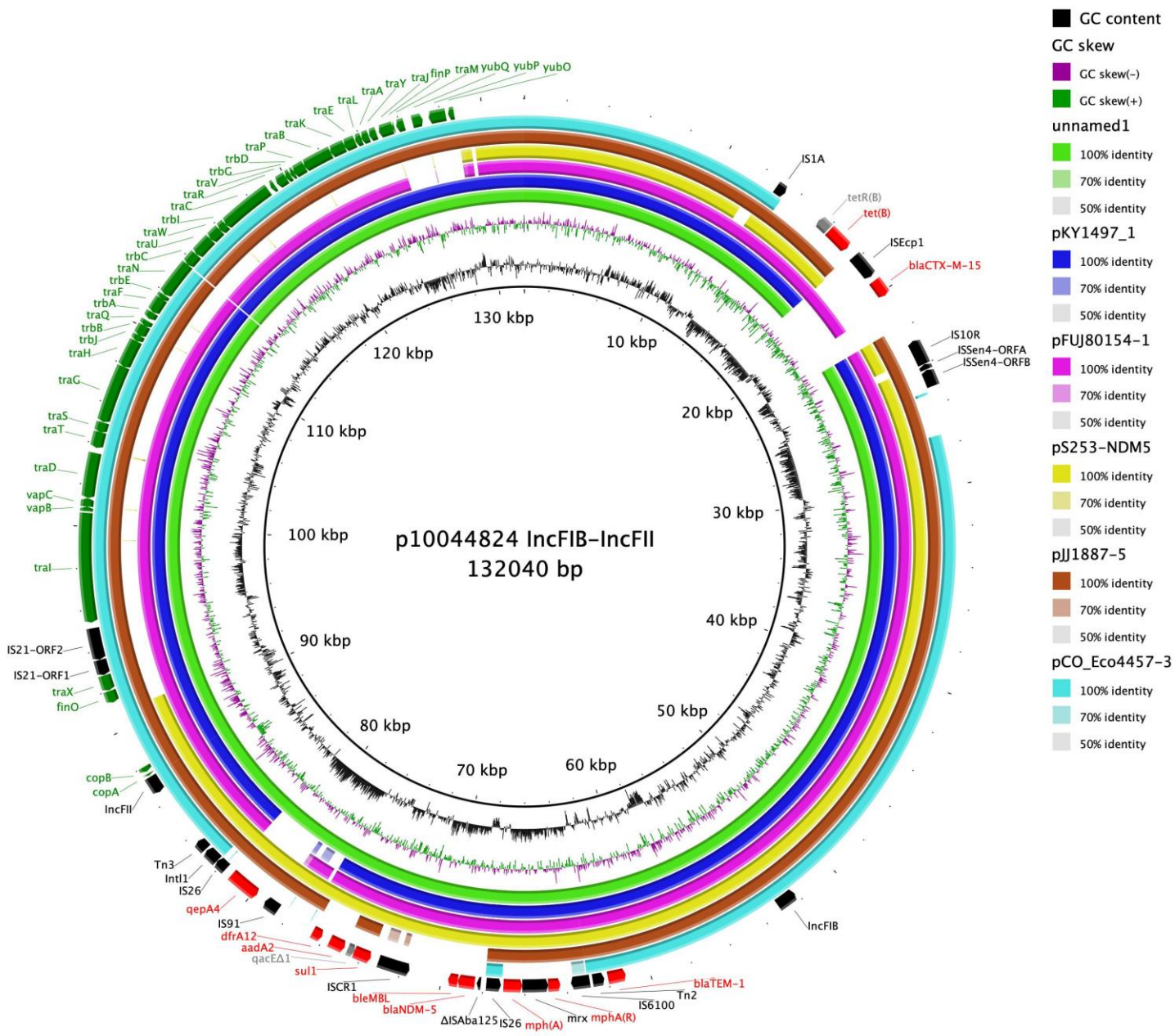

- IncFIB-IncFII hybrid plasmid contains numerous resistance genes:
  - *bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$
  - *sul1*, *dfrA12*, *qepA4*, *tet*(B), *mph*(A), aadA2
- Increased risk of co-selection and of the plasmid being maintained by selective pressure
- genetic context of *bla*$_{NDM-5}$ is highly conserved
  - Seen in IncF-type and IncX3 plasmids, indicating capacity for dissemination

Draft figure

Comparison with related plasmids from clinical cases in USA, Germany, Japan and New Zealand

Comments

- The human-associated *bla*$_{NDM-5}$-carrying *E. coli* in meat is of a great concern
  - contribute to the transmission of carbapenemase-producing bacteria to humans via food-producing environments or foods
- Multidrug resistance gene package on plasmid is of great concern
- The findings of three clonally related *E. coli* in different types of retail meat indicate human contamination in slaughterhouse/retail level
- Emerging resistance to carbapenems is seen in the primary production of animals in several European countries, but also transmission on retail and slaughterhouse level should be considered

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Questions or comments?