



Assessment of two selective agar media to isolate colistin-resistant bovine *Escherichia coli*: Correlation with minimal inhibitory concentration and presence of *mcr* genes

Damien Thiry^a, Anis Berrah^a, Julien Evrard^b, Jean-Noël Duprez^a, Jacques G. Mainil^{a,*}, Marc Saulmont^b

^a Bacteriology, Department of Infectious Diseases, Faculty of Veterinary Medicine, FARAH (Fundamental and Applied Research for Animals and Health) Centre, University of Liège, Liège B-4000, Belgium

^b ARSIA (Association Régionale de Santé et d'Identification Animale), Ciney B5590, Belgium



ARTICLE INFO

Keywords:

Escherichia coli
Colistin resistance
Selective agar
Minimal inhibitory concentration
mcr genes
Disk diffusion assay
Cattle

ABSTRACT

The identification of colistin-resistant enterobacteria in veterinary medicine is impaired by the absence of first-line reliable phenotypic assay. The purpose of this study was to assess two selective agar media for the detection of colistin-resistant bovine pathogenic *Escherichia coli*. A total of 158 *E. coli* (46 R < resistant >, 96 I < intermediate > and 16 S < sensitive > at the disk diffusion assay) isolated between 2013 and 2018 from < 3 month-old calves suffering enteritis or septicæmia, were (i) tested by the broth dilution assay to determine colistin Minimal Inhibitory Concentrations (MIC); (ii) streaked on CHROMID® Colistin_R and CHROMagar™ COL-APSE agar plates; (iii) submitted to a pentaplex PCR to identify the presence of *mcr-1* to *mcr-5* genes. Of the 92 *E. coli* growing on both agar media, 90 had a MIC > 2.0 µg/ml as had the 3 *E. coli* that grew only on the CHROMID® Colistin_R agar medium and one *E. coli* that grew on neither agar media. Therefore, the positive predictive values of the CHROMID® Colistin_R and CHROMagar™ COL-APSE agar media were both 0.98 whereas their negative predictive values were 0.98 and 0.94, respectively. Also noteworthy 43 of the 46 R isolates had a MIC > 2.0 µg/ml and grew on both selective media as did half of the 96 I isolates and only 1 of the S isolates. Conversely, only 30 of the 90 isolates that grew on both agar media and with a MIC > 2.0 µg/ml tested positive for the *mcr-1* or *mcr-2* genes with the pentaplex PCR. These two selective agar media can be used to reliably detect colistin-resistant *E. coli*. Positive growth was highly correlated with R results at the disk diffusion assay, but not with the presence of *mcr* genes.

1. Introduction

Polymyxins are bactericidal antibiotics produced by *Paenibacillus polymyxa* with activity against most Gram-negative bacteria, via an interaction with the lipid A moiety of the lipopolysaccharide (Dowling, 2013; Stansly and Schlosser, 1947). Polymyxin E, or colistin, has been used for decades in veterinary medicine more especially against *Escherichia (E.) coli* infections in farm animals (Dowling, 2013; Kempf et al., 2016). A few years ago, human medicine began to also use colistin against the carbapenemase-producing *Enterobacteriaceae* in hospitals (Caniaux et al., 2017). Unfortunately it was not long before some species such as *Klebsiella (K.) pneumoniae*, developed resistance due to mutations in chromosomal genes required for the synthesis of lipid A.

The mechanism of resistance is a modification of the negative charge of lipid A that leads to a decreased interaction with the positively-charged polymyxins (Caniaux et al., 2017; Olaitan et al., 2014).

In November 2015, the first plasmid-located transferable mechanism of resistance to colistin was identified in a porcine isolate of *E. coli* in China (Liu et al., 2016). During the following months and years the *mcr-1* (after “mobilized colistin resistance”) gene was identified by PCR in *E. coli* isolates from cattle, humans, piglets, poultry in several countries in different continents and in several other species of the *Enterobacteriaceae* family, including *Salmonella (S.) enterica* and *K. pneumoniae* (Sun et al., 2018). Moreover other *mcr* genes have been since described: *mcr-2* in a porcine *E. coli* isolate in Belgium (Xavier et al., 2016); *mcr-3* in a porcine *E. coli* isolate in China (Yin et al., 2017); *mcr-4*

* Corresponding author at: Bacteriology, Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Liège, Campus du Sart Tilman B43a, Quartier Vallée II, Avenue de Cureghem 6, 4000 Liège, Belgium.

E-mail address: jg.mainil@uliege.be (J.G. Mainil).

<https://doi.org/10.1016/j.mimet.2019.03.004>

Received 28 January 2019; Received in revised form 5 March 2019; Accepted 7 March 2019

Available online 08 March 2019

0167-7012/ © 2019 Published by Elsevier B.V.

in a porcine *S. enterica* isolate in Italy (Carattoli et al., 2017); *mcr-5* in a poultry *S. enterica* in Germany (Borowiak et al., 2017); *mcr-6*, originally named *mcr-2.2*, in a porcine *Moraxella pleuranimalium*-like isolate in Great Britain (AbuOun et al., 2017); *mcr-7* in a poultry *K. pneumoniae* isolate in China (Yang et al., 2018); and *mcr-8* in a porcine *K. pneumoniae* isolate in China (Wang et al., 2018).

Identifying acquired resistance of pathogenic bacteria in most routine diagnostic laboratories classically relies on the disk diffusion assay but is not suitable for polymyxins as these poorly diffuse in agar. Actually, the most reliable method for polymyxins is the determination of the Minimal Inhibitory Concentration (MIC) by the broth dilution assay but this procedure is not compatible with the workload of veterinary first-line diagnostic laboratories. Therefore, three selective media for the detection of Gram negative pathogens with acquired resistance to colistin have been commercialized and assessed in human medicine: SuperPolymyxin or Rapid Polymyxin NP (ELITech MICRO-BIO, Signes, France), ChromID® Colistin R (BioMérieux, Lyon, France) and CHROMagar™ COL-APSE (CHROMagar, Paris, France) (Abdul Momin et al., 2017; Girlich et al., 2018). As far as the authors know, only the SuperPolymyxin medium has been assessed in veterinary medicine for the detection of porcine colistin-resistant *E. coli* and *K. pneumoniae* (Kieffer et al., 2017).

Since *E. coli* is the most frequent pathogenic bacteria isolated from young calves with enteritis or septicaemia (Mainil and Fairbrother, 2014), the purpose of this study was to assess two of those selective agar media, ChromID® Colistin R and CHROMagar™ COL-APSE for the routine detection of colistin-resistant *E. coli* in a veterinary diagnostic laboratory by correlating the bacterial growth with the MIC and with the presence of *mcr* genes.

2. Materials and methods

2.1. *E. coli* isolates

Since the disk diffusion assay is still routinely carried out with colistin at ARSIA, a total of 158 *E. coli* were chosen based on the diameter of the inhibition zones according to the breakpoints of enterobacteria (AFNOR, 2012; CA_SFM, 2017): 16 isolates were classified as susceptible (S), 46 as resistant (R) and 96 as intermediate (I). They were isolated between 2013 and 2018 from faeces, intestinal contents, blood, and internal organs, of young calves (< 3 months of age) suffering enteritis or septicaemia and identified following classical procedure.

Briefly, clinical samples were streaked on Columbia blood and Gassner (Thermo Fisher Scientific, Merelbeke, Belgium) agar plates. Lactose-fermenting colonies from faecal materials and intestinal contents were identified to *E. coli* using the Maldi Biotyper (Bruker Daltonics, Bremen, Germany) and tested by agglutination for the production of F5, F17a or CS31A antigens (Ceva Biovac, Angers, France) (Mainil and Fairbrother, 2014). One F5-, F17a- or CS31A-positive colony per calf was subsequently tested by the disk diffusion assay. When a pure culture was obtained from heart blood or internal organ samples, one lactose-fermenting colony per calf was similarly identified to *E. coli* and tested by the disk diffusion assay. All 158 isolates were subsequently studied for the minimal inhibitory concentration of colistin, growth on selective agar plates and presence of *mcr* genes. Isolates with contradictory results were tested a second time.

2.2. Minimal inhibitory concentration (MIC)

The determination of the colistin MIC of these 158 *E. coli* isolates was performed by the broth dilution assay in FRCOL 96-well microtitre plates, according to the manufacturer's instructions (Sensititre, Fischer Scientific, Hampton, USA). Each row comprises 11 wells with final colistin concentrations ranging from 128.0 µg/ml to 0.125 µg/ml and one negative control well. Briefly, a colony from an overnight growth on Columbia agar plates was suspended in sterile saline and the

turbidity was adjusted to 0.5 MacFarland. The suspension was diluted in Mueller Hinton broth to obtain a final concentration of 5.10⁵ CFU/ml that was distributed into 12 wells per isolate (50 µl par well). The plates were incubated 18 ± 2 h at 35 ± 1 °C aerobically. The susceptibility MIC was ≤ 2.0 µg/ml and the resistance MIC was > 2.0 µg/ml according to EUCAST breakpoint tables (EUCAST, 2018). *E. coli* ATCC 25922 was the quality control strain.

2.3. Selective agar media

The 158 *E. coli* were streaked on the two selective agar media: CHROMID® Colistin_R purchased from BioMérieux (Lyon, France) and CHROMagar™ COL-APSE kindly received from CHROMagar (Paris, France). Growth was classified in two categories: positive (+) with growth on the whole surface of the plate and negative (–) when some growth was only observed at the inoculation area or when no growth was observed.

2.4. Polymerase chain reaction (PCR)

After DNA extraction by the alkaline-boiling method from 1 ml of an overnight growth in Luria-Bertani (LB) broth at 37 °C (Ooka et al., 2009), the pentaplex PCR was performed using the FastGene® Optima HotStart ReadyMix (Filter Service, Eupen, Belgium) following the protocol of Rebelo et al. (2018) with one modification: the number of amplification cycles was 30 instead of 25. The amplified fragments were detected by electrophoresis in 1.5% agarose gels. The PCR positive controls were one *S. enterica* from poultry for *mcr-1* gene (kindly provided by Sciensano, Brussels, Belgium) (Garcia-Graells et al., 2018), one *E. coli* from a pig for *mcr-2* gene (Mainil et al., 2017), DNA from *E. coli* strains ZTA15/01169-1 EB1 from a bovine and ZTA15/00750 EB1 from a pig for *mcr-3* and *mcr-4* genes, respectively (kindly provided by Visavet health surveillance centre, Madrid, Spain) (Carattoli et al., 2017) and the *S. enterica* 13-SA01718 strain for the *mcr-5* gene (kindly provided by the Federal Institute for Risk Assessment, Berlin, Germany) (Borowiak et al., 2017). The PCR-negative control was the HS *E. coli* strain isolated from human faeces (O'Brien et al., 1982).

3. Results

3.1. MIC vs disk diffusion assay

Of the 158 *E. coli* isolates studied 94 had a MIC > 2.0 µg/ml, 5 had a MIC of 2.0 µg/ml and 59 had a MIC < 2.0 µg/ml. Forty-five of the 46 R isolates (98%) at the disk diffusion assay had a MIC > 2.0 µg/ml and of 15 of the 16 S isolates (94%) had a MIC < 2.0 µg/ml while 48 of the 96 I isolates (50%) had a MIC > 2.0 µg/ml and 44 a MIC < 2.0 µg/ml (44%) (Table 1). The remaining R and 4 I isolates had a MIC of 2.0 µg/ml while the remaining S isolate had a MIC of 4.0 µg/ml.

Table 1

Disk diffusion assay results and Minimal Inhibitory Concentrations (MIC) of the 158 *E. coli* isolates assayed for resistance and susceptibility to colistin.

S/I/R ^a (No. Isolates)	Minimal Inhibitory Concentrations (MIC): No. Isolates (%)		
	> 2.0 µg/ml	= 2.0 µg/ml	< 2.0 µg/ml
S (16)	1 (6%) ^b	–	15 (94%)
I (96)	48 (50%)	4 (4%)	44 (46%)
R (46)	45 (98%)	1 (2%)	–
Total (158)	94	5	59

^a S = susceptible, I = intermediate, R = resistant at the disk diffusion assay.

^b This isolate had a MIC of 4.0 µg/ml.

Table 2

Disk diffusion assay results and growth on selective media of the 158 *E. coli* isolates studied for resistance and susceptibility to colistin.

S/I/R ^a (No. Isolates)	Growth on CHROMagar™ and CHROMID®: No. Isolates (%)	Growth on CHROMID® only: No. Isolates (%)	No growth: No. Isolates (%)
S (16)	1 (6%) ^b	0	15 (94%)
I (96)	48 (50%) ^c	2 (2%) ^d	46 (48%) ^e
R (46)	43 (94%)	1 (2%) ^f	2 (4%) ^g
Total (158)	92	3	63

^a S = susceptible, I = intermediate, R = resistant at the disk diffusion assay.

^b This isolate had a Minimal Inhibitory Concentration (MIC) of 4.0 µg/ml.

^c Including 2 isolates with a MIC of 2.0 µg/ml.

^d These 2 isolates had a MIC of 8.0 µg/ml.

^e Including two isolates with a MIC of 2.0 µg/ml.

^f This isolate had a MIC of 4.0 µg/ml.

^g These 2 isolates had a MIC of 2.0 or 4.0 µg/ml.

3.2. Growth on selective agar media vs disk diffusion assay

Of the 158 studied *E. coli* isolates, 92 grew (+) on the whole surface of both selective agar plates and 3 more grew only on CHROMID® Colistin_R agar plates, while 63 gave negative results (–). Forty-three of the 46 R isolates (94%), 48 of the 96 I isolates (50%) and one of the 16 S isolates (6%) at the disk diffusion assay grew on both selective media (Table 2). The 3 isolates growing only on CHROMID® Colistin_R agar medium were R (1 isolate) or I (2 isolates) at the disk diffusion assay.

3.3. Pentaplex PCR for *mcr-1* to *mcr-5* genes vs disk diffusion assay

Of the 158 isolates, 22 were positive using the *mcr-1* PCR and 14 using the *mcr-2* PCR, but none at the *mcr-3*, *mcr-4* or *mcr-5* PCR. Nine (20%) of the 46 R isolates tested positive with the *mcr-1* PCR and 7 others (15%) with the *mcr-2* PCR, as did 12 (13%) and 7 (7%) of the 93 I isolates, respectively, and one (6%) of the 16 S isolates with the *mcr-1* PCR (Table 3).

3.4. Combination of all results (Table 4)

Isolates with any contradictory result were tested a second time with identical results.

Of the 92 *E. coli* isolates growing on the CHROMagar™ COL-APSE medium (43/46 R, 48/96 I and 1/16 S isolates at the disk diffusion assay), 90 had a MIC > 2.0 µg/ml (98%) and 2 had a MIC of 2.0 µg/ml (2%). Conversely, of the 66 isolates showing no growth, 2 had a MIC of 8.0 µg/ml (3%), 2 a MIC of 4.0 µg/ml (3%) and 3 a MIC of 2.0 µg/ml (5%). Taking into account the MIC breakpoint (> 2.0 µg/ml) to define

Table 3

Disk diffusion assay and *mcr* gene PCR results of the 158 *E. coli* isolates studied for resistance and susceptibility to colistin.

S/I/R ^a (No. Isolates)	PCR: No. Isolates (%)		
	<i>mcr-1</i> -positive	<i>mcr-2</i> -positive	<i>mcr</i> -negative
S (16)	1 (6%) ^b	0	15 (94%)
I (96)	12 (13%) ^c	7 (7%) ^d	77 (80%)
R (46)	9 (20%)	7 (15%)	30 (65%)
Total (158)	22	14	122

^a S = susceptible, I = intermediate, R = resistant at the disk diffusion assay.

^b This isolate had a Minimal Inhibitory Concentration (MIC) of 4.0 µg/ml.

^c 2 isolates had a MIC of 2.0 µg/ml; one of them grew on neither selective media.

^d 1 isolate had a MIC of 2.0 µg/ml; another isolate had a MIC of 1.0 µg/ml and grew on neither selective media.

resistance, the positive predictive value is 0.98 and the negative predictive value is 0.94 for this selective medium.

Of the 95 *E. coli* isolates growing on the CHROMID® Colistin_R agar medium (44/46 R, 50/96 I and 1/16 S isolates at the disk diffusion assay), 93 had a MIC > 2.0 µg/ml (98%), including the 3 isolates growing only on CHROMID® Colistin_R agar medium, and 2 a MIC of 2.0 µg/ml (2%). One of the 63 isolates with no growth had a MIC of 4.0 µg/ml (1.6%) and 3 a MIC of 2.0 µg/ml (5%). Taking into account the same MIC breakpoint (> 2.0 µg/ml), the positive predictive value is 0.98 and the negative predictive value is 0.98 for this selective medium.

Twenty-one *mcr-1*-positive and 13 *mcr-2*-positive isolates grew on both selective media: 20 and 12, respectively had a MIC > 2.0 µg/ml and 2 had a MIC of 2.0 µg/ml of colistin. In addition, 2 isolates (3%) not growing on either media were also positive with the *mcr-1* PCR (MIC of 2.0 µg/ml) or *mcr-2* PCR (MIC of 1.0 µg/ml).

4. Discussion

In diagnostic laboratories, the disk diffusion assay is routinely used to identify antibiotic resistance profiles of pathogenic bacteria, but is considered unreliable for polymyxins that do not diffuse efficiently in agar media. Unfortunately, other tests, like MIC determination by micro-dilution are not compatible with high turnover veterinary diagnostic laboratories. Nevertheless, there is today a need for a reliable first-line test for colistin in as much chromosome- and plasmid-mediated acquired resistance recently became a problem in human hospitals and in food-producing animals (Caniaux et al., 2017; El Garch et al., 2018; Kempf et al., 2016). One possibility is the development of selective agar media (Caniaux et al., 2017; Sun et al., 2018). Up-to-date, the SuperPolymyxin or Rapid Polymyxin NP (ELITech MICROBIO, Signes, France), ChromID® Colistin R (BioMérieux, Lyon, France) and CHROMagar™ COL-APSE (CHROMagar, Paris, France) media have been assessed in human, but not in veterinary medicine for the detection of Gram negative pathogens with acquired resistance to colistin (Abdul Momin et al., 2017; Girlich et al., 2018). Of them *E. coli* is the most frequent pathogen in young calves, responsible for diarrhoea and septicemia and colistin is among the most frequently used antibiotics, especially in intestinal infections (Kempf et al., 2016; Mainil and Fairbrother, 2014; Sun et al., 2018). The main purpose of this study was therefore to compare the growth on ChromID® Colistin R and/or CHROMagar™ COL-APSE selective media and the MIC of bovine *E. coli* isolated from young calves between 2013 and 2018 at a veterinary diagnostic laboratory in Belgium.

Taking into account the official MIC > 2.0 µg/ml, the positive predictive values of the two selective agar media tested in this study are both 0.98, whereas their negative predictive values are 0.98 for CHROMID® Colistin R medium and 0.94 for CHROMagar™ COL-APSE medium, respectively. However, 2 of the 4 isolates with a MIC of 2.0 µg/ml also grow on the selective media. Therefore taking into account MIC ≥ 2.0 µg/ml, the positive predictive values of the two selective agar media tested in this study both become 1.0, whereas their negative predictive values decrease to 0.94 for CHROMID® Colistin R medium and 0.89 for CHROMagar™ COL-APSE medium, respectively.

Though considered as unreliable, the disk diffusion assay remains predictive of the MIC for R and S *E. coli*, as recently published (ANSES, 2016). Indeed (i) all but one of the 46 R isolates have a MIC > 2.0 µg/ml and all but one grow on both selective media; (ii) only one of the 16 S isolates has a MIC > 2.0 µg/ml and grow on both selective media. This test is however totally unreliable for the 96 I isolates since ca. half of them have a MIC > 2.0 µg/ml and grow on the selective media, while the second half do not (Tables 1 and 2).

Conversely neither growth on the selective agar plates nor a MIC > 2.0 µg/ml, nor the results of the disk diffusion assay is predictive of the presence of *mcr* genes (Tables 3 and 4), as already observed by others (El Garch et al., 2018). Interestingly enough, only the *mcr-1* and *mcr-2* genes are detected in the 36 PCR-positive bovine *E.*

Table 4

Minimal Inhibitory Concentration (MIC), growth on selective media, disk diffusion assay results and *mcr* gene PCR results of the 158 *E. coli* isolates assayed for resistance and susceptibility to colistin.

Minimal Inhibitory Concentration (MIC): µg/ml	Growth on (Disk Diffusion Assay Result ^a : No. Isolates)		
	CHROMagar™ and CHROMID®	CHROMID® only (not on CHROMagar™)	No growth
0.125	–	–	2 (I: 2) ^a
0.25	–	–	21 (I: 14; S: 7)
0.5	–	–	33 (I: 25; S: 8)
1.0	–	–	3 (I: 3)
2.0	2 (I: 2) ^a	–	3 (R: 1; I: 2)
4.0	26 (R: 8; I: 17; S: 1)	1 (R: 1) ^a	1 (R: 1)
8.0	50 (R: 25; I: 25)	2 (I: 2)	–
16.0	12 (R: 10; I: 2)	–	–
32.0	2 (I: 2)	–	–
Total isolates	92 (R: 43; I: 48; S: 1)	3 (R: 1; I: 2)	63 (R: 2; I: 46; S: 15)
Total <i>mcr</i> -1 PCR +	21 ^b (R: 9; I: 11; S: 1)	–	1 ^c (I: 1)
Total <i>mcr</i> -2 PCR +	13 ^b (R: 7; I: 6)	–	1 ^d (I: 1)

^a S = susceptible, I = intermediate, R = resistant at the disk diffusion assay.

^b 1 *mcr*-1-positive and 1 *mcr*-2-positive isolates had a Minimal Inhibitory Concentration (MIC) of 2.0 µg/ml; the MIC of the other 32 isolates ranged from 4.0 µg/ml to 32.0 µg/ml.

^c MIC of 2.0 µg/ml.

^d MIC of 1.0 µg/ml.

coli, although the other three *mcr* genes have already been detected among bovine, porcine and/or avian *E. coli* in other European countries (Alba et al., 2018). The PCR-negative *E. coli* may of course harbour one the even more recently described *mcr*-6, *mcr*-7 and *mcr*-8 genes (AbuOun et al., 2017; Wang et al., 2018; Yang et al., 2018), which have not been identified in bovine *E. coli* so far. Whichever the actual reason it is only a question of time before Belgian bovine *E. coli* also become positive for those other *mcr* genes via strain and/or plasmid transfers (Caniaux et al., 2017; El Garch et al., 2018; Sun et al., 2018). Of course the presence of even other still undescribed *mcr* genes or of chromosome-mediated resistance mechanisms cannot be formerly ruled out either (Sato et al., 2018; Sun et al., 2018). Further studies with for instance Whole Genome Sequencing and/or plasmid transfer by conjugation should bring answers to these questions.

5. Conclusion

These two selective media can be recommended as a first line test in veterinary routine diagnostic laboratories to detect bovine colistin-resistant *E. coli*. The MIC of all isolates growing on either selective media is ≥ 2.0 µg/ml whereas the MIC of 98% and 94% of the isolates not growing on CHROMID® Colistin R medium and CHROMagar™, respectively is ≤ 2.0 µg/ml. Nevertheless, the disk diffusion assay remains an interesting first line assay in identifying the resistance/susceptibility to colistin of *E. coli* when the results are R or S, respectively. Conversely, MIC testing and/or growth on selective media are necessary for isolates with I results at the disk diffusion assay.

Acknowledgments

The authors thank the Sciensano Institute (Brussels, Belgium), the Visavet Health Surveillance Centre (Madrid, Spain) and the Institute for Risk Assessment (Berlin, Germany) for providing positive control strains or DNA for the PCR detection of the *mcr*-1, *mcr*-3, 4 and 5 genes. This study was financially supported, in part, by the “Federal Public Service of Health, Food Chain Safety and Environment”.

Declaration of conflict of interest

The authors declare no conflict of interest.

References

- Abdul Momin, M.H.F., Bean, D.C., Hendriksen, R.S., Haenni, M., Phee, L.M., Wareham, D.W., 2017. CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. *J. Med. Microbiol.* 66, 1554–1561. <https://doi.org/10.1099/jmm.0.000602>.
- AbuOun, M., Stubberfield, E.J., Duggett, N.A., Kirchner, M., Dormer, L., Nunez-Garcia, J., Randall, L.P., Lemma, F., Crook, D.W., Teale, C., Smith, R.P., Anjum, M.F., 2017. *mcr*-1 and *mcr*-2 (*mcr*-6.1) variant genes identified in *Moraxella* species isolated from pigs in Great-Britain from 2014 to 2015. *J. Antimicrob. Chemother.* 10, 2745–2749. <https://doi.org/10.1093/jac/dkx286>. Addendum in Doi: 10.1093/jac/dky272.
- AFNOR (Association française de Normalisation), 2012. Guide to performing antibiograms using the diffusion method in an agar medium. In: Standard UN 47-107. <https://www.boutique.afnor.org/norme/nf-u47-107/methodes-d-analyse-en-sante-animale-guide-de-realisation-des-antibiogrammes-par-la-methode-de-diffusion-en-milieu-gelose/article/806054/fa170310>.
- Alba, P., Leekitcharoenphon, P., Franco, A., Feltrin, F., Ianzano, A., Caprioli, A., Stravino, F., Hendriksen, R.S., Bortolaia, V., Battisti, A., 2018. Molecular epidemiology of *mcr*-encoded colistin resistance in *Enterobacteriaceae* from food-producing animals in Italy revealed through the EU harmonized antimicrobial resistance monitoring. *Front. Microbiol.* 9, 1217. <https://doi.org/10.3389/fmicb.2018.01217>.
- ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), 2016. Résapath - Réseau d'épidémiologie de l'antibiorésistance des bactéries pathogènes animales, bilan 2015, Lyon et Ploufragan-Plouzané, France. In: Rapport novembre 2016, pp. 47–50. <http://www.resapath.anses.fr>.
- Borowiak, M., Fischer, J., Hammerl, J.A., Hendriksen, R.S., Szabo, I., Malorny, B., 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr*-5, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. *J. Antimicrob. Chemother.* 72, 3317–3324. <https://doi.org/10.1093/jac/dkx327v>.
- CA_SFM (Antibiogram Committee of the French Society for Microbiology), 2017. Veterinary Antibiogram. http://www.sfm-microbiologie.org/page/page/showpage/page_id/90.html.
- Caniaux, I., van Belkum, A., Zambardi, G., Poirel, L., Gros, M.F., 2017. MCR: modern colistin resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* 36, 415–420. <https://doi.org/10.1007/s10096-016-2846-y>.
- Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., Pezzotti, G., Magistrali, C.F., 2017. Novel plasmid-mediated colistin resistance *mcr*-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill.* 22 (31). <https://doi.org/10.2807/1560-7917.pii=30589>.
- Dowling, P.M., 2013. Peptide antibiotics: polymyxins, glycopeptides, bacitracin, and fosfomycin. In: Giguère, S., Prescott, J.F., Dowling, P.M. (Eds.), *Antimicrobial Therapy in Veterinary Medicine*, 5th edition. Wiley Blackwell, pp. 189–192 ISBN 978-0-470-96302-9.
- El Garch, F., de Jong, A., Bertrand, X., Hocquet, D., Sauguet, M., 2018. *mcr*-1-like detection in commensal *Escherichia coli* and *Salmonella* spp. from food-producing animals at slaughter in Europe. *Vet. Microbiol.* 213, 42–46. <https://doi.org/10.1016/j.vetmic.2017.11.014>.
- EUCAST (European Committee on Antimicrobial Susceptibility Testing), 2018. Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 8.1. <http://www.eucast.org>.
- Garcia-Graells, C., De Keersmaecker, S.C.J., Vanneste, K., Pochet, B., Vermeersch, K., Roosens, N., Dierick, K., Botteldoorn, N., 2018. Detection of plasmid-mediated colistin resistance, *mcr*-1 and *mcr*-2 genes, in *Salmonella* spp. isolated from food at retail in Belgium from 2012 to 2015. *Foodborne Pathog. Dis.* 15, 114–117. <https://doi.org/>

- 10.1089/fpd.2017.2329.
- Girlich, D., Naas, T., Dortet, L., 2018. Comparison of the Superpolymyxin and ChromID Colistin R screening media for the detection of colistin-resistant *Enterobacteriaceae* from spiked rectal swabs. *Antimicrob. Agents Chemother.* 63 <https://doi.org/10.1128/AAC.01618-18>. pii: e01618-18.
- Kempf, I., Jouy, E., Chauvin, C., 2016. Colistin use and colistin resistance in bacteria from animals. *Int. J. Antimicrob. Agents* 48, 598–606. <https://doi.org/10.1016/j.ijantimicag.2016.09.016>.
- Kieffer, N., Aires-de-Sousa, M., Nordmann, P., Poirel, L., 2017. High rate of MCR-1 producing *Escherichia coli* and *Klebsiella pneumoniae* among pigs, Portugal. *Emerg. Infect. Dis.* 23, 2023–2029. <https://doi.org/10.3201/eid2312.170883>.
- Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J., 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16, 161–168. [https://doi.org/10.1016/S1473-3099\(15\)00424-7](https://doi.org/10.1016/S1473-3099(15)00424-7).
- Mainil, J., Fairbrother, J.M., 2014. Pathogenic *Escherichia coli* in domestic mammals and birds. In: Morabito, S. (Ed.), *Pathogenic Escherichia coli: Molecular and Cellular Microbiology*. Horizon Scientific Press and Caister Academic Press, Norwich, UK, pp. 19–44 ISBN 978-1-908230-99-7.
- Mainil, J., Muylaert, A., Saulmont, M., Vanrobaeys, M., Duprez, J.-N., Ngassam Tchamba, C., Dierick, K., Vanneste, K., Verhaegen, B., Thiry, D., Botteldoorn, N., 2017. Identification de souches bovines et porcines d'*Escherichia coli* résistantes à la colistine et porteuses des gènes *mcr-1* ou *mcr-2*. In: Mainil, J. (Ed.), *Compte-rendu du 7^{ème} Colloque international francophone de Microbiologie animale (CIFMA)*. Presses de la Faculté de Médecine vétérinaire de l'Université de Liège, Liège, Belgium, pp. 27 Abstract CO.02. ISBN 978-2-87543-102-8.
- O'Brien, A.D., La Veck, G.D., Thompson, M.R., Formal, S.B., 1982. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. *J. Infect. Dis.* 146, 763–769 PMID: 6754826.
- Olaitan, A.O., Morand, S., Rolain, J.-M., 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front. Microbiol.* 5, 1–18. <https://doi.org/10.3389/fmicb.2014.00643>.
- Ooka, T., Terajima, J., Kusumoto, M., Iguchi, A., Kurokawa, K., Ogura, Y., Asadulghani, M., Nakayama, K., Murase, K., Ohnishi, M., Iyoda, S., Watanabe, H., Hayashi, T., 2009. Development of a multiplex PCR-based rapid typing method for enterohemorrhagic *Escherichia coli* O157 strains. *J. Clin. Microbiol.* 47, 2888–2894. <https://doi.org/10.1128/JCM.00792-09>.
- Rebello, A.R., Bortolaia, V., Kjeldgaard, J.S., Pedersen, S.K., Leekitcharoenphon, P., Hansen, I.M., Guerra, B., Malorny, B., Borowiak, M., Hammerl, J.A., Battisti, A., Franco, A., Alba, P., Perrin-Guyomard, A., Granier, S.A., De Frutos Escobar, C., Malhotra-Kumar, S., Villa, L., Carattoli, A., Hendriksen, R.S., 2018. 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.* 23 (6). <https://doi.org/10.2807/1560-7917>. pii = 17.00672.
- Sato, T., Shiraishi, T., Hiyama, Y., Honda, H., Shinagawa, M., Usui, M., Kuronuma, K., Masumori, N., Takahashi, S., Tamura, Y., Yokota, S.I., 2018. Contribution to a novel amino acid alteration in PmrA or PmrB to colistin resistance in *mcr*-negative *Escherichia coli* clinical isolates including major multi-drug resistant lineages O25b:H4-ST131-H30Rx and non-x. *Antimicrob. Agents Chemother.* 62 (9). <https://doi.org/10.1128/AAC.00864-18>. pii: e00864-18.
- Stansly, P.G., Schlosser, M.E., 1947. Studies on polymyxin: isolation and identification of *Bacillus polymyxa* and differentiation of polymyxin from certain known antibiotics. *J. Bacteriol.* 54, 549–556 PMID: 16561391.
- Sun, J., Zhang, H., Liu, Y.-H., Feng, Y., 2018. Towards understanding MCR-like colistin resistance. *Trends Microbiol.* 2, 794–808. <https://doi.org/10.1016/j.tim.2018.02.006>.
- Wang, X., Wang, Y., Zhou, Y., Li, J., Yin, W., Wang, S., Zhang, S., Shen, J., Shen, Z., Wang, Y., 2018. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg. Microbes Infect.* 7, 122. <https://doi.org/10.1038/s41426-018-0124-z>.
- Xavier, B.B., Lammens, C., Ruhel, R., Kumar-Singh, S., Butaye, P., Goossens, H., Malhotra-Kumar, S., 2016. Identification of a novel plasmid-mediated colistin resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill.* 21 (27). <https://doi.org/10.2807/1560-7917>. pii = 30280.
- Yang, Y.-Q., Li, Y.-X., Lei, C.-W., Zhang, A.-Y., Wang, H.-N., 2018. Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. *J. Antimicrob. Chemother.* 73, 1791–1795. <https://doi.org/10.1093/jac/dky111>.
- Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., Zhang, R., Walsh, T.R., Shen, J., Wang, Y., 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *mBio* 8 <https://doi.org/10.1128/mBio.00543-17>. e00543-17. Erratum in Doi: 10.1128/mBio.01166-17.