International Journal of Food Microbiology 291 (2019) 87–90

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu Province

X. Zhang[a,b,*], B. Zhang[a,b], Y. Guo[a,b], J. Wang[a,b], P. Zhao[c], J. Liu[c], K. He[a,b,*]

[a] Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Key Laboratory of Engineering Research of Veterinary Bio-products of Agricultural Ministry, Jiangsu Key Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base of Ministry of Science and Technology, Nanjing, China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China
[c] College of Veterinary Medicine, Henan University of Animal Husbandry and Economy, Zhengzhou, China

ARTICLE INFO

Keywords:
Colistin
Mcr gene
Bacteria
E. coli
Intensive breeding farms

ABSTRACT

The global dissemination of colistin resistance has received a great deal of attention. Recently, the plasmid-mediated colistin resistance encoded by *mcr* genes in *Escherichia coli* (*E. coli*) strains from animals, food, and patients in China has been reported frequently. To investigate the colistin resistance and *mcr*-1 *and mcr*-2 genes spread in domestic animals in Jiangsu Province, we collected fecael swabs from pigs, chicken and cattle at different ages distributed in intensive feeding farms. The selective chromogenic agar and *mcr*-PCR were used to screen the colistin resistance and *mcr* gene carriage. Colistin resistant *E. coli* colonies were identified in 54.25% (440/811), 35.96% (443/1232), and 26.92% (42/156) faecal swabs from pigs, chickens, and cattle, respectively. The prevalence of *mcr*-1 in colistin resistant *E. coli* isolates from pigs, chickens and cattle was 68.86% (303/440), 87.58% (388/443), and 71.43% (30/42), respectively, compared to *mcr*-2 which was present in 46.82% (206/440), 14.90% (66/443), and 19.05% (8/42) of the colistin-resistant *E. coli* isolated from pigs, chickens and cattle, respectively. Co-occurrence of *mcr*-1 and *mcr*-2 was identified in 20% (88/440) in pigs, 7.22% (32/443) in chickens, and 9.52% (4/42) in cattle. Interventions and alternative options are necessary to minimise further dissemination of *mcr* between food-producing animals and human.

1. Introduction

Colistin is recognized one of the last defence lines for the treatment of highly resistant bacteria, but the emergence of resistance to this vital antibiotic that is conferred by transferable plasmid-encoded mobilized colistin resistance genes (*mcr*-1 and *mcr*-2) is extremely disturbing. In November 2016 in *the Lancet Infectious Diseases*, Liu et al. reported finding a transferable plasmid-mediated *mcr* gene (*mcr*-1) in *E. coli* isolates from animal foods in China (Liu et al., 2016). After *mcr*-1 was reported, additional four *mcr* genes were discovered sequentially: *mcr*-2 was found in *E. coli* from calves and piglets in Belgium (Xavier et al., 2016), *mcr*-3 in *E. coli* from pigs in China (Yin et al., 2017), *mcr*-4 in *E. coli* and *Salmonella enterica* serovar Typhimurium from pigs in Italy, Spain and Belgium (Carattoli et al., 2017) and *mcr*-5 in *Salmonella* Paratyphi B from poultry in Germany (Borowiak et al., 2017). Among these findings, *mcr*-1 and *mcr*-2 explained majority of colistin resistance of multidrug-resistant *E. coli*. The prevalence of *mcr*-3 among *E. coli* isolates was lower than that of *mcr*-1 and *mcr*-2 (Hernández et al., 2017; Roer et al., 2017).

Actually, the mechanism of colistin resistance can be generally classified as *mcr*-independent or *mcr*-dependent. Compared with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, in *E. coli* colistin resistance was rarely mediated by chromosomal mutations, suggesting that these mutations might impose a fitness cost (Nicolet et al., 2016). This implies that *mcr* genes might be the major mechanism of colistin resistance in *E. coli*, and could promote colistin resistance transmission among bacteria by plasmid transfer and chromosomal recombination. The aim of this study is to determine the prevalence of colistin resistance and the molecular epidemiology of *mcr*-1 and *mcr*-2 mediated colistin resistance genes among farms in Jiangsu Province.

2. Materials and methods

2.1. Sample collection

From March 2015 to December 2016, a surveillance of colistin

* Corresponding authors at: Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, China.
E-mail address: liuxuehan1996@hotmail.com (X. Zhang).

https://doi.org/10.1016/j.ijfoodmicro.2018.11.013
Received 8 May 2018; Received in revised form 26 October 2018; Accepted 16 November 2018
Available online 22 November 2018
0168-1605/ © 2018 Elsevier B.V. All rights reserved.

Table 1
Categories of samples and numbers of colistin resistance-positive *E. coli*. and *mcr*-positive *E. coli*. in this study.

	Total	Colistin resistance	*mcr*-1 +	*mcr*-2 +	*mcr*-1 + *mcr*-2 +	[a]Others
Swine faecal swabs	811	440	303	206	88	19
Suckling piglets	199	38	23	14	3	4
Weaned piglets	211	86	52	34	8	8
Fattening pigs	220	162	111	80	33	4
Sows	181	154	117	78	44	3
Chicken faecal swabs	1232	443	388	66	32	21
Chicks	400	80	67	8	4	9
Egg-laying growers	406	152	134	19	7	6
Laying hens	426	211	187	39	21	6
Cattle faecal swabs	156	42	30	8	4	8
Calves	50	7	4	2	0	1
Growing cows	51	16	12	2	1	3
Milking cows	55	19	14	4	3	4

[a] colistin-resistant *E. coli* colonies were not positive amplification for *mcr*-1 and *mcr*-2 gene.

resistant *E. coli* was conducted in Jiangsu Province, China. A total of 2199 faecal swab samples (Table 1) were randomly collected from apparently healthy pigs, chicken and cattle. 811 faecal swab samples were collected from suckling piglets, weaned piglets, fattening pigs, and sows at 4 farms. 1232 faecal swab samples were collected from chicks, egg-laying growers and laying hens at 5 farms. These pig and poultry farms have been used colistin before it was banned on April 1st, 2017 as a therapeutic drug and feed additive to animals by Chinese government. 156 faecal samples were collected from calves, growing cows and milking cows at 2 farms that seldom have been used colistin, but one of them close to a poultry farm, and one of them is located the downwind area of a poultry farm.

2.2. Colistin resistance screening

E. coli has been identified as an index for monitoring drug resistance (Guyonnet et al., 2010; Rhouma et al., 2016a, 2016b). Here, we used *E. coli* selective chromogenic agar with 10 μg/mL of colistin sulphate (MMWR, 2016) to test drug resistance to *E. coli* in domestic animal faeces. Each swab was dipped in 2 mL PBS for two hours at 4 °C, and then mixed by vortex. The homogenates were centrifuged at 23 RCF for 15 min to remove plant debris. After the aspirated supernatants were centrifuged at 12,000 RCF 13523 for 5 min, the pellets were suspended with 1 mL PBS. One-hundred microliters of tenfold dilution series of the suspended pellets were plated onto *E. coli* selective chromogenic agar (HopeBio Biotech Corp., China) containing colistin sulphate. After overnight incubation at 37 °C, the blue-green colonies were considered presumptive *E. coli* isolates (HopeBio Biotech Corp., China). If necessary, the faecal swabs were dropped into Tryptic Soy Broth (TSB) with antibiotics for enrichment and then bacterial culture was plated onto *E. coli* selective chromogenic agar (HopeBio Biotech Corp., China).

2.3. Confirmation of E. coli isolates and screening mcr-1 and mcr-2

All blue-green colonies were picked into Luria-Bertani (LB) broth for 6 h growth, and bacterial cultures were used to prepare DNA template by conventional boiling method. For the *E. coli* colonies identified, PCR was used to verify them by primer pairs P1-F and P1-R (Miyaguchi et al., 1996) from the 16S rRNA gene. For *mcr-1* gene and *mcr*-2 screening, primer pairs P2-F/R (MMWR, 2016) and P3-F/R (Xavier et al., 2016), respectively, were used for PCR amplification. All amplicons, from *mcr* genes and 16S rRNA, were sequenced by Genscript Corporation (Nanjing, China). Primers used in this study were listed in Table 2.

Table 2
Primers used in this study.

Primer	Nucleotide sequences (5′-3′)	Gene name	Length (bp)
P1-F/R	agagtttgatcatggctcag aaggaggtgatccaaccgca	16srRNA	1543
P2-F/R	atgatgcagcatacttctgtgtggt tcagcggatgaatgcggtgcggtc	*mcr*-1	1626
P3-F/R	tggtacagccccttttatt gcttgagattgggttatga	*mcr*-2	1747

3. Result

3.1. Plate screening for colistin-resistant E. coli colonies

All blue-green colonies from *E. coli* selective chromogenic agar were considered presumptive *E. coli* isolates and confirmed by PCR amplification and sequencing of a part of the 16S rRNA gene. In pigs, colistin resistant colonies were identified from 19.10% (38/199) of sucking piglets, 40.76% (86/211) of weaned piglets, 73.64% (162/220) of fattening pigs, and 85.08% (154/181) of sows. In chickens, colistin resistant colonies were identified from 20% (80/400) of chicks, 37.44% (152/406) of egg-laying growers, and 49.53% (211/426) of laying hens. In cattle, colistin resistant colonies were identified from 14% (7/50) of calve, 31.37% (16/51) of growing cows, and 34.55% (19/55) of milking cows. Data on prevalence of colistin resistance in swab samples from all ages of domestic animals are presented in Table 2.

3.2. Prevalence of mcr-1

The *mcr*-1 was identified in colistin-resistant *E. coli* colonies from all ages of pigs, chickens, and cattle. *E. coli* yielding a *mcr*-1 PCR product was isolated 68.86% (303/440) in pigs, 87.58% (388/443) in chicken, and 71.43% (30/42) in cattle (Table 1). For pigs, the *mcr*-1 PCR product was amplified from *E. coli* isolated from 60.53% (23/38) of suckling piglets, 60.47% (52/86) of weaned piglets, 68.52% (111/162) of fattening pigs, and 75.97% (117/154) of sows. For chickens, the *mcr*-1 PCR product was amplified from *E. coli* isolated from 83.75% (67/80) of chicks, 88.16% (134/152) of egg-laying growers, and 88.63% (187/211) of laying hens. For cattle, the *mcr*-1 PCR product was amplified from *E. coli* isolated from 57.14% (4/7) of calves, 75.00% (12/16) of growing cows, and 73.68% (14/19) of milking cows.

3.3. Prevalence of mcr-2

The *mcr*-2 was identified in colistin-resistant *E. coli* colonies from all ages of pigs, chickens, and cattle. *E. coli* yielding a *mcr*-2 PCR product was isolated 46.82% (206/440) in pigs, 14.90% (66/443) in chicken,

and 19.05% (8/42) in cattle (Table 1). For pigs, the *mcr*-2 PCR product was amplified from *E. coli* isolated from 36.84% (14/38) of suckling piglets, 39.53% (34/86) of weaned piglets, 49.38% (80/162) of fattening pigs, and 50.65% (78/154) of sows. For chickens, the *mcr*-2 PCR product was amplified from *E. coli* isolated from 10% (8/80) of chicks, 12.50% (19/152) of egg-laying growers, and 18.48% (39/211) of laying hens. For cattle, the *mcr*-2 PCR product was amplified from *E. coli* isolated from 28.57% (2/7) of calves, 12.50% (2/16) of growing cows, and 21.05% (4/19) of milking cows.

3.4. Co-occurrence of mcr-1 and mcr-2

E. coli yielding both *mcr*-1 and *mcr*-2 PCR product was isolated 20% (88/440) in pigs, 7.22% (32/443) in chickens, and 9.52% (4/42) in cattle (Table 1). Dual positivity was identified in 7.89% (3/38) of suckling piglets, 9.30% (8/86) of weaned piglets, 20.37% (33/162) of fattening pigs, 28.57% (44/154) of sows, 5.00% (4/80) of chicks, 4.61% (7/152) of egg-laying growers, 9.95% (21/211) of laying hens, 6.25% (1/16) of growing cows, and 15.79% (3/19) of milking cows, but not in calves.

4. Discussion

In the 1960s colistin was introduced into food animal production in several countries for growth promotion, therapeutics and prophylactics purposes to control *Enterobacteriaceae* infections, particularly for those caused by *E. coli* (Guyonnet et al., 2010; Rhouma et al., 2016a). However, with the recent discovery of plasmid-mediated colistin resistance encoded by the *mcr* genes (Liu et al., 2016; Yassin et al., 2017; Zhang et al., 2018) and the higher prevalence of samples harboring these genes in animal isolates compared to other origins, livestock and poultry has been recognized as the major reservoir for colistin resistance amplification and spread (Hoelzer et al., 2017).

During 2015–2016, we collected 2199 faecal swabs from pigs, chicken and cattle to determine the prevalence of colistin resistance in intensive breeding farms of Jiangsu Province. Our study using selective chromogenic agar with colistin showed that *E. coli* resistance to colistin occurred widely in pigs (54.25%), poultry (35.96%) and cattle (26.92%), suggesting that colistin resistance was considerably serious, especially in pigs. From 2013 to 2014, it was reported that a high frequency of colistin resistance in *E. coli* from pigs (26.5%), from chickens (14.0%), and from cattle (0.9%) on farms in different geographic areas of China, including Jiangsu Province (CVDA, 2014). Increasing use of colistin in fodder in recent years may be the reason of the high prevalence of colistin resistance in these food animals. Here, in 811 pig samples, colistin resistant colonies were identified from 85.08% (154/181) of sows and 73.64% (162/220) of fattening pigs, significantly higher than 19.10% (38/199) of sucking piglets, 40.76% (86/211) of weaned piglets. The same patterns were also observed in chicken and cattle samples. The highest proportions of resistant *E. coli* colonies were identified from the adult animals, implying that the long-term selective pressure resulted in not only the highest prevalence of colistin resistance among *E. coli* isolates from adult animals found in this study, but also bacterial evolution and adaptation from the piglet groups to adult groups (Jans et al., 2018). Compared with the isolates from pigs and chickens recovered during 2013–2014, *E. coli* isolates collected during 2007–2008 (5.5%) and 2010–2011 (12.4%) showed significantly lower frequency of colistin resistance (Liebana et al., 2013). A high frequency of colistin resistance in *E. coli* from pigs on farm (24.1%) and from chickens on farm (14.0%) led to a high prevalence of colistin at pig slaughter (24.3%) and chicken slaughter (9.5%) in 2013–2014 (Liebana et al., 2013). As adult animals generally entered the slaughter house and the food chains, drug-resistant *E. coli* potentially were transmitted to consumers, increasing risk of disease. Sows appear to be a significant reservoir for colistin-resistant *E. coli*, they give not only life to piglets, but also resistant strains to them, which promote drug resistance circulation among Chinese farms (Olaitan et al., 2015). The link between animals and humans in terms of colistin resistant *E. coli* strain transfer following direct contact has recently been confirmed (Rhouma et al., 2016b). The overuse of antibiotics will promote the unrestricted expansion and circulation of drug-resistant strains among human-animals-environment.

While colistin is a last-line antibiotic used to treat multidrug resistant Gram-negative bacteria isolated from food animals, raw meat, and humans in several countries (Rhouma et al., 2015), its efficacy is being compromised by the mobile colistin resistance genes, *mcr*-1 first detected at the end of 2015 (Liu et al., 2016), and subsequently *mcr*-2, *mcr*-3, *mcr*-4, *mcr*-5 (Xavier et al., 2016; Fukuda et al., 2018). Of all the colistin resistant *E. coli* colonies, the *mcr*-1 was the predominant gene for the colistin resistance of *E. coli*, higher than *mcr*-2. In our study, the *mcr*-1 prevalence was 68.86% (303/440) in pigs, 87.58% (388/443) in chicken, and 71.43% (30/42) in cattle, compared with *mcr*-2 prevalence of 46.82% (206/440) in pigs, 14.90% (66/443) in chicken, and 19.05% (8/42) in cattle. Directly testing samples with the *mcr*-PCRs (Zhang et al., 2018) gave far higher prevalence than testing isolates from samples, the *mcr*-1 and *mcr*-2 occurred more widely than other *mcr* variants in pigs and poultry of Chinese farms (Zhang et al., 2018; Yassin et al., 2017). Except harboring the *mcr* genes, a *mcr*-independent mechanism behind the remaining colistin resistant *E. coli* colonies, for example, lipopolysaccharide modification (Guerin et al., 2016), other (transferable) colistin-resistant mechanisms. The *mcr*-independent and *mcr*-dependent mechanisms together promote colistin-resistant wide spread in *E. coli*, even in enteric bacteria, which will cause fatal drug resistance to animals, even humans. Since 1 April 2017, the Chinese government has implemented the withdrawal of colistin as a food additive for growth promotion in food animal (Wang et al., 2017). This policy is in line with One Health framework (FAO et al., 2017).

5. Conclusion

Our study will provide new data about colistin resistance prevalence worldwide. Colistin resistant *E. coli* was recovered from food animals of all ages at a high frequency and the *mcr*-dependent mechanism dominated in *E. coli*. We also found that the older and adult animals were a reservoir of resistant strains, suggesting a potential food safety issue and greater public health problems.

Author contributions

Zhang XH and He KW conceived and designed the experiments. Zhang BC analyzed the data. Yu ZY, Guo YY, Wang J, Zhao PD, and Liu JJ performed the experiments; Zhang XH and Zhang BC wrote the paper.

Acknowledgements

Funding: This work was supported by the Jiangsu Key Research and Development plan (BE2017341-1), National Natural Science Foundation of China (31572503) and the National Key Research and Development Program of China (2018YFD0500500). *Competing interests*: The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethical approval: Ethical approval was granted by the Ethics Committee of the Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences (Nanjing, Jiangsu, China) [20150212].

References

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.

Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., 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, 30589.

China Veterinary Drug Association (CVDA), 2014. Annual Report on Development of Veterinary Medicine Industry in China (2012). China Agriculture Press, Beijing.

Food and Agriculture Organization (FAO), World Animal Health Organization (OIE), World Health Organization (WHO), 2017. Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment. Geneva, Switzerland, 1–5 December 2003. http://www.who.int/foodsafety/publications/amr-management/en/, Accessed date: 26 October 2017.

Fukuda, A., Sato, T., Shinagawa, M., Takahashi, S., Asai, T., Yokota, S-i., Usui, M., Tamura, Y., 2018. High prevalence of *mcr-1*, *mcr-3*, and *mcr-5* in *Escherichia coli* derived from diseased pigs in Japan. Int. J. Antimicrob. Agents 51 (1), 163–164.

Guerin, F., Isnard, C., Sinel, C., Morand, P., Dhalluin, A., Cattoir, V., Giard, J.C., 2016. Cluster-dependent colistin hetero-resistance in Enterobacter cloacae complex. J. Antimicrob. Chemother. 71 (11), 3058–3061.

Guyonnet, J., Manco, B., Baduel, L., Kaltsatos, V., Aliabadi, M.H., Lees, P., 2010. Determination of adosage regimen of colistin by pharmacokinetic/pharmacodynamics integration and modeling for treatment of G.I.T. disease in pigs. Res. Vet. Sci. 88, 307–314.

Hernández, M., Iglesias, M.R., Rodríguez-Lázaro, D., Gallardo, A., Quijada, N., Miguela-Villoldo, P., 2017. Co-occurrence of colistin-resistance genes *mcr-1* and *mcr-3* among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. Euro Surveill. 22, 30586.

Hoelzer, K., Wong, N., Thomas, J., Talkington, K., Jungman, E., Coukell, A., 2017. Antimicrobial drug use in food-producing animals and associated human health risks: what, and how strong, is the evidence? BMC Vet. Res. 13 (1), 211.

Jans, C., Sarno, E., Collineau, L., Meile, L., Stärk, K.D.C., Stephan, R., 2018. Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. Front. Microbiol. 9, 362.

Liebana, E., Carattoli, A., Coque, T.M., Hasman, H., Magiorakos, A.P., Mevius, D., Peixe, L., Poirel, L., Schuepbach-Regula, G., Torneke, K., Torren-Edo, J., Torres, C., Threlfall, J., 2013. Public health risks of enterobacterial isolates producing extendedspectrum beta-lactamases or AmpC beta-lactamases in food and food producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. Clin. Infect. Dis. 56, 1030–1037.

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 (2), 161–168.

Miyaguchi, H., Narita, H., Sakamoto, K., 1996. An antibiotic-binding motif of an RNA fragment derived from the A site related region of *E. coli* 16S rRNA. Nucleic Acids Res. 24, 3700–3706.

Morbidity and Mortality Weekly Report (MMWR), 2016. Investigation of *E. coli* Harboring the *mcr*-1Resistance Gene — Connecticut, 2016. 65(36). pp. 979–980.

Nicolet, S., Goldenberger, D., Schwede, T., Page, M., Creus, M., 2016. Plasmid-mediated colistin resistance in a patient infected with *Klebsiella* pneumoniae. Lancet Infect. Dis. 16 (9), 998–999.

Olaitan, A.O., Thongmalayvong, B., Akkhavong, K., Somphavong, S., Paboriboune, P., Khounsy, S., Morand, S., Rolain, J.M., 2015. Clonal transmission of a colistin-resistant *Escherichia coli* from a domesticated pig to a human in Laos. J. Antimicrob. Chemother. 70, 3402–3404.

Rhouma, M., Beaudry, F., Thériault, W., Bergeron, N., Laurent-Lewandowski, S., Fairbrother, J.M., Letellier, A., 2015. Gastric stability and oral bioavailability of colistin sulfate in pigs challenged or not with *Escherichia coli* O149: F4(K88). Res. Vet. Sci. 102, 173–181.

Rhouma, M., Beaudry, F., Letellier, A., 2016a. Resistance to colistin: what is the fate for this antibiotic in pig production? Int. J. Antimicrob. Agents 48, 119–126.

Rhouma, M., Beaudry, F., Thériault, W., Letellier, A., 2016b. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front. Microbiol. 11, 7.

Roer, L., Hansen, F., Stegger, M., Sönksen, U.W., Hasman, H., Hammerum, A.M., 2017. Novel *mcr-3* variant, encoding mobile colistin resistance, in an ST131 *Escherichia coli* isolate from bloodstream infection, Denmark, 2014. Euro Surveill. 22, 22846.

Wang, X., Liu, Y., Qi, X., Wang, R., Jin, L., Zhao, M., Zhang, Y., Wang, Q., Chen, H., Wang, H., 2017. Molecular epidemiology of colistin-resistant Enterobacteriaceae in inpatients and avian from China: high prevalence of *mcr*-negative *Klebsiella pneumoniae*. Int. J. Antimicrob. Agents 50 (4), 536–541.

Xavier, B.B., Lammens, C., Ruhal, 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).

Yassin, A.K., Zhang, J., Wang, J., Chen, L., Kelly, P., Butaye, P., Lu, G., Gong, J., Li, M., Wei, L., Wang, Y., Qi, K., Han, X., Price, S., Hathcock, T., Wang, C., 2017. Identification and characterization of *mcr* mediated colistin resistance in extra-intestinal *Escherichia coli* from poultry and livestock in China. FEMS Microbiol. Lett. 364 (24).

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. MBio 8 (e00543-17).

Zhang, J., Chen, L., Wang, J., Yassin, A.K., Butaye, P., Kelly, P., Gong, J., Guo, W., Li, J., Li, M., Yang, F., Feng, Z., Jiang, P., Song, C., Wang, Y., You, J., Yang, Y., Price, S., Qi, K., Kang, Y., Wang, C., 2018. Molecular detection of colistin resistance genes (*mcr*-1, *mcr*-2 and *mcr*-3) in nasal/oropharyngeal and anal/cloacal swabs from pigs and poultry. Sci. Rep. 8 (1), 3705.