Animal & Plant Health Agency

mcr-1 and *mcr-2* variant genes identified in *Moraxella* spp. isolated from pigs.

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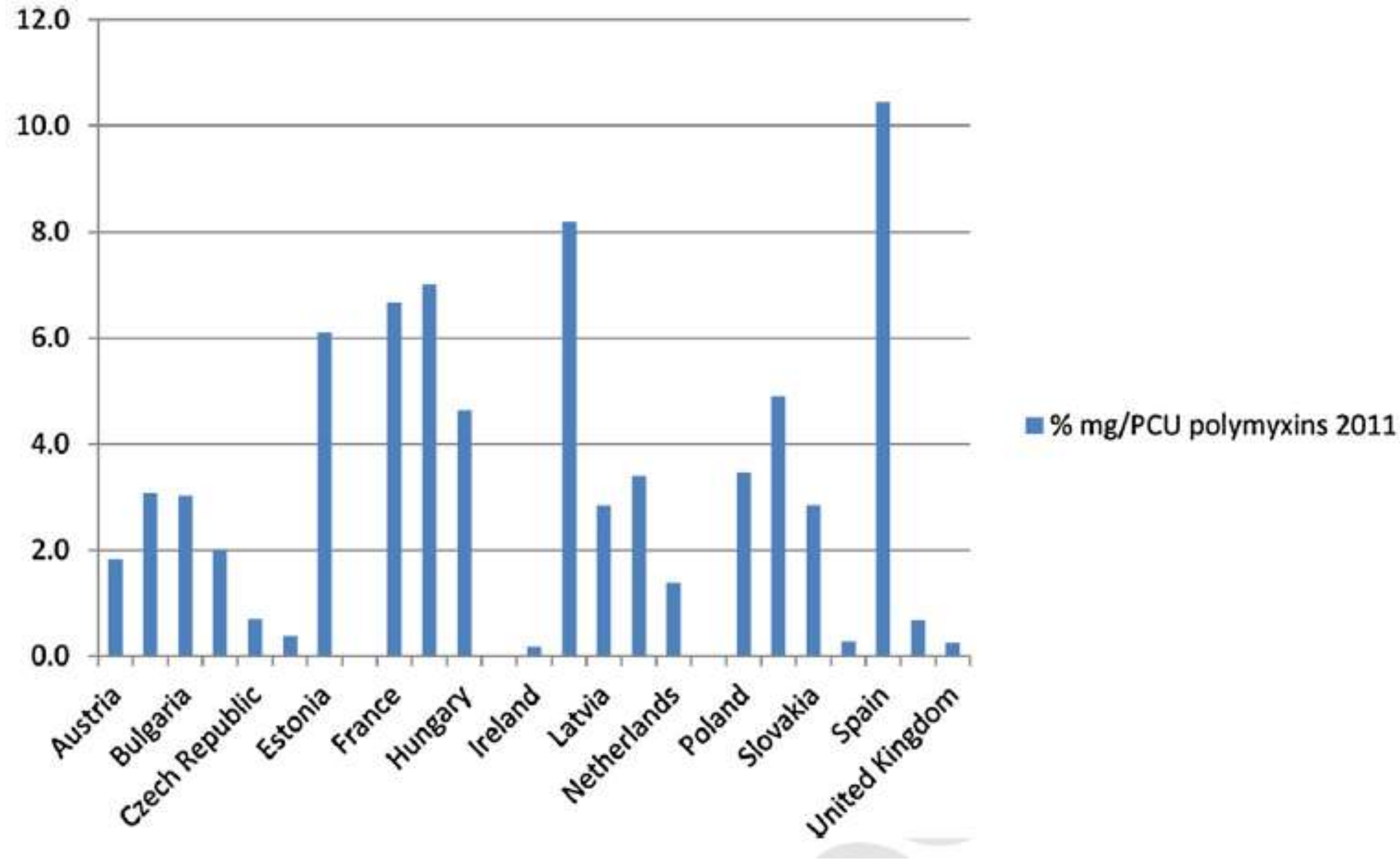
The colistin usage in animals

- It is authorised for usage in animals for treatment of gastrointestinal infections caused by *Escherichia coli* in livestock.
- It was first authorised in the UK in 2004 for veterinary medicine and has been used in animal husbandry globally.
- Until recently, only chromosomal mutations associated with colistin resistance and no evidence of transfer of colistin resistance in bacteria from animals to humans and vice versa.
- Transferable colistin resistance gene *mcr-1* on a plasmid reported by Liu et al. 2015 in The Lancet Infectious Disease.

Polymyxin usage levels reported in 2011 in animals across Europe:



Occurrence of *mcr-1* in animals 2015-16 in GB:

- APHA takes part in anonymised surveillance of healthy animals and scanning surveillance, through veterinary diagnostic submissions on behalf of the UK Government.

- The *mcr-1 E. coli* was detected on two pig farms in GB through anonymised surveillance of 387 caecal samples collected in 2015 from pigs at slaughter from 313 different herds, thus 0.6% of those pig herds sampled were positive[1].

- *mcr-1 E. coli* was also detected on 2/105 (1.9%) of pig farms from which archived *E. coli* isolates from veterinary diagnostic investigations in 2015/16 were available[1,2].

[1](Duggett et al, 2017, Journal of Antimicrobial Chemotherapy 72:691)

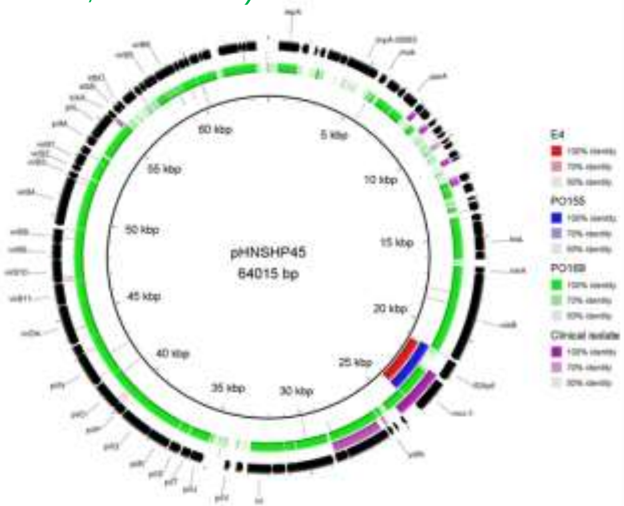
[2](Anjum et al, 2016, Journal of Antimicrobial Chemotherapy 71:2306)

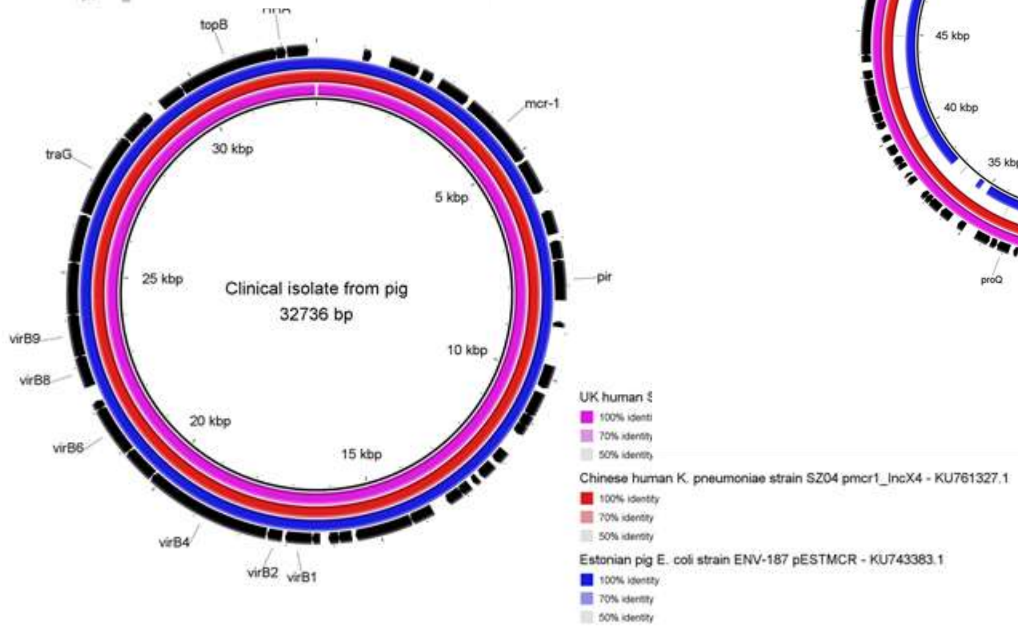
Characterising of *mcr-1* isolates by WGS using APHA SeqFinder pipeline (Duggett et al, JAC 2017).

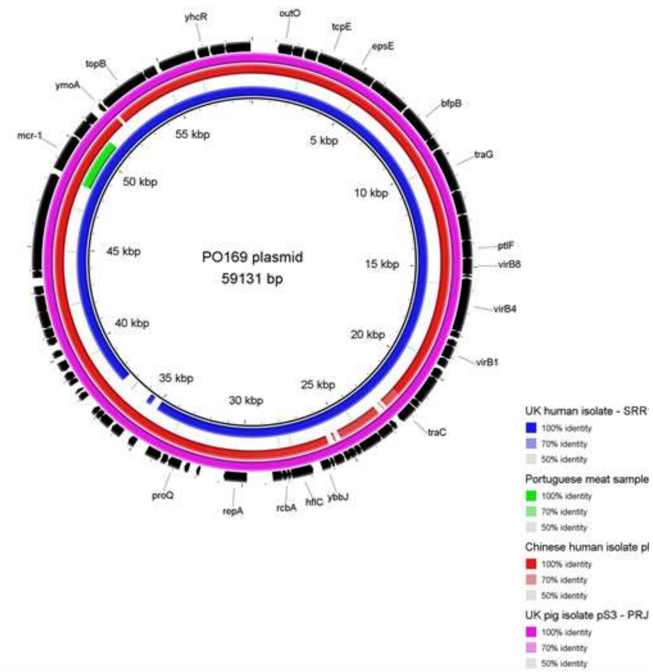
Isolate and origin	**Inc-types present**	**Estimated size of *mcr-1* containing plasmid (kb)****	**Similarity to pHNSHP45 (%)**	**Maximum similarity to publicly available *mcr-1* plasmids (%)**	**Colistin resistance genes**	**Other AMR genes**
E4 # O139:K82 2015 Veterinary submission [4]	IncX4, IncIFIB(K), repB, **pO111**, IncI1, IncX1, IncFIA(HI1), IncA/C2	78.5	16	*89*	*pmrA*, pmrB*, phoP*, phoQ*, etk** and *mcr-1*	*aadA2, aac3-Iva, aph4-Ia, aph3-Ib, aph6-Id, blaTEM-1, blaLAT-1, cml, dfrA12, sul1, sul2, tet(A), gyrA**
Clinical isolate ^ O149:H10 2015 Veterinary submission	Inc1, **IncX4**, IncFII(pCoo), IncFIB(AP001918), IncFIC(FII), IncY	32.7	28	*99*	*phoP*, phoQ*, pmrA*, etk** and *mcr-1*	*aadA1b, ant3-Ia, dfrA1, folP*, sul2*
PO155 ^ -:H56 2015 surveillance study	Incl1, Col8282, **pO111**, IncX1	91.2	19	*90*	*mcr-1*	*aac3-IVa, aadA2, ant3-Ia, aph3-Ib, aph4-Ia, aph6-Id, blaTEM-1, cml, dfrA12, inuF, sul2, tetA*
PO169 ^-:H2 2015 surveillance study	IncX1, **IncI2**, IncFII(pCoo), IncB/O/K/Z	59.2	90	*97*	*acrR*, phoP** and *mcr-1*	*blaTEM-1, gyrA*, qnrS1, tetA*

Comparison of *mcr-1* plasmids from animal isolates in GB to human clinical and food isolates.

(Duggett et al, JAC 2017)







Presence of *mcr-1* and *mcr-2* in other Gram-negative bacteria.

- As part of continued surveillance for detection of plasmid mediated colistin resistance, we examined presence of *mcr-1* and *-2* in other Gram-negative bacteria present in our collection.

- 657 Gram-negative bacteria isolated from caecal contents of pigs at slaughter from 57 pig farms in Great Britain were included.
  - MALDI-TOF or 16S rRNA gene sequencing was performed for bacterial identification.

- Whole genome sequencing was performed and presence of colistin resistance genes, *mcr-1* or *mcr-2*, determined using the APHA SeqFinder pipeline.

- Only isolates from *Moraxella* sp. showed presence of homologs for these genes.

Moraxella species: Background

- *Moraxella* species are Gram negative bacteria normally present in the oropharynx, mucous membranes, skin, and genital tract in humans.

- Isolated from conjunctivae and nasal cavities of cattle, sheep, horses, saliva of dogs and cats, mouth and pharynx of guinea pigs and rabbits, nasal cavity of goats, genital tract of pigs, intestines of goats, and genital tract and brain of sheep and cattle.

- *M. bovis* is an established animal pathogen responsible for ocular and respiratory infections
- *M. porci* isolated from brain of pig with meningitis
- *M. pluranimalium* isolated from nasal turbinate of an apparently healthy pig

Colistin resistance gene variants in *Moraxella* species from pigs.

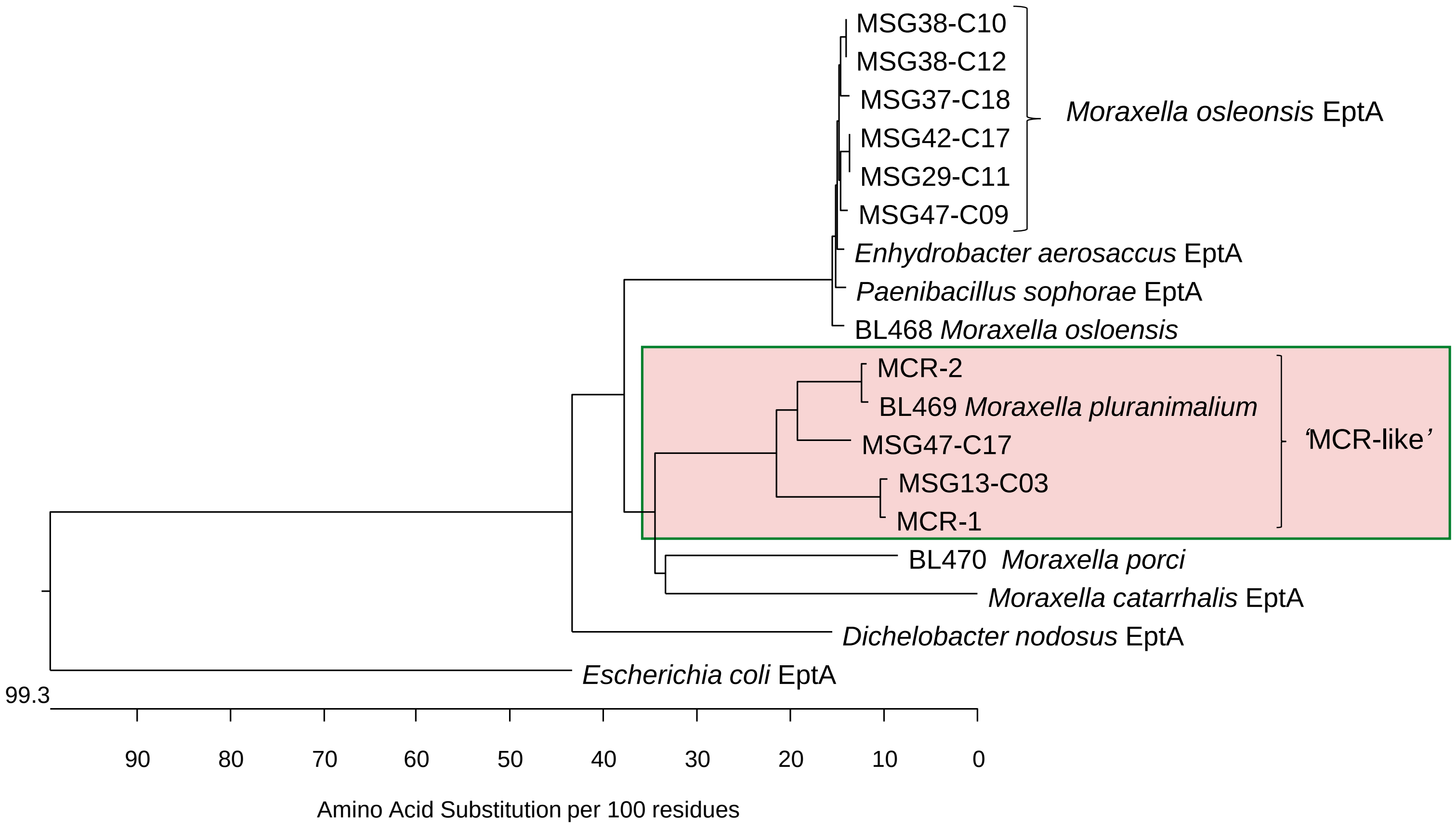
- 6 *Moraxella* isolates identified as *M. osleonsis* , 1 isolate as *M.porci*-like and 1 isolate as *M.pluranimalium*-like.

- The 6 *M. osleonsis* isolates harboured the phosphoethanolamine transferases (EptA) gene (~98% aa identity). These show 62-64% aa identity to MCR.

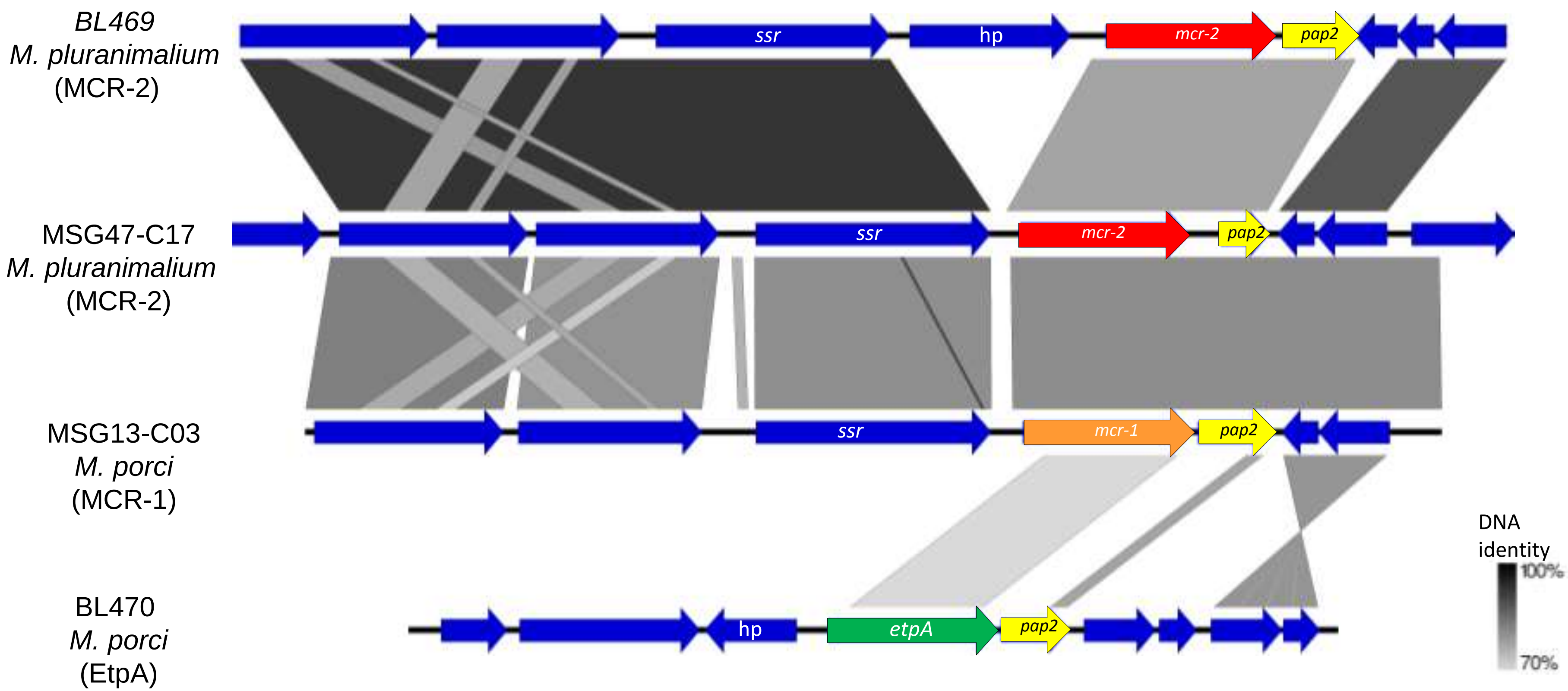
- *M.porci*-like isolate MSG13-C03 showed 98.7% aa identity of MCR-1 in *E. coli* - only 7 amino acid substitutions.

- *M.pluranimalium*-like MSG47-C17 showed 87.9% aa identity to MCR-2 in *E. coli*.
- 8 aa important for catalytic activity were conserved in all the MCR-1, MCR-2 and EptA sequences.

Phylogenetic tree of the amino acid sequence of EptA, MCR-1-like and MCR-2-like sequences identified in *Moraxella species*



Location of *eptA*, *mcr*-1-like and *mcr*-2-like genes



Minimum Inhibitory Concentrations (MIC) for colistin

	Gene	*Moraxella osloensis*						*Moraxella porci like*	*Moraxella pluranimalium*	*Moraxella osloensis*	*Moraxella pluranimalium*	*Moraxella porci*
		MSG29-C11	MSG37-C18	MSG38-C10	MSG38-C12	MSG42-C17	MSG47-C09	MSG13-C03	MSG47-C17	H091500600 (BL468)	DSM-22804 (BL469)	DSM-25326 (BL470)
	eptA	+	+	+	+	+	+			+		+
Colistin	*mcr-1*							+				
	mcr-2								+		+	
Colistin MIC (1µg/ml)		4	4	4	2	2	2	2	1	8	2	64

Conclusion

- Following the detection of *mcr-1* in *E. coli* from pigs in GB, we surveyed the presence of *mcr-1* and *mcr-2* in other Gram-negative bacteria isolated from pigs.
- Analysis of WGS of 657 Gram-negative bacteria identified the presence of *mcr* homologs in 8 *Moraxella* isolates.
- Six isolates were identified as *M. osleonsis* and harboured EptA (~98% aa identity) and showed colistin MIC 2-4 mg/L (up to 64 for *M. porci* reference strain).
- One isolate identified as *M.porci*-like and 1 as *M.pluranimalium*-like harboured MCR-1 and MCR-2, respectively; their colistin MIC was 1-2 mg/L.
- Phylogenetic analysis indicated EptA present in *Moraxella* is distinct from MCR-1 and -2, but they have probably evolved from a common ancestor.

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