JAC-Antimicrobial Resistance

Colistin-resistant Enterobacterales among veterinary healthcare workers and in the Dutch population

C. M. Dierikx[1]*, A. P. Meijs[1], P. D. Hengeveld[1], F. R. M. van der Klis[1], J. van Vliet[1], E. F. Gijsbers[1], M. Rozwandowicz[1], A. H. A. M. van Hoek[1], A. P. A. Hendrickx[1], J. Hordijk[1] and E. Van Duijkeren[1]

[1]National Institute for Public Health and the Environment (RIVM), Centrum for Infectious Disease Control, Bilthoven, The Netherlands

*Corresponding author. E-mail: cindy.dierikx@rivm.nl

Objectives: Plasmid-mediated colistin resistance can be transferred from animals to humans. We investigated the prevalence of carriage of *mcr*-mediated colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* (ColR-E/K) in veterinary healthcare workers and in the general population in the Netherlands.

Methods: Two cross-sectional population studies were performed: one among veterinary healthcare workers and one in the general population. Participants sent in a faecal sample and filled in a questionnaire. Samples were analysed using selective enrichment and culture. Mobile colistin resistance genes (*mcr*) were detected by PCR and ColR-E/K were sequenced using Illumina and Nanopore technologies.

Results: The prevalence of *mcr*-mediated ColR-E/K was 0.2% (1/482, 95% CI 0.04%–1.17%) among veterinary personnel and 0.8% (5/660, 95% CI 0.3%–1.8%) in the population sample. *mcr-1* was found in *E. coli* from four persons, *mcr-8* in *K. pneumoniae* from one person and another person carried both *mcr-1* and *mcr-8* in a *K. pneumoniae* isolate. *mcr-1* was found on different plasmid types (IncX4, IncI1 and IncI2), while *mcr-8* was found on IncF plasmids only.

Conclusions: *mcr*-mediated ColR-E/K resistance was uncommon in both populations. Professional contact with animals does not increase the chance of carriage of these bacteria in the Netherlands at present. *mcr-8* was found for the first time in the Netherlands. Surveillance of colistin resistance and its underlying mechanisms in humans, livestock and food is important in order to identify emerging trends in time.

Introduction

Colistin or polymyxin E is an antibiotic belonging to the class of polymyxins and has been on the market since the 1950s.[1] In human medicine, it is a last-resort drug for treatment of MDR Gram-negative bacterial infections and its use has increased during the last decade.[2] Polymyxins are categorized as highest priority critically important antimicrobials by WHO.[3]

Colistin is also used in animals for treatment of gastrointestinal infectious diseases mainly in calves, pigs and poultry.[4,5] For a long time it was believed that the mechanism of resistance to colistin was limited to chromosomal mutations. However, in 2015 a mobile colistin resistance gene (*mcr-1*) on a plasmid was found in Enterobacterales from pigs, retail meat and an inpatient in China.[6] The emergence of plasmid-mediated, transmissible colistin resistance is of great concern as it facilitates the spread between different bacterial isolates and species as well as between animals and humans. To date, 10 *mcr* genes (*mcr-1* to *mcr-10*) and many subvariants have been described.[7] These genes have been detected in zoonotic pathogens and it is likely that they are transferred between animals and humans.[1,8] The more frequent isolation of *mcr* genes among animal isolates compared with human ones, together with the higher use of colistin in livestock compared with human medicine in China has been suggestive of the direction of transmission from animals to humans.[6]

Although prevalence data and colistin use suggest that plasmid-mediated colistin resistance is transferred from animals to humans, it is unknown whether individuals with professional animal contact carry colistin-resistant Enterobacterales more often. Therefore, the objective of the present study was to investigate the prevalence of *mcr*-mediated colistin resistant *Escherichia coli* and *Klebsiella pneumoniae* (ColR-E/K) carriage among the Dutch population as well as among veterinary healthcare workers.

Material and methods

Ethics

Samples from two population studies were used: one study was part of the Antibiotic Resistant Bacteria in Dutch Veterinarians study (Dutch acronym: AREND) and the other study was part of the national sero-epidemiological study (Dutch acronym: PIENTER3). The University Medical Centre Utrecht designated the AREND study not to be subject to the Medical Research Involving Human Subjects Act or WMO (Decision number 18-389/C), while the medical research ethics committee Noord-Holland approved PIENTER3 (METC number M015-022). All participants signed an informed consent form.

Population studies

The AREND study was conducted between August 2018 and March 2019 and included 482 veterinarians, veterinary technicians and veterinary assistants. Participants were recruited at the annual Dutch veterinary conference in 2018, via articles in newsletters and journals for veterinarians, and by information about the study sent directly to veterinary clinics by postal mail. Criteria for inclusion were age 18 years or older and working in veterinary care. Persons working with companion animals, horses and/or livestock were included. Participants were asked to complete a web-based questionnaire about their contact with animals at work and at home, hygiene, health and medication use and leisure activities such as travel behaviour. Furthermore, they were asked to collect a faecal sample at home and send it to the laboratory by regular mail. On the day of arrival, the faecal samples were kept in the fridge (4°C) until further processing, which took place within 2 days.

The PIENTER3 study was conducted in 2016–17.[9] This was a nationwide cross-sectional population study in the Netherlands, to investigate the protection level against infectious diseases included in the National Immunization Programme. For that purpose, a two-stage cluster sampling technique was used to draw a random sample of persons aged 0–89 years from Dutch municipalities. Subpopulations were oversampled: these included people living in low vaccination coverage areas, people with a non-Western migration background and people with a migration background from Suriname, Aruba and the former Dutch Antilles (SAN). A total of 7600 persons in the Netherlands were enrolled in the study. Participants were invited to give a blood sample and fill in a general questionnaire, and in addition they were asked to join extra surveys. One of these extra surveys consisted of an additional questionnaire with in-depth questions regarding antibiotic use, contact with animals, food consumption patterns and leisure activities. Participants of this extra survey were among others also asked to collect a faecal sample in 15% glycerol-physiological salt solution, which was packed in a plastic bag directly after collection and kept in the freezer at home until they were brought in cold packs to the survey location by the participant. The faecal sample was transported under frozen conditions to the laboratory, where the samples were kept at −70°C. For more information about the study design of PIENTER3 see Verberk *et al.,* 2019.[9] In the present study, samples derived from participants aged ≥18 years and stored in the −70°C freezer were investigated. No further selection criteria were applied. The samples included 660 faecal samples, derived from twenty municipalities, from a total of 2779 persons from 48 municipalities in the Netherlands that had provided a faecal sample.[9]

Laboratory methods

To determine ColR-E/K presence in the faecal samples a sterile cotton swab (AREND) or a 10 μL loop (PIENTER3) of the faecal material was suspended in a sterile glass tube with 5 mL of Buffered Peptone Water (BioTrading) with 2 mg/L colistin (colistin sulphate salt, Sigma). After overnight incubation at 37°C, the enrichment was inoculated on ChromID Colistin R Agar (bioMérieux) with a 10 μL inoculation loop. The plates were incubated overnight at 37°C, and afterwards one colony per colour (pink to burgundy or blue/green) per person was isolated and inoculated on Colombia Agar with sheep blood (Oxoid). Species were confirmed using MALDI-TOF MS (Bruker) according to the manufacturer's instructions. For ColR-E/K suspected isolates the MICs were analysed by broth microdilution using MIC colistin strips (Merlin) according to the manufacturer's instruction, following ISO 20776-1:2019.[10] The clinical breakpoint of >2 mg/L recommended by CLSI and EUCAST was used to determine resistance. The ColR-E/K suspected isolates were screened for *mcr-1* to *-9* genes by two multiplex PCRs. If *mcr* genes were suspected, the PCR was repeated in singleplex format with the same primers for confirmation. The primers are described previously.[11–16]

Genome analysis of mcr-mediated ColR-E/K

One colony was inoculated in 1.5 mL BHI broth and incubated overnight at 37°C. The overnight culture was centrifuged at 13 000 g for 3 min. After removal of the supernatant, the cell pellet was washed in 500 μL physiological saline (0.9%) and subsequently centrifuged again for 3 min. The cell pellet was resuspended in 450 μL DNA/RNA-Shield (Zymo Research) and stored at 4°C. Genomic DNA was isolated using a Zymo Research kit (BaseClear, Leiden, the Netherlands). An Illumina genomic Nextera XT 2 × 150 bp paired-end DNA library was prepared and sequenced on an Illumina NovaSeq 6000 (BaseClear). Raw reads were trimmed and *de novo* assembled using SPAdes 3.10.0.[17]

The isolates were analysed by long read Nanopore sequencing as described in Hendrickx *et al.,* 2020.[18] Illumina and Nanopore sequences were combined for a hybrid assembly using Unicycler v0.4.8[19] with modified settings for depth_filter (set to 0.1) and min_fasta_length (set to 1000). Plasmids 260-1 and 132-1 were assembled with the depth_filter set to 0.01. Phylogenetic groups, serotypes and STs were assigned using in-house implemented task templates[20] in Ridom SeqSphere+[Version 7.1.0 (2020-06)]. Plasmids were characterized with Abricate v0.9.3 (https://github.com/tseemann/abricate), using the PlasmidFinder[21] database. Resistance genes were assigned with ResFinder version 4.0[22] using >98% identity and 100% coverage of genes. Plasmids were compared using blastn.

All the genomic sequences are available at the European Nucleotide Archive at the European Molecular Biology Laboratory (accession no. PRJEB45559, see Table S1, available as Supplementary data at *JAC-AMR* Online).

Results

The 482 veterinary healthcare workers that participated in the AREND study were aged between 20 and 70 years, the average age was 40 years (median 38 years) and 85% were female. The 660 included participants from PIENTER3 study were aged between 18 and 85 years, the average being 50 years (median 52 years). Thirty-three percent were born in a non-Western country, 61% were female and 7.8% reported direct contact with pets and/or farm animals at work.

mcr genes were detected in an *E. coli* isolate from a veterinary technician participating in the AREND study [0.2% (1/482, 95% CI 0.04%–1.17%)] and in three *E. coli* and two *K. pneumoniae* isolates from five persons from the PIENTER3 population [0.8% (5/660, 95% CI 0.3%–1.8%)], including four with a migration background from Suriname. Three of these six persons reported travel to Asia, two did not report any travel in the past 6 months and for one person the travel history was unknown.

All *E. coli* isolates harboured *mcr-1* (three on IncX4 plasmids, and one on an IncI2 plasmid). One *K. pneumoniae* isolate

harboured *mcr-8* on an IncF plasmid, while the other *K. pneumoniae* isolate carried *mcr-1* as well as *mcr-8* on IncI1 and IncF plasmids, respectively. An overview of the characteristics of the *mcr*-positive isolates, the participants and the potential risk factors is shown in Table 1.

The three IncX4 plasmids found in *E. coli* isolates in this study were not identical to each other and were 33, 34 and 41 kb in size. The *mcr-1* genes were organized in the same commonly found embedding structure: IS*26*-hypothetical protein (hp)-hp-*parA*-hp-hp-*mcr-1*-*pap2*-hp-hp-*pir*. This genetic structure was previously found on a 33 kb IncX4 plasmid (pLV23529) in an *E. coli* isolate originating from swine cecum in Portugal.[23] Plasmid 260-1 in addition to *mcr-1* carried *bla*$_{TEM-1B}$.

The two IncF plasmids 91-1 and 137-1_IncF in *K. pneumoniae* (Table 1) were different in size (103 kb and 161 kb, respectively). Both of them contained an IncFIIK replicon and plasmid 91-1 an IncFIA as well. The genetic region around *mcr-8* of plasmid 137-1_incF was identical to the region in a 300 kb *K. pneumoniae* plasmid pk9 isolated from a patient in Lebanon (*sasA-CopR-mcr-8*-hp-hp).[24] This region in plasmid 91-1 was almost identical (*sasA-CopR-mcr-8*-hp-IS*Kpn43*) to that of pk9 and 137_1_incF. Plasmid 137-1_IncF carried, besides the *mcr-8* gene, resistance determinants to aminoglycosides (*aadA2*), β-lactams (*bla*$_{TEM-1B}$) macrolides [*mph*(A)], sulphonamides (*sul1*), tetracycline [*tet*(A)] and trimethoprim (*dfrA12*). Plasmid 91-1 did not carry additional antibiotic resistance genes.

The IncI1 plasmid 137-1_IncI1 of plasmid ST8, carrying the *mcr-1* gene, was 51 kb in size. This plasmid differed in size from an *mcr-1*-carrying IncI1 plasmid (pMCR-E2899) recently isolated from turkey meat (107 kb) in the Netherlands.[25] The genetic region around *mcr-1* of plasmid 137-1_IncI1 was identical to the region in pMCR-E2899 (IS*Apl1*-*Δpap2*-*mcr1.1*-IS*Apl1*). Besides *mcr-1*, plasmid 137-1_IncI carried genes for resistance to aminoglycosides (*aadA12*), macrolides [*erm*(B)] and sulphonamides (*sul1*) in contrast to the published pMCR-E2899, which harboured resistance genes to only β-lactams (*bla*$_{TEM-1B}$) and colistin (*mcr-1*).

IncI2 plasmid 88-1 (Table 1) was 60 kb in size and carried the *mcr-1.1* gene also surrounded by the genes *nikB* and *pap2* as described in a 64 kb plasmid pEC26 carrying *mcr-1.9* found in an enterotoxigenic *E. coli* isolate from a patient in China.[26] Plasmid 88-1 like pEC26 only carried resistance to colistin (i.e. *mcr-1.1*).

Besides *mcr* and the other resistance genes carried on the identified plasmids, multiple additional antibiotic resistance determinants were present in several of the isolates investigated (Table 1) either on the chromosome or on other plasmids present.

Discussion

In the present culture-based cross-sectional study, the prevalence of intestinal carriage of *mcr*-mediated ColR-E/K resistance was low in veterinary healthcare workers as well as in a subset of the general Dutch population. This might be a result of low veterinary colistin usage in the Netherlands. Consequently, *mcr*-mediated resistance is identified only incidentally in *E. coli* from different livestock species (<2% in 2019).[27] Colistin is almost never used in companion animals in the Netherlands,[28] therefore carriage of ColR-E/K in pets is also expected to be low. This indicates that at present professional contact with animals is not an important transmission route for humans in the Netherlands.

The Dutch population might also be exposed to *mcr* genes through consumption of (imported) food products. This was illustrated in 2018, when a marked difference was found between the *mcr-1* prevalence among caecal samples of broilers fattened in Germany but slaughtered in the Netherlands (24.4%) compared with the *mcr* prevalence among Dutch broilers (0.3%).[29] To date, the prevalence of *mcr*-positive *E. coli* in meat sold in the Netherlands is low, except for turkey meat (13.3% in 2019).[27] Additional studies are needed to evaluate the transfer of *mcr* genes from animals and food to humans in different countries with different prevalence.

The *mcr*-prevalence in our study is comparable to that found in patients in other studies from Europe. Among patients attending a tertiary care hospital in the Netherlands prevalence was 0.35% (2/576 persons *mcr-1* positive) in 2014–15[30] and among inpatients in hospitals in Paris it was 0.57% (7/1217 patients *mcr-1* positive).[31] In a study among 1091 healthy individuals in Switzerland no *mcr*-related resistance was found.[32] In China, however, much higher prevalence of carriage of *mcr-1*-mediated colistin resistance in *E. coli* was reported in persons visiting hospitals for routine examinations. In 2006, this prevalence was found to be 14.6%. In 2019, after the ban of the use of colistin as growth promotor in animals in China, it declined to 6.3%, which is still much higher than in our study.[33]

Three of the six persons carrying *mcr*-mediated ColR-E/K in the present study had travelled to Asia during the 6 months prior to sampling. In a study among inpatients in hospitals in Paris three of the seven persons had been hospitalized abroad in Asia in the previous year.[31] Travel to Asia, Africa and Latin America has been identified as a risk factor for carriage of other MDR bacteria, such as ESBL-producing Enterobacterales,[34] and this might play a role for *mcr*-producing isolates as well.[35]

Four of the six persons carrying *mcr* genes (including the two *mcr-8* carriers) in our study were first generation immigrants from Suriname, but none of them reported travelling to Suriname during the last 6 months. It should be noted that first generation immigrants from Suriname were oversampled in the PIENTER3 population study and this might partly explain our finding. To illustrate: in 2019, 3.0% of the population at large were first or second generation immigrants from Suriname, Aruba and the former Dutch Antilles, against 29.2% of PIENTER3 participants.[36]

In the present study, the *mcr-1* genes were found on different plasmid types, namely IncI2 and IncX4 in *E. coli* and IncI1 in *K. pneumoniae*. The plasmid families IncHI2, IncI2 and IncX4 account for more than 90% of plasmids carrying the *mcr-1* genes from different sources worldwide.[37] IncI1 plasmids carrying *mcr-1* are less commonly reported, although they have been isolated recently from wastewater samples of German pig and poultry slaughterhouses.[38] Interestingly, *mcr-1* in a similar resistance region on another IncI1 plasmid has been reported in an *E. coli* isolate originating from a retail turkey meat sample in the Netherlands in 2015.[25] All plasmids described in this study differ from each other, but have *mcr*-surrounding genes that have been described previously in similar plasmid families all over the world.[23,25,26]

To the best of our knowledge this is the first time that *mcr-8* genes were found in the Netherlands. We found only two *mcr-8* isolates and therefore no conclusions can be drawn about the origin. However, until now *mcr-8* has not been described in Europe,

Table 1. Characteristics of *mcr*-positive isolates and participants carrying these colistin-resistant *E. coli/K. pneumoniae*

Isolates	PIENTER3					AREND
	1	2	3		4	5	6
Species	*K. pneumoniae*	*E. coli*	*K. pneumoniae*		*E. coli*	*E. coli*	*E. coli*
ST	ST4065	ST106	ST231		ST636	ST93	ST93
Serotype	O2v2: KL-	O17:H18	O1v2:KL51		O21:H7	O5:H4	O7:H4
E. coli phylogenetic group	n.a.	D1	n.a.		B23	A0	A0
Colistin MIC, mg/L	8	4	32		4	8	4
ID number *mcr*-carrying plasmid	91-1	132-1	137-1		88-1	260-1	308-1
mcr gene type	*mcr-8.1*	*mcr-1.1*	*mcr-1.1*	*mcr-8.1*	*mcr-1.1*	*mcr-1.1*	*mcr-1.1*
Plasmid type	IncF	IncX4	IncI1	IncF	IncI2	IncX4	IncX4
Plasmid size, kb	103	33	51	161	60	34	41
Additional resistance genes on this plasmid	None	None	*ermB, sul1*	bla_{TEM-1B}, *dfrA12, mph(A), sul1*	None	bla_{TEM-1B}	None
Additional resistance genes in the isolate[a]	$bla_{SHV-172}$-like[a], *oqxA, oqxB, tet*(A), *strA, strB*	None	$bla_{SHV-212}$-like[a], *oqxA, oqxB*		bla_{TEM-1B}, *dfrA1, strA, strB, sul2, tet*(B)	bla_{TEM-1B}, *cmIA1, mdf*(A), *sul3, tet*(B)	bla_{TEM-1B}, *mdf*(A)
Participants							
Age (category), years	70–89	60–69	60–69		50–59	30–39	30–39
Sex	Male	Male	Female		Male	Male	Female
Country of birth	Suriname	Suriname	Suriname		Suriname	Netherlands	Netherlands
Ethnicity	First gen. Suriname	First gen. Suriname	First gen. Suriname		First gen. Suriname	Second gen. other Western	UNK
Urbanization level[b]	Very high	Very high	Very high		Very high	Very high	Very low
Has children attending daycare centre	No	No	No		UNK	No	No
Job	Retired	Retired	Healthcare		Maritime industry	Government	Veterinary technician
Has weekly or more often contact with animals at work	No	No	No		No	No	Yes (dog, cat rabbit/guinea pig/ hamster, horse)
Travel in last 6 months	No travel	Southern Asia	Western Asia		UNK	Eastern Asia	No travel
Contact with animals in last 4 weeks	No contact	No contact	Cat		UNK	Dog, guinea pig	Dog, cat, horse
Use of stomach protectors	Yes	Yes	No		No	No	No
Antibiotic use last 3 months	No	Yes	Yes		Yes	No	No
Which type of antibiotic?	n.a.	Amoxicillin	Co-trimoxazole		UNK	n.a.	n.a.
Hospital visit in last 6 months	Yes	Yes	No		UNK	No	UNK
Hospitalized in last 6 months	No	No	No		UNK	No	Yes

n.a. , not applicable; gen., generation; UNK, unknown.
[a]ResFinder results with 100% coverage and >98% identity. The $bla_{SHV-172}$-like variant differs by two non-synonymous SNPs from the reference sequence: A256C (Q86K) and T756G (D252E). The $bla_{SHV-212}$-like variant differs by one non-synonymous SNP to the reference sequence: A238G (M80V).
[b]Very high: ≥2500 addresses/km^2; high/moderate: 1000–2500 addresses/km^2; low/very low: <1000 addresses/km^2.

except for one patient in France, who was repatriated from Morocco.[39] The *mcr-8* gene was first described by Wang *et al.*[15] in 2018 in *K. pneumoniae* isolates on IncFII conjugative plasmids derived from chickens and pigs in China and in a patient in a hospital. To date *mcr-8* has been described in *K. pneumoniae* and other bacteria (*Stenotrophomonas* sp. and *Raoultella ornithinolytica*) isolated from poultry, sewage water from a poultry farm, pigs and patients in China.[40–43] In addition, besides the patient in France mentioned earlier, *mcr-8* was also found in Algeria (in a patient), Laos (in healthy humans) and Bangladesh (in patients), often located on IncF plasmids.[44–46]

Generally, the *mcr*-carrying plasmids reported in this study did not harbour as many resistance determinants as some plasmids reported in previous studies that have a similar *mcr* gene genetic environment. Nevertheless, nearly all isolates carried (multiple) other antibiotic resistance genes. This could complicate treatment in case of an infection.

In conclusion, professional contact with animals does not increase the chance of *mcr* carriage in humans in the Netherlands at present. *mcr-8* was detected for the first time in the Netherlands. Although carriage of *mcr*-positive ColR-E/K is still very rare in both populations studied, different *mcr* genes were carried on four different plasmid types. Therefore surveillance of colistin resistance and its underlying mechanisms in food, humans and livestock is important in order to identify emerging trends in time.

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

References

1 Catry B, Cavaleri M, Baptiste K *et al.* Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *Int J Antimicrob Agents* 2015; **46**: 297–306.

2 Li J, Nation RL, Milne RW *et al.* Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. *Int J Antimicrob Agents* 2005; **25**: 11–25.

3 WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). Critically Important Antimicrobials for Human Medicine: 6th Revision. 2018. https://www.who.int/publications/i/item/9789241515528.

4 EMA. Updated Advice on the Use of Colistin Products in Animals Within the European Union: Development of Resistance and Possible Impact on Human and Animal Health: Report Number EMA/CVMP/CHMP/231573/2016. 2016. https://www.ema.europa.eu/en/documents/scientific-guideline/updated-advice-use-colistin-products-animals-within-european-union-development-resistance-possible_en-0.pdf.

5 Kempf I, Jouy E, Chauvin C. Colistin use and colistin resistance in bacteria from animals. *Int J Antimicrob Agents* 2016; **48**: 598–606.

6 Liu YY, Wang Y, Walsh TR *et al.* 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* 2016; **16**: 161–8.

7 Wang C, Feng Y, Liu L *et al.* Identification of novel mobile colistin resistance gene *mcr-10*. *Emerg Microbes Infect* 2020; **9**: 508–16.

8 Gharaibeh MH, Shatnawi SQ. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: a review. *Vet World* 2019; **12**: 1735–46.

9 Verberk JDM, Vos RA, Mollema L *et al.* Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands. *BMC Infect Dis* 2019; **19**: 470.

10 International Organization for Standardization. *ISO 20776-1:2019 Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices—Part 1: Broth Micro-Dilution Reference Method for Testing the In Vitro Activity of Antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases*. ISO, 2019.

11 AbuOun M, Stubberfield EJ, Duggett NA *et al. 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* 2018; **73**: 2904.

12 Borowiak M, Fischer J, Hammerl JA *et al.* 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* 2017; **72**: 3317–24.

13 Kieffer N, Royer G, Decousser JW *et al. mcr-9*, an Inducible Gene Encoding an Acquired Phosphoethanolamine Transferase in *Escherichia coli*, and Its Origin. *Antimicrob Agents Chemother* 2019; **63**: e00965-19.

14 Rebelo AR, Bortolaia V, Kjeldgaard JS *et al.* 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**: 17-00672.

15 Wang X, Wang Y, Zhou Y *et al.* Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg Microbes Infect* 2018; **7**: 122.

16 Yang Y-Q, Li Y-X, Lei C-W *et al.* Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2018; **73**: 1791–5.

17 Bankevich A, Nurk S, Antipov D *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012; **19**: 455–77.

18 Hendrickx APA, Landman F, de Haan A *et al.* Plasmid diversity among genetically related *Klebsiella pneumoniae* bla$_{KPC-2}$ and bla$_{KPC-3}$ isolates collected in the Dutch national surveillance. *Sci Rep* 2020; **10**: 16778.

19 Wick RR, Judd LM, Gorrie CL *et al.* Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 2017; **13**: e1005595.

20 van Hoek A, van Veldhuizen JNJ, Friesema I *et al.* Comparative genomics reveals a lack of evidence for pigeons as a main source of *stx2f*-carrying *Escherichia coli* causing disease in humans and the common existence of hybrid Shiga toxin-producing and enteropathogenic *E. coli* pathotypes. *BMC Genomics* 2019; **20**: 271.

21 Carattoli A, Zankari E, Garcia-Fernandez A *et al. In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014; **58**: 3895–903.

22 Bortolaia V, Kaas RS, Ruppe E *et al.* ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500.

23 Manageiro V, Clemente L, Romao R *et al.* IncX4 plasmid carrying the new *mcr-1.9* gene variant in a CTX-M-8-producing *Escherichia coli* isolate recovered from swine. *Front Microbiol* 2019; **10**: 367.

24 Salloum T, Panossian B, Bitar I *et al.* First report of plasmid-mediated colistin resistance *mcr-8.1* gene from a clinical *Klebsiella pneumoniae* isolate from Lebanon. *Antimicrob Resist Infect Control* 2020; **9**: 94.

25 Brouwer MSM, Goodman RN, Kant A *et al.* Mobile colistin resistance gene *mcr-1* detected on an IncI1 plasmid in *Escherichia coli* from meat. *J Glob Antimicrob Resist* 2020; **23**: 145–8.

26 Liu H, Zhu B, Liang B *et al.* A novel *mcr-1* variant carried by an IncI2-type plasmid identified from a multidrug resistant enterotoxigenic *Escherichia coli*. *Front Microbiol* 2018; **9**: 815.

27 Nethmap/MARAN 2020. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2019. https://www.wur.nl/nl/show/Nethmap-MARAN-2020.htm.

28 van Dijk M, Taverne F. Antibioticumgebruik bij Gezelschapsdieren: Uitkomsten van een Survey Onder Dierenartspraktijken Over de Jaren 2012 t/m 2014. 2016. https://cdn.i-pulse.nl/autoriteitdiergeneesmiddelen/userfiles/Publicaties/def-sda-rapport-antibioticumgebruik-bij-gezelschapsdieren.pdf.

29 Nethmap/MARAN 2019. Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2018. https://www.wur.nl/nl/show/MARAN-2019-rapport.htm.

30 Terveer EM, Nijhuis RHT, Crobach MJT *et al.* Prevalence of colistin resistance gene (*mcr-1*) containing Enterobacteriaceae in feces of patients attending a tertiary care hospital and detection of a *mcr-1* containing, colistin susceptible *E. coli*. *PLoS One* 2017; **12**: e0178598.

31 Bourrel AS, Poirel L, Royer G *et al.* Colistin resistance in Parisian inpatient faecal *Escherichia coli* as the result of two distinct evolutionary pathways. *J Antimicrob Chemother* 2019; **74**: 1521–30.

32 Zurfluh K, Stephan R, Widmer A *et al.* Screening for fecal carriage of MCR-producing Enterobacteriaceae in healthy humans and primary care patients. *Antimicrob Resist Infect Control* 2017; **6**: 28.

33 Wang Y, Xu C, Zhang R *et al.* Changes in colistin resistance and *mcr-1* abundance in *Escherichia coli* of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study. *Lancet Infect Dis* 2020; **20**: 1161–71.

34 van den Bunt G, van Pelt W, Hidalgo L *et al.* Prevalence, risk factors and genetic characterisation of extended-spectrum β-lactamase and carbapenemase-producing Enterobacteriaceae (ESBL-E and CPE): a community-based cross-sectional study, the Netherlands, 2014 to 2016. *Euro Surveill* 2019; **24**: 1800594.

35 Arcilla MS, van Hattem JM, Matamoros S *et al.* Dissemination of the *mcr-1* colistin resistance gene. *Lancet Infect Dis* 2016; **16**: 147–9.

36 StatLine Bevolking. Kerncijfers 2019. https://opendata.cbs.nl/statline/#/CBS/nl/dataset/37296NED/table? fromstatweb.

37 Matamoros S, van Hattem JM, Arcilla MS *et al.* Global phylogenetic analysis of *Escherichia coli* and plasmids carrying the *mcr-1* gene indicates bacterial diversity but plasmid restriction. *Sci Rep* 2020; **10**: 2963.

38 Savin M, Bierbaum G, Blau K *et al.* Colistin-resistant Enterobacteriaceae isolated from process waters and wastewater from German poultry and pig slaughterhouses. *Front Microbiol* 2020; **11**: 575391.

39 Bonnin RA, Bernabeu S, Jaureguy F *et al.* MCR-8 mediated colistin resistance in a carbapenem-resistant *Klebsiella pneumoniae* isolated from a repatriated patient from Morocco. *Int J Antimicrob Agents* 2020; **55**: 105920.

40 Li J, Liu S, Fu J *et al.* Co-occurrence of colistin and meropenem resistance determinants in a *Stenotrophomonas* strain isolated from sewage water. *Microb Drug Resist* 2019; **25**: 317–25.

41 Ma K, Feng Y, Liu L *et al.* A cluster of colistin- and carbapenem-resistant *Klebsiella pneumoniae* carrying bla_{NDM-1} and *mcr-8.2*. *J Infect Dis* 2020; **221** Suppl 2: S237–42.

42 Wang X, Wang Y, Zhou Y *et al.* Emergence of colistin resistance gene *mcr-8* and its variant in *Raoultella ornithinolytica*. *Front Microbiol* 2019; **10**: 228.

43 Yang X, Liu L, Wang Z *et al.* Emergence of *mcr-8.2*-bearing *Klebsiella quasipneumoniae* of animal origin. *J Antimicrob Chemother* 2019; **74**: 2814–7.

44 Farzana R, Jones LS, Barratt A *et al.* Emergence of mobile colistin resistance (*mcr-8*) in a highly successful *Klebsiella pneumoniae* sequence type 15 clone from clinical infections in Bangladesh. *mSphere* 2020; **5**: e00023-20.

45 Hadjadj L, Baron SA, Olaitan AO *et al.* Co-occurrence of variants of *mcr-3* and *mcr-8* genes in a *Klebsiella pneumoniae* isolate from Laos. *Front Microbiol* 2019; **10**: 2720.

46 Nabti LZ, Sahli F, Ngaiganam EP *et al.* Development of real-time PCR assay allowed describing the first clinical *Klebsiella pneumoniae* isolate harboring plasmid-mediated colistin resistance *mcr-8* gene in Algeria. *J Glob Antimicrob Resist* 2020; **20**: 266–71.