Retrospective Screening and Analysis of mcr-1 and bla_{NDM} in Gram-Negative Bacteria in China, 2010–2019

Rong Fan\(^1\*,\) Chuchu Li\(^{1,2}†,\) Ran Duan\(^1†,\) Shuai Qin\(^1†,\) Junrong Liang\(^1,\) Meng Xiao\(^1,\)
Dongyue Lv\(^1,\) Huaiqi Jing\(^1\) and Xin Wang\(^{1,*}\)

\(^1\) State Key Laboratory of Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases – National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, \(^2\) Department of Acute Infectious Disease Control and Prevention, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China

Currently, Gram-negative bacteria have developed multidrug and broad-spectrum drug resistance, and the numbers of species and strains carrying \(mcr\) or \(bla_{NDM}\) genes are increasing. In this study, \(mcr-1\) and \(bla_{NDM}\) distribution of 12,858 Gram-negative bacteria isolated from wildlife, patients, livestock, poultry and environment in 14 provinces of China from 2010 to 2019 and the antibiotics resistance in regard to polymyxins (polymyxin B and colistin) and carbapenems of positive strains were investigated. A total of 70 strains of 10 species carried the \(mcr-1\) gene, positive rates of patients, livestock and poultry, and environmental strains were 0.62% (36/5,828), 4.07% (29/712), 5.43% (5/92), respectively. Six strains of 3 species carrying the \(bla_{NDM}\) gene all came from patients 0.10% (6/5,828). Two new \(mcr-1\) gene variants (GenBank: MK965883, MK965884) were identified, one of which contains premature stop codon. The drug susceptibility results showed that all \(mcr-1\) carriers were sensitive to carbapenems, among which, 66 strains were resistant and 4 were sensitive to polymyxins. The strains with the \(bla_{NDM}\) gene had different degrees of resistance to carbapenems and were sensitive to polymyxins. The findings that species carrying \(mcr-1\) or \(bla_{NDM}\) genes were limited and mostly normal flora of opportunistic or low pathogenic organisms indicated that transfer of \(mcr-1\) and \(bla_{NDM}\) genes between bacteria was relatively limited in China. The none detection among wildlife compared with other sources supports the speculation that the emergence of and increase in polymyxins and carbapenem-resistant strains was mainly related to the selective pressure of antibiotics.

Keywords: MCR, NDM, polymyxin, carbapenem, Gram-negative

INTRODUCTION

Bacterial resistance has been a global public health concern (Nolte, 2014; Alos, 2015). Currently, Gram-negative bacteria are developing multidrug resistance and broad-spectrum drug resistance, and the available antibiotics used in clinical treatment, agricultural and livestock production are gradually decreasing (Abdelaouf et al., 2017; Theuretzbacher, 2017). Polymyxins and carbapenems are among the antibiotics of last resort to treat Gram-negative bacteria infections. In 2009 and 2016,
the superbug that carried the New Delhi metallo-beta-lactamase gene (\textit{bla}\textsubscript{NDM–1}) (Yong et al., 2009; KumaraSamy et al., 2010) and the \textit{Escherichia. coli} that carried the colistin resistance gene (\textit{mcr–1}) (Liu et al., 2016) were identified. Until now, 9 subtypes of \textit{mcr} (Liu et al., 2016; Xavier et al., 2016; AbuOun et al., 2017; Borowiak et al., 2017; Carattoli et al., 2017; Yin et al., 2017; Wang X. et al., 2018; Yang et al., 2018; Carroll et al., 2019) and 21 subtypes of \textit{bla}\textsubscript{NDM} (Liu et al., 2018) have been published with papers. The discovery of these two types of resistance genes that can be horizontally transferred via plasmids has made researchers aware of the post-antibiotic era. Such plasmids usually carry other resistance genes, encoded for aminoglycosides and quinolones for instance (Carattoli, 2013; Rozwandowicz et al., 2018). The rapid horizontal spread of drug-resistant genes by plasmids is one of the reasons for the increasing number of multidrug resistant bacteria, however, the transfer range of bacteria species is unknown. Studies have confirmed that many countries and regions have isolated Gram-negative bacteria with \textit{mcr–1} gene (Fernandes et al., 2016; Hadjadj et al., 2017; Liu et al., 2017a), the \textit{bla}\textsubscript{NDM–1} gene (Wang et al., 2014; Michael et al., 2015; Zenati et al., 2016; Abderrahim et al., 2017; Madec et al., 2017; Riazzo et al., 2017; Yu et al., 2017), or both (Zheng et al., 2016; Liu et al., 2017b; Wang R. et al., 2018), from humans, the environment and animals. Whether isolates from antibiotics-free and depopulated areas, wildlife, carried \textit{mcr} or \textit{bla}\textsubscript{NDM} genes is undiscovered.

To explore the transfer range of polymyxins and carbapenems resistance genes, 12,858 Gram-negative isolates of ≥188 species were retrospectively collected. Considering limited genes can be screened for the 12,858 isolates, only the \textit{mcr–1} and \textit{bla}\textsubscript{NDM} genes were chosen, for their wide dissemination around the world and across species (López et al., 2019). To confirm the effect of antibiotics on the emergence of resistant strains, strains isolated from wildlife, patients, livestock, poultry, and environment from past 10 years were screened. Strains positive with either of the gene were confirmed by open reading frame (ORF) sequencing, and were tested for antimicrobial susceptibility.

**MATERIALS AND METHODS**

**Bacteria Isolation and Identification**

We retrospectively collected 12,858 Gram-negative bacteria that were isolated from wildlife (6,226/12,858), patients (5,828/12,858), livestock and poultry (712/12,858), and environment (92/12,858) in 14 provinces (Anhui, Beijing, Gansu, Guangxi, Guizhou, Hainan, Hunan, Jiangxi, Ningxia, Qinghai, Sichuan, Tianjin, Yunnan, Zhejiang) of China from 2010 to 2019. The study was approved by the ethics review committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. Informed consent was obtained from participants. All strains were identified using VITEK II Compact system (bioMérieux, France) or API 20E strips (bioMérieux, France). The \textit{mcr–1} or \textit{bla}\textsubscript{NDM} gene positive strains were identified again by VITEK II Compact system (bioMérieux, France), the results of which were consistent with the original ones.

**Screening and ORF Sequencing of \textit{mcr–1} or \textit{bla}\textsubscript{NDM} Positive Strains**

DNA templates were extracted by using TIANamp Bacteria DNA Kit. Positive controls were used for PCR. All 12,858 Gram-negative bacteria were screened for \textit{mcr–1} and \textit{bla}\textsubscript{NDM} gene by screening primers (Table 1), and ORF of positive strains were further amplified, cloned and sequenced. The \textit{mcr–1} or \textit{bla}\textsubscript{NDM} positive strains were confirmed only if ORF sequences were obtained.

**Antimicrobial Susceptibility Testing**

The minimum inhibitory concentrations (MICs) of polymyxins (polymyxin B and colistin) and carbapenems of 70 strains with \textit{mcr–1} and 6 strains with \textit{bla}\textsubscript{NDM} were determined by broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2019). Replication of sensitivity testing was conducted. Quality controls and breakpoints were in accordance with the European Committee on Antimicrobial Susceptibility Testing (2019) and the Clinical and Laboratory Standards Institute (2019) for polymyxins and carbapenems, respectively.

**RESULTS**

**Distribution of \textit{mcr–1} or \textit{bla}\textsubscript{NDM} Positive Strains**

A total of 70 strains with \textit{mcr–1} and 6 strains with \textit{bla}\textsubscript{NDM} were confirmed. \textit{mcr–1} positive strains were isolated each year from 2010 to 2019. Strains with \textit{bla}\textsubscript{NDM} were isolated in 2012, 2013, 2014, and 2016 respectively.

| Primer | Sequence (5′ → 3′) | Product size (bp) | Annealing temperature(°C) |
|---|---|---|---|
| \textit{mcr–1} | MCR-1_CLRS-F CCGGTCGACAGATGAGCGG 309\textsuperscript{a} | 54 |
| | MCR-1_CLRS-R TCTGTGCTGTGCTAGGG | |
| \textit{bla}\textsubscript{NDM} | NDM-1_17U-F AGACACACCTTCTATCTT 292\textsuperscript{a} | 54 |
| | NDM-1_17U-R CCGCAACCACCTCCTT | |
| \textit{mcr–1} | mcr–1FL-F AGAAGACCTGGGTGTAGATAT 2189\textsuperscript{b} | 54 |
| | mcr–1FL-R GCCATGACAAGAGCGATACGATA | |
| \textit{mcr–1} | FR-mcr-FL-F CATCAATCACTGAGGCGCG 2060\textsuperscript{b} | 54 |
| | FR-mcr-FL-R CTGATCTGCAAGAGGAGGC | |
| \textit{andra} | DR-mcr-FL-F GCAAGATATGGCCTGAAT 1841\textsuperscript{a} | 50 |
| | DR-mcr-FL-R CGACGTGCTGCTAACAGGG | |
| \textit{bla}\textsubscript{NDM} | 21U-FL-F TGCACAACACGCTCTG 1007\textsuperscript{b} | 54 |
| | 21U-FL-R GAAATGTCGACACTCAT | |

\textsuperscript{a}Screening, \textsuperscript{b}ORF amplification.

Frontiers in Microbiology | www.frontiersin.org 2 February 2020 | Volume 11 | Article 121
since 2012. The positive rate from 2012 to 2019 was 0.31% (2/649), 0.09% (1/1,169), 0.63% (7/11,105), 0.36% (9/2,520), 0.64% (34/5334), 0.28% (4/1,420), 2.12% (9/424), and 8.16% (4/49), respectively. The isolation rates of \( \text{bla}_{\text{NDM}} \) positive strains were 0.08% (2/2,520) in 2015, 0.06% (3/5,334) in 2016, and 0.24% (1/424) in 2018 (Table 2). Among the 70 strains with \( mcr-1 \), 36 were isolated from patients, with a positive rate of 0.62% (36/5,828) (Table 3). Thirty-five were isolated from diarrheal stool and 1 was from the sputum of an acute-pancreatitis patient. Twenty-nine strains were isolated from livestock and poultry specimens, with a positive rate of 4.07% (29/712), of which 24 isolates were from pig feces and 5 were from chicken feces. Five strains were isolated from environmental specimens, related to chicken slaughter, with a positive rate of 4.07% (29/712), 1 strain of \( \text{Enterobacter} \) pneumoniae ssp. pneumoniae, 2 strains of \( \text{Klebsiella pneumoniae} \) ssp. pneumoniae and 1 strain of \( \text{Klebsiella oxytoca} \).

**Table 2** | The positive rate of strains with \( mcr-1 \) or \( \text{bla}_{\text{NDM}} \) isolated in different years.

| Year | \( mcr-1 \) positive strains (%) | \( \text{bla}_{\text{NDM}} \) positive strains (%) |
|------|---------------------------------|---------------------------------|
| 2010 | None                            | None                            |
| 2011 | None                            | None                            |
| 2012 | 2 (0.31)                        | None                            |
| 2013 | 1 (0.09)                        | None                            |
| 2014 | 7 (0.63)                        | None                            |
| 2015 | 9 (0.36)                        | 2 (0.08)                        |
| 2016 | 34 (0.64)                       | 3 (0.06)                        |
| 2017 | 4 (0.28)                        | None                            |
| 2018 | 9 (2.12)                        | 1 (0.24)                        |
| 2019 | 4 (8.16)                        | None                            |
| Total| 70 (0.54)                       | 6 (0.05)                        |

None: no strain positive for the \( mcr-1 \) or \( \text{bla}_{\text{NDM}} \) gene.

**Table 3** | The positive rate of strains with \( mcr-1 \) or \( \text{bla}_{\text{NDM}} \) isolated from different sources.

| Source                | No. strains | \( mcr-1 \) positive strains (%) | \( \text{bla}_{\text{NDM}} \) positive strains (%) |
|-----------------------|-------------|---------------------------------|---------------------------------|
| Wildlife              | 6,226       | None                            | None                            |
| Livestock and poultry | 712         | 29 (4.07)                       | None                            |
| Environment           | 92          | 5 (5.43)                        | None                            |
| Patients              | 5,828       | 36 (0.62)                       | 6 (0.10)                        |
| Total                 | 12,858      | 70 (0.54)                       | 6 (0.05)                        |

None: no strain positive for the \( mcr-1 \) or \( \text{bla}_{\text{NDM}} \) gene.

**Sequence Analysis of \( mcr-1 \) and \( \text{bla}_{\text{NDM}} \)**

The sequence alignment of the ORF showed that 68 of 70 \( mcr-1 \) positive strains possessed an identical sequence compared to the reference sequence of the \( mcr-1 \) gene, 1626 bp (NCBI Reference Sequence: KP347127, region: 22413-24038) (Figure 1). Among these strains, 65 were resistant while 3 were sensitive to polymyxins. The additional 2 \( mcr-1 \) positive strains had a single base mutation compared to the reference sequence. One possessed \( mcr-1.21 \) (GenBank: MK965883), an \( Escherichia coli \) isolated from pig feces in Qinghai in 2016, which mutated at 1234nt of reference sequence from C to T, resulting in a proline to serine change at the amino acid level. This strain shows resistance to polymyxins. The other strain possessed MK965884, an AEAC isolated from the stool of a diarrhea patient in Beijing in 2012. A base mutation site was located at 1344 nt of the reference sequence from G to A, resulting in a stop codon. The susceptibility results showed sensitivity to polymyxins.

Of 6 \( \text{bla}_{\text{NDM}} \) positive strains, 3 had identical sequences compared to the 813 bp reference sequence in the ORF of the \( \text{bla}_{\text{NDM}}-1 \) gene (NCBI Reference Sequence: FN396876 REGION: 2420-3232), 2 were identical to the \( \text{bla}_{\text{NDM}}-3 \) gene (NCBI Reference Sequence: JQ734687 REGION: 1-813), and 1 was identical to the 813 bp reference sequence in the ORF of the \( \text{bla}_{\text{NDM}}-5 \) gene (NCBI Reference Sequence: JN104597 REGION: 115-927).

**Antimicrobial Susceptibility Result**

According to clinical breakpoint of carbapenem antibiotics, CLSI, all 70 strains carrying \( mcr-1 \) showed sensitivity to ertapenem, imipenem, and meropenem. Among the 6 strains with \( \text{bla}_{\text{NDM}} \), 5 were resistant to ertapenem (MIC > 4 \( \mu \)g/ml), imipenem (3 strains MIC > 4 \( \mu \)g/ml, 2 strains MIC = 4 \( \mu \)g/ml) and meropenem (MIC > 4 \( \mu \)g/ml), while the other strain was resistant to ertapenem (MIC > 4 \( \mu \)g/ml) but intermediate to imipenem (MIC = 2 \( \mu \)g/ml) and meropenem (MIC = 2 \( \mu \)g/ml). According to clinical breakpoint of EUCAST, among the 70 strains with the \( mcr-1 \) gene, 66 were resistant to polymyxins (polymyxin B: 6 strains MIC = 4 \( \mu \)g/ml, 33 strains MIC = 8 \( \mu \)g/ml, 27 strain MIC > 8 \( \mu \)g/ml. colistin: 9 strains MIC = 4 \( \mu \)g/ml, 47 strains MIC = 8 \( \mu \)g/ml, 10 strain MIC > 8 \( \mu \)g/ml) and the other four were sensitive to polymyxins (polymyxin B: 3 strains MIC = 1 \( \mu \)g/ml, 1 strain MIC = 0.5 \( \mu \)g/ml colistin: 4 strains MIC = 0.5 \( \mu \)g/ml) (Table 4). Six strains with \( \text{bla}_{\text{NDM}} \) were sensitive to polymyxins (Figure 3). The sensitivity data for all strains referred to Supplementary Table S1.
**DISCUSSION**

The increase in Gram-negative bacterial strains carrying the *mcr* or *bla*NDM genes has led global health workers to reconsider infections and the treatment of bacteria infection (Kumarasamy et al., 2010). A total of 12,858 strains from ≥118 species of Gram-negative bacteria were screened, only to find 10 species carrying the *mcr-1* gene and 3 species carrying *bla*NDM gene,
indicating the species carrying these two genes and interspecific transfer of the two resistance genes were limited. The bacteria carrying these two resistance genes were mainly normal flora of opportunistic or low pathogenic organisms. Bacterial resistance is an ecological feature to maintain the lineage extension of the bacteria. The emergence of drug-resistant strains has a relationship with the widespread use of antibiotics (Liu et al., 2016). In fact, drug-resistant strains have existed for a long time (Haenni et al., 2016). Our study shows that the use of antibiotics may change the number of resistant strains, but it does not have an essential effect on the frequency of gene transfer. For example, metallo-β-lactamases itself is crucial in defining bacterial host specificity. The wider dissemination of NDM among different bacterial hosts, compared to VIM-2 and SPM-1, is mainly due to unique and singular features of this protein (López et al., 2019). In Gram-positive pathogens, the emergence of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) were alarming (Centers for Disease Control and Prevention, 2002). However, as of May 2015, only 14 VRSA infections have been reported in patients from the United States (Walters et al., 2015).

The total detection rate of this study was lower than other studies, mainly because 48.42% (6,226/12,858) of the strains were isolated from wildlife, while neither mcr-1 nor blaNDM genes were positive. Among the wildlife, mammals, plateau pika and bats live away from humans in this study. Rodents besides marmot (such as Rattus flavipespect and Rattus norvegicus) and birds mainly live between human surroundings and lands away from humans. The existence of antibiotic selective pressure away from humans is minimal, where no strain was found carrying mcr-1 or blaNDM gene. Among other sources, the highest detection rate of mcr-1 was found in strains from the environment, which related to chicken slaughter (5.43%, 5/92), followed by livestock and poultry (4.07%, 29/712). Colistin has been used as a feed additive for livestock and poultry for growth promotion and disease prevention, especially for the treatment of gastrointestinal infections in livestock and poultry (Catry et al., 2015), which may cause widespread resistance of strains in livestock and poultry, further resulting in pollution of the environment, food, water and so on. The mcr-1 gene was detected in bacteria isolated from imported chickens in Denmark (Hasman et al., 2015), food (Kuo et al., 2016; Yang et al., 2019) and wastewater (Zhao and Zong, 2016; Zhao et al., 2017) in China. In patients, the strains carrying the mcr-1 gene (0.62%, 36/5,828) or blaNDM gene (0.10%, 6/5,828) are possibly related to the use of polymyxins or carbapenems in hospitals and in-hospital transmission of drug-resistant strains. Therefore, it is possible that the increase in these two kinds of resistant strains is mainly due to the use of polymyxins or carbapenems antibiotics.

Different antibiotics usage may lead to various isolates selection. No resistant strains in this study co-harbor mcr-1 and blaNDM−1 gene, while in other studies from China, such strains were seen (Zheng et al., 2016; Liu et al., 2017b; Wang R. et al., 2018). For instances, in patients, different antibiotics treatment were adopted according to different illness and severity. For livestock and poultry, antibiotics usage may also varied with breeding scales and farms. In addition to mcr-1 and blaNDM−1 gene, other genotype combination were also reported from China (Kong et al., 2017; Liu et al., 2017b; Chavda et al., 2018; Wang Q. et al., 2018; Wang R. et al., 2018). The mcr and blaNDM genes co-harbor isolates from China are often E. coli., and E. cloacae, etc., occasionally. Further restrictions should be made on antibiotics usage for relevant occasions.

Compared with the ORF of the mcr-1.1 gene, it was found that two strains have variants. One variant mcr-1.21 (GenBank: MK965883), changed from C to T at locus 1234 nt, which changed the codon from proline to serine but did not affect the polymyxin-resistant phenotype of the strain. The other variant (GenBank: MK965884) changed from G to A at locus 1344 nt, resulting in a codon change from tryptophan to stop codon. Strain of this variant was sensitive to polymyxins. It was speculated that the translation of this mcr-1 gene was terminated.
when containing premature stop codon and the functional mcr-1 protein was not completely expressed, it truncated MCR family phosphoethanolamine – lipid A transferase. These two strains were isolated from pig and diarrhea patients, respectively, implying that mutations may be in response to antibiotic selection and certain environmental stresses. Some researchers have also detected variants of the mcr-1 gene from bacteria isolated in environment or healthy individuals (Lu et al., 2017). In addition, three strains with the mcr-1 gene were found to be sensitive to polymyxins, suggesting that the gene does not play a role in certain strains (Quan et al., 2017), which may be related to the metabolism of the strains. Another possible mechanism is the inhibition of mcr-1 gene expression or the inactivation of phosphoethanolamine transferase it encodes (Liassine et al., 2016). In addition, multiple primers were designed to amplify the ORF of mcr-1 positive strains, indirectly reflecting that the flanking regions of the ORF in this study may be variable. Variations did exist in the upstream of the ORF, compared with reference plasmid (KP347127): sequences amplified by the primer mcr-1 FL showed T to C mutation at 22377nt. As to sequences amplified by primer FR-mcr-1 FL, shortly after the forward primer (KP347127:22091-22107) was a homologous fragments of ≥256 bp, which should be located at 20897-21152 of KP347127, prior to the location of forward primer.

Many genes are responsible and important for polymyxins or carbapenems resistance, e.g., blaOXA (Evans and Amyes, 2014) and blaKPC (Pitout et al., 2015). To focus on both mobile and widespread genes, only mcr-1 and blanDM genes were screened for 12,858 strains, which is on the other hand, the limitation of the study. There may exist other important resistance mechanisms or genes in our strains. We would further resolve it using strains in this study and isolated afterward. The none detection of wildlife isolates from depopulated areas, in sharp contrast with positive findings of isolates from antibiotics-using areas, emphasized the importance of antibiotics management. On the other hand, the majority of strains collected from wildlife and patients is also the limitation of the study and should be considered when making conclusion.

The mcr-1 and blanDM genes, mainly encoded by plasmids (Kumarasamy et al., 2010; Liu et al., 2016), have caused harm to livestock, poultry, humans and the environment, and therefore active measures should be taken against the bacteria. Our study have demonstrated that the transfer of mcr-1 or blanDM genes between bacteria may be limited in China, however, the emergence of and increase in polymyxins and carbapenem-resistant strains was mainly related to the selective pressure of antibiotics. When using polymyxins and carbapenems antibiotics for disease prevention and control in the clinical setting, poultry, livestock and environment, strict management of usage is essential (Walsh and Wu, 2016; Al-Tawfiq et al., 2017), to prevent further intra- and interspecies dissemination of resistance genes and to control the spread of a broad-spectrum drug resistant or multidrug resistant bacteria.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the https://www.ncbi.nlm.nih.gov/pathogens/isolates#/refgene/gene_family:(mcr-1), MK965884.

AUTHOR CONTRIBUTIONS

XW and HJ contributed to the conception and design of the work. RF, CL, SQ, JL, MX, and DL performed the experiments. RF, CL, and RD drafted the manuscript. RF and RD performed the analysis and interpretation of the data. XW supervised the work. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank American Journal Experts for their critical editing and helpful comments regarding our manuscript (Sub ID S86HSZSZC).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00121/full#supplementary-material

REFERENCES

Abdelraouf, K., Linder, K. E., Naailor, M. D., and Nicolau, D. P. (2017). Predicting and preventing antimicrobial resistance utilizing pharmacodynamics: part II Gram-negative bacteria. Expert. Opin. Drug Metab. Toxicol. 13, 705–714. doi: 10.1080/17425355.2017.1329417

Abderrahim, A., Djahmi, N., Pujol, C., Nedjai, S., Bentakouk, M. C., Kirane-Gacemi, D., et al. (2017). First Case of NDm-1-Producing Klebsiella pneumoniae in annaba university hospital. Algeria. Microb. Drug Resist. 23, 895–900. doi: 10.1080/mdr.2016.0213

AbuOun, M., Stubberfield, E. J., Duggett, N. A., Kirchner, M., Dormer, L., Nunez-Garcia, J., et al. (2017). mcr-1 and mcr-2 variant genes identified in Moraxella

species isolated from pigs in great britain from 2014 to 2015. J. Antimicrob. Chemother. 72, 2745–2749. doi: 10.1093/jac/dkx286

Alos, J. I. (2015). Antibiotic resistance: a global crisis. Enferm. Infecc. Microbiol. Clin. 33, 692–699.

Al-Tawfiq, J. A., Laxminarayan, R., and Mendelson, M. (2017). How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? Int. J. Infect. Dis. 54, 77–84. doi: 10.1016/j.ijid.2016.11.415

Borowiak, M., Fischer, J., Hammerl, J. A., Hendriksen, R. S., Szabo, I., and 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. doi: 10.1093/jac/dkx327
Liassine, N., Assouvie, L., Descombes, M.-C., Dénervaud Tendon, V., Kieffer, Liu, L., Feng, Y., McNally, A., and Zong, Z. (2018). blaNDM-21, a new variant of Klebsiella pneumoniae, a Key Pathogen Set for Global Nosocomial Dominance. Emerg. Microbes Infec. 7:122. doi: 10.1038/s41426-018-0078-z

Luo, X., Hu, Y., Luo, M., Zhou, H., Wang, X., Du, Y., et al. (2017). MCR-1, A New MCR Variant Carried by an IncP Plasmid in a Colistin-Resistant Salmonella enterica Serovar Typhimurium Isolates from a Healthy Individual. Antimicrob. Agents Chemother. 61.e02632-16.. doi: 10.1128/AAC.02632-16

Mox, J., Haeni, M., Nordmann, P., and Poirel, L. (2017). Extended-spectrum beta-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? Clin. Microbiol. Infect. 23, 826–833. doi: 10.1016/j.cmi.2017.01.013

Michael, G. B., Freitag, C., Wendlandt, S., Eidam, C., Fessler, A. T., Lopes, G. V., et al. (2015). Emerging issues in antimicrobial resistance of bacteria from food-producing animals. Future Microbiol. 10, 427–443. doi: 10.2217/fmb.14.93

Nolte, O. (2014). Antimicrobial resistance in the 21st century: a multifaceted challenge. Protein Pept. Lett. 21, 330–335. doi: 10.2174/0929865113510660106

Pitout, J. D., Nordmann, P., and Poirel, L. (2015). Carbapenemase-Producing Klebsiella pneumoniae, a Key Pathogen Set for Global Nosocomial Dominance. Antimicrob. Agents Chemother. 59, 5873–5884. doi: 10.1128/AAC.01919-15

Quan, J., Li, X., Chen, Y., Jiang, Y., Zhou, Z., Zhang, H., et al. (2017). Prevalence of mcr-1 in Escherichia coli and Klebsiella pneumoniae recovered from bloodstream infections in China: a multicentre longitudinal study. Lancet Infect. Dis. 17, 400–410. doi: 10.1016/S1473-3099(16)30528-6

Rizoo, C., Lopez-Cerero, L., Rojo-Martin, M. D., Hoyos-Mallecot, Y., Fernandez-Cuenca, F., Martin-Ruiz, J. L., et al. (2017). First report of NDM-1-producing clinical isolate of Lectaricia aerobacteroless in Spain. Diagn. Microbiol. Infect. Dis. 88, 268–270. doi: 10.1016/j.diagmicrobio.2017.04.013

Rozwandowicz, M., Brouwer, M. S. M., Fischer, J., Wagenaar, J. A., Gonzalez-Zorn, B., Guerra, R., et al. (2018). Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J. Antimicrob. Chemother. 73, 1121–1137. doi: 10.1093/jac/dkx448

Theureultz, U. (2017). Global antimicrobial resistance in Gram-negative pathogens and clinical need. Curr. Opin. Microbiol. 39, 106–112. doi: 10.1016/j.mib.2017.10.028

Walsh, T. R., and Wu, Y. (2016). China bans colistin as a feed additive for animals. Lancet Infect. Dis. 16, 1102–1103. doi: 10.1016/S1473-3099(16)30329-2

Walters, M. S., Eggers, P., Albrecht, V., Travis, T., Lonsdaw, D., Hovan, G., et al. (2015). Vancomycin-resistant staphylococcus aureus - delaware. Euro. Surveill. 20, 1–5. doi: 10.2649/EU.2015.10.008

Wang, Q., Zhang, P., Zhao, D., Jiang, Y., Zhao, F., Wang, Y., et al. (2018). Emergence of tigecycline resistance in Escherichia coli co-producing MCR-1 and NDM-5 during tigecycline salvage treatment. Infect. Drug Resist. 11, 2241–2248. doi: 10.2147/IDR.S179618

Wang, R., Liu, Y., Zhang, Q., Jin, L., Wang, Q., Zhang, Y., et al. (2018). The prevalence of colistin resistance in Escherichia coli and Klebsiella pneumoniae isolated from food animals in China: coexistence of mcr-1 and blaNDM with low fitness cost. Int. J. Antimicrob. Agents. 51, 739–744. doi: 10.1016/j.ijantimicag.2018.01.023

Wang, X., Wang, Y., Zhou, Y., Li, Y., Yin, W., and Wang, S. (2018). Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg. Microbes Infect. 7:122. doi: 10.1038/s41426-18-0124-z

Wang, X., Zhang, Z., Hao, Q., Wu, J., Xiao, J., and Jing, H. (2014). Complete genome sequence of Acinetobacter baumannii ZW85-1. Genome Announc. 2:e00183-13. doi: 10.1128/genomeA.00183-13

Xavier, B. B., Lammens, C., Ruhlal, R., Kumar-Singh, S., Butaye, P., Goossens, H., et al. (2016). Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli. Belgium. June 2016. Euro. Surveill. 21:30280. doi: 10.2807/1560-7917.ES.2016.21.27.30280

Yang, F., Shen, C., Zheng, X., Liu, Y., El-Sayed Ahmed, M. A. E., Zhao, Z., et al. (2019). Plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli and
*Klebsiella pneumoniae* isolated from market retail fruits in Guangzhou, China. *Infect. Drug Resist.* 12, 385–389. doi: 10.2147/IDR.S194635

Yang, Y. Q., Li, Y. X., Lei, C. W., Zhang, A. Y., and Wang, H. N. (2018). Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. *J. Antimicrob. Chemother.* 73, 1791–1795. doi: 10.1093/jac/dky111

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., et al. (2017). Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *mBio* 8:e00543-17.. doi: 10.1128/mBio.00543-17

Yong, D., Toleman, M. A., Guske, C. G., Cho, H. S., Sundman, K., Lee, K., et al. (2009). Characterization of a new metallo-beta-lactamase gene, *bla*(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* 53, 5046–5054. doi: 10.1128/AAC.00774-09

Yu, J., Wang, Y., Chen, Z., Zhu, X., Tian, L., Li, L., et al. (2017). Outbreak of nosocomial *NDM-1*-producing *Klebsiella pneumoniae* ST1419 in a neonatal unit. *J. Glob. Antimicrob. Resist.* 8, 135–139. doi: 10.1016/j.jgar.2016.1 0.014

Zenati, K., Touati, A., Bakour, S., Sahli, F., and Rolain, J. M. (2016). Characterization of *NDM-1*- and *OXA-23*-producing *Acinetobacter baumannii* isolates from inanimate surfaces in a hospital environment in Algeria. *J. Hosp. Infect.* 92, 19–26. doi: 10.1016/j.jhin.2015.09.020

Zhao, F., and Zong, Z. (2016). *Kluyvera ascorbata* strain from hospital sewage carrying the *mcr-1* colistin resistance gene. *Antimicrobial. Agents Chem.* 60, 7498–7501.

Zhao, F., Feng, Y., Liu, X., McNally, A., and Zong, Z. (2017). IncP plasmid carrying colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* from hospital sewage. *Antimicrobial. Agents Chem.* 61:e02229-16.. doi: 10.1128/AAC.02229-16

Zheng, B., Dong, H., Xu, H., Lv, J., Zhang, J., Jiang, X., et al. (2016). Coexistence of *MCR-1* and *NDM-1* in Clinical *Escherichia coli* Isolates. *Clin. Infect. Dis.* 63, 1393–1395. doi: 10.1093/cid/ciw553

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Fan, Li, Duan, Qin, Liang, Xiao, Lv, Jing and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.