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Review

Plasmid-mediated colistin resistance in animals: current status and future directions

Jian Sun[1]†, Ximin Zeng[2]†, Xing-Ping Li[1], Xiao-Ping Liao[1], Ya-Hong Liu[1]* and Jun Lin[2]*

[1] *National Risk Assessment Laboratory for Antimicrobial Resistance of Animal Original Bacteria, South China Agricultural University, Guangzhou, China*
[2] *Department of Animal Science, The University of Tennessee, 2506 River Drive, Knoxville, TN 37996, USA*

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Abstract

Colistin, a peptide antibiotic belonging to the polymyxin family, is one of the last effective drugs for the treatment of multidrug resistant Gram-negative infections. Recent discovery of a novel mobile colistin resistance gene, *mcr-1*, from people and food animals has caused a significant public health concern and drawn worldwide attention. Extensive usage of colistin in food animals has been proposed as a major driving force for the emergence and transmission of *mcr-1*; thus, there is a worldwide trend to limit colistin usage in animal production. However, despite lack of colistin usage in food animals in the USA, *mcr-1*-positive *Escherichia coli* isolates were still isolated from swine. In this paper, we provided an overview of colistin usage and epidemiology of *mcr-1* in food animals, and summarized the current status of mechanistic and evolutionary studies of the plasmid-mediated colistin resistance. Based on published information, we further discussed several non-colistin usage risk factors that may contribute to the persistence, transmission, and emergence of colistin resistance in an animal production system. Filling the knowledge gaps identified in this review is critical for risk assessment and risk management of colistin resistance, which will facilitate proactive and effective strategies to mitigate colistin resistance in future animal production systems.

Keywords: Colistin resistance, MCR-1, epidemiology, animal, risk factors.

Introduction

Colistin, a polymyxin antibiotic, is one of the last effective drugs for the treatment of multidrug-resistant (MDR) Gram-negative infections in human beings. Resistance to colistin used to be considered non-transmissible and most forms are intrinsic properties of bacteria. At the end of 2015, a novel plasmid-mediated colistin resistance gene, *mcr-1*, was identified as the single determinant to confer polymyxin resistance in *Escherichia coli* isolates from people and food animals (Liu *et al.*, 2016). More alarmingly, *mcr-1* could be mobilized among Enterobacteriaceae at a rather high frequency by conjugation and stably persisted (Liu *et al.*, 2016). Subsequently, Gram-negative bacteria harboring the transmissible *mcr-1*-bearing plasmids were reported in five continents, which has caused a significant public health concern and drawn worldwide attention.

The *mcr-1* gene exists in the bacterial isolates from both food animals (e.g. pig, poultry, and cattle) and human beings. Extensive usage of colistin in food animals has been proposed as a major driving force for the emergence and transmission of *mcr-1* (Rhouma *et al.*, 2016). Thus, recently, use of colistin in animal production has been proposed to be re-evaluated and regulated (Rhouma *et al.*, 2016). In 2016, the Chinese government responded quickly and released an announcement to ban colistin usage as an in-feed growth promoter (Walsh and Wu, 2016). In the USA, although colistin has never been used

*Corresponding author. E-mail: jlin6@utk.edu and lyh@scau.edu.cn
†The authors contributed equally to this paper.

in food animals, *mcr-1*-positive *E. coli* isolates were still identified in two swine intestinal samples (Meinersmann *et al.*, 2017). Emergence of colistin resistance in US animal production is still a mystery, but clearly suggests that non-colistin usage risk factors exist and contribute to the persistence, transmission, and emergence of colistin resistance in an animal production system. Addressing this issue is imperative to prevent and control the transmissible colistin resistance in future animal production in the USA and other countries.

In this paper, we briefly reviewed historical status of colistin applications, colistin resistance development as well as recent emergence of *mcr-1*; additional in-depth information for these topics can be found in several of recent reviews (Li *et al.*, 2006a; Nation and Li, 2009; Yahav *et al.*, 2012; Kempf *et al.*, 2016; Rhouma *et al.*, 2016). We also comprehensively summarized recent extensive epidemiological studies focused on the prevalence of *mcr* gene in food animals, companion animals, and wildlife. In addition, on the basis of published science-based information, we identified and discussed several non-colistin usage risk factors that may contribute to the persistence, transmission, and emergence of colistin resistance in an animal production system.

History and applications of polymyxins

Colistin (also known as 'polymyxin E') and polymyxin B are bacteria-derived peptide antibiotics that have been used to treat infections with Gram-negative bacteria (Kwa *et al.*, 2007). Colistin was discovered in 1949, and was produced by *Bacillus polymyxa* subspecies *colistinus* (Koyama *et al.*, 1950; Komura and Kurahashi, 1979). Polymyxins contain a strong positive charge and a hydrophobic acyl side chain, which display a similar mode of action as many antimicrobial peptides (AMPs) which kill bacteria by forming pores in the membrane (Wanty *et al.*, 2013). Due to potential nephrotoxicity and neurotoxicity (Brown *et al.*, 1970; Koch-Weser *et al.*, 1970; Nation and Li, 2009), polymyxins normally are used for topical human infections, but have not been used as a routine clinical human practice for decades. However, recent emergence of MDR Gram-negative bacteria have made polymyxins regain attention, by becoming the new last line of defense against fatal MDR bacterial infections, because MDR pathogens, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, are still susceptible to polymyxins (Bratu *et al.*, 2005; Yahav *et al.*, 2012).

Two forms of colistin are commercially available for human clinical usage: (1) colistin methanesulfonate sodium (or colistimethate sodium) for parenteral use and aerosol therapy, and (2) colistin sulfate for oral and topical use (Li *et al.*, 2006a). In aqueous solutions colistin methanesulfonate sodium is spontaneously hydrolyzed to a mixture of partially sulfomethylated derivatives and colistin (Barnett *et al.*, 1964; Li *et al.*, 2003). Colistin methanesulfonate sodium is less toxic than colistin sulfate when administered parenterally (Schwartz *et al.*, 1959) and was considered an inactive prodrug of colistin (Bergen *et al.*, 2006). In food animals, colistin sulfate has been frequently used as a veterinary medicine or as an in-feed antibiotic growth promoter, which is reviewed in the section below.

Use of colistin in animal production

Colistin has been extensively used in animal production for prophylactic and therapeutic purposes as well as for growth promotion in some countries, particularly in Asia and Europe (Rhouma *et al.*, 2016). In the USA, colistin is in fact not used in food animals, although colistin has been approved for use by the FDA. Driven by China, the largest user of colistin in agriculture, global demand for colistin in agriculture was estimated to reach 11,942 tons per year by the end of 2015 (Liu, *et al.*, 2016). Colistin has been heavily used as an in-feed antibiotic growth promoter to improve feed efficiency and body weight gain in food animals in Asian countries, which include China, India, Japan, and Vietnam (Kempf *et al.*, 2016). In Brazil, colistin is also widely used in animal feed as a growth promoter in livestock, mainly in pigs and poultry (Fernandes *et al.*, 2016).

As a veterinary medicine, colistin has been used worldwide for decades, especially in swine and veal calves (Catry *et al.*, 2015; Kempf *et al.*, 2016; Rhouma *et al.*, 2016). In Korea, colistin was used for prevention and treatment of animal disease and the annual usage of colistin in food animals ranged from 6 to16 tons from 2005 to 2015 (Lim *et al.*, 2016). In Europe, colistin was used to treat infections caused by Enterobacteriaceae in many animals, such as pigs, broilers, veal and beef cattle, sheep and goats (Catry *et al.*, 2015). In 2011, Germany, Portugal, Italy, and Estonia had higher colistin sales than many other European countries (Irrgang *et al.*, 2016).

Extensive usage of colistin in food animals has been recognized as a major risk factor for the emergence and transmission of the plasmid-borne colistin resistance gene *mcr-1* (Rhouma *et al.*, 2016). Therefore, the usage of colistin in animal production has been proposed to be re-evaluated and regulated (Rhouma *et al.*, 2016). Notably, the Chinese government responded quickly and released an announcement regarding the cessation of colistin as a growth promoter at 7 months after the discovery of *mcr-1* (Walsh and Wu, 2016). However, this ban only prohibits the use of colistin as a feed additive, and does not limit colistin use as a therapeutic agent for disease control in animals, which still raises a concern for the risk of colistin usage in animals (Walsh and Wu, 2016). Similarly, the Brazilian government also announced ban of colistin usage as a feed additive in food animals on 30 November 2016 (http://www.brasil.gov.br/economia-e-emprego/2016/11/mapa-proibe-uso-de-substancia-antimicrobiana-em-racoes).

Colistin resistance and recent emergence of *mcr-1*

In some countries, such as China, colistin has been extensively used in animal production, which is consistent with the increasing colistin resistance observed in recent years (Shen *et al.*, 2016; Huang *et al.*, 2017). Similarly, a high rate of colistin resistance in

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Acinetobacter baumannii was reported in Korea recently (Ko *et al.*, 2007). Colistin hetero-resistance also has been widely reported in MDR *A. baumannii* (Li *et al.*, 2006b; Hawley *et al.*, 2008; Yau *et al.*, 2009), *K. pneumoniae* (Poudyal *et al.*, 2008; Meletis *et al.*, 2011) and *P. aeruginosa* (Bergen *et al.*, 2011). Not surprisingly, given that colistin is an AMP, mechanisms of colistin resistance are similar to those of other AMPs (Ernst *et al.*, 2001; Yeaman and Yount, 2003; Kraus and Peschel, 2006; Peschel and Sahl, 2006), which are primarily mediated through non-transmissible modification of cell surface with intrinsic genetic determinants located in the chromosome or in chromosomal mutations (Baron *et al.*, 2016; Schwarz and Johnson, 2016).

Mobile polymyxin resistance was not reported until the plasmid-mediated colistin-resistant gene, *mcr-1*, was recently reported in China (Liu *et al.*, 2016). The *mcr-1* was identified as the single determinant to confer polymyxin resistance in commensal *E. coli* from food animals (Liu *et al.*, 2016). More alarmingly, the *mcr-1* could be mobilized among Enterobacteriaceae at a rather high frequency by conjugation and then stably persisted in transformed bacteria (Liu *et al.*, 2016). The MCR-1 has phosphoethanolamine transferase activity, which is involved in lipid A modification, consequently providing protection of *E. coli* against colistin in an *in vivo* mouse infection model (Liu *et al.*, 2016).

In the past 2 years, *mcr-1* has been found in *E. coli* (Arcilla *et al.*, 2016; Liu *et al.*, 2016; Olaitan *et al.*, 2016), *Klebsiella* (Liu *et al.*, 2016; Stoesser *et al.*, 2016), *Salmonella* (Campos *et al.*, 2016; Figueiredo *et al.*, 2016; Webb *et al.*, 2016), *Enterobacter aerogenes* (Zeng *et al.*, 2016; Wang *et al.*, 2017b), *Enterobacter cloacae* (Zeng *et al.*, 2016; Wang *et al.*, 2017b), *Cronobacter sakazakii* (Liu *et al.*, 2017a), *Shigella sonnei* (Pham Thanh *et al.*, 2016), *Kluyvera* spp. (Zhao and Zong, 2016), and *Citrobacter* spp.(Li *et al.*, 2017; Sennati *et al.*, 2017). The *mcr-1* gene has been reported to exist in different food animals, such as in pigs (Meinersmann *et al.*, 2017; Roschanski *et al.*, 2017), poultry (Lima Barbieri *et al.*, 2017; Monte *et al.*, 2017) and cattle (Huang *et al.*, 2017). This gene also has been detected in bacteria from different environment niches and other commodities, such as coastal water (Fernandes *et al.*, 2017), rivers and lakes (Zurfuh *et al.*, 2016), hospital sewage (Zhao *et al.*, 2017), wells (Sun *et al.*, 2017c), meats and vegetables (Hasman *et al.*, 2015; Zurfuh *et al.*, 2016).

In people, *mcr-1* was detected in approximately 1% of *E. coli* and *K. pneumoniae* isolates (of more than 20,000 isolates) in two recent reports from China (Quan *et al.*, 2017; Wang *et al.*, 2017b). The *mcr-1* gene was found in pathogens from human bloodstream infections (Hasman *et al.*, 2015; Wang *et al.*, 2017a), in patients after colistin treatment (Beyrouthy *et al.*, 2017), in travelers (Arcilla *et al.*, 2016) and in pilgrims (Leangapichart *et al.*, 2016). The Centers for Disease Control and Prevention (CDC), the US Food and Drug Administration (FDA), and the US Department of Agriculture (USDA) started tracking *mcr-1* prevalence in the USA immediately after the report from China (https://www.cdc.gov/drugresistance/tracking-mcr1.html). Less than 6 months after the first identification of *mcr-1* in China, a human bacterial isolate carrying *mcr-1* was isolated from a Pennsylvania patient by the Department of Defense, while *mcr-1* was identified in two swine intestinal samples that were collected as part of the National Antimicrobial Resistance Monitoring System, a shared project of the USDA, FDA, and CDC (Meinersmann *et al.*, 2017). To date, there have been 14 human isolates and two animal isolates reported to carry MCR-1 for colistin resistance in the USA (https://www.cdc.gov/drugresistance/tracking-mcr1.html). In 2017, plasmid-mediated colistin-resistant *K. pneumoniae* were associated with an outbreak in China, leading to six infections and two deaths (Tian *et al.*, 2017). Thus, the recent emergence of the plasmid-mediated colistin-resistant Enterobacteriaceae in both food animals and in human beings has drawn worldwide attention and fears.

Prevalence of *mcr-1* in animals

Recent epidemiological studies of colistin resistance have shown the *mcr-1* positive Enterobacteriaceae isolates are highly prevalent in various animal hosts worldwide (Summarized in Table 1). Unregulated usage of colistin in food animals is believed to be the important driving force for the emergence and transmission of the *mcr-1* (Liu *et al.*, 2016; Rhouma *et al.*, 2016). The following paragraphs summarize findings from recent extensive epidemiological studies for the prevalence of *mcr-1* in livestock, companion animals, and wildlife.

Livestock

In China, Liu *et al.* (2016) first reported 21 and 15% positive rates of *mcr-1* in *E. coli* of pig and retail meat origin, respectively. In a retrospective study on 1611 *E. coli* isolates of chicken origin, collected from the early 1970s to 2014 in China, only three *mcr-1*-positive *E. coli* isolates were identified in the 1980s; for almost two decades afterward, *mcr-1* was not detected until in 2004 and 2006 when sporadic occurrences of *mcr-1* were observed. The ratio of *mcr-1*-positive *E. coli* increased from 5.2% (6/115) in 2009 to 30.0% (15/50) in 2014 (Shen *et al.*, 2016). A similar trend was also observed in another study in China, in which 4438 *E. coli* isolates of food animal origin were tested for colistin resistance and the presence of *mcr-1* (Huang *et al.*, 2017). Approximately 16.8% of *E. coli* isolates from pigs and chickens recovered during 2013–2014 displayed colistin resistance, which is significantly higher than those isolated during 2007–2008 (5.5%) and 2010–2011 (12.4%). The increasing prevalence of *mcr-1*-harboring *E. coli* in the past decade (Shen *et al.*, 2016; Huang *et al.*, 2017) is coincident with the fact that China started to introduce colistin in the early 2000s and became one of the world's largest users of colistin in agriculture by the end of 2015 (Liu *et al.*, 2016). Among the Shiga toxin-producing *E. coli* isolated from pigs in China, 10.8% (10/93) were *mcr-1* positive (Bai *et al.*, 2016). In Chinese poultry production, Wang *et al.* (2017c) found that 37 (23.0%) carbapenem-resistant isolates were positive for *mcr-1*. Yang *et al.* (2017) reported a 5.11% *mcr-1*-positive rate in *E. coli* of chicken origin in 2010–2015. Compared with the extensive

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Table 1. Characteristics of *mcr*-positive isolates from food animals, people and other sources

Country/ Region	Sample source	Type of *mcr*-like gene	Species	Year	Number of isolates	Number of colistin resistance isolates	Number of *mcr-1*-positive isolates (n)	Plasmids	Other resistance gene(s)	MLST	Reference
China	Food animals	*mcr-1*	*E coli*	2011.04–2014.11	804		166 (21%)				Liu *et al.* (2016)
China	Raw meat	*mcr-1*	*E coli*	2011.04–2014.11	523		78 (15%)				Liu, *et al.* (2016)
China	Meat products	*mcr-1*	*E coli*	2014.07			15%	IncI2	bla_{NDM-9}, $bla_{CTX-M-65}$, *rmtB* and *fosA3*		Yao *et al.* (2016)
China	Chicken	*mcr-1* *mcr-1.3*	*E coli*	2010–2015	1136		*mcr-1* (5.11% (58/1136)) *mcr-1.3* (1)	IncI2	bla_{CTX-M}	40 distinct sequence types, ST48, ST616, ST10, ST88 were the most common	Yang *et al.* (2017)
China	Food animals	*mcr-1*	*E. coli*	1970–2014	1611		104				Shen *et al.* (2016)
China	Chicken, pig	*mcr-1*	*E. coli*	2015.07–2016.06	262		6 (Chicken), 7 (pig)	A Phage-like IncY		ST877 (pig)	Zhang *et al.* (2017)
China	Food animals	*mcr-1*	*E. coli*	2013–2014	4089	763 (18.7%)	182 (Of the 200 randomly selected colistin-resistant isolates)				Huang *et al.* (2017)
China	Farm animals	*mcr-1*	*E. coli*		2330		54 (2.3 %)				Chen *et al.* (2017)
China	Swine	*mcr-1*	*E. coli*	2011 to 2013		16	6				Li *et al.* (2016c)
China	Chicken	*mcr-1*	*E. coli*, *C. sakazakii*	2015	3		3 (1 *E. coli*, 2 *C. sakazakii*)	Two different IncI2 plasmids	*fosA3*-bla_{NDM-9} in *E. coli* from the same diseased chicken		Liu *et al.* (2017a)
China	Chicken	*mcr-1*	*E. coli*	2015	78 *E. coli*		53 (67.9%)	IncI2 (co-spread of bla_{NDM-4} and *mcr-1* in a single IncHI2/ ST3 plasmid in Enterobacteriaceae species.)	bla_{NDM}	ST297, ST156, ST2973, ST2847, ST117, ST101, ST617, ST10, ST1011, and ST2944.	Liu *et al.* (2017b)
China	Swine	*mcr-1*	*E. coli*	2015.10	105		16	IncX4 (*mcr-1*), IncX3 (bla_{NDM-5})	bla_{NDM-5}		Kong *et al.* (2017)
China	Duck	*mcr-1*	*E. coli*	2015.05			2	IncHI2 and IncI2 (*mcr-1*), IncX3 (bla_{NDM-5})	bla_{NDM-5}, bla_{TEM-1}, $bla_{CTX-M-55}$, *fosA3*, and *aac(6′)-Ib*	ST648 and ST156	Yang *et al.* (2016a)

Table 1. (*Cont.*)

Country/ Region	Sample source	Type of *mcr*-like gene	Species	Year	Number of isolates	Number of colistin resistance isolates	Number of *mcr-1*-positive isolates (n)	Plasmids	Other resistance gene(s)	MLST	Reference
China	Pigs	*mcr-1*	*E coli*	2011.05, 2012.08	93	10	10	IncI2, IncHI2, IncHI2A, IncA/C, IncFIB, IncFIC, IncX1, IncFI; IncFIC, IncHI2, IncHI2A, IncN, IncFIB, IncX4	*aadA1, aadA2, aph (3')-Ia, strAB, sul1, sul2, sul3, tet(A), dfrA12, ermB, mphA, cmlA1, flo; aadA1, aadA2, aph(3')-Ia, strAB, tet(A), dfrA12, dfrA14, cmlA1, floR, qnrS1, oqxA*	ST88, ST3628	Bai *et al.* (2016)
China	Poultry, dogs, sewage, wild birds and flies	*mcr-1*	*E. coli*	2014–2015	245		37 (23.0%) hatchery farms, chicken cloacae, caeca and meat, and extremely high percentages of positive samples		Co-transfer of bla_{NDM} and *mcr-1* was observed in five strains	ST101 ($n = 19$), ST156 ($n = 13$) and ST746 ($n = 13$)	Wang *et al.* (2017c)
China		*mcr-1*	X4 positive *E. coli*		43		13	IncX4, IncHI2, IncI2	$bla_{CTX-M-55}$ $bla_{CTX-M-130}$		Sun *et al.* (2017a)
China	Chicken	*mcr-1*	*E. coli*	2012.08				IncI2	$bla_{CTX-M-55}$		Sun *et al.* (2016a)
China	Chicken	*mcr-1*	*Salmonella* spp.	2014–2015	53		7.5% (4)	IncI2	$bla_{CTX-M-55}$		Yang *et al.* (2016b)
China	Food animals	*mcr-1*	*Salmonella*	2007–2015	276	22	5	IncI2, IncHI2	*aac(6')-Ib-cr*	ST34	Li *et al.* (2016b)
China	Pig	*mcr-1*	*Salmonella enterica*	2013.07–2014.05	142		21 (14.8%)	IncHI2-like plasmids, IncF, IncFIB	*floR, oqxAB*		Yi *et al.* (2017)
China	Pig	*mcr-1*	*Citrobacter freundii*	2012			1	IncHI2			Li *et al.* (2017)
China	Pig	*mcr-3*	*E. coli*	2015				IncHI2	*aac(6')-Ib-cr floR, cmlA1, catB3 strA, strB, aadA1, aadA2 aac(3)-Iva arr3 bla_{OXA-1} sul1, sul2, sul3 tet(A)*	ST1642	Yin *et al.* (2017)
Taiwan, China	Humans and food animals	*mcr-1*	*Salmonella*	2010–2015	6386 Humans (5178) animals (1208)	1917	19 humans (10), pigs (7), and chickens (2)	IncI2 IncX4			Chiou *et al.* (2017)
Taiwan, China	Humans and retail meats	*mcr-1*	*E. coli*	2010, 2012 and 2014	4589 (1136, 1752 and 1701 from 2010, 2012 and 2014), 18 (retail meats)		32 (14 patients, 18 retail meats)		All ST38 isolates carried CTX-M-Group 1 ESBL	MLST revealed 18 distinct STs, ST38 and ST117 beingmost common, ST701, ST744, ST428, ST131, et al	Kuo *et al.* (2016)

Japan	Healthy Food animals		*E. coli*	2000–2014	9308	90	2				Suzuki *et al.* (2016)
Japan	Healthy food animals	*mcr-1*	*E. coli*	2000–2014	9306	(7.9%) 732	39 (5, 20, and 14 isolated from cattle, swine and broiler, respectively)	IncI2			Kawanishi *et al.* (2017)
Japan	Swine	*mcr-1*	Pathogenic *E. coli*	2007–2014	684	309 (45%)	90 (13%)				Kusumoto *et al.* (2016)
Japan	Retail meat	*mcr-1*	*E. coli*	2015–2016				IncI2	$bla_{CTX-M-1}$		Ohsaki *et al.* (2017)
South Korea	Chicken, pig	*mcr-1*	*E. coli*	2013 and 2015	10,576	154 (1.46%)	11 (10 chicken,1 pig)	IncI2	$bla_{CTX-M-1}$ (ST162)	ST410, ST156, ST10, ST101, ST1, ST226, ST162, ST88, ST2732, ST1741	Lim *et al.* (2016)
Malaysia	Human, animal and environmental	*mcr-1*	*E. coli*	2013	900		6 (chicken, pig, water, human, Chicken feed)			ST3489 (Chicken), ST3014 (Pig), ST117 (Chicken),	Yu *et al.* (2016)
Vietnam	Chicken	*mcr-1*	*E. coli*	2012–2013			59%	IncHI2, IncI2, IncHI2 and IncHI2A			Trung *et al.* (2017)
Vietnam	Pig and chicken	*mcr-1*	*E. coli*	2014–15	24		9		All produce extended-spectrum β-lactamase		Malhotra-Kumar *et al.* (2016)
Vietnam	Chicken and pig	*mcr-1*	*E. coli*	2013–2014	180		37				Nguyen *et al.* (2016)
Laos, Algeria	Pig	*mcr-1*	*E. coli*	2012, 2015	19		3				Olaitan *et al.* (2016)
Germany	Livestock	*mcr-1*	*E. coli*	2012–2013		129	3	IncX4 and IncHI2	$bla_{CTX-M-1}$	ST410	Falgenhauer *et al.* (2016)
Germany	Livestock and food	*mcr-1*	*E. coli*	2010–2015	10,609	505	402				Irrgang *et al.* (2016)
German	Boot swabs, flies, dog faeces and manure from three pig farms	*mcr-1*	*E. coli*	2011–2012	35		7 (2 barn, 3 manure, 1 dog, 1 stable fly)	IncX4		ST10, ST1011, ST1140, ST5281 and ST342	Guenther *et al.* (2017)
Belgium	Porcine and bovine	*mcr-2*	*E. coli*	2016				IncX4		ST10 and ST167	Xavier *et al.* (2016b)
Belgium	Pig	*mcr-1*	*E. coli*	2011–2012	53		7	IncFII		ST10 (n¼42), ST100 (n¼42), ST90 (n¼41) and novel STs (n¼42)	Xavier *et al.* (2016a)

Table 1. (*Cont.*)

Country/ Region	Sample source	Type of *mcr*-like gene	Species	Year	Number of isolates	Number of colistin resistance isolates	Number of *mcr-1*-positive isolates (n)	Plasmids	Other resistance gene(s)	MLST	Reference
France and Germany	Diseased food animals	*mcr-1*	*E. coli* and *Salmonella* spp.	2004 and 2014	*E. coli* ($n = 218$) and *Salmonella* spp. ($n = 74$)		42 *E. coli* and three *Salmonella* spp.				El Garch *et al.* (2017)
Spain	Poultry and swine	*mcr-1*	*E. coli and Salmonella enterica*	2010 and 2011			6 (swine: 3 *Salmonella enterica* 2 *E. coli*)				Quesada *et al.* (2016)
Italy	Humans, animals, food, environment	*mcr-1*	*Salmonella enterica*	2012–2015	4473 (3294 humans, 1143 veterinary sources, 36 environmental)	269 (6%)	25, 10 from humans and 15 from veterinary sources (2 Poultry,9 Swine, Pork 4)				Carnevali *et al.* (2016)
Italy	Pig	*mcr-1*	*E. coli*	2016				IncX4	$bla_{OXA-181}$	ST641	Pulss *et al.* (2017)
Italy	Pig	*mcr-1*	*E. coli*	2015–2016		51	37 (72.5%)				Curcio *et al.* (2017)
Portugal	Retail meat	*mcr-1*	*Salmonella enterica*	2011–2012	258		37 isolates (14%)	IncHI2			Figueiredo *et al.* (2016)
Portugal	Food	*mcr-1*	*Salmonella enterica*	2011							Hu *et al.* (2016)
Portugal	Human clinical cases, food products, food-animal production settings and aquatic environments	*mcr-1*	*Salmonella*	2011–2015	1010		11	IncX4, IncHI2			Campos *et al.* (2016)
Portugal	Food	*mcr-1*	*S Typhimurium*	2011				IncHI2			Tse and Yuen (2016)
France and Germany		*mcr-1*	*E. coli*	2004–2010	150	3	1	IncHI2			Brennan *et al.* (2016)
France	Calves	*mcr-1*	*ESBL*-producing *E. coli*	2006–2014	885		From 4.76% in 2006 to 21.28% in 2014				Haenni *et al.* (2016)
France	Broilers, pigs and turkeys	*mcr-1*	*E. coli*	2007–2014	1696	23	23		bla_{CMY-2}		Perrin-Guyomard *et al.* (2016)
France	Agricultural food sector	*mcr-1*	*salmonella*	2012–2013	8684		4				Webb *et al.* (2016)
France and Laos	animals and humans	*mcr-1*	*K. pneumoniae*	2012–2013		32	6 (4 Humans)			ST1319, ST37, ST491, ST39, ST1310, ST8	Rolain *et al.* (2016)

Netherlands	Calves (n¼15), broilers (n¼10) and turkey	*mcr-1*	*E. coli, Salmonella*		26 E. coli, 13 *Salmonella*		20 *E. coli*, 13 *Salmonella*	IncHI2 and IncX4, IncHI2/P		ST4	Veldman *et al.* (2016)
Netherland	Humans, food animals and food	*mcr-1*	*Enterobacteriaceae*	2009–2015	2471		3 (*E. coli*, chicken meat)			ST2079, ST117	Kluytmans-van den Bergh *et al.* (2016)
England and Wales	Humans and 2 isolates from poultry meat	*mcr-1*	*Salmonella enterica, E. coli, Klebsiella* spp., *Enterobacter* spp., *Campylobacter* spp. and *Shigella* spp.	2012–2015	~24,000		15 (10 human *S. enterica*;3 *E. coli*; 2 *Salmonella Paratyphi B var Java* from poultry meat)	IncHI2, IncI2 and IncX4		ST34, ST457, ST16, ST36, ST28, ST42,	Doumith *et al.* (2016)
Estonia	Humans, animals environment	*mcr-1*	*E. coli, Pseudomonas aeruginosa*	2011–2014	347 *E. coli*, 237 *Pseudomonas aeruginosa*		3 *E. coli* (pig)	IncX4			Brauer *et al.* (2016)
Great Britain	Pig	*mcr-1*	*E. coli* (2) and *Salmonella* (1)		3		2 (*E. coli* and *Salmonella*)	repB (pO111) (*E. coli*), IncI2 (*Salmonella*)			Anjum *et al.* (2016)
Wallonia and Flanders	Calves, piglets	*mcr-1*	*E. coli*	2011–2012		105	13	IncP			Malhotra-Kumar *et al.* (2016)
United States	Cattle, chickens, swine, turkeys	*mcr-1*	*E coli*	2016			2 (Swine)	IncI2		ST3234, ST132	Meinersmann *et al.* (2017)
Brazil	Chicken	*mcr-1*	*E.coli*	2003–2015	280		5% (14)		$bla_{CTX-M-1}$, $bla_{CTX-M-8}$ and $bla_{CTX-M-15}$, bla_{CMY-2}		Fernandes *et al.* (2016)
Brazil	Swine			2012–2014	113		1.8% (2)				Fernandes *et al.* (2016)
Brazil	Chicken meat	*mcr-1*	*E. coli*	2016	breasts ($n = 20$), thighs ($n = 20$), and liver ($n = 1$)		8	IncX4	bla_{CTX-M}	ST132, ST48, ST4419, ST522 and ST10	Monte *et al.* (2017)
Brazil	Chicken	*mcr-1*	*E. coli*	2015	343		10				Lentz *et al.* (2016)
Venezuela	Swine	*mcr-1*	*Enterobacteriaceae*	2015	93		2	IncI2		novel ST452	Delgado-Blas *et al.* (2016)
South Africa	Broiler	*mcr-1*	*E. coli*	2008–2015	4934		19				Perreten *et al.* (2016)
Tunisia	Food animals (birds)	*mcr-1*	*E. coli*	2015	37		29 (20% on farm A, 17% on farm B and 83% on farm C)	IncHI2/ST4	$bla_{CTX-M-1}$		Grami *et al.* (2016)
Egypt	Animals	*mcr-1*	*E. coli*	2014	185		1		bla_{TEM-1}	ST10	Khalifa *et al.* (2016)
China and Egypt	Birds (including broilers, layers, breeder flocks, ducks and geese)	*mcr-1*	Avian Pathogenic *E. coli* (APEC)		980		12 8 (chicken, China) 4 (chicken, Egypt)	FIB, IncI2, I1	bla_{TEM}, bla_{SHV}, bla_{CMY}, bla_{CTX-M} and bla_{OXA}		Lima Barbieri *et al.* (2017)
USA, Vietnam and Uzbekistan		*mcr-1*	*E. coli, Klebsiella* spp., *Citrobacter* spp. *and Enterobacter* spp.	2013–2014	142 *E. coli*, 138 *Klebsiella* spp., 180 *Citrobacter* spp. and 86 *Enterobacter* spp.	8	2 *E. coli*			ST117, ST1011	Unger *et al.* (2017)

Table 1. (*Cont.*)

Country/ Region	Sample source	Type of *mcr*-like gene	Species	Year	Number of isolates	Number of colistin resistance isolates	Number of *mcr-1*-positive isolates (n)	Plasmids	Other resistance gene(s)	MLST	Reference
Lithuania	Larus argentatus	*mcr-1*	*E. coli*	2016	117						Ruzauskas and Vaskeviciute (2016)
	Kelp gulls	*mcr-1*		2012				IncI2	$bla_{CTX-M-2}$, $bla_{CTX-M-14}$	ST10, ST744	Liakopoulos *et al.* (2016)
Pakistan	Eurasian coot	*mcr-1*	*E. coli*	2014			1	IncI2	$bla_{CTX-M-15}$	ST354	Mohsin *et al.* (2016)
Brazil	Magellanic penguins	*mcr-1*	*E. coli*	2006–2014			1	IncX4		ST10	Sellera *et al.* (2017)
China	companion animals	*mcr-1*	*E. coli*	2015–2016	39 dogs and 14 cats		4 from dogs and 2 from cats		bla_{IMP-4}, bla_{TEM-1}, $bla_{CTX-M-55}$, *fosA3*, *rmtB*, *qepA1*, *qepA*, bla_{SHV-12}, $bla_{CTX-M-15}$, *fosA3*, *rmtB*, *qnrS*, *aac(6')-Ib-c*	ST93, ST354	Zhang *et al.* (2016)
China	Companion animals	*mcr-1*	*E. coli*	2012–2016	566	79/566 (14.0%)	4 dogs and 2 cats				Lei *et al.* (2017)
China	pet cat	*mcr-1*	*E. coli*	2015				IncX3–X4		ST156	Sun *et al.* (2016b)
China	pet	*mcr-1*			108 samples		8 (7.4%)		bla_{NDM-5}		Chen *et al.* (2017)

surveys focused on *E. coli*, to date, limited information is available for *mcr-1*-carrying *Salmonella* in China. The ST34 *Salmonella* isolate was reportedly involved in the spread of the *mcr-1* in *Salmonella enterica* from food animals in China (Li *et al.*, 2016b; Yi *et al.*, 2017).

In Japan, a high proportion of colistin-resistant (45%) pathogenic and *mcr-1*-positive (13%) *E. coli* were reported in swine production (Kusumoto *et al.*, 2016). However, in Japan, the rates of the colistin-resistant and *mcr-1*-positive *E. coli* strains isolated from healthy animals were low (1.00 and 0.02%, respectively) (Suzuki *et al.*, 2016). Kawanishi *et al.* (2017) reported that the prevalence of *mcr-1* in *E. coli* from healthy animals actually increased slightly over the years. In Vietnam, a high prevalence (59.4%) of *mcr-1* in fecal samples from chickens was observed on farms routinely using colistin, suggesting that emergence and transmission of *mcr-1*-carrying bacteria were associated with colistin usage in chickens (Trung *et al.*, 2017). In Korea, although the *mcr-1*-positive rate in *E. coli* was low (0.1%), the prevalence of *mcr-1* has risen since 2013 (Lim *et al.*, 2016). The *mcr-1*-carrying pig isolates were also reported in Laos (Olaitan *et al.*, 2016).

In Europe, *mcr-1* was reported in Germany (Brennan *et al.*, 2016; Irrgang *et al.*, 2016; Guenther *et al.*, 2017), Belgium (Xavier *et al.*, 2016a, b), Spain (Quesada *et al.*, 2016), Italy (Carnevali *et al.*, 2016), Portugal (Campos *et al.*, 2016; Figueiredo *et al.*, 2016; Hu *et al.*, 2016; Tse and Yuen, 2016), France (Brennan *et al.*, 2016; El Garch *et al.*, 2017), The Netherlands (Kluytmans-van den Bergh *et al.*, 2016; Veldman *et al.*, 2016), Estonia (Brauer *et al.*, 2016), Great Britain (Anjum *et al.*, 2016), Lithunia, Wallonia and Flanders (Malhotra-Kumar *et al.*, 2016). Based on a retrospective survey in Germany, *mcr-1* was found in 402 of 10,600 (3.79%) *E. coli* isolates from livestock and food (Irrgang *et al.*, 2016). The *mcr-1*-positive rate ranged between 5.3 and 7.8% in broilers while the highest prevalence of *mcr-1* was found in turkeys (>10%) from 2010 to 2014 (Irrgang *et al.*, 2016). In pigs, *mcr-1* was detected with a prevalence of 1.5% in Germany (Irrgang *et al.*, 2016). In Belgium, a new colistin-resistant gene, *mcr-2*, was discovered from *E. coli* in pigs, and the prevalence of *mcr-2* in colistin-resistant *E. coli* (11/53) was higher than that of *mcr-1* (7/53) (Xavier *et al.*, 2016b).

In the USA, low prevalence of *mcr-1* was reported in food animals at slaughter (0.1%) and in swine at slaughter (0.35%) (Meinersmann *et al.*, 2017). It is interesting that two *mcr-1*-mediated colistin-resistant *E. coli* isolates were identified in swine in the USA (Meinersmann *et al.*, 2017), because colistin has not been used in animals in the USA. Emergence of colistin resistance in US animal production is still a mystery.

In South America, *mcr-1*-harboring *E. coli* isolates have been present at least since 2012. In Brazil, *mcr-1* was detected in 5% (14/280) and 1.8% (2/113) of *E. coli* of chicken and pig origin, respectively (Fernandes *et al.*, 2016); interestingly, some colistin-susceptible *E. coli* strains were observed to carry the *mcr-1* (Fernandes *et al.*, 2016). In Venezuela, two swine *E. coli* isolates were *mcr-1* positive (Delgado-Blas *et al.*, 2016).

In South Africa, colistin-resistant Avian-Pathogenic *E. coli* has significantly increased from an average of 4.5% (from 2008 to

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2014) to 13.6% in 2015 (Perreten *et al.*, 2016). Grami *et al.* (2016) reported a high prevalence (17–83%) of MCR-1 and CTX-M-1-producing *E. coli* in chicken farms in Tunisia, emphasizing the significant impact of food animal trade on the spread of *mcr-1*. In Egypt, only one *E. coli* from a cow was identified to carry *mcr-1* (Khalifa *et al.*, 2016). In another study, three avian-pathogenic *E. coli* isolates from chickens in Egypt were also reported to be *mcr-1* positive (Lima Barbieri *et al.*, 2017).

Companion animals

The *mcr-1*-positive Enterobacteriaceae also have been identified from companion animals. In China, an *E. coli* strain from a cat was found to carry a mobile IncX3-X4 hybrid plasmid bearing both *mcr-1* and bla_{NDM-5} genes (Sun *et al.*, 2016b). Transmission of *E. coli* strains harboring *mcr-1* between companion animals and human beings was also observed in a recent report in China (Lei *et al.*, 2017). In addition, a man who worked in a pet store was reported to carry *mcr-1* gene-harboring *E. coli* (Zhang *et al.*, 2016). Using real-time polymerase chain reaction (PCR), 7.4% (8/108) of fecal samples from companion animals were *mcr-1* positive (Chen *et al.*, 2017).

Wildlife

Recently, *mcr-1* was discovered in *E. coli* isolated from migratory birds in Asia (Mohsin *et al.*, 2016), Europe (Ruzauskas and Vaskeviciute, 2016) and South America (Liakopoulos *et al.*, 2016). In addition, Magellanic penguins were reported to carry *mcr-1*-positive *E. coli* in Brazil (Sellera *et al.*, 2017). The lifestyle of wildlife allows them to disseminate pathogenic and resistant microorganisms despite country borders, which may serve as an important risk factor for the spread of *mcr-1* (Ruzauskas and Vaskeviciute, 2016).

Genomic features of *mcr-1* and *mcr-1*-bearing plasmids

To date, sequences of the globally prevalent *mcr-1* gene from diverse bacterial strains are almost identical. At least six additional variants of the MCR-1 were identified; these variants only differ from MCR-1 by a single amino acid (aa), which include MCR-1.2 (Gln3→Leu) (Di Pilato *et al.*, 2016), MCR-1.3 (Ile37→Leu) (Yang *et al.*, 2017), MCR-1.4 (Asp439→Asn) (Yin *et al.*, 2017), MCR-1.5 (His451→Tyr) (Yin *et al.*, 2017), MCR-1.6 (Arg535→His) (Lu *et al.*, 2017) and MCR-1.7 (Ala214→Thr) (Yin *et al.*, 2017). The sequences of the MCR-1 identified from recent extensive studies have confirmed the same high aa identity to the first reported MCR-1 (Shen *et al.*, 2016; Lima Barbieri *et al.*, 2017; Meinersmann *et al.*, 2017; Roschanski *et al.*, 2017), including the one located in the contig N009A from a healthy human microbiome (Ye *et al.*, 2016). Strikingly, the three *mcr*-1 genes identified from *E. coli* isolates collected in the 1980s are 100% identical to the first reported *mcr-1* (Shen *et al.*, 2016).

In contrast to the high-level identity of MCR-1 sequences, the plasmids bearing *mcr-1* is fairly heterogeneous (Poirel *et al.*, 2017), such as those belong to IncI2 (Gao *et al.*, 2016; Liu *et al.*, 2016; Yao *et al.*, 2016; Meinersmann *et al.*, 2017), X4 (Hasman *et al.*, 2015; Gao *et al.*, 2016; Webb *et al.*, 2016), HI2 (Tse and Yuen, 2016) and P (Webb *et al.*, 2016). These findings suggest that *mcr-1* might be phylogenetically young and is rapidly spreading through horizontal gene transfer, either via whole plasmid conjugation (Liu *et al.*, 2016; Meinersmann *et al.*, 2017) or possible *mcr-1* cassette recombination between different plasmids (Li *et al.*, 2016a). In fact, it was shown that *mcr-1*, often neighbored by one or two copies of the insertion sequence ISApl1 (IS30 family), forms a 2600-bp cassette containing promoter sequences (Di Pilato *et al.*, 2016; Poirel *et al.*, 2016). The role of this 2600-bp cassette in the mobilization of the *mcr-1* gene was recently confirmed (Poirel *et al.*, 2017).

mcr-like genes

Besides *mcr-1*-like genes, a plasmid-borne colistin resistance gene *mcr-2*, which shared 76.7% nucleotide identity to *mcr-1*, was identified in colistin-resistant *E. coli* isolates of porcine and bovine origin in Belgium (Xavier *et al.*, 2016b). Prevalence of *mcr-2* in porcine colistin-resistant *E. coli* (11/53) in Belgium was reported to be higher than that of *mcr-1* (7/53) (Xavier *et al.*, 2016b). To date, the *mcr-2* was only reported in Belgium; a large survey showed that the *mcr-2* was not detected in 436 samples from German pig-fattening farms (Roschanski *et al.*, 2017) and in 1200 Avian Pathogenic *Escherichia coli* isolates (Lima Barbieri *et al.*, 2017).

Another plasmid-borne colistin resistance gene, *mcr-3*, was discovered in a conjugative plasmid from *E. coli* of pig origin in China (Yin *et al.*, 2017). The *mcr-3* gene displayed 45.0 and 47.0% nucleotide sequence identity to *mcr-1* and *mcr-2*, respectively (Yin *et al.*, 2017).

A recent phylogenetical analysis revealed that MCR-1 is highly homologous to its counterpart PEA lipid A transferase from *Paenibacillus* spp., which are known producers of polymyxins (Gao *et al.*, 2016). Interestingly, within the PEA transferase family, the plasmid-borne MCR-1 is closely clustered to the chromosomally encoded LptA from *Neisseria* spp., suggesting a parallel evolutionary path for MCR-1 and LptA (Gao *et al.*, 2016). Notably, despite low a sequence identity between MCR-1 and LptA (<30%), expression of LptA in the pBAD24/MG1655 system can confer colistin resistance to the level observed for MCR-1 (Gao *et al.*, 2016). In addition, *Moraxella* spp., mainly animal pathogens, were reported as potential reservoirs of *mcr*-like colistin-resistant genes (Kieffer *et al.*, 2017).

Similar to the findings from the *mcr-1*-bearing plasmids as summarized above, two IS1595-like insertion sequences were observed to be adjacent to another mobile colistin-resistant gene, *mcr-2*, in the IncX4 plasmid, pKP37-BE (Xavier *et al.*, 2016b). The IS1595-like element (714 bp) carries a transposase gene (654 bp) flanked by two 18-bp inverted repeats (Sun *et al.*,

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2017b). The formation of a circularized intermediate of *mcr-2* in the presence of the bracketing IS*Ec69* elements (Partridge, 2017; Sun *et al.*, 2017b), indicates the IS*Ec69* might be involved in the mobilization of *mcr-2*. These findings further suggest a transpositional mechanism in the spread of *mcr* at the molecular level.

Risk factors contributing to colistin resistance in animal production systems

Epidemiological studies on *mcr-1*-mediated colistin and emergence of *mcr*-like genes in animals suggest that colistin usage in food animals exert a selection pressure and serve as a major risk factor contributing to the emergence and transmission of *mcr-1*. It is important to mention that colistin is not absorbed by animal gastrointestinal tracts (Guyonnet *et al.*, 2010; Rhouma *et al.*, 2015); thus, accumulation of colistin or its metabolites in manure may significantly increase colistin resistant, *mcr-1*-harboring bacteria in agricultural ecosystems (Kruse and Sorum, 1994; Thanner *et al.*, 2016). Some studies have suggested a significant impact of livestock trade or the international trade with exotic animals, such as reptiles, on the spread of *mcr-1*-mediated colistin resistance (Grami *et al.*, 2016; Unger *et al.*, 2017).

Currently, there is a worldwide trend to limit and even ban colistin usage in animal production. Clearly, limitation of colistin usage is expected to greatly reduce selection pressure, consequently controlling transmissible colistin resistance. However, to simply limit or ban colistin in animal production will not fully solve this serious and challenging issue, because this antibiotic resistance issue can be influenced by complex multi-level factors. One example is that *mcr-1*-positive *E. coli* isolates have been identified in two swine intestinal samples in the USA (Meinersmann *et al.*, 2017), even though colistin has not been used in US animal production. Emergence of the transmissible colistin resistance in US animal production is still a mystery, but clearly suggests there may exist non-colistin usage risk factors contributing to the persistence, transmission and emergence of colistin resistance in an animal production system. Based on published information, here we discuss several potential non-colistin usage risk factors, which may represent several significant knowledge gaps impeding development of an effective mitigation strategy to control colistin resistance.

Co-selection of the mcr-1 *with specific clinical antibiotics*

Based on published complete genomes of *mcr-1* gene-harboring strains, some *mcr-1*-bearing plasmids were observed to also carry other AR genes (Malhotra-Kumar *et al.*, 2016). For example, there are several resistance-encoding genes to trimethoprim (*dfrA1*), tetracycline (*tetA*), aminoglycoside (*aadA1*, *aph(6)-Id* or *strA*, and *aph(3')-Ib*/*strB*) and sulphonamide (*sul1*) antibiotics co-residing in the *mcr-1*-bearing plasmid pKH-457-3-BE (Malhotra-Kumar *et al.*, 2016). In one *mcr-1*-positive *E. coli* strain, plasmid sequencing identified multiple genes encoding resistance to trimethoprim (*dfrA12*), tetracycline (*tetA*), aminoglycoside (*aadA3*, *aph(3')-IA*), phenicol (*cmlA1*), quinolone (*qnrS1*, *oqxA*), lincosamide (*lnu(F)*), sulphonamide (*sul2*, *sul3*) and β-lactam (extended-spectrum β-lactamase $bla_{\text{CTX-M55}}$) antibiotics (Malhotra-Kumar *et al.*, 2016). The co-existence of the *mcr-1* gene with other AR genes in a single plasmid would enable co-selection of *mcr-1* gene by using certain antibiotics, leading to persistence and transmission of the *mcr-1* gene-bearing plasmid in the absence of colistin selection pressure. To date, there is not any published information addressing this issue. Unbiased evaluation of this risk factor needs an appropriate animal model system in conjunction with comprehensive epidemiological studies to examine antibiogram, plasmid profile, and complete genome sequences of diverse *mcr-1*-bearing strains from the animal production system, which is highly warranted in the future.

Factors enhancing horizontal gene transfer

The *mcr-1*-bearing plasmid can transfer among different enteric bacteria via conjugation with variable frequencies, depending on the donor or recipient strains (Liu *et al.*, 2016; Denervaud Tendon *et al.*, 2017). According to published information (Carnevali *et al.*, 2016; Poirel *et al.*, 2016) and our phylogenetic analysis of diverse *mcr-1* positive *E. coli* strains with pig and chicken origins (unpublished data), *mcr-1*-harboring strains do not have clonal relationship and *mcr-1*-bearing plasmids display significant diversity in terms of antibiogram, plasmid profiles and genetic contents. We speculate that any factors that can enhance bacterial conjugation may promote transmission of *mcr-1*, consequently providing new opportunities for *mcr-1* to persist in the host or other niches in the absence of colistin selection pressure. For example, it has been reported that some antibiotics, such as beta-lactams and fluroquinolones, can serve as DNA damaging agents for induction of SOS response, consequently enhancing conjugation efficiency (Barr *et al.*, 1986; Beaber *et al.*, 2004). Thus, using such SOS-inducing antibiotics in animals may promote the horizontal transfer of the *mcr-1* gene-bearing plasmid. This hypothesis is partly supported by a recent case-control study showing infections caused by *mcr-1*-positive *E. coli* were associated with prior use of carbapenems and fluoroquinolones (Wang *et al.*, 2017b).

Cross-resistance between polymyxins and other AMPs

Natural antimicrobial peptides (AMPs) have been recognized as a novel class of antimicrobials to combat increasing MDR in bacterial pathogens (Hancock and Chapple, 1999; Shryock, 2004; Cotter *et al.*, 2005; Toke, 2005). AMPs are typically cationic, short, amphipathic, and microbicidal peptides that can be found in virtually all species of life (Riley and Wertz, 2002; Zasloff, 2002; Brogden *et al.*, 2003; Yeaman and Yount, 2003; Brogden, 2005; Wehkamp *et al.*, 2007). The endogenous AMPs could be derived from both metazoan hosts (e.g. defensins and cathelicidins) and bacteria (e.g. bacteriocins). AMPs display a broad spectrum of antimicrobial activity and

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have been increasingly recognized as a novel class of antibiotics (peptide antibiotics) to control pathogens (Hancock and Chapple, 1999; Shryock, 2004; Cotter *et al.*, 2005; Rossi *et al.*, 2008; Sit and Vederas, 2008). Although bacteria co-evolved with the host innate defense and developed means to curtail the effect of endogenous AMPs such as defensins, cathelicidins and bacteriocins (Ernst *et al.*, 2001; Yeaman and Yount, 2003; Kraus and Peschel, 2006; Peschel and Sahl, 2006), bacteria have not developed highly effective AMP resistance mechanisms during millions of years of co-evolution with endogenous AMPs. This intriguing phenomenon is likely due to multiple activities and pleotropic effects of natural AMPs (Peschel and Sahl, 2006; Wehkamp *et al.*, 2007).

Notably, as a bacterium-derived AMP, polymyxin has been widely and successfully used as model AMP (or AMP surrogate) to study AMP resistance in bacteria, and polymyxin bears some structural resemblance to many other AMPs. Therefore, acquisition of polymyxin resistance has been observed to result in cross-resistance to different types of AMPs (Groisman *et al.*, 1997; Bengoechea and Skurnik, 2000; Gunn *et al.*, 2000; McCoy *et al.*, 2001; McPhee *et al.*, 2003; Campos *et al.*, 2004; Chen *et al.*, 2004; Shi *et al.*, 2004; Winfield and Groisman, 2004). Based on these extensive AMP studies, the transmissible colistin resistance determinant MCR-1 may also confer a certain level of cross-resistance to some AMPs, raising a serious concern for use or development of AMP-based interventions against bacterial infections. If MCR-1 displays cross-resistance to some AMPs that have been used or are being targeted for developing new antimicrobials, such AMPs may serve as another non-colistin usage factor to promote persistence and transmissibility of *mcr-1*.

Recently, Dobias *et al.* (2017) addressed this cross-resistance issue and observed that MCR-1 did not confer cross-resistance to human cathelicidin LL-37, α-defensin 5, and β-defensin 3 in *E. coli* and *K. pneumoniae*. We also observed that MCR-1 did not confer cross-resistance to chicken host AMPs in *E. coli* either (unpublished data). Therefore, the transmissible colistin resistance determinant MCR-1 apparently does not confer cross-resistance to common host defense peptides, which greatly mitigates safety and sustainability concerns for some recent efforts to develop AMP-based intervention, such as using host AMP-inducing compounds as an innovative non-antibiotic approach to control bacterial infections (Sunkara *et al.*, 2011, 2014; van der Does *et al.*, 2012). However, to better address this cross-resistance issue, in the future, more extensive studies are needed to examine if MCR-1 can confer cross-resistance to AMPs from various sources, which will provide critical information for risk assessment and management of AMP-based anti-infectives.

Existence and emergence of novel colistin-resistant genes in ecosystem

Recent metagenomics and functional genomics studies have provided compelling evidence that antibiotic resistance genes are widespread; the novel and immensely diverse resistance genes exist in various ecosystems such as the intestinal tracts of people and food animals, agriculture (e.g., animal manure, soil, water and wastewater lagoons), and even ancient soils (Aminov and Mackie, 2007; Pehrsson *et al.*, 2013; Davies, 2014). Functional screening of metagenomic libraries constructed from fecal samples from human beings (Sommer *et al.*, 2009), chickens (Zhou *et al.*, 2012), pigs (Kazimierczak *et al.*, 2009), gulls (Martiny *et al.*, 2011) and dairy cows (Wichmann *et al.*, 2014) have demonstrated that majority of antibiotic-resistant genes in gut microbial communities are novel and share low identity (40–60%) with the previously identified resistance genes. These novel antibiotic-resistance genes have potential to emerge if the opportunity arises (Aminov and Mackie, 2007; Pehrsson *et al.*, 2013; Davies, 2014). Based on these metagenomic discoveries, novel colistin-resistance genes may also exist in ecosystems and have potential to emerge if the opportunity arises; this speculation has been partly supported by recent identification of LptA (Gao *et al.*, 2016) as well as MCR-2 (Xavier *et al.*, 2016b) and MCR-3 (Yin *et al.*, 2017) discussed in above sections.

Identification of novel colistin-resistance genes is important for combating the colistin resistance threat, because elucidation of the colistin resistome will greatly facilitate development of molecular diagnostic tools to effectively monitor colistin resistance in agricultural ecosystems. In addition, further in-depth characterization of colistin-resistance genes will improve our understanding of the evolution and molecular basis of colistin resistance, consequently providing insights into new interventions by targeting resistance mechanisms. Finally, identification of novel colistin-resistance genes using a functional cloning approach will complement the modern high-throughput sequencing of metagenomes by greatly improving annotation power. Therefore, identification of a novel colistin resistome from an animal production system using a functional metagenomics screening approach is also highly warranted in the future.

Conclusion

Recent emergence of the transmissible colistin resistance gene, *mcr-1*, has drawn worldwide attention and fears. Epidemiological studies suggest that use of colistin in animals is a major risk factor for the emergence and transmission of *mcr-1*. Therefore, there is a worldwide trend to limit colistin usage in animal production. Although the regulated use of colistin would greatly reduce selection pressure, consequently controlling the transmissible colistin resistance in an animal production system, other non-colistin usage risk factors may also exist for colistin resistance development. In this review, in addition to a comprehensive review of epidemiological studies for prevalence of *mcr* genes in animals, we also summarized published information to support the existence of several non-colistin usage factors that may contribute to the persistence, transmission and emergence of colistin resistance in an animal production system. Comprehensive examination of these non-colistin usage factors using both *in vitro* and *in vivo* systems in the future will generate critical information for risk assessment and risk management of transmissible colistin resistance, leading to proactive and effective strategies for mitigation of colistin resistance in animal production system worldwide.

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