12th EURL-AR Workshop

The EURL protocol on *mcr-1, -2, -3, -4, -5*

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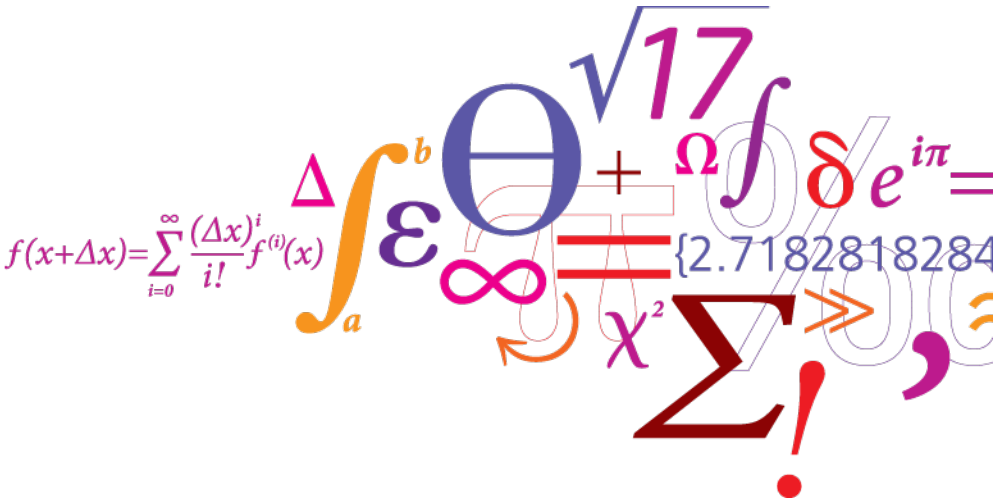


DTU Food
National Food Institute

Colistin resistance in *Enterobacteriaceae*

Mechanisms

pmrA/pmrB mutations – not totally defined at this moment

mcr genes

Epidemiology

mcr-genes more prevalent in animal isolates

Distributed all over the globe

100+ cases of *mcr-1, mcr-3* and *mcr-4* in human infection

mcr genes in *Enterobacteriaceae*

Genes	Species	Variants in *Enterobacteriaceae*	
mcr-1	*E. coli*	*mcr-1.2* (Q3L) - *K. pneumoniae* *mcr-1.3* (I38V) - *E. coli* *mcr-1.4* (D440N) - *E. coli* *mcr-1.5* (H452Y) - *E. coli* *mcr-1.6* (R536H) - *Salmonella*	*mcr-1.7* (A215T) - *E. coli* *mcr-1.8* (Q3R) - *E. coli* *mcr-1.9* (V413A) - *E. coli* *mcr.-1.11* (V7_W8insV) – *E. coli* *mcr-1.12* (Q3H) - *E. coli*
mcr-2.1	*E. coli*		
mcr-3.1	*E. coli*	*mcr-3.2* (T488I) - *Shigella sonnei* *mcr-3.4* (G373V) - *K. pneumoniae* *mcr-3.5* (M23V/A457E/T488I) - *E. coli* *mcr-3.10* (V122G/R297L/I313V/E337K/H341Y/D358E/Q468K) - *E. coli* *mcr-3.11* (G373V/Q468T) - *E. coli*	
*mcr-3** (D295E)	*K. pneumoniae*		
*mcr-3** (M23V)	*Citrobacter freundii*		
mcr-4.1	*Salmonella*	*mcr-4.2* (Q331R) – *Salmonella* *mcr-4.2*** (V179G/V236F) - *Enterobacter cloacae* *mcr-4.3* (V236F) – *Salmonella*	
mcr-5.1	*Salmonella*	*mcr-5.2* (E234del) – *E. coli*	
mcr-6.1	*Moraxella* sp.		
mcr-7.1	*K. pneumoniae*		

* NCBI RefSeq project contains three different sequences named *mcr-3* or *mcr-3.1*. The mutations described for these two genes and for *mcr-3* variants are in relation to the *mcr-3.1* gene described in the first published paper (Yin W, 2017; accession KY924928)

** GenBank contains two different sequences named *mcr-4.2*

Phenotypic testing

Uneven diffusion in agar

Liquid media composition affects diffusion and stability

Some laboratory materials promote adsorption

Only microbroth dilution recommended by international standards

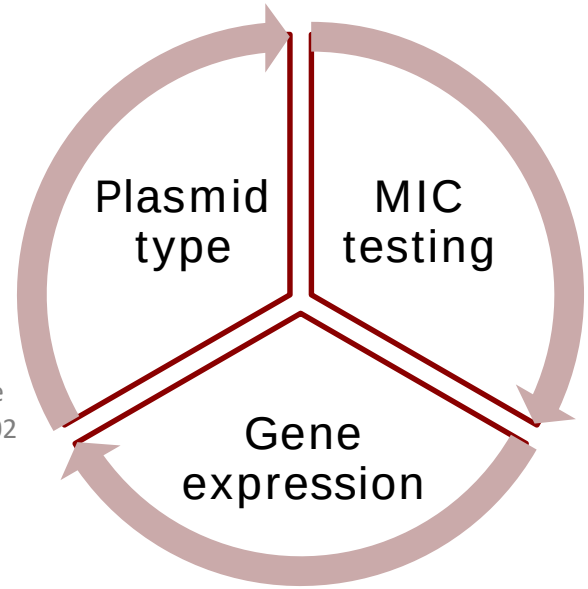
Still some problems with reproducibility of MIC results

Phenotypic testing

Gene present in phenotypically susceptible isolates (MIC ≤2)

Year	Species	Host	Colistin MIC	PCR mcr-1
2009	*E. coli*	Poultry	2; 4*	+
2009	*E. coli*	Poultry	2; 4*	+
2009	*E. coli*	Poultry	2; 2*	+
2009	*E. coli*	Poultry	2; 2*	+
2008	*E. coli*	Poultry	2; 2*	+
2008	*E. coli*	Poultry	2; 4*	+
2008	*E. coli*	Poultry	2; 2*	+
2008	*E. coli*	Poultry	2; 4*	+
2008	*E. coli*	Poultry	2; 2*	+
2008	*E. coli*	Poultry	2; 4*	+

Zhang, H., et al (2017). Expression characteristics of the plasmid-borne mcr-1 colistin resistance gene. *Oncotarget*, *8*(64), 107596–107602



Yang Q., et al (2017). Balancing mcr-1 expression and bacterial survival is a delicate equilibrium between essential cellular defence mechanisms. *Nature Communications*, *8*(1), 2054

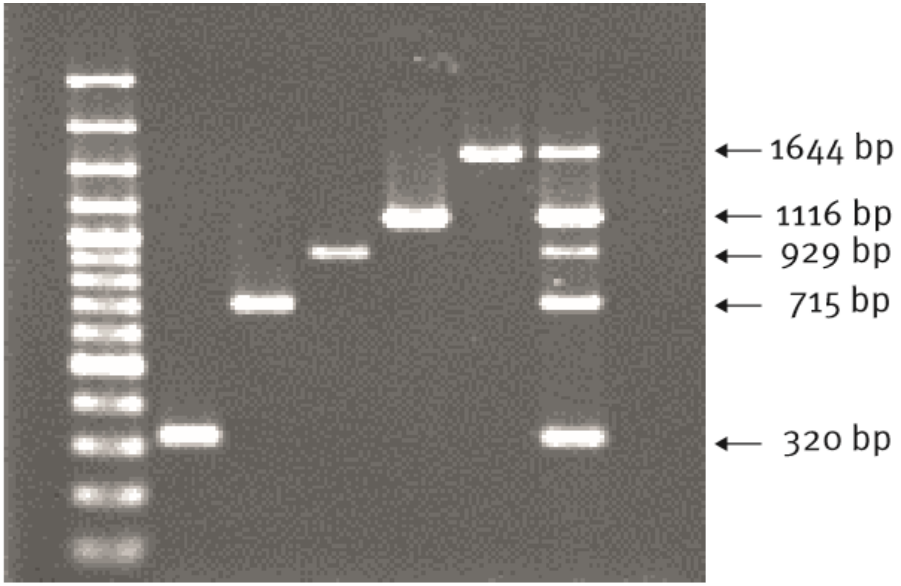
Multiplex PCR

Target	Primers	Control strain
mcr-1	>mcr1_320bp_fw AGTCCGTTTGTTCTTGTGGC >mcr1_320bp_rev AGATCCTTGGTCTCGGCTTG	*Escherichia coli* 2012-60-1176-27
mcr-2	>mcr2_700bp_fw CAAGTGTGTTGGTCGCAGTT >mcr2_700bp_rev TCTAGCCCGACAAGCATACC	*Escherichia coli* KP37
mcr-3	>mcr3_900bp_fw AAATAAAAATTGTTCCGCTTATG >mcr3_900bp_rev AATGGAGATCCCCGTTTTT	*Escherichia coli* 2013-SQ352
mcr-4	>mcr4_1100bp_fw TCACTTTCATCACTGCGTTG >mcr4_1100bp_rev TTGGTCCATGACTACCAATG	*Escherichia coli* DH5α
mcr-5	>MCR5_fw ATGCGGTTGTCTGCATTTATC >MCR5_rev TCATTGTGGTTGTCCTTTTCTG	*Salmonella* 12-SA01718

PCR conditions: Denaturation at 94 C – 15 m
25 cycles
Denaturation at 94 C – 30 sec
Annealing at 58 C – 90 s
Elongation at 72 C – 60 s
Elongation at 72 C – 10 m

DreamTaq Green PCR Master Mix
0.5 µL of each primer solution (10 µM)

Multiplex PCR



Variants detected *in silico:*

All *mcr-1, mcr-3, mcr-4* and *mcr-5* variants described to date in *Enterobacteriaceae*

3 additional sequences (*mcr-3**, *mcr-3** and *mcr-4.2***)

Total: 5 genes and 21 variants

Test isolates for validation

49 isolates from EURL-AR/EFSA confirmatory testing

MIC determination by broth microdilution and WGS by Illumina Hi-Seq

42 *E. coli* and 7 *Salmonella*

Spain (n=19)

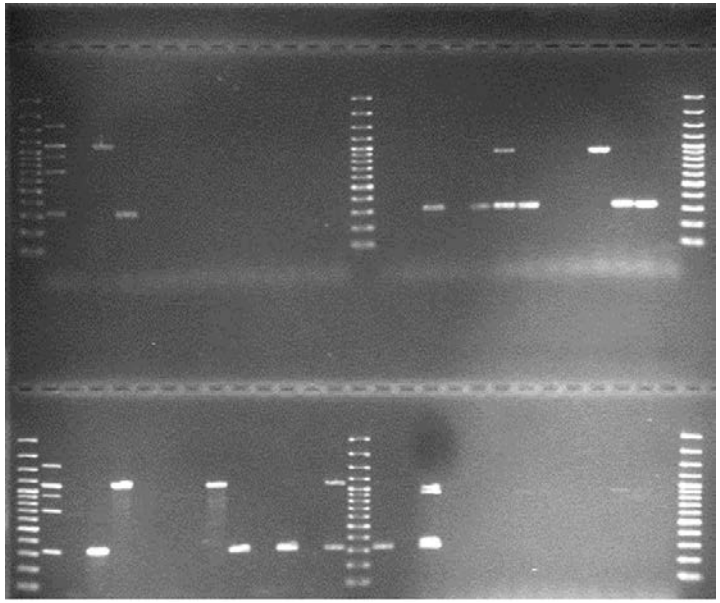
Germany (n=16)

France (n=10)

Italy (n=4)

17 COL-resistant isolates

MIC 4 – 8 mg/L



mcr PCR detection	WGS	Nr. of isolates
mcr-1	*mcr-1, mcr-1.xx*	10 (one with MIC=2)
mcr-3	*mcr-3.2*	1
mcr-4	*mcr-4.2, mcr-4.3* (V236F)	3
mcr-1 and *mcr-3*	*mcr-1* and *mcr-3.2*	2
mcr-1 and *mcr-4*	*mcr-1* and *mcr-4* (441delT)	1
None	*PmrB* D283G/Y358N	1

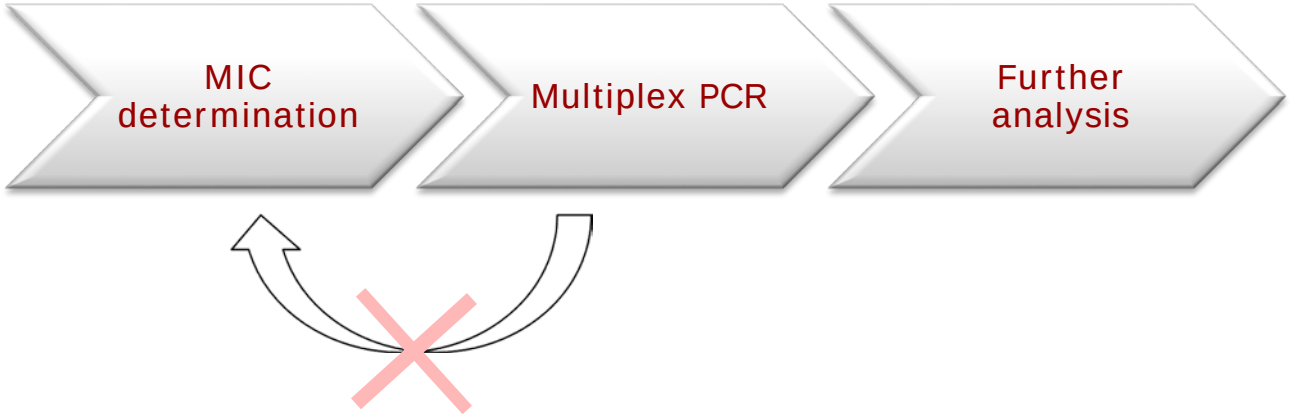
Advantages

Fast – <24 h

Easy and accessibe for most laboratories

Detects *mcr* genes in phenotypically susceptible isolates

Suggested workflow



mcr-6 and *mcr-7*

Described after the development of the protocol

In silico analysis: Current primers do not anneal

Future prospects

Clone *mcr-1, mcr-2, mcr-3* and *mcr-5* into non-pathogenic vectors

Validate the protocol in clinical isolates

Continuously update of the protocol: Design and validate primers to detect *mcr-7*

<u>Other possibility</u>

Validate the protocol for other species:
Expand the protocol for genes and variants not present in *Enterobacteriaceae*

Thank you

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Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* for surveillance purposes. *Euro Surveill*. 2018;23(6):pii=17-00672. https://doi.org/10.2807/1560-7917.ES.2018.23.6.17-00672

EURL-AR Laboratory Protocol: PCR for plasmid-mediated colistin resistance genes, *mcr-1, mcr-2, mcr-3, mcr-4, mcr-5* and variants (multiplex), Version 3, February 2018
https://www.eurl-ar.eu/protocols.aspx