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The prevalence of *mcr*-1 and resistance characteristics of *Escherichia coli* isolates from diseased and healthy pigs

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Running title: *mcr*-1 of *E. coli* from diseased pigs.

Abstract

Colistin has been used as the last-line antibiotic for *Escherichia coli* infections. Herein, we collected 102 *E. coli* isolates from diseased pigs and 204 from healthy ones in Henan province of China. Then, we screened antimicrobial resistance and *mcr*-1 of bacteria. There was 25.5% (78/306) *mcr*-1 positive porcine *E. coli*, in which 46 isolates (45.1%, 46/102) obtained from diseased pigs, the others (15.7%, 32/204) collected from healthy pigs (45.1% versus 15.7%, P=0.000). Meanwhile, the former presented more serious resistance to colistin, ceftiofur, cefquinome, gentamicin, amikacin, doxycycline, florfenicol, enrofloxacin, and olaquindox than those from healthy pigs, which were similar to the relations between isolates with or without *mcr*-1, except for amikacin and doxycycline. Also, the resistance profiles of *mcr*-1 positive *E. coli* were more extensive than those of *mcr*-1 negative isolates.

Key words: *mcr*-1; Porcine *Escherichia coli*; Resistance characteristics; Resistance profiles; Diseased pigs; Healthy pigs

Introduction

Colistin has been routinely used for treatments of farm animals, like swine and poultry, from the early 1980s in the world (Tängdén and Giske, 2015). The plasmid-mediated *mcr* colistin resistance genes (from *mcr*-1 to *mcr*-5) have been described in *Escherichia coli, Klebsiella pneumonia, Salmonella spp.*, *Enterobacter aerogenes, Enterobacter cloacae,* and *Pseudomonas spp* (Borowiak *et al*., 2017; Carattoli *et al*., 2017; Quesada *et al*., 2016; Torpdahl *et al*., 2017; Wang *et al*., 2017; Xavier *et al*., 2016; Yin *et al*., 2017; Zeng *et al*., 2016) isolated from several origins, including humans, animals, retail meat and the environment (Bai *et al*., 2016; Liu *et al*., 2016), since *mcr*-1 was initially discovered in 2015 (Liu *et al*., 2016). Meanwhile, the coexistence of *mcr* genes and other resistance genes, such as $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{NDM}, bla_{KPC}, *fos*A, *qnr*S, *flo*R and *oqx*AB (Bi *et al*., 2017; Lai *et al*., 2017; Li *et al*., 2017; Sun *et al*., 2016), is of great concern. So, the emergence and dissemination of *mcr* genes in *Enterobacteriaceae* is worrying as it could limit the usefulness of colistin and severely affect the possibility of treating infections caused by multidrug-resistant, extensively drug-resistant and pan-resistant gram-negative pathogens globally (Wang *et al*., 2017).

To date, although *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5 colistin resistance genes have been identified persistently, *mcr*-1 remains the predominant in China. Significantly varying levels of *mcr*-1 carriage in different *E. coli* samples were reported, which have apparently associated with origins (Wang *et al*., 2017). In 2015, Liu et al. proved that the *mcr*-1 prevalence in *E*. *coli* were 1% of inpatients with infection, 15% of raw meat and 21% of healthy pigs (Liu *et al*., 2016). Rhouma &Letellier thought a historical link has existed between *mcr*-1, extended-spectrum β-lactamase (ESBL) and carbapenemase (Rhouma and Letellier, 2017). But, little research has demonstrated whether the *mcr*-1 prevalence of *E. coli* is associated with isolates from healthy or diseased animals or not. Therefore, this study was conducted to evaluate the occurrence of *mcr*-1 in *E. coli* isolated from healthy or diseased pigs in Henan province of China.

Material and methods

Bacterial isolates

We collected 306 porcine *E. coli* isolates from diseased or healthy pigs in Henan provinces from June 1, 2016 to Feb 28, 2017. The 204 *E. coli* from healthy pigs were randomly collected from pig farms by rectal swab. No more than five samples were collected from the same pig farm. The 102 *E. coli* from diseased pigs were collected from animal hospitals. All samples were aseptically obtained from liver swabs as soon as sick pigs died, which were diagnosed as colibacillosis and had received

antimicrobial treatment for 3 or more days, and seeded in macconkey agar immediately.

Antimicrobial susceptibility testing

We calculated minimum inhibitory concentrations (MICs) of colistin, amoxicillin, ceftiofur, cefquinome, gentamicin, amikacin, oxycycline, doxycycline, florfenicol, sulfamethoxazole/trimethoprim(5/1), enrofloxacin, olaquindox, and mequindox for all isolates using the broth microdilution method in accordance with the Clinical & Laboratory Standards Institute guidelines, and we interpreted the results in accordance with the CLSI breakpoints (CLSI, 2016).

Screening

We screened all colistin-resistance isolates (MIC≥2 μg/ml) for the presence of *mcr*-1 using PCR with primers *mcr*-1-forward (5′-GCTCGGTCAGTCCGTTTG-3′) and *mcr*-1-reverse (5′-GAATGCGGTGCGGTCTTT-3′). The resulting amplicons were subsequently sequenced.

Statistical analysis

The resistance rates and resistance profiles of *E. coli* with or without *mcr*-1, the resistance rates of *E. coli* from diseased or healthy pigs were documented in Statistical Packages of Social Sciences (SPSS) software for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Statistical analysis was performed using descriptive statistics, and χ^2-test was used for testing group differences, with $P<0.05$ set as the level of significant differences, $P<0.01$ set as the level of extremely significant differences.

Results

The MIC profiles of *E. coli* from diseased or healthy pigs

In the present study, the resistance rates was significantly higher in *E. coli* isolates from diseased pigs (n=102) than those from healthy pigs (n=204) for colistin (P=0.000), ceftiofur (P=0.000), cefquinome (P=0.000), gentamicin (P=0.000), amikacin (P=0.000), doxycycline (P=0.001), florfenicol (P=0.005), enrofloxacin (P=0.000), and olaquindox (P=0.000) (Table 1). There were large differences in MIC_{50} between isolates from diseased pigs and those from healthy pigs, with differences of more than 1024-fold for ceftiofur, 256-fold for cefquinome, 64-fold for gentamicin, and 64-fold for enrofloxacin. Moreover, the MIC_{90} values of isolates from diseased pigs were also apparently higher than those from healthy pigs, which were more than eight-fold for gentamicin, more than 128-fold for amikacin and 16-fold for enrofloxacin.

Prevalence of *mcr*-1

Although 81 of 306 porcine *E. coli* (46 from diseased pigs and 35 from health) conferred resistance to colistin from table 1, the *mcr*-1 carriages were 25.5% (78/306). There were three colistin-resistance isolates which did not carry *mcr*-1 gene but probably harbored the *mcr*-1 variants (Borowiak *et al*., 2017; Carattoli *et al*., 2017; Xavier *et al*., 2016; Yin *et al*., 2017). In 78 *mcr*-1 positive isolates, we found 46 (45.1%, 46/102) *E. coli* obtained from diseased pigs, the others (15.7%, 32/204) collected from rectal swabs of healthy pigs, which were significantly less (15.7%

Table 1 The MIC profiles of *E. coli* from healthy or not, and *mcr*-1 (+/-).

Antibiotics	Not healthy (n=102)			Healthy (n=204)			P value	*mcr*-1 (+/-) R/%		P value
	MIC_{50}	MIC_{90}	R /%	MIC_{50}	MIC_{90}	R /%		+ (n=78)	- (n=228)	
colistin	<0.5	2	45.1(46)	<0.5	2	17.2(35)	0.000	100.0(78)	1.3(3)	0.000
amoxicillin	>512	>512	100.0(102)	>512	>512	100.0(204)	-	100.0(78)	100.0(228)	-
ceftiofur	>512	>512	61.8(63)	<0.5	>512	13.2(27)	0.000	52.6(41)	21.5(49)	0.000
cefquinome	128	>512	58.8(60)	<0.5	128	11.8(24)	0.000	50.0(39)	19.7 (45)	0.000
gentamicin	32	>512	64.7(66)	<0.5	64	15.7(32)	0.000	48.7(38)	25.4(58)	0.000
amikacin	2	>512	10.8(11)	2	4	1.0(2)	0.000	5.1(4)	3.9(9)	0.904
oxycycline	128	>512	100.0(102)	128	256	96.1(196)	0.100	100.0(78)	96.5(220)	0.206
doxycycline	16	64	75.5(77)	16	32	55.4(113)	0.001	71.8(56)	58.8(134)	0.056
florfenicol	128	256	82.4(84)	64	128	66.2(135)	0.005	92.3(72)	64.5(147)	0.000
sulfamethoxazole /trimethoprim(5/1)	>512	>512	99.0(101)	>512	>512	94.6(193)	0.118	96.2(75)	96.1(219)	1.000
enrofloxacin	32	128	67.6(69)	<0.5	8	15.7(32)	0.000	57.7(45)	24.6(56)	0.000
olaquindox	16	64	27.5(28)	8	32	7.8(16)	0.000	33.3(26)	7.5(17)	0.000
mequindox	8	32	3.9(4)	8	32	2.9(6)	0.909	5.1(4)	2.6(6)	0.483

R, resistance rates.

versus 45.1%, P=0.000) than the former. At the same time, the differences of resistance rates between *mcr*-1 positive and *mcr*-1 negative *E. coli* were extremely statistical significance (P=0.000), similar to the relations between isolates from diseased pigs with healthy animals, except for amikacin and doxycycline (Table 1).

The resistance profiles of *E. coli* with or without *mcr*-1

The resistance profiles of *mcr*-1 positive *E. coli* were significantly more extensive than those of *mcr*-1 negative isolates (Table 2). For *mcr*-1 positive *E. coli*, all isolates possessed not less than 4 drugs resistance profiles, while about 86.4% (197/228) *mcr*-1 negative ones did (P=0.000), that is, there were 31 *mcr*-1 negative isolates which showed resistance to not more than 3 drugs. In addition, about half of *mcr*-1 positive strains (47.4%, 37/78) presented resistance to at least nine drugs, but only

Table 2 The resistance profiles of *E. coli* with or without *mcr*-1

Resistance profiles	*mcr*-1 positive (n=78)	*mcr*-1 negative (n=228)	P value
≥12 drugs	6.4(5)	0.0(0)	0.001
≥11 drugs	16.7(13)	0.0(0)	0.000
≥10 drugs	30.8(24)	2.6(6)	0.000
≥9 drugs	47.4(37)	5.7(13)	0.000
≥8 drugs	61.5(48)	11.0(25)	0.000
≥7 drugs	66.7(52)	21.1(48)	0.000
≥6 drugs	87.2(68)	37.3(85)	0.000
≥5 drugs	98.7(77)	60.1(137)	0.000
≥4 drugs	100.0(78)	86.4(197)	0.000
≥3 drugs	100.0(78)	96.9(221)	0.197
≥2 drugs	100.0(78)	99.6(227)	1.000

5.7% (13/228) *mcr*-1 negative isolates did (P=0.000). Meanwhile, 16.7% (13/78) *mcr*-1 positive isolates demonstrated 11 to 12 drugs resistance profiles, but not any *mcr*-1 negative *E. coli* did (P=0.000).

Discussion

The *mcr*-1 carriages have apparently associated with origins. In the initial report on the discovery of *mcr*-1 in China, Liu and colleagues reported a high prevalence of the *mcr*-1among *E. coli* (21%) collected from healthy pigs (Liu *et al*., 2016), which were similar to those from pigs in our results (25.5%, 78/306), but a little more than those from healthy pigs (15.7%, 32/204). Meanwhile, the *mcr*-1 carriages of *E. coli* was about 1% from patients (Liassine *et al*., 2016; Liu *et al*., 2016; Quan *et al*., 2017; Wang *et al*., 2017), 3.5% from ESBL-producing strains of human faecal samples (Bi *et al*., 2017), which were less than Shiga toxin-producing *E. coli* from healthy pigs (10.7%) (Bai *et al*., 2016), extremely less than those from pigs in our results (25.5%). Colistin has not yet been approved for use in human beings in China, but it has been used in animals as a therapeutic drug and feed additive since the early 1980s. Since April, 2017, colistin has been formally banned from animal feeds in China because of the *mcr*-1 emergence and quickly spread. However, the high *mcr*-1 carriages in samples from diseased pigs (45.1%) were still surprising, which suggested that previous antimicrobial agents exposure was strongly associated with the presence of *mcr*-1.

The *mcr*-1 positive *E. coli* presented more serious resistance to ceftiofur, cefquinome,

gentamicin, florfenicol, enrofloxacin, and olaquindox, than the *mcr*-1 negative isolates (P=0.000). On the one hand, many reports have verified that *mcr*-1 could coexist the other resistance genes on the same plasmids, for example bla_{NDM}, bla_{CTX-M}, bla_{TEM}, bla_{CMY}, *fos*A, *qnr*S, *flo*R and *oqx*AB (Bi *et al*., 2017; Lai *et al*., 2017; Li *et al*., 2017; Sun *et al*., 2016). On the other hand, colistin commonly used as the last-line antibiotic for the treatment of infections caused by multidrug-resistant and extensively drug-resistant gram-negative pathogens in many countries, such as carbapenem-resistant *Enterobacteriaceae* (Tängdén and Giske, 2015), which could lead to isolates coselection of colistin with other antibiotics. Counterintuitively, porcine *E. coli* with or without *mcr*-1 showed similar resistance to amikacin and doxycycline, although *E. coli* from diseased pigs had more resistance to them than from healthy pigs (for amikacin, 10.8% versus 1.0%, P=0.000; for doxycycline, 75.5% versus 55.4%, P=0.001; respectively). Further studies will be necessary to determine the characteristics of plasmid carring the *mcr*-1 in our isolates.

Conclusions

In conclusion, the *mcr*-1 carriages in porcine *E. coli* were extremely significant differences (45.1% versus 15.7%, P=0.000) between isolates from diseased pigs with from healthy pigs. Meanwhile, *E. coli* from diseased pigs had more resistance to ceftiofur, cefquinome, gentamicin, amikacin, doxycycline, florfenicol, enrofloxacin, and olaquindox than those from healthy pigs, which were similar to the relations between isolates with or without *mcr*-1, except for amikacin and doxycycline.

Author contributions

LY and GH conceived of the study, and participated in its design. XL and PD isolated the isolates, XL, BL and FL carried out the antibiotics susceptibility testing; LY and GH performed the statistical analysis and wrote the manuscript.

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Ethical approval

Not required.

Conflict of interest

The authors have no conflicts of interest to disclose.

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