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*

Elisabeth M. Terveer*, Roel H. T. Nijhuis, Monique J. T. Crobach, Cornelis W. Knetsch*, Karin E. Veldkamp, Jairo Gooskens, Ed J. Kuijper, Eric C. J. Claas

Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands

Current address: DDL Diagnostic Laboratory, Rijswijk, the Netherlands  
* e.m.terveer@lumc.nl

Abstract

The emergence of the plasmid-mediated *mcr* colistin resistance gene in the community poses a potential threat for treatment of patients, especially when hospitalized. The aim of this study was to determine the prevalence of all currently known *mcr* mediated colistin resistance gene in fecal samples of patients attending a tertiary care hospital. From November 2014 until July 2015, fecal samples of patients attending the Leiden University Medical Center were collected and screened for presence of *mcr* using real-time PCR. Two of 576 patients were positive for *mcr-1*, resulting in a prevalence of 0.35%, whereas no *mcr-2* was found. One of these samples was culture negative, the second sample contained a *bla*CMY-2 and *mcr-1* containing *E. coli*. This strain belonged to Sequence Type 359 and serotype O177:H21. The *mcr-1* containing *E. coli* was phenotypically susceptible to colistin with a MIC of 0.25mg/l, due to a 1329bp transposon IS10R inserted into the *mcr-1* gene as identified by WGS. This prevalence study shows that *mcr-1* is present in low levels patients out of the community attending a hospital. Furthermore the study underlines the importance of phenotypical confirmation of molecular detection of a *mcr-1* gene.

Introduction

Colistin, also known as polymyxin E, is highly active against most Gram-negative bacteria [1]. However, its nephrotoxicity and neurotoxicity has prevented the use of colistin in regular patient treatment [2]. Therefore, colistin usage was mainly limited to veterinary medicine for treatment of gastrointestinal infections in food producing animals [3]. In the Netherlands, colistin is frequently used for selective gut decontamination in Intensive Care Unit (ICU) and stem cell transplantation patients [4, 5]. Colistin regained new worldwide interest after the emergence of multi-drug resistant (MDR) *Enterobacteriaceae* and is nowadays used as a last
resort antibiotic for infections caused by MDR Enterobacteriaceae. The recent finding of a plasmid harbouring a novel colistin resistance gene, mcr-1 and mcr-2, is therefore of concern [6, 7].

The mcr-1 colistin resistance gene is predominantly found in Enterobacteriaceae, and results in a moderate level of resistance, with MIC values varying from 4 to 16 mg/l [8, 9]. The prevalence of mcr-1 varies considerably and ranges from 0.02% to 20.6% in livestock, 1.3% to 19% in retail meat and 0.08% to 2% in hospitalized patients [10–14]. The worldwide distribution of the mcr-1 gene and a relatively high prevalence of mcr-1 mediated colistin resistance in livestock and retail meat suggests food animals as reservoir for transmission to humans [8]. Until now, almost exclusively Extended Spectrum Beta-Lactamase (ESBL) producing or colistin resistant isolates have been screened for the presence of mcr-1. A number of reports of mcr-1 in the United States of clinical and ESBL-negative strains indicate that the true extent of mcr-1 prevalence amongst unselected Gram-negatives may be highly underestimated [15, 16]. The mcr-2 colistin resistance gene had 76.7% nucleotide identity to mcr-1 and had so far only been found in colistin-resistant E. coli isolates identified from porcine and bovine [7].

Studies about human fecal carriage of mcr-1 in the community are limited, and so far only been described in China; in healthy volunteers (prevalence of 19 of 2923 = 0.65%), in a public bacterial metagenome dataset before 2010 (prevalence of 3/1267 = 0.24%) [17, 18], and in Dutch travellers returning from Asia, South America or Africa (prevalence of 0.95%–4.9%) [19, 20]. Recently, no mcr genes could be detected in the stool of 1091 healthy Swiss individuals [21]. Epidemiological data on the prevalence of mcr-1 in the community attending a hospital are lacking and the risk of colonized patients to spread mcr-1 positive bacteria is unknown. Therefore, the aim of this study was to determine the prevalence of mcr mediated colistin resistance gene in fecal samples of patients attending a tertiary care hospital.

Material/methods

Patients and specimens

Between November 2014 and July 2015, fecal samples were obtained from patients on admission to internal medicine and surgical wards, and from patients attending the kidney transplant outpatient clinic of the Leiden University Medical Center (LUMC) in the Netherlands. These wards were selected for their relative high patient turn-over, enabling more rapid inclusion of sufficient patients attending our hospital. The fecal samples were originally used for a study to define the role of Clostridium difficile in asymptomatic colonised patients at admission to the hospital. The samples were processed within 72 hours of arrival at the laboratory and were subsequently stored at -20°C, without addition of glycerol. These samples obtained for C. difficile screening were also used for screening of the mcr gene. The medical ethical committee “Medisch Ethische Toetsings Commissie” of the LUMC waived the need for consent for the additional analysis on these fecal samples.

DNA extraction and real-time PCR

After thawing the stored fecal samples, DNA extraction was performed using the MagnaPure96 system (Roche Diagnostics, Almere, Netherlands). In short, approximately 0.3 to 0.4 gram (half a pea) feces was resuspended in 1mL S.T.A.R. buffer (Roche Diagnostics, Almere, The Netherlands), supplemented with Precellys beads (Bertin Technology, France), mixed thoroughly by shaking on a Vibra Shaker (5 min, 2200rpm) and centrifuged for 1min at 14000 rpm. Of the supernatant, 200μl was used for nucleic acid (NA) extraction using the MP96 system and Viral NA Small volume kit (Roche Diagnostics) yielding a final eluate of 100μl. To monitor the NA extraction process and the presence of potential PCR inhibitors in
the eluate, an universal internal control Phocine Herpes Virus (PhHV) was used [22]. Initially real-time PCR for the specific detection of the mcr-1 gene was tested in a multiplex assay with PhHV as described previously [23]. After the report of Xavier et al., describing mcr-2, a generic mcr real-time PCR assay for the detection of both mcr-1 and mcr-2 was developed and used to screen for the presence of additional mcr-2 containing samples (Table 1) [7].

Culture and colistin susceptibility testing

To further characterize mcr containing isolates, mcr positive fecal samples were cultured on commercially available sheep blood-, CNA- (colistin and naladixic acid containing agar) and CLED- (cysteine lactose electrolyte deficient) medium (BioMérieux, Marcy l’Etoile, France) both directly and after enrichment in a Tryptic Soy Broth with and without colistin (2 mg/l). All morphological different aerobic Gram-negative bacteria were identified by MALDI-TOF MS (Microflex, Bruker Daltonics, Bremen, Germany) and tested for the presence of mcr-1 by real-time PCR as described earlier. All bacterial isolates were also tested for colistin resistance with VITEK2 (card N199, BioMérieux, Marcy l’Etoile, France) and Sensititre colistin microdilution assay (Sensititre, TREK Diagnostic Systems, Inc., Cleveland, OK), using EUCAST breakpoints for Enterobacteriaceae, which interprets a MIC of ≤ 2 mg/l as susceptible and > 2mg/l as resistant.

Whole Genome Sequence analysis

Whole Genome Sequence analysis of mcr-1 containing isolates was performed to further characterize the E. coli strain including the plasmid carrying the mcr-1 gene and other genes associated with antimicrobial resistance [6]. The genome sequence of the mcr-1 containing isolate was determined using the Pacific Biosciences RSII system from DNA prepared by the Qiagen Genomic Tip 500/G kit (Qiagen, Hilden, Germany) following the manufacturer’s recommendations. De novo assembly was performed using SMRT Analysis v2.3.0 (PacBio’s bioinformatics software suite) with expected genome size of 5 Mbp and coverage of 30. The assembled sequence was analysed using Geneious software V8.0.5 (Biomatters, Auckland, New Zealand) and the online tools Resfinder, MLST, SeroTypeFinder and Plasmidfinder (http://genomicepidemiology.org/). The plasmid sequence was analysed in DNA plotter to generate a circular DNA map.

Results

Mcr prevalence and culture of mcr containing isolates

A total of 621 fecal samples of 576 unique patients were screened for presence of the mcr genes by real-time PCR. The median age of patients at submission of their stool was 62 years (range 18–93). Two samples of two different patients (0.35%) were positive for mcr-1 in real-time PCR with quantification cycle (Cq) values of 31 and 17, respectively (S1 Table). Additional testing with the mcr-generic real-time PCR assay confirmed this finding and did not find extra positive samples. A mcr-1 containing E.coli isolate was cultured from the second fecal sample.
(Cq 17) only, in subcultures of the enrichment broth without colistin. Remarkably, despite the presence of mcr-1 gene sequences, this E.coli isolate tested colistin susceptible (MIC <0.25 mg/l), which was confirmed in triplicate by both VITEK2 and the Sensititre assay. The antimicrobial susceptibility results and the corresponding genes coding for resistance are depicted in Table 2. Because of the decreased susceptibility to cephalosporins, the production of ESBL was tested phenotypically using the combination disk diffusion test, with a negative result. Subsequent testing for an AmpC β-lactamase gene by an in-house developed real-time PCR assay showed the presence of the blaCIT gene.

Whole Genome Sequence analysis

WGS analysis showed that the mcr-1 gene found in the colistin susceptible E. coli isolate had a homology of 100% with the first published mcr-1 gene sequence [6]. However, the reading frame was disrupted by a 1329bp long IS10R transposon (Fig 1). WGS analysis of the E.coli resulted into six contigs with a total length of ~5.5 Mbp (accession numbers: CP016546-CP016551, S2 Table). The largest contig was ~5.1 Mbp, covering the expected E. coli genome size, whereas analysis of the remaining five contigs (length between ~7.3 kb and ~126 kb) with PlasmidFinder 1.3 indicated the presence of plasmids IncX4 (~50kb), IncI2 (~86kb), IncB/O/K/Z (~91kb) and IncY (~126kb). WGS analysis also revealed the presence of two identical IS10R containing mcr-1 genes located on the same IncX4 plasmid. Multi Locus Sequence Typing (MLST) and serotype analysis showed that the E.coli belonged to Sequence Type (ST) 359 and serotype O177:H21. With ResFinder, the AmpC belonging to the CIT-group, as detected by the in-house AmpC real-time PCR, was confirmed to be present as blaCMY-2, located on the plasmid designated as IncB/O/K/Z. Additional genes associated with antimicrobial resistance detected in the sequence with their resulting antimicrobial phenotype are depicted in Table 2.

Table 2. Antibiotic phenotype with the corresponding molecular resistance of cultured mcr-1 containing E.coli.

| Antibiotic                          | MIC (mg/l) | Interpretation | Encoding resistance genes                  |
|-------------------------------------|------------|----------------|--------------------------------------------|
| Ampicillin                          | ≥ 32       | R              | blaTEM-1B                                  |
| Amoxicillin/Clavulanic acid         | ≥ 32       | R              | blaTEM-1B                                  |
| Cefuroxime                          | 32         | R              | blaCMY-2                                   |
| Cefotaxime                          | 4          | R              | blaCMY-2                                   |
| Cefoxitin                           | ≥ 32       | R              | blaCMY-2                                   |
| Ceftazidime                         | 16         | R              | blaCMY-2                                   |
| Cefepine                            | ≤ 1        | S              |                                            |
| Ciprofloxacin                       | ≥ 4        | R              |                                            |
| Colistin                            | ≤ 0.25     | S              | Mcr-1 inserted by IS10R transposon          |
| Gentamicin                          | ≤ 1        | S              | aph(3')-lc, strB, strA, aadA5               |
| Meropenem                           | ≤ 0.25     | S              |                                            |
| Nitrofurantoin                      | ≥ 320      | S              |                                            |
| Piperacillin/Tazobactam             | ≤ 4        | S              |                                            |
| Tetracycline                        | 128        | R              | tetB                                       |
| Tobramycin                          | ≤ 1        | S              | aph(3')-lc, strB, strA, aadA5               |
| Trimethoprim/Sulfamethoxazole       | ≥ 4        | R              | sul1, sul2, dfraA17                        |

The phenotype was tested with VITEK2 and a colistin microdilution assay, using EUCAST breakpoints. Molecular resistance determined with whole genome sequencing.

https://doi.org/10.1371/journal.pone.0178598.t002
Both *mcr-1* positive patients were kidney transplant patients. The *mcr-1* positive stool sample from which no *mcr-1* containing isolate could be cultured belonged to a patient admitted to the acute care ward due to bacteremia with a colistin resistant *Salmonella enterica* serotype *Dublin* (MIC ≥4 mg/l). The *S. enterica* isolate tested negative with the specific *mcr-1* PCR. The feces with the colistin susceptible *mcr-1* *E. coli* belonged to a patient attending the kidney transplant outpatient clinic. No epidemiologic link could be established between the two patients. The patients did not have a history of recent travelling and had not been treated with colistin recently. Also, none of the patients had developed an infection with a *mcr-1* containing isolate.


Discussion

To assess the risk of mcr introduction into our academic tertiary care hospital, the prevalence of mcr in fecal samples obtained from patients attending our hospital was investigated and found to be 0.35% (n = 2) of the 576 tested patients for mcr-1, whereas no mcr-2 was found. This low prevalence is in accordance with earlier studies performed in asymptomatic carriers in the European community, ranging from 0% to 0.92% [19–21]. However, studies on mcr-1 prevalence in asymptomatic carriers attending a hospital are lacking. Infections in hospitalized patients with mcr-1 positive isolates have been reported in a number of countries, ranging from 0.24% to 1.4% depending on the used denominator [6, 10, 24]. In line with earlier studies, no mcr-2 containing samples were detected in this study [21, 25].

One of the mcr-1 positive fecal samples from the current study could not be confirmed by culture, most likely due to the fecal storage without glycerol at -20˚C for one year which reduces the viability of Gram-negative bacteria. The fecal sample of the second patient contained a mcr-1 positive E.coli with a colistin MIC of <0.25 mg/l. WGS analysis of the isolate revealed the presence of IS10R, encoding for an active transposon commonly found in Enterobacteriaceae [26]. Introduction of this IS10R into the mcr-1 gene resulted in a non-functional mcr-1 gene. Interestingly, two identical mcr-1 genes with IS10R duplicates were located on IncX4, a plasmid that has been frequently observed in combination with mcr-1 [10, 12, 27, 28].

The mcr-1 containing E.coli belonged to ST359, this ST with a very similar antimicrobial resistance pattern is earlier described on chicken retail meat in Denmark [10]. Though we tested all morphological different Gram negative Enterobacteriaceae for the presence of mcr-1, we cannot exclude the possibly that more than one mcr-1 containing bacterial species was present in the positive tested feces samples.

Pham Thanh et al. reported the first mcr-1 positive but colistin susceptible isolate, a Shigella sonnei, that was based on a truncated mcr-1 gene caused by a 22bp duplication [29]. A colistin susceptible mcr-1 containing E.coli isolate with unknown cause of the susceptibility was reported in August 2016 by Liassine et al. [25]. Although the altered mcr-1 gene of the Shigella sonnei could be re-activated by conjugation experiments resulting in a colistin resistant phenotype, the mcr-1 gene interrupted with IS10R containing E.coli of this study cannot be re-activated, as upon removal of an IS transposon two remaining nucleotides would disrupt the reading frame of the gene [30]. These studies underline the importance of phenotypical confirmation after molecular screening, as respectively the E.coli and Shigella sonnei isolate showed colistin susceptibility despite the presence of mcr-1 gene sequences that had been detected by PCR amplification.

The mcr-1 positive E.coli isolate showed resistance to third generation cephalosporins due to the presence of a AmpC β-lactamase gene, blaCMY-2, as has previously been found by Prim et al. [24]. As almost all earlier studies only screened for the presence of mcr-1 in ESBL producing isolates, the true extent of the mcr-1 prevalence may be underestimated [10, 11, 14, 19, 31, 32].

Most likely, the kidney transplant patients acquired the mcr-1 gene in the community, for instance by consumption of mcr-1 containing retail meat [6, 10, 12, 13, 32]. Spread of the mcr-1 gene in the community and successively in the hospital would pose a threat to patients developing an infection with mcr-1 containing multidrug resistant isolates. Enterobacteriaceae resistant to both carbapenems and colistin by the presence of plasmid mediated mcr-1 have already been reported [17, 31, 33–35]. Therefore, screening for and isolation of mcr-1 containing patients should be considered. Prudence and close monitoring is necessary, especially when selective gut decontamination with colistin for ICU and hematological stem cell patients is common practice.
In conclusion, the current prevalence of mcr-1 suggests that spread from the community into the hospital environment is low, but cannot be excluded. Furthermore the finding of a colistin susceptible, mcr-1 containing E.coli underlines the importance of phenotypical confirmation after molecular screening.

Supporting information
S1 Table. Mcr real-time PCR and culture results of all 621 screened fecal samples.
(XLSX)

S2 Table. Accession numbers of the six contig containing mcr-1 positive, colistin susceptible E.coli.
(XLSX)

Acknowledgments
Preliminary results from this study were presented at the European Congress of Clinical Microbiology and Infectious Diseases, April 9–12, 2016, Amsterdam, the Netherlands [36].

Author Contributions
Conceptualization: EMT RHTN ECJC EJK.
Data curation: EMT RHTN MJTC CWK.
Formal analysis: EMT RHTN MJTC CWK.
Funding acquisition: MJTC EJK.
Investigation: EMT.
Methodology: EMT RHTN EJK ECJC.
Project administration: EMT EJK ECJC.
Resources: EMT MJTC.
Supervision: EJK ECJC.
Validation: EMT RHTN MJTC.
Visualization: EMT RHTN CWK MJTC.
Writing – original draft: EMT RHTN CWK MJTC.
Writing – review & editing: EMT RHTN CWK MJTC KEV JG ECJC EJK.

References
1. Grayson ML, Crowe SM, McCarthy JS, Mills J, Mouton JW, Ragnar NS, et al. Kucers’ The use of antibiotics. sixth edition ed. L NR, editor. London: Hachette UK; 2010. 3210 p.
2. Falagas ME, Rafailidis PI, Matthaiou DK. Resistance to polymyxins: Mechanisms, frequency and treatment options. Drug resistance updates: reviews and commentaries in antimicrobial and anticancer chemotherapy. 2010; 13(4–5):132–8. Epub 2010/09/17.
3. Catry B, Cavaleri M, Baptiste K, Grave K, Grein K, Holm A, 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. International journal of antimicrobial agents. 2015; 46(3):297–306. Epub 2015/07/29. https://doi.org/10.1016/j.ijantimicag.2015.06.005 PMID: 26215780
Mcr-1 prevalence in patients attending a tertiary care hospital, the finding of a mcr-1 containing, colistin susceptible E. coli

4. Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van der Geest S, van Tiel FH, et al. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. American journal of respiratory and critical care medicine. 2001; 164(3):382–8. Epub 2001/08/14. https://doi.org/10.1164/ajrccm.164.3.2005003 PMID: 11500337

5. Oostdijk EA, Keseicoglju J, Schultz MJ, Visser CE, de Jonge E, van Essen EH, et al. Effects of decontamination of the oropharynx and intestinal tract on antibiotic resistance in ICU: a randomized clinical trial. Jama. 2014; 312(14):1429–37. Epub 2014/10/02. https://doi.org/10.1001/jama.2014.7247 PMID: 25271544

6. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. The Lancet Infectious diseases. 2016; 16(2):161–8. https://doi.org/10.1016/S1473-3099(15)00424-7 PMID: 26603172

7. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Gooresses H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin. 2016; 21(27). Epub 2016/07/16.

8. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin. 2016; 21(9). Epub 2016/03/12.

9. Zeng KJ, Dui Y, Patil S, Huang X, Tian GB. Emergence of plasmid-mediated mcr-1 gene in colistin-resistant Enterobacter aerogenes and Enterobacter cloacae. Antimicrobial agents and chemotherapy. 2016. Epub 2016/03/16.

10. Haesman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersoo Y, et al. Detection of mcr-1 encoding plasmid-mediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro surveillance: bulletin European sur les maladies transmissibles = European communicable disease bulletin. 2015; 20(49).

11. Malhotra-Kumar S, Xavier BB, Das AJ, Lammens C, Hoang HT, Pham NT, et al. Colistin-resistant Escherichia coli harbouring mcr-1 isolated from food animals in Hanoi, Vietnam. The Lancet Infectious diseases. 2016.

12. Webb HE, Granier SA, Marault M, Millemann Y, den Bakker HC, Nightingale KK, et al. Dissemination of the mcr-1 colistin resistance gene. The Lancet Infectious diseases. 2016; 16(2):144–5. https://doi.org/10.1016/S1473-3099(15)00538-1 PMID: 26711363

13. Vasquez AM, Montero N, Laughlin M, Dancy E, Melmed R, Sosa L, et al. Investigation of Escherichia coli Harboring the mcr-1 Resistance Gene—Connecticut, 2016. MMWR Morbidity and mortality weekly report. 2016; 65(36):979–80. Epub 2016/09/16. https://doi.org/10.15585/mmwr.mm6536e3 PMID: 27631346

14. McGann P, Snersrud E, Maybank R, Corey B, Ong AC, Clifford R, et al. Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First Report of mcr-1 in the United States. Antimicrobial agents and chemotherapy. 2016; 60(7):4420–1. Epub 2016/05/28. https://doi.org/10.1128/AAC.01103-16 PMID: 27230792

15. Du H, Chen L, Tang YW, Kreiswirth BN. Emergence of the mcr-1 colistin resistance gene in carbapenem-resistant Enterobacteriaceae. The Lancet Infectious diseases. 2016.

16. Wang Y, Tian GB, Zhang R, Shen Y, Tyrrell JM, Huang X, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study. The Lancet Infectious diseases. 2017. Epub 2017/02/01.

17. Arcilia MS, van Hattem JM, Matamoros S, Melles DC, Penders J, de Jong MD, et al. Dissemination of the mcr-1 colistin resistance gene. The Lancet Infectious diseases. 2016; 16(2):147–9.

18. von Wintersdorff CJ, Wolffs PF, van Niekerk JM, Beuken E, van Alphen LB, Stobberingh EE, et al. Detection of the plasmid-mediated colistin-resistance gene mcr-1 in faecal metagenomes of Dutch travellers. The Journal of antimicrobial chemotherapy. 2016; 71(12):3416–9. Epub 2016/08/26. https://doi.org/10.1093/jac/dkw328 PMID: 27559117

19. Zurfluh K, Stephan R, Widmer A, Poirel L, Nordmann P, Nuesch HJ, et al. Screening for fecal carriage of MCR-producing Enterobacteriaceae in healthy humans and primary care patients. Antimicrobial
resistance and infection control. 2017; 6:28. Epub 2017/03/21. PubMed Central PMCID: PMCPMC5351167. https://doi.org/10.1186/s13756-017-0186-z PMID: 28316780

22. van Doornum GJ, Guldenmezester J, Osterhaus AD, Niesters HG. Diagnosing herpesvirus infections by real-time amplification and rapid culture. Journal of clinical microbiology. 2003; 41(2):576–80. Epub 2003/02/08. PubMed Central PMCID: PMCPmc149665. https://doi.org/10.1128/JCM.41.2.576-580.2003 PMID: 12574249

23. Nijhuis RH, Veldman KT, Schelfaut J, Van Essen-Zandbergen A, Wessels E, Claas EC, et al. Detection of the plasmid-mediated colistin-resistance gene mcr-1 in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay. The Journal of antimicrobial chemotherapy. 2016. Epub 2016/06/05.

24. Prim N, Rivera A, Rodriguez-Navarro J, Espanol M, Turbau M, Coll P, et al. Detection of mcr-1 colistin resistance gene in polyclonal Escherichia coli isolates in Barcelona, Spain, 2012 to 2015. Euro surveillance bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2016; 21(13). Epub 2016/04/09.

25. Liassine N, Assouvie L, Descombes MC, Tendon VD, Kieffer N, Poirel L, et al. Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract Enterobacteriaceae in Switzerland. International journal of infectious diseases: IJD: official publication of the International Society for Infectious Diseases. 2016; 51:4–5. Epub 2016/10/26.

26. Matsutani S. Multiple copies of IS10 in the Enterobacter cloacae MD36 chromosome. Journal of bacteriology. 1991; 173(24):7802–9. Epub 1991/12/01. PubMed Central PMCID: PMCPmc212570. PMID: 1660455

27. Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X, et al. Complete sequences of mcr-1-harboring plasmids from extended spectrum beta-lactamase (ESBL)- and carbapenemase-producing Enterobacteriaceae (CPE). Antimicrobial agents and chemotherapy. 2016. Epub 2016/04/20.

28. Doumith M, Godbole G, Ashton P, Larkin L, Dallman T, Day M, et al. Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales. The Journal of antimicrobial chemotherapy. 2016; 71(8):2300–5. Epub 2016/04/20. https://doi.org/10.1093/jac/dkw093 PMID: 27090630

29. Pham Thanh D, Thanh Tuyen H, Nguyen Thi Nguyen T, Chung The H, Wick RR, Thwaites GE, et al. Inducible colistin resistance via a disrupted plasmid-borne mcr-1 gene in a 2008 Vietnamese Shigella sonnei isolate. The Journal of antimicrobial chemotherapy. 2016. Epub 2016/06/02.

30. Mahillon J, Chandler M. Insertion sequences. Microbiology and molecular biology reviews: MMBR. 1998; 62(3):725–74. Epub 1998/09/06. PubMed Central PMCID: PMCPmc98933. PMID: 9729608

31. Faigenhauer L, Waezsada SE, Yao Y, Imirzalioglu C, Kasbohrer A, Roesler U, et al. Colistin resistance gene mcr-1 in extended-spectrum beta-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. The Lancet Infectious diseases. 2016.

32. Zurufku K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stephan R. Occurrence of the Plasmid-Borne mcr-1 Colistin Resistance Gene in Extended-Spectrum beta-Lactamase-Producing Enterobacteriaceae in River Water and Imported Vegetable Samples in Switzerland. Antimicrobial agents and chemotherapy. 2016; 60(4):2594–5. Epub 2016/02/18. PubMed Central PMCID: PMCPMC4808203. https://doi.org/10.1128/AAC.00066-16 PMID: 26883696

33. Yao X, Doi Y, Zeng L, Lv L, Liu JH. Carbapenem-resistant and colistin-resistant Escherichia coli co-producing NDM-9 and MCR-1. The Lancet Infectious diseases. 2016.

34. Poirel L, Kieffer N, Liassine N, Thanh D, Nordmann P. Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of Escherichia coli. The Lancet Infectious diseases. 2016; 16(3):281. Epub 2016/01/18.

35. Mulvey MR, Mataseje LF, Robertson J, Nash JH, Boerlin P, Toye B, et al. Dissemination of the mcr-1 colistin resistance gene. The Lancet Infectious diseases. 2016; 16(3):289–90. Epub 2016/03/15. https://doi.org/10.1016/S1473-3099(16)00067-0 PMID: 26973304

36. Terveer EM, Nijhuis RHT, Crobach MJ, Veldkamp KE, Goossens J, E.J. K, et al. Prevalence of colistin resistance gene (mcr-1) containing Enterobacteriaceae in feces of patients admitted to a tertiary care hospital in the Netherlands. Abstract ID 7590 Oral presentation 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, the Netherlands. 2016; April 9–12.