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Prevalence of Extended-Spectrum Beta-Lactamase-Producing Gram-Negative Bacilli and Emergence of *mcr-1* Colistin Resistance Gene in Lebanese Swine Farms

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Livestock are considered reservoirs of multidrug-resistant organisms that can be transferred to humans through direct/indirect routes. Once transmitted, these organisms can be responsible for infections with therapeutic challenges. The aim of this study was to determine the prevalence of extended-spectrum cephalosporin and colistin-resistant Gram-negative bacilli in Lebanese swine farms. In May 2017, 114 fecal samples were collected from swine farms in south Lebanon. Separate media supplemented with cefotaxime, ertapenem, and colistin were used for the screening of resistant organisms. Double-disk synergy test and ampC disk test were performed to detect extended-spectrum beta-lactamase (ESBL) and ampC producers, respectively. Detection of beta-lactamase and *mcr* genes was performed using real time PCR. Of 114 fecal samples, 76 showed growth on the medium with cefotaxime. In total, 111 strains were isolated with 94.5% being *Escherichia coli*. Phenotypic tests showed that 98, 6, and 7 strains were ESBL, ampC, and ESBL/ampC producers, respectively. CTX-M and CMY were the main beta-lactamase genes detected. On the medium with colistin, 19 samples showed growth. In total, 23 colistin-resistant *E. coli* strains harboring the *mcr-1* gene were isolated. This is the first study in Lebanon determining multidrug resistance epidemiology in pigs. The prevalence of ESBLs is high and the emergence of colistin resistance is alarming.

Keywords: ampC, ESBL, *E. coli*, *mcr-1*, pigs

Introduction

Resistance in Gram-negative bacilli toward the most common antibiotics administered in the human medicine, that is, beta-lactams has significantly increased in the past decade.[1] Resistance to beta-lactams and carbapenems in Gram-negative bacteria is mainly mediated through the production of extended-spectrum beta-lactamases (ESBLs), ampC beta-lactamases, and carbapenemases.[1] Genes encoding these enzymes are often colocalized on plasmids harboring resistance genes to other commonly prescribed antibiotics in human medicine such as aminoglycosides and quinolones.[1] Dissemination of resistant organisms often results in reducing the efficacy of beta-lactam antibiotics, thus limiting treatment options of infectious diseases.[2]

This is currently emphasized with the recent emergence of colistin resistance in Gram-negative bacilli. Colistin belongs to the polymyxin antibiotics family that acts on the lipopolysaccharide (LPS) chain of the bacteria and leads to increased permeability of the outer membrane and subsequent cellular leakage followed by cell death.[3] In human medicine history, colistin was abandoned because of its nephrotoxicity and neurotoxicity inside human body.[4] However, because of the widespread multidrug-resistant (MDR) organisms, mainly carbapenem-resistant organisms, colistin was reintroduced in clinical settings.[5] This antibiotic revival had to face the emergence of colistin resistance in bacteria of human and animal origin.[6]

Before 2015, colistin resistance was thought to be only mediated through chromosomal mutations that leads to the alteration of the lipid A subunit of the LPS chain by the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolamine (PEtN),[6] thus resulting in a reduced binding to colistin and subsequently bacterial resistance.[6] However, in 2016, Liu *et al.* reported the first detection of a transferable phosphoenolamine transferase named *mcr-1* gene in *Escherichia coli* strains isolated from pigs and meat.[7] In this context, *mcr-1* was reported from clinical and animal isolates across all continents. Furthermore, *mcr* variants, that is, *mcr-2*,[8] *mcr-3*,[9] *mcr-4*,[10] and *mcr-5*[11] have also emerged.

Nowadays, farm animals are considered reservoirs of antimicrobial resistance.[12] The unregulated use of antibiotics is

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considered among the most common drivers for the emergence of resistance in livestock.[13] Indeed, antibiotics are given not only for treatment but are also prescribed for prophylaxis and administered as growth promoters.[13] The major public health concern about multidrug resistance spread in animals is the potential transmission to humans through direct contact or indirectly through the consumption of undercooked/uncooked animal-origin food.[14] Once transmitted, these organisms can cause infections with limited therapeutic options, especially those cross-resistant to antibiotics frequently used in the human medicine.[15]

In Lebanon, the dissemination of MDR organisms in the clinical settings is well documented[16–20]; however, studies addressing multidrug resistance in animals remain scarce. One study carried by Diab *et al.* showed a relatively high prevalence of the CTX-M-15 ESBL type in *E. coli* of cattle origin in Lebanon.[21] More recently, a nationwide study conducted in Lebanese chicken farms reported an elevated level of ESBL/ampC-producing Gram-negative bacilli intestinal carriage.[22] Recently, our group reported the first detection of an *E. coli* isolated from poultry in south Lebanon harboring the *mcr-1* colistin resistance gene in addition to the TEM-135-like ESBL gene.[23] In pigs, only one study reported the detection of an OXA-23-producing *Acinetobacter baumannii* in northern Lebanon.[24] The prevalence of MDR organisms in the Lebanese swine farms remains unknown. In collaboration with the ministry of agriculture, the aim of this study was to determine the prevalence of extended-spectrum cephalosporin and colistin-resistant Gram-negative bacilli in Lebanese swine farms.

Materials and Methods

Ethics statement and collection of samples

The Ministry of Agriculture in Lebanon approved the collection of fecal samples from swine farms. The sampling was realized in compliance with the national guidelines for animal safety. On May 30, 2017, 111 fecal samples were randomly collected from 3 different swine farms located in south Lebanon. In addition, three fecal samples were taken from three wild pigs living in the same region. The number of samples collected was relatively proportional to the farms size that ranged from 20 to 120 pigs per farm (Table 1). The fecal samples were collected using sterile urine cups and directly placed in a portable refrigerator; then when taken to the university laboratory, they were stored at −80°C until being used.

Screening of resistant organisms and identification

Each fecal sample was mixed in a sterile container and then a swab was used to subculture a considerable amount on MacConkey agars supplemented separately with cefotaxime (2 μg/mL), ertapenem (1 μg/mL), and colistin (4 mg/L) for the screening of resistant Gram-negative bacilli. After overnight incubation at 37°C, isolated colonies with different morphologies were separately taken from each plate and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a score value ≥1.9 using the Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany).[25] Thereafter, the strains were conserved in 40% glycerol aliquots at −80°C for further tests.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines 2017.[26] A total of 16 antibiotics were tested involving 11 beta-lactams (ampicillin, amoxicillin–clavulanic acid, aztreonam, cefotaxime, ceftazidime, cefoxitin [FOX], cefepime, piperacillin–tazobactam, ertapenem, meropenem, imipenem) and 5 non-beta-lactams (colistin, gentamicin, ciprofloxacin, trimethoprim–sulfamethoxazole and tigecycline; Bio-Rad, Marnes-la-Coquette, France). The phenotypic detection of ESBL was performed using the double-disk synergy test by placing an amoxicillin–clavulanic acid disk between cefepime, ceftazidime, and aztreonam. Formation of a keyhole effect was considered as a phenotypic indication of ESBL production. Regarding screening of ampC beta-lactamase and carbapenemase production, ampC disk test and carba NP test were performed, respectively, as previously described.[27,28] Furthermore, all isolates having a narrow diameter zone of inhibition around the colistin disk were subjected to colistin broth microdilution test as previously described.[26] An isolate is termed as MDR if this latter was resistant to three different classes of antibiotics at least.[29]

Table 1. Distribution of Extended-Spectrum Beta-Lactamase/ampC-Producing and Colistin-Resistant Gram-Negative Bacilli per Farm

	AB used	*Collected samples,* n	*ESBLs/ampCs samples,* n	*ESBLs/ampCs isolates,* n	*Species*	*Col/R samples,* n	*Col/R isolates,* n	*Species*
Farm 1 ($n=120$)	Enrofloxacin	60	42	65	60 *Escherichia coli,* 4 *Escherichia fergusonii,* 1 *Klebsiella pneumoniae*	8	8	*E. coli*
Farm 2 ($n=20$)	Unknown	15	8	9	8 *E. coli*, 1 *K. pneumoniae*	4	5	*E. coli*
Farm 3 ($n=100$)	Unknown	36	24	35	*E. coli*	7	10	*E. coli*
WP ($n=3$)	Unknown	3	2	2	*E. coli*	0	0	

AB, antibiotic; Col/R, colistin resistant; ESBLs, extended-spectrum beta-lactamases; WP, wild pigs.

PCR identification of beta-lactamase genes

All isolates showing a keyhole effect or having resistance to both cefoxitin and cefepime were subjected to real-time PCR analysis for blaCTX-M, blaSHV, and blaTEM genes screening.[30] Furthermore, all strains found positive to the ampC disk test were also tested for genes encoding ampC beta-lactamases FOX, MOX, ACC, EBC, DHA, and CMY using simplex PCRs.[31] DNA extraction was performed using EZ1 DNA extraction kit (Qiagen, Courtaboeuf, France), following manufacturer instructions with an EZ1 Advanced XL biorobot.

Molecular characterization of mcr-1 *colistin resistance gene*

All strains having a colistin minimum inhibitory concentration (MIC) ≥2 μg/mL were subjected to standard PCR amplification and sequencing for the detection of *mcr-1* colistin resistance gene. DNA extraction was carried out using an EZ1 DNA extraction kit (Qiagen) with an EZ1 Advanced XL biorobot. Primers used in molecular analysis were previously described in other studies.[32]

Results

Prevalence of beta-lactamase producers and colistin-resistant Gram-negative bacilli

Of 114 fecal samples collected, 76 (66.5%) showed positive growth on the selective medium supplemented with cefotaxime. In total, 111 MDR strains were isolated according to the following distribution: 65 strains in farm 1, 9 in farm 2, 35 in farm 3, and 2 isolates from the wild pigs. MALDI-TOF MS identification revealed that *E. coli* made up to 94.5% of isolated MDR strains, *Escherichia fergusonii* 3.5%, and *Klebsiella pneumoniae* 2% (Table 1). Besides, 23 colistin-resistant *E. coli* strains isolated from 19 fecal samples were obtained. No carbapenemase producers were detected in this study.

Phenotypic profiles of beta-lactamase producers

The resistance profiles of isolated ESBL and/or ampC-producing Gram-negative bacilli are summarized in Table 2. All ESBL/ampC-producing strains were susceptible to colistin and carbapenems. Carba np test, double-disk synergy test, and ampC disk test revealed the absence of carbapenemase producers, 98 isolates (88.5%) were categorized as ESBL producers, 7 (6%) as ESBL/ampC coproducers, and 6 strains (5.5%) as solely ampC producers. *K. pneumoniae* isolates were only ESBL producers, whereas three *E. fergusonii* were categorized as ampC producers and one as an ESBL producer. Coproduction of ESBL and ampC was only detected in *E. coli* isolates. Regarding non-beta-lactam antibiotics resistance in the aforementioned strains, one isolate was coresistant to all non-beta-lactams tested: tigecycline, gentamicin, ciprofloxacin, and trimethoprim–sulfamethoxazole, 32 (29%) were co-resistant to 3 non-beta-lactams, 59 (53%) to 2 non-beta-lactams, 16 (14%) to 1 non-beta-lactam, and three strains were susceptible to all non-beta-lactam antibiotics. Overall, 83% of beta-lactamase-producing Gram-negative bacilli in this study were co-resistant to at least two non-beta-lactams.

TABLE 2. RESISTANCE PROFILES OF EXTENDED-SPECTRUM BETA-LACTAMASE/AMPC-PRODUCING GRAM-NEGATIVE BACILLI

	Antibiotic susceptibility testing											*Phenotype*		
Species	*AMP*	*CTX*	*AZT*	*FOX*	*CAZ*	*AUG*	*FEP*	*PTZ*	*TGC*	*SXT*	*CIP*	*GNT*	*% of ESBL*	*% of ampC*	*% of ESBL/ampC*
E. coli ($n=105$)	103 (98)	70 (67)	45 (43)	25 (24)	44 (42)	48 (46)	57 (54)	1 (1)	1 (1)	97 (92)	82 (78)	44 (42)	90	3	7
E. fergusonii ($n=4$)	4 (100)	2 (50)	3 (80)	4 (100)	4 (100)	4 (100)	0 (0)	0 (0)	0 (0)	1 (20)	4 (100)	3 (80)	20	80	
K. pneumoniae ($n=2$)	2 (100)	2 (100)	1 (50)	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	100		

Resistance profiles are presented as number (percentage).
AMP, ampicillin; AUG, amoxicillin–clavulanic acid; AZT, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GNT, gentamicin; SXT, trimethoprim–sulfamethoxazole; TGC, tigecycline; PTZ, piperacillin–tazobactam.

Molecular characterization of beta-lactamase genes

A total of 105 Gram-negative bacilli having ESBL phenotypes were subjected to real-time PCR analysis for the screening of CTX-M-, TEM-, and SHV-encoding genes. CTX-M was detected in 83 (79%) ESBL isolates, TEM in 57 (54%), and SHV in 9 (8.5%). In total, 12 strains (11%) showed the coexistence of the 3 *bla* genes together, 43 (41%) showed the coexistence of 2 *bla* genes, and 57 (54%) harbored only 1 beta-lactamase gene. In addition, CMY was the only ampC-encoding gene detected in ampC and ESBL/ampC coproducers.

Colistin-resistant isolates: resistance profiles and genotype

The detailed profile of the resistance of *E. coli* colistin-resistant strains isolated in this study is given in Fig. 1. To summarize, 4 of the 23 strains were colistin resistant and also ESBL producers, whereas the remaining strains (19 isolates) were susceptible to all beta-lactams tested, except for ampicillin. Resistance rates toward non-beta-lactam antibiotics varied: eight strains were co-resistant to gentamicin, ciprofloxacin, and trimethoprim–sulfamethoxazole, seven strains were resistant to two non-beta-lactams, two were resistant to only one non-beta-lactam antibiotic and six strains were susceptible to all non-beta-lactams tested. Colistin MICs of the 23 *E. coli* isolates ranged between 4 and 16 μg/mL except 1 strain having an MIC of 256 μg/mL. Standard PCR and sequencing revealed that all the strains were *mcr-1* positive. In the four ESBL *mcr-1*-positive resistant isolates, CTX-M was detected in two strains, whereas SHV and TEM were detected in all four strains (Fig. 1).

Discussion

Antimicrobial resistance is rapidly evolving and disseminating worldwide. In the context of antimicrobial resistance in the one health concept, livestock (*i.e.*, pigs, poultry, and cattle) is now considered as a major reservoir of MDR organisms and antibiotic resistance genes.[12] In Lebanon, few studies have been conducted to determine the prevalence of MDR organisms in Lebanese livestock[21]; however in pork, only one study reported the detection of a carbapenemase-producing *A. baumannii* isolate from a pig in northern Lebanon.[24] To the best of our knowledge, our study is the first in Lebanon to describe the epidemiology of beta-lactamase-producing Gram-negative bacilli in Lebanese swine farms. It is worth mentioning that the number of samples collected was not relatively high because only few swine farms are accessible in Lebanon. The role of the Ministry of Agriculture was essential to carry out this study because it provided the legal permission to access and sample the different sites.

In our investigation, ESBL/ampC-producing Gram-negative bacilli were detected in 66.5% of the collected fecal samples (Table 1). Compared with other epidemiological studies investigating pigs worldwide, the prevalence in Lebanon is not far from what is reported in Belgium (75%)[33] and Germany (88%)[34] but is still much higher than those reported in China (32%),[35] United Kingdom (23%),[36]

	Isolate	Colistin MIC (μg/ml)	AMP	FOX	ATM	CTX	PTZ	FEP	AUG	CAZ	Carb	GNT	SXT	CIP	TGC	*bla* genes
Farm 1	*E.coli* (1)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (2)	4	R	S	S	S	S	S	R	S	S	S	R	R	S	
	E.coli (3)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (4)	16	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (5)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (6)	8	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (7)	8	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (8)	4	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (9)	4	R	S	S	S	S	S	S	S	S	R	R	R	S	
	E.coli (10)	4	R	R	S	S	S	S	R	S	S	R	R	S	S	SHV/TEM
Farm 2	*E.coli* (11)	8	R	S	S	S	S	S	R	S	S	S	R	S	S	
	E.coli (12)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (13)	8	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (14)	8	R	S	S	S	S	S	S	S	S	S	S	S	S	
	E.coli (15)	8	R	R	S	S	S	S	R	S	S	S	S	S	S	SHV/TEM
Farm 3	*E.coli* (16)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (17)	8	R	S	S	S	S	S	R	S	S	R	R	R	S	
	E.coli (18)	4	R	S	S	S	S	S	S	S	S	S	R	R	S	
	E.coli (19)	8	R	S	S	S	S	S	S	S	S	S	R	S	S	
	E.coli (20)	8	R	S	S	S	S	S	S	S	S	S	R	S	S	
	E.coli (21)	16	R	R	S	S	S	S	R	R	S	S	R	S	S	CTX-M/SHV/TEM
	E.coli (22)	8	R	S	S	S	S	S	S	S	S	S	S	R	S	
	E.coli (23)	up 256	R	S	S	R	S	R	S	R	S	S	S	R	S	CTX-M/SHV/TEM

FIG. 1. Resistance profiles of *mcr-1* colistin-resistant *Escherichia coli* isolates. AMP, ampicillin; AUG, amoxicillin–clavulanic acid; AZT, aztreonam; bla, beta-lactamase; Carb, carbapenems; CAZ, ceftazidime; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; GNT, gentamicin; R, resistant; S, sensitive; SXT, trimethoprim–sulfamethoxazole; TGC, tigecycline; PTZ, piperacillin–tazobactam.

Denmark (18.5%),[37] Switzerland (15%),[38] and Thailand (2.4%).[39] Differences in the number of samples and screening methodologies, in addition to the level and type of antibiotics prescribed in the farms of each country could explain these differences.[3] The aforementioned concept applies also to prevalence of *mcr-1*-positive *E. coli* strains detected in our previous study (17%) compared with other international studies: Portugal (98%),[40] Vietnam (37.5%),[41] China (20.6%),[7] Japan (1%),[42] France (0.5%)[43] and United States (0.35%).[44]

In this study, 83% of ESBL/ampC producers were co-resistant to at least two non-beta-lactam antibiotics with the highest level of resistance being observed against trimethoprim–sulfamethoxazole and ciprofloxacin. During our samples collection, we tried hard to collect correct data on the types and quantities of antibiotics used in the different farms, a mission nearly impossible. Indeed, despite the official presence of the Ministry of Agriculture, the cooperation of the farm owners was not easy to get, and there was no clear distinction between different uses of antibiotic in farms investigated (treatment of infections, prevention on infection, and growth enhancement). Unofficially, we were informed that enrofloxacin is frequently administered to pork in Lebanon. In fact, it has been reported that in pigs, penicillins are used to treat necrotic enteritis, whereas cephalosporins such as cefquinome and ceftiofur are prescribed for polyarthritis, septicemia, polyserositis, and respiratory infections.[2] Use of non-beta-lactams such as gentamicin, fluoroquinolones, aminoglycosides, and colistin was also reported.[45,46]

On the contrary, it is not clear to us to which extent international guidelines and recommendations for hygiene and waste management in pig farms are applied in our country. Questionable hygiene, poor feed quality, and bad waste management imply another important drive in the emergence of multidrug resistance in pigs in addition to the overuse of antibiotics that facilitates the transmission of resistant organisms from pigs to their surrounding environment and vice versa. At the molecular level, the most commonly detected beta-lactamase gene was the CTX-M. This gene was highly reported in Lebanon in the clinical settings[16,47] and in cattle[21] and poultry.[22] CTX-M is also the main ESBL type reported globally in farm animals.[36,37,39,48] As for ampC producers, this study showed that CMY was the only ampC beta-lactamase gene detected in swine farms in Lebanon. The same observation was also made in chicken farms (data not given). It has been shown worldwide that this gene is the most common ampC beta-lactamase gene detected in poultry,[49,50] food-producing animals,[51,52] and healthy pets.[53,54] In this study, it has not escaped our attention that no carbapenemase producers were detected. This is in accordance with another study performed by our group in poultry farms[22] reflecting that carbapenemase producers are really scarce in Lebanese livestock.

Furthermore, in this study we report for the first time the detection of *mcr-1* in pork in Lebanon. In this country, *mcr-1* gene was first reported in chicken during an epidemiological study aiming at determining the prevalence of MDR organisms in Lebanese chicken farms.[23] The MIC values of colistin in *mcr-1*-producing *E. coli* isolates in this study range between 4 and 16 μg/mL. These results are in accordance with other studies showing that *mcr-1*-harboring isolates do not usually have elevated colistin MICs.[55,56] Some reports showed that *mcr-1*-positive *E. coli* isolate could have a colistin MIC as low as 2 μg/mL.[57] In our collection of *mcr-1* strains, only one ESBL-producing *E. coli* had a colistin MIC of 256 μg/mL. This elevated MIC might be attributed to additional chromosomal mutations in the *phoP/Q*, *pmrA/B*, and *mgrB* genes as reported previously in the literature.[5] However, further genomic analysis is needed to explore this possibility. Delannoy *et al.* reported the isolation of *E. coli* strains harboring *mcr-1* and having amino acid mutations in the *phoP/Q*, *pmrA/B*, and *mgrB* genes from diseased pigs in France.[57]

Furthermore, it is worth mentioning that, as given in Fig. 1, none of the colistin-resistant isolates was pandrug resistant, but rather remained susceptible to the majority of the tested antibiotics, except four strains that were ESBL producers. The coexistence of *mcr-1*- and ESBL/carbapenemase-encoding genes was previously reported in several studies in the literature.[58,59] Resistance profiles of *mcr-1* strains in this study possibly illustrate an overestimated fear of colistin resistance. *E. coli* colistin-resistant isolates will pose therapeutic challenges only if transmission of MDR strains to humans occurs.

In conclusion, this study describes the epidemiology of ESBL/ampC-producing Gram-negative bacilli in Lebanese swine farms. The emergence of *mcr-1* in pigs is alarming. The level of antibiotic consumption in Lebanese swine farms remains unknown; a more transparent policy should be adopted in this context. Therefore, the surveillance and control programs addressing antibiotic consumption in Lebanese farms, especially in pigs, are urgently needed. Future studies should not only focus on antimicrobials usage but also on the risk factors associated with the carriage of MDR organisms in pigs.

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Disclosure Statement

No competing financial interests exist.

References

1. Ruppe, E., P.L. Woerther, and F. Barbier. 2015. Mechanisms of antimicrobial resistance in gram-negative bacilli. Ann. Intensive Care 5:21.
2. Seiffert, S.N., M. Hilty, V. Perreten, and A. Endimiani. 2013. Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: an emerging problem for human health? Drug Resist. Updat. 16:22–45.
3. Rhouma, M., F. Beaudry, and A. Letellier. 2016. Resistance to colistin: what is the fate for this antibiotic in pig production? Int. J. Antimicrob. Agents 48:119–126.
4. Olaitan, A.O., and J. Li. 2016. Emergence of polymyxin resistance in gram-negative bacteria. Int. J. Antimicrob. Agents 48:581–582.

5. Olaitan, A.O., S. Morand, and J.M. Rolain. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front. Microbiol. 5:643.
6. Baron, S., L. Hadjadj, J.M. Rolain, and A.O. Olaitan. 2016. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int. J. Antimicrob. Agents 48:583–591.
7. Liu, Y.Y., Y. Wang, T.R. Walsh, L.X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L.F. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J.H. Liu, and J. Shen. 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. 16:161–168.
8. Xavier, B.B., C. Lammens, R. Ruhal, S. Kumar-Singh, P. Butaye, H. Goossens, and S. Malhotra-Kumar. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium. Euro. Surveill. 21:1–6.
9. Yin, W., H. Li, Y. Shen, Z. Liu, S. Wang, Z. Shen, R. Zhang, T.R. Walsh, J. Shen, and Y. Wang. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. MBio 8:pii:e00543-17.
10. Carattoli, A., L. Villa, C. Feudi, L. Curcio, S. Orsini, A. Luppi, G. Pezzotti, and C.F. Magistrali. 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:pii:30589.
11. Borowiak, M., J. Fischer, J.A. Hammerl, R.S. Hendriksen, I. Szabo, and B. Malorny. 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.
12. Szmolka, A., and B. Nagy. 2013. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Front. Microbiol. 4:258.
13. Barton, M.D. 2014. Impact of antibiotic use in the swine industry. Curr. Opin. Microbiol. 19:9–15.
14. Dahms, C., N.O. Hubner, F. Wilke, and A. Kramer. 2014. Mini-review: epidemiology and zoonotic potential of multiresistant bacteria and *Clostridium difficile* in livestock and food. GMS Hyg. Infect. Control 9:Doc21.
15. Overdevest, I., I. Willemsen, M. Rijnsburger, A. Eustace, L. Xu, P. Hawkey, M. Heck, P. Savelkoul, C. Vandenbroucke-Grauls, K. van der Zwaluw, X. Huijsdens, and J. Kluytmans. 2011. Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg. Infect. Dis. 17:1216–1222.
16. Baroud, M., I. Dandache, G.F. Araj, R. Wakim, S. Kanj, Z. Kanafani, M. Khairallah, A. Sabra, M. Shehab, G. Dbaibo, and G.M. Matar. 2013. Underlying mechanisms of carbapenem resistance in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates at a tertiary care centre in Lebanon: role of OXA-48 and NDM-1 carbapenemases. Int. J. Antimicrob. Agents 41:75–79.
17. El-Herte, R.I., G.F. Araj, G.M. Matar, M. Baroud, Z.A. Kanafani, and S.S. Kanj. 2012. Detection of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* producing NDM-1 in Lebanon. J. Infect. Dev. Ctries. 6: 457–461.
18. Moghnieh, R., N. Estaitieh, A. Mugharbil, T. Jisr, D.I. Abdallah, F. Ziade, L. Sinno, and A. Ibrahim. 2015. Third generation cephalosporin resistant enterobacteriaceae and multidrug resistant gram-negative bacteria causing bacteremia in febrile neutropenia adult cancer patients in Lebanon, broad spectrum antibiotics use as a major risk factor, and correlation with poor prognosis. Front. Cell. Infect. Microbiol. 5:11.
19. Daoud, Z., E. Salem Sokhn, K. Masri, K. Cheaito, N. Haidar-Ahmad, G.M. Matar, and S. Doron. 2015. Corrigendum: *Escherichia coli* isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. Front. Med. (Lausanne) 2:66.
20. Al Atrouni, A., M. Hamze, T. Jisr, C. Lemarie, M. Eveillard, M.L. Joly-Guillou, and M. Kempf. 2016. Wide spread of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* belonging to clonal complex II in different hospitals in Lebanon. Int. J. Infect. Dis. 52: 29–36.
21. Diab, M., M. Hamze, J.Y. Madec, and M. Haenni. 2016. High prevalence of non-ST131 CTX-M-15-producing *Escherichia coli* in healthy cattle in Lebanon. Microb. Drug Resist. 23:261–266.
22. Dandachi, I., E.S. Sokhn, E. Dahdouh, E. Azar, B. El-Bazzal, J. Rolain, and Z. Daoud. 2018. Prevalence and characterization of multi-drug-resistant gram-negative bacilli isolated from Lebanese poultry: a nationwide study. Front. Microbiol. 9:550.
23. Dandachi, I., T. Leangapichart, Z. Daoud, and J.M. Rolain. 2018. First detection of *mcr-1* plasmid mediated colistin resistant *E. coli* in Lebanese poultry. J. Glob. Antimicrob. Resist. 12:137–138.
24. Al Bayssari, C., F. Dabboussi, M. Hamze, and J.M. Rolain. 2015. Emergence of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in livestock animals in Lebanon. J. Antimicrob. Chemother. 70:950–951.
25. Seng, P., J.M. Rolain, P.E. Fournier, B. La Scola, M. Drancourt, and D. Raoult. 2010. MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol. 5:1733–1754.
26. European Committee on Antimicrobial Susceptibility Testing. 2017. Breakpoint tables for interpretation of MICs and zone diameters, version 7.1. Available at www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf (accessed December 12, 2017).
27. Black, J.A., E.S. Moland, and K.S. Thomson. 2005. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in enterobacteriaceae lacking chromosomal AmpC beta-lactamases. J. Clin. Microbiol. 43: 3110–3113.
28. Bakour, S., V. Garcia, L. Loucif, J.M. Brunel, A. Gharout-Sait, A. Touati, and J.M. Rolain. 2015. Rapid identification of carbapenemase-producing enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using a modified carba NP test. New Microbes New Infect. 7: 89–93.
29. Magiorakos, A.P., A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, S. Harbarth, J.F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D.L. Paterson, L.B. Rice, J. Stelling, M.J. Struelens, A. Vatopoulos, J.T. Weber, and D.L. Monnet. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268–281.

30. Roschanski, N., J. Fischer, B. Guerra, and U. Roesler. 2014. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT-type AmpCs in enterobacteriaceae. PLoS One 9:e100956.
31. Dallenne, C., A. Da Costa, D. Decre, C. Favier, and G. Arlet. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in enterobacteriaceae. J. Antimicrob. Chemother. 65:490–495.
32. Bachiri, T., R. Lalaoui, S. Bakour, M. Allouache, N. Belkebla, J.M. Rolain, and A. Touati. 2017. First report of the plasmid-mediated colistin resistance gene *mcr-1* in *Escherichia coli* ST405 isolated from wildlife in Bejaia, Algeria. Microb. Drug Resist. [Epub ahead of print]; DOI: 10.1089/mdr.2017.0026.
33. Van Damme, I., C. Garcia-Graells, W. Biasino, T. Gowda, N. Botteldoorn, and L. De Zutter. 2017. High abundance and diversity of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in faeces and tonsils of pigs at slaughter. Vet. Microbiol. 208:190–194.
34. Dahms, C., N.O. Hubner, A. Kossow, A. Mellmann, K. Dittmann, and A. Kramer. 2015. Occurrence of ESBL-producing *Escherichia coli* in livestock and farm workers in Mecklenburg-western Pomerania, Germany. PLoS One 10:e0143326.
35. Hu, Y.Y., J.C. Cai, H.W. Zhou, D. Chi, X.F. Zhang, W.L. Chen, R. Zhang, and G.X. Chen. 2013. Molecular typing of CTX-M-producing *Escherichia coli* isolates from environmental water, swine feces, specimens from healthy humans, and human patients. Appl. Environ. Microbiol. 79:5988–5996.
36. Randall, L.P., F. Lemma, J.P. Rogers, T.E. Cheney, L.F. Powell, and C.J. Teale. 2014. Prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* from pigs at slaughter in the in 2013. J. Antimicrob. Chemother. 69:2947–2950.
37. Hammerum, A.M., J. Larsen, V.D. Andersen, C.H. Lester, T.S. Skovgaard Skytte, F. Hansen, S.S. Olsen, H. Mordhorst, R.L. Skov, F.M. Aarestrup, and Y. Agersø. 2014. Characterization of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. J. Antimicrob. Chemother. 69:2650–2657.
38. Geser, N., R. Stephan, and H. Hachler. 2012. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet. Res. 8:21.
39. Sinwat, N., S. Angkittitrakul, K.F. Coulson, F.M. Pilapil, D. Meunsene, and R. Chuanchuen. 2016. High prevalence and molecular characteristics of multidrug-resistant *Salmonella* in pigs, pork and humans in Thailand and Laos provinces. J. Med. Microbiol. 65:1182–1193.
40. Kieffer, N., M. Aires-de-Sousa, P. Nordmann, and L. Poirel. 2017. High rate of *MCR-1*-producing *Escherichia coli* and *Klebsiella pneumoniae* among pigs, Portugal. Emerg. Infect. Dis. 23:2023–2029.
41. Malhotra-Kumar, S., B.B. Xavier, A.J. Das, C. Lammens, H.T. Hoang, N.T. Pham, and H. Goossens. 2016. Colistin-resistant *Escherichia coli* harbouring *mcr-1* isolated from food animals in Hanoi, Vietnam. Lancet Infect. Dis. 16: 286–287.
42. Kawanishi, M., H. Abo, M. Ozawa, M. Uchiyama, T. Shirakawa, S. Suzuki, A. Shima, A. Yamashita, T. Sekizuka, K. Kato, M. Kuroda, R. Koike, and M. Kijima. 2016. Prevalence of colistin resistance gene *mcr-1* and absence of *mcr-2* in *Escherichia coli* isolated from healthy food-producing animals in Japan. Antimicrob. Agents Chemother. 61:pii:e02057-16.
43. Perrin-Guyomard, A., M. Bruneau, P. Houee, K. Deleurme, P. Legrandois, C. Poirier, C. Soumet, and P. Sanders. 2016. Prevalence of *mcr-1* in commensal *Escherichia coli* from French livestock, 2007 to 2014. Euro. Surveill. 21:1–3.
44. Meinersmann, R.J., S.R. Ladely, J.R. Plumblee, K.L. Cook, and E. Thacker. 2017. Prevalence of *mcr-1* in the cecal contents of food animals in the United States. Antimicrob. Agents Chemother. 61:1–4.
45. Tian, G.B., H.N. Wang, A.Y. Zhang, Y. Zhang, W.Q. Fan, C.W. Xu, B. Zeng, Z.B. Guan, and L.K. Zou. 2012. Detection of clinically important beta-lactamases in commensal *Escherichia coli* of human and swine origin in western China. J. Med. Microbiol. 61(Pt 2):233–238.
46. Rhouma, M., F. Beaudry, W. Theriault, and A. Letellier. 2016. Colistin in pig production: chemistry, mechanism of antibacterial action, microbial resistance emergence, and one health perspectives. Front. Microbiol. 7:1789.
47. Sokhn, S.E., E. Dahdouh, and Z. Daoud. 2013. Resistance of gram-negative bacilli in Lebanon. ISRN Infect. Dis. 2013:Article ID 759208.
48. Wang, J., J.F. Gibbons, K. McGrath, L. Bai, F. Li, F.C. Leonard, R. Stephan, and S. Fanning. 2016. Molecular characterization of blaESBL-producing *Escherichia coli* cultured from pig farms in Ireland. J. Antimicrob. Chemother. 71:3062–3065.
49. Dierikx, C.M., J.A. van der Goot, H.E. Smith, A. Kant, and D.J. Mevius. 2013. Presence of ESBL/AmpC-producing *Escherichia coli* in the broiler production pyramid: a descriptive study. PLoS One 8:e79005.
50. El-Shazly, D.A., S.A. Nasef, F.F. Mahmoud, and D. Jonas. 2017. Expanded spectrum beta-lactamase producing *Escherichia coli* isolated from chickens with colibacillosis in Egypt. Poult. Sci. 96:2375–2384.
51. Aguilar-Montes de Oca, S., M. Talavera-Rojas, E. Soriano-Vargas, J. Barba-Leon, and J. Vazquez-Navarrete. 2015. Determination of extended spectrum beta-lactamases/AmpC beta-lactamases and plasmid-mediated quinolone resistance in *Escherichia coli* isolates obtained from bovine carcasses in Mexico. Trop. Anim. Health Prod. 47:975–981.
52. Sato, T., T. Okubo, M. Usui, S. Yokota, S. Izumiyama, and Y. Tamura. 2014. Association of veterinary third-generation cephalosporin use with the risk of emergence of extended-spectrum-cephalosporin resistance in *Escherichia coli* from dairy cattle in Japan. PLoS One 9:e96101.
53. Donati, V., F. Feltrin, R.S. Hendriksen, C.A. Svendsen, G. Cordaro, A. Garcia-Fernandez, S. Lorenzetti, R. Lorenzetti, A. Battisti, and A. Franco. 2014. Extended-spectrum-beta-lactamases, AmpC beta-lactamases and plasmid mediated quinolone resistance in *Klebsiella* spp. from companion animals in Italy. PLoS One 9:e90564.
54. Liu, X., K. Thungrat, and D.M. Boothe. 2016. Occurrence of OXA-48 carbapenemase and other beta-lactamase genes in ESBL-producing multidrug resistant *Escherichia coli* from dogs and cats in the United States, 2009–2013. Front. Microbiol. 7:1057.

55. Bai, L., D. Hurley, J. Li, Q. Meng, J. Wang, S. Fanning, and Y. Xiong. 2016. Characterisation of multidrug-resistant Shiga toxin-producing *Escherichia coli* cultured from pigs in China: co-occurrence of extended-spectrum beta-lactamase- and *mcr-1*-encoding genes on plasmids. Int. J. Antimicrob. Agents 48:445–448.
56. El Garch, F., M. Sauget, D. Hocquet, D. LeChaudee, F. Woehrle, and X. Bertrand. 2017. *Mcr-1* is borne by highly diverse *Escherichia coli* isolates since 2004 in food-producing animals in Europe. Clin. Microbiol. Infect. 23: 51.e1–51.e4.
57. Delannoy, S., L. Le Devendec, E. Jouy, P. Fach, D. Drider, and I. Kempf. 2017. Characterization of colistin-resistant *Escherichia coli* isolated from diseased pigs in France. Front. Microbiol. 8:2278.
58. Brauer, A., K. Telling, M. Laht, P. Kalmus, I. Lutsar, M. Remm, V. Kisand, T. Tenson. 2016. Plasmid with colistin resistance gene *mcr-1* in extended-spectrum-beta-lactamase-producing *Escherichia coli* strains isolated from pig slurry in Estonia. Antimicrob. Agents Chemother. 60:6933–6936.
59. Kong, L.H., C.W. Lei, S.Z. Ma, W. Jiang, B.H. Liu, Y.X. Wang, R. Guan, S. Men, Q.W. Yuan, G.Y. Cheng, W.C. Zhou, and H.N. Wang. 2017. Various sequence types of *Escherichia coli* isolates coharboring bla*NDM-5* and *mcr-1* genes from a commercial swine farm in China. Antimicrob. Agents Chemother. 61:1–5.

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