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Emergence of *mcr-3 carrying Escherichia coli* in Diseased Pigs in South Korea

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Abstract: We examined the prevalence and molecular characteristics of *mcr-3* carrying colistin-resistant *Escherichia coli* among cattle, pig, and chicken isolates in South Korea. Among a total of 185 colistin-resistant *E. coli* isolates determined in this study (47 from cattle, 90 from pigs, and 48 from chicken), PCR amplification detected *mcr-3* genes in 17 isolates predominantly from diseased pigs. The *mcr-3* genes were characterized as *mcr-3.1* in 15 isolates and *mcr-3.5* in 2 isolates. The *mcr-3* gene was transferred to the *E. coli* J53 recipient strain from more than 50% of the *mcr-3*-carrying isolates. The *mcr-3.1* and *mcr-3.5* genes were identified predominantly in IncHI2 and IncP plasmids, respectively. Multi-locus sequence typing analysis revealed eight previously reported sequence types (ST), including ST1, ST10, and ST42. We identified isolates with similar pulsed-field gel electrophoresis patterns from diseased pigs in three farms. Besides, the isolates carried various virulence factors and demonstrated resistance to multiple antimicrobials, including β-lactams and quinolones. Further, the *mcr-3.5* encodes three amino acid substitutions compared with *mcr-3.1*. To the best of our knowledge, this is the first report of pathogenic *E. coli* carrying *mcr-3.5* in South Korea, which implies that *mcr-3* variants may have already been widely spread in the pig industry.

Keywords: colistin; *Escherichia coli*; *mcr-3* gene; plasmid; resistance

1. Introduction

Colistin is considered one of the last-resort antimicrobial agents against multi-drug resistant Gram-negative bacterial infections. The emergence of *mcr*-harboring colistin-resistant *Escherichia coli* presented a serious public health risk. Among the ten *mcr* genes identified so far i.e., *mcr-1* up to *mcr-10*, the *mcr-3* genes have been distributed worldwide [1]. Recent studies in South Korea (Korea), identified *mcr-3*-carrying *E. coli* isolates from food-producing animals [2,3]. However, both studies investigated limited numbers of isolates from specific provinces. Besides, although the *mcr-3* gene is subjected to constant evolution due to the impacts of unknown selective pressure in the environment, animals, and humans [4], no attempt has been made so far to determine the *mcr-3* variants in bacteria isolated in Korea. Consequently, we undertook this study to provide new knowledge on the prevalence and molecular characteristics of *mcr-3* variants in *E. coli* isolated from food-producing animals throughout Korea between 2005 and 2018.

2. Materials and Methods

2.1. Identification of Colistin-Resistant E. coli

E. coli isolates were recovered from healthy and diseased animals (i.e., cattle, chicken, and pigs), and their carcasses during a nationwide surveillance study on antimicrobial susceptibility conducted between 2005 and 2018. The minimum inhibitory concentration (MIC) of colistin was determined by the broth microdilution method [5] in KRNV5F Sensititre Panel following the manufacturer's instruction (Trek Diagnostic Systems, Waltham, MA, USA). The MIC values were interpreted according to the EUCAST breakpoint (>2 µg/mL). PCR amplification was performed to investigate the *mcr-3* gene carriage of isolates exhibiting colistin resistance using primer pairs and PCR conditions described previously [6].

2.2. Conjugation Assay

Conjugation was performed using a filter mating method with azide-resistant *E. coli* J53 as the recipient strain [7]. The transconjugants were confirmed by PCR detection of the *mcr-3* genes and were investigated for their MICs as described above.

2.3. Molecular Characterization of mcr-3 carrying E. coli

A PCR-based replicon typing kit (Diatheva, Fano, Italy) and a multiplex PCR assay [8] were used to identify the plasmid replicon types and virulence factor genes, respectively. Pulsed-field gel electrophoresis of *mcr-3* positive isolates was conducted using genomic DNA prepared in agarose blocks, digested with Xbal enzyme (TaKaRa, Shiga, Japan), as described previously [9]. The banding profiles were analyzed using Bionumerics software and the genetic relatedness of the isolates was calculated using the unweighted pair-group method. Besides, molecular typing of *mcr-3* carrying isolates was carried out according to the protocols specified at the *E. coli* multilocus sequence typing website [10].

2.4. Whole-Genome Sequencing

Whole-genome sequencing was conducted to investigate the immediate genetic environment and amino acid sequences of *mcr-3* genes (PacBio RSII platform, Pacific Biosciences, Menlo Park, CA, USA). Complete sequences of the chromosomes and plasmids of strain V01-E02-025, V01-E02-51, and V01-R02-053 have been deposited into GenBank under the accession no. (CP049943, CP049944), (CP049299, CP049300), and (CP049086, CP049087), respectively.

3. Results and Discussion

A total of 14,631 *E. coli* isolates were obtained from healthy and diseased animals (i.e., cattle, chicken, and pigs), and their carcasses during 2005−2018 (Table 1). The overall prevalence of colistin-resistant *E. coli* was less than 5%. In our previous study [11], colistin resistance was identified only in 1.3% of isolates recovered from food-producing animals between 2005 and 2015. This study demonstrated that the colistin resistance rate was maintained below 2% for three consecutive years after 2015. Indeed, the prevalence of colistin-resistant isolates in this study was consistent with previous reports in Poland [12] but lower than other reports from Japan (48%) [13], China (42%) [14], and Cambodia (20%) [1].

Table 1. Prevalence of *mcr-3* in colistin-resistant *Escherichia coli* obtained from food-producing animals and animal carcasses from Korea.

Year (No. of Isolates)	Prevalence (%) of *mcr-3* Gene (no. of *mcr-3* Positive Isolates/ no. of Colistin-Resistant Isolates)												
	Cattle (*n* = 47)				Pigs (*n* = 90)				Chicken (*n* = 48)				Total (*n* = 185)
	Healthy	Carcasses	Diseased	Subtotal	Healthy	Carcasses	Diseased	Subtotal	Healthy	Carcasses	Diseased	Subtotal	
2005 (*n* = 693)	0 (0/1)	0 (0/0)	0 (0/0)	0 (0/1)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/1)
2006 (*n* = 744)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/5)	0 (0/0)	0 (0/0)	0 (0/5)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/5)
2007 (*n* = 744)	0 (0/12)	0 (0/0)	0 (0/0)	0 (0/12)	0 (0/17)	0 (0/0)	0 (0/0)	0 (0/17)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/29)
2008 (*n* = 559)	0 (0/16)	0 (0/0)	0 (0/0)	0 (0/16)	0 (0/6)	0 (0/0)	0 (0/0)	0 (0/6)	0 (0/1)	0 (0/0)	0 (0/0)	0 (0/1)	0 (0/23)
2009 (*n* = 641)	0 (0/5)	0 (0/0)	0 (0/0)	0 (0/5)	0 (0/7)	0 (0/0)	0 (0/0)	0 (0/7)	0(0/2)	0 (0/0)	0 (0/0)	0(0/2)	0 (0/14)
2010 (*n* = 1101)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/2)	0 (0/1)	0 (0/1)	0 (0/4)	0 (0/8)	0 (0/0)	0 (0/2)	0 (0/10)	0 (0/14)
2011 (*n* = 1276)	0 (0/4)	0 (0/2)	0 (0/0)	0 (0/6)	0 (0/2)	0 (0/1)	50 (1/2)	20 (1/5)	0 (0/3)	0 (0/4)	0 (0/5)	0 (0/12)	4.3 (1/23)
2012 (*n* = 1242)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0))	50 (1/2)	0 (0/1)	0 (0/0)	33.3 (1/3)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	33.3 (1/3)
2013 (*n* = 1078)	0 (0/2)	0 (0/0)	0 (0/0)	0 (0/2)	0 (0/0)	50 (1/2)	0 (0/3)	20 (1/5)	0 (0/2)	0 (0/1)	0 (0/0)	0 (0/3)	10 (1/10)
2014 (*n* = 1329)	0 (0/1)	0 (0/0)	0 (0/1)	0 (0/2)	0 (0/1)	0 (0/0)	50 (3/6)	42.9 (3/7)	0 (0/3)	0 (0/5)	0 (0/4)	0 (0/12)	14.3 (3/21)
2015 (*n* = 1169)	0 (0/0)	0 (0/0)	0 (0/1)	0 (0/1)	0 (0/0)	0 (0/0)	42.9 (3/7)	42.9 (3/7)	0 (0/2)	0 (0/1)	0 (0/0)	0 (0/3)	27.3 (3/11)
2016 (*n* = 1794)	0 (0/2)	0 (0/0)	0 (0/0)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/0)	0 (0/4)	0 (0/1)	0 (0/0)	0 (0/0)	0 (0/1)	0 (0/7)
2017 (*n* = 1218)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/5)	0 (0/1)	0 (0/0)	0 (0/6)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/6)
2018 (*n* = 1043)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	50 (1/2)	0 (0/0)	58.3 (7/12)	57.1(8/14)	0 (0/0)	0 (0/1)	0 (0/3)	0 (0/4)	44.4 (8/18)
Total	0 (0/43)	0 (0/2)	0 (0/2)	0 (0/47)	3.9 (2/51)	12.5 (1/8)	45.2 (14/31)	18.9 (17/90)	0 (0/22)	0 (0/12)	0(0/14)	0(0/48)	9.2 (17/185)

Among a total of 185 colistin-resistant *E. coli* isolates determined in this study (47 from cattle, 90 from pigs, and 48 from chicken), PCR amplification detected *mcr-3* genes in 17 isolates: 2 from healthy pigs, 1 from a pig carcass, and 14 from diseased pigs (Table 1). *mcr-1* was the major colistin resistance determinant in *E. Couple* isolated from livestock, especially chickens in Korea since 2013 [11]. However, this study exhibited the emergence of *mcr-3* in livestock, especially in pigs since 2011. Notably, the majority of the *mcr-3* carrying isolates from diseased pigs were found between 2014 and 2018, highlighting a recent rise in prevalence compared to previous years. Recent studies reported a 38% [1] and 13% [15] prevalence of *mcr-3* among colistin-resistant pig isolates in Cambodia and Brazil, respectively. In addition, Fukuda et al. [13] identified the *mcr-3* gene in 8% of *E. coli* isolated from diseased pigs in Japan. *mcr-3*-carrying plasmids can stably persist by lowering fitness cost [16], suggesting careful monitoring of the *mcr-3* gene in Korean livestock.

The *mcr-3* genes were characterized as *mcr-3.1* in 15 isolates and *mcr-3.5* in 2 isolates (Table 1). Both of the *mcr*-3.5 isolates were identified in 2018. To the best of our knowledge, this is the first report of *mcr-3.5*-carrying *E. coli* in Korea, while these genes have been identified in *E. coli* from pigs and other sources in Europe and other Asian countries [17–19]. The *mcr-3* gene was transferred to *E. coli* J53 recipient strain from 53% of the *mcr-3*-carrying isolates as indicated by filter mating assay (Table 2), which is lower than Belaynehe et al. [2]. Agreeing with Zurfluh et al. [19], all *mcr-3*-carrying isolates were multi-drug resistant (MDR). Notably, the two *mcr-3.5*-carrying isolates from diseased pigs were resistant to ceftiofur. In addition, MDR in five *mcr-3*-carrying isolates was transferred to a recipient *E. coli*. Although we did not investigate other antimicrobial resistance genes, the co-existence of multiple resistant genes in the same or different plasmids could confer resistance to a broad range of antimicrobials [20].

Table 2. Characteristic of the *mcr-3* positive *Escherichia coli* from healthy and diseased pigs, and pig carcasses in Korea.

Isolates	Source	Farm ID	Year	Province	MIC of Colistin (μg/mL)	MCR-3 Variant Type	Resistance Pattern [a,b]	Transfer-Ability	Replicon Type of Transconjugant Plasmid	Multiloccus Sequence Type	PULSOTYPE	Virulence Factors
V08-R02-015	diseased	GB-1	2011	Gyeongbuk	16	*mcr*-3.1	AMP CHL CIP GEN NAL STR FIS TET SXT	/		3523	A	F18/LT/STb/EAST
V04-A02-010	healthy	CN-1	2012	Chungnam	8	*mcr*-3.1	AMP CHL GEN STR FIS TET SXT	+	HI2	4532	B	
V05-S02-016	carcass	CN-2	2013	Chungnam	8	*mcr*-3.1	CHL NAL STR FIS TET	/		101	C	EAST
14D084	diseased	GB-2	2014	Gyeongbuk	16	*mcr*-3.1	AMP CHL GEN NAL FIS TET SXT	+	HI2, I1-α	1	D	F18/Stx2e /AIDA
14D084-2	diseased	GB-2	2014	Gyeongbuk	16	*mcr*-3.1	AMP GEN NAL FIS TET SXT	/		1	D	F18/Stx2e /AIDA
14D085	diseased	GB-3	2014	Gyeongbuk	16	*mcr*-3.1	AMP CHL GEN NAL FIS TET SXT	/		1	D	F18/Stx2e /AIDA
V01-R02-019	diseased	CB-4	2015	Chungbuk	16	*mcr*-3.1	AMP CHL STR FIS TET SXT	+	HI2	ND [c]	E	
V01-R02-020	diseased	CB-4	2015	Chungbuk	8	*mcr*-3.1	AMP CIP CHL NAL STR FIS TET SXT	/		10	- [d]	
V01-R02-053	diseased	GG-1	2015	Gyeonggi	16	*mcr*-3.1	AMP CHL FIS TET SXT	+	M	1	D-1	F18/Stx2e/AIDA
V01-A02-017	healthy	GN-3	2018	Gyeongnam	16	*mcr*-3.1	AMP CHL CIP NAL STR FIS TET SXT	/		10	L	LT/ STb/EAST
V01-E02-088	diseased	GN-4	2018	Gyeongnam	16	*mcr*-3.1	AMP CHL CIP GEN NAL STR FIS TET SXT	/		10	L	LT/ STb/EAST
V01-E02-090	diseased	GN-4	2018	Gyeongnam	>16	*mcr*-3.1	AMP CHL CIP GEN NAL STR FIS TET SXT	+	HI2, I1-α, N	29	M	eae/paa
V01-E02-023	diseased	GB-4	2018	Gyeongbuk	4	*mcr*-3.5	AMP CHL XNL STR FIS TET SXT	+	P1, I1-α	42	N	F18/LT/STb/EAST
V01-E02-025	diseased	GB-4	2018	Gyeongbuk	8	*mcr*-3.5	AMP CHL XNL STR FIS TET SXT	+	P1	42	N	F18/LT/STb/EAST
V01-E02-049	diseased	GN-5	2018	Gyeongnam	>16	*mcr*-3.1	AMC AMP FOX CHL CIP GEN NAL STR FIS TET SXT	/		10	L	F18/LT/STb/EAST/AIDA
V01-E02-050	diseased	GN-5	2018	Gyeongnam	8	*mcr*-3.1	AMP CHL GEN STR FIS TET SXT	+	HI2	641	O	STb/EAST/AIDA
V01-E02-051	diseased	GN-5	2018	Gyeongnam	8	*mcr*-3.1	AMP CHL GEN STR FIS TET SXT	+	HI2	641	O	STb/EAST/AIDA

[a] AMC, amoxicillin/clavulanic acid; AMP, ampicillin; FOX, cefoxitin, XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole. [b] The underlined resistance markers were transferred to the recipient *E. coli* J53 strain by conjugation. [c] Not determined. [d] *Xbal* macrorestriction analysis yielded no DNA banding patterns in V01-R02-020 *E. coli* strain due to constant autodigestion of the genomic DNA during agarose plug preparation, and thus, a cluster formed by this strain is excluded. +: *mcr*-3 gene was transferred to E. coli J53 recipient strain. /: *mcr-3* gene was not transferred to E. coli J53 recipient strain.

PCR analysis presented five replicon types (IncP1, HI2, I1-α, IncM, and IncN). The *mcr-3.1* gene was identified predominantly in the IncHI2 plasmid, which is associated with the spread of MDR, including β-lactams and quinolones [14,21]. Agreeing with Li et al. [16], the *mcr-3.5* genes belonged to IncP plasmid. The less frequent plasmid replicon types in our study, such as IncI1-α, IncM, IncN, and IncP, were reported to co-harbor genes resistant to aminoglycosides, β-lactams, quinolones, and tetracyclines in *Enterobacteriaceae* [17,21,22].

Multi-locus sequence typing analysis revealed eight previously reported ST types: four ST1s, four ST10s, two ST42s, two ST641s, and each of ST29, ST101, ST3523, and ST4532 (Table 2). *E. coli* ST1, ST42, and ST641 isolates from diseased pigs of three farms showed similar patterns in the pulse-field gel electrophoresis results (Figure S1). Besides, ST1 and ST10 isolates carrying *mcr-3.1* gene were identified from farms located in different provinces. ST1, ST10, ST101, and ST641 *E. coli* isolates have already been identified in food-producing animals in several countries [11,12,15], suggesting its widespread distribution. Thus, a combination of clonal expansion and dissemination of plasmids carrying *mcr-3* variants contributed to the rise in the prevalence of *mcr-3* carrying *E. coli*.

We identified a total of 13 different virulence factor genes, with up to 5 of those in a single isolate (Table 2). The predominant virulence factors include the fimbrial adhesins (F18), the heat-labile (LT) or heat-stable (STb) enterotoxins, and virulence factors involved in diffuse adherence of *E. coli* (AIDA). *Mcr-3* positive strains isolated from diseased pigs were associated with enterotoxigenic *E. coli* (47.1%) and Shiga toxin-producing *E. coli* (23.5%), both expressing fimbrial adhesion (F18). These toxins and fimbrial genes are associated with porcine diarrhea and edematous disease [23,24].

Whole-genome sequencing demonstrated that plasmids pK18EC051 (GenBank accession no. CP049300, 270.3 kb) and pK15EC053 (CP049087, 96.2 kb) shared a similar *mcr-3.1*-carrying region with plasmid pZR10 from pigs in China, but a gene encoding for 5-nitroimidazole based antimicrobials (*nimC*) was excluded from downstream of *mcr-3.1* gene in pK15EC053 (Figure 1). In contrast, only diacylglycerol kinase (*dgkA*) and transposase encoding genes were identified in the immediate downstream and upstream of the *mcr-3.5* gene, respectively, in pK18EC025 (CP049944, 60.2kb). The *mcr-3.5* variant in pK18EC025 differed from the *mcr-3.1* gene variant found in this study (pK18EC051 and pK15EC053) as well as the original *mcr-3* variant from China (pWJ1) by three amino acid substitutions (M23V, A457E, and T488I). Although the MIC of colistin was not altered by these substitutions, Yang et al. [16] demonstrated that *mcr-3.5* has higher fitness than *mcr-3.1*.

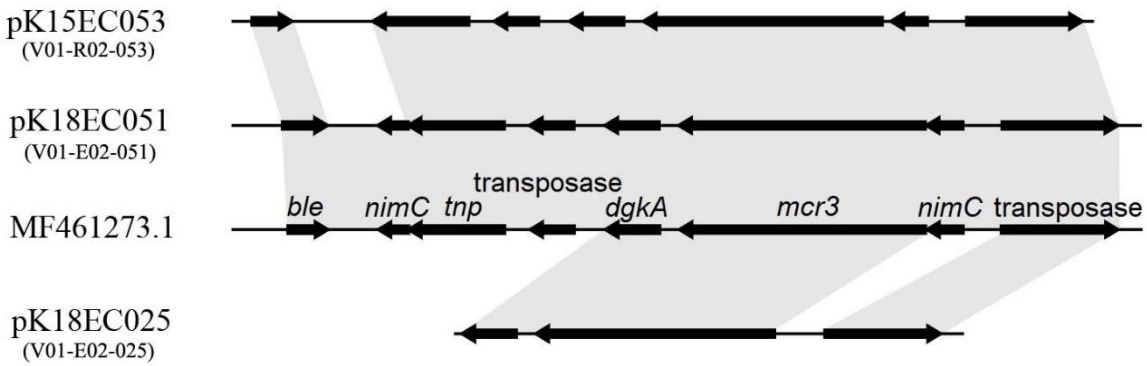

Figure 1. Comparison of the genetic environment of the *mcr-3* gene in plasmids pK18EC025, pK18EC051, and pK15EC053 with pWZR10 (MF461273.1). Arrows indicate the positions and directions of the genes. Regions with >99% homology are indicated by grey shading.

In conclusion, the proportion of *mcr-3*-carrying isolates is increasing in diseased pigs, presumably due to the horizontal and clonal dissemination. Therefore, active surveillance of *mcr*-carrying isolates is vital for preventing the spread of colistin resistance. In addition, a guideline that ensures prudent use of antimicrobials in pigs is urgently needed.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-2607/8/10/1538/s1. **Figure S1**: *Xbal*-digested pulsed-field gel electrophoresis patterns of *mcr-3* carrying *E. coli* strains isolated from healthy pigs, pig carcasses, and diseased pigs in Korea. *Xbal* macrorestriction analysis yielded no DNA banding patterns in V01-R02-020 *E. coli* strain due to constant autodigestion of the genomic DNA during agarose plug preparation, and thus, a cluster formed by this strain is excluded. (ND, not determined).

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References

1. Storm, H.G.; Borjesson, S.; Sokerya, S.; Sothyra, T.; Magnusson, U. Detection of *mcr*-mediated colistin resistance in *Escherichia coli* isolates from pigs in small scale farms in Cambodia. *Antimicrob. Agents. Chemother.* **2019**, *63*, e02241-18.
2. Belaynehe, K.M.; Shin, S.W.; Park, K.Y.; Jang, J.Y.; Won, H.G.; Yoon, I.J.; Yoo, H.S. Emergence of *mcr-1* and *mcr-3* variants coding for plasmid-mediated colistin resistance in *Escherichia* isolates from food-producing animals in South Korea. *Int. J. Infect. Dis.* **2018**, *72*, 22–24. [CrossRef]
3. Do, K.H.; Park, H.E.; Byun, J.W.; Lee, W.K. Virulence and antimicrobial resistance profiles of *Escherichia coli* encoding *mcr* gene from diarrheic weaned piglets in Korea during 2007–2016. *J. Glob. Antimicrob. Resist.* **2019**, *19*, 30243–30247.
4. Sun, J.; Zhang, H.; Liu, Y.H.; Feng, Y. Towards understanding *mcr*-like colistin resistance. *Trends Microbial.* **2018**, *26*, 794–808. [CrossRef]
5. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*; Twentieth Informational Supplement; Document M100; CLSI: Wayne, PA, USA, 2018.
6. Wang, X.; Wang, Y.; Zhou, Y.; Li, J.; Yin, W.; Wang, S.; Zhang, S.; Shen, J.; Shen, Z.; Wang, Y. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg. Microbes. Infect.* **2018**, *7*, 122. [CrossRef]
7. Tamang, M.D.; Gurung, M.; Kang, M.S.; Nam, H.M.; Moon, D.C.; Jang, G.C.; Jung, S.C.; Park, Y.H.; Lim, S.K. Characterization of plasmids encoding CTX-M-β lactamase and their addiction systems in *Escherichia coli* isolates from animals. *Vet. Microbiol.* **2014**, *174*, 456–462. [CrossRef] [PubMed]
8. Buyn, J.W.; Jung, B.Y.; Kim, H.Y.; Fairbrother, J.M.; Lee, W.K. O-serogroups, virulence genes of pathogenic *Escherichia coli* and pulse-filed gel electrophoresis (PFGE) patterns of O149 isolates from diarrheic piglets in Korea. *Vet. Med.* **2013**, *58*, 468–476.
9. Gautom, R.K. Rapid pulse-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in day 1. *J. Clin. Microbiol.* **1997**, *35*, 2977–2980. [CrossRef] [PubMed]
10. Escherichia coli MLST Database. Available online: http://mlst.warwick.ac.uk/mlst/dbs/Ecoli (accessed on 9 January 2020).
11. Lim, S.K.; Kang, H.Y.; Lee, K.; Moon, D.C.; Lee, H.S.; Jung, S.C. First detection of the *mcr-1* gene in *Escherichia coli* isolated from livestock between 2013 and 2015 in South Korea. *Antimicrob. Agents. Chemother.* **2016**, *60*, 6991–6993. [CrossRef] [PubMed]
12. Zajac, M.; Sztromwasser, P.; Bortolaia, V.; Leekitcharoenphon, P.; Cavaco, L.M.; Zietek-Barszcz, A.; Hendriksen, R.S.; Wasly, D. Occurrence and characterization of *mcr-1*-positive *E. coli* isolated from food-producing animals in Poland, 2011–2016. *Front. Microbiol.* **2019**, *10*, 1753. [CrossRef]
13. Fukuda, A.; Sato, T.; Shinagawa, M.; Takahashi, S.; Asai, T.; Yokota, S.I.; Usui, M.; Tamura, Y. High prevalence of *mcr-1*, *mcr-3* and *mcr-5* in *Escherichia coli* derived from diseased pigs. *Int. J. Antimicrob. Agents.* **2018**, *51*, 163–164. [CrossRef] [PubMed]

14. Zhang, X.; Zhang, B.; Guo, Y.; Wang, J.; Zhao, P.; Liu, J.; He, K. Colistin resistance prevalence in *Escherichia coli* from domestic animals in intensive breeding farms of Jiangsu province. *Int. J. Food. Microbiol.* **2019**, *16*, 87–90. [CrossRef] [PubMed]
15. Kieffer, N.; Nordmann, P.; Moreno, A.M.; Moreno, L.Z.; Chaby, R.; Breton, A.; Tissieres, P.; Poirel, L. Genetic and functional characterization of an *mcr-3*-like enzyme-producing *Escherichia coli* isolate recovered from swine in Brazil. *Antimicrob. Agents. Chemother.* **2018**, *62*, e00278-18. [CrossRef] [PubMed]
16. Yang, Q.E.; Maclean, C.; Papkou, A.; Pitchard, M.; Powell, L.; Thomas, D.; Andrey, D.O.; Li, M.; Spiller, B.; Yang, W.; et al. Compensatory mutations modulate the competitiveness and dynamics of plasmid-mediated colistin resistance in *Escherichia coli* clones. *ISME* **2020**, *14*, 861–865. [CrossRef]
17. Li, J.; Hulth, A.; Nilsson, L.E.; Bo¨rjesson, S.; Chen, B.; Bi, Z.; Wang, Y.; Schwarz, S.; Wu, C. Occurrence of the mobile colistin resistance gene *mcr-3* in *Escherichia coli* from household pigs in rural areas. *Antimicrob. Chemother.* **2018**, *73*, 1721–1723. [CrossRef]
18. Wise, M.G.; Estabrook, M.A.; Sahm, D.F.; Stone, G.G.; Kazmierczak, K.M. Prevalence of *mcr*-type genes among colistin-resistant *Enterobacteriaceae* collected in 2014–2016 as part of the INFORM global surveillance program. *PLoS ONE.* **2018**, *13*, e01952. [CrossRef]
19. Zurfluh, K.; Stevens, M.J.A.; Bucher, M.; Poirel, L.; Nordmann, P.; Stephan, R. Full genome sequence of pT3, a multi-resistant plasmid carrying the *mcr-3.5* colistin resistance gene, recovered from an extended-spectrum β-lactamase-producing *Escherichia coli* isolate from crickets sold as food. *Microbiol. Resour. Announc.* **2019**, *8*, e00647-19. [CrossRef]
20. Sun, J.; Li, X.P.; Fang, L.X.; Sun, R.Y.; He, Y.Z.; Lin, J.; Liu, Y.H. Co-occurrence of *mcr-1* in the chromosome and on an IncHI2 plasmid: Persistence of colistin resistance in *Escherichia coli*. *Int. J. Antimicrob. Agents.* **2018**, *51*, 842–847. [CrossRef]
21. Bortolaia, V.; Guardabassi, L.; Trevisani, M.; Bisgaard, M.; Venturi, L.; Bojesen, A.M. High diversity of extended-spectrum β-lactamases in *Escherichia coli* isolates from Italian broiler flocks. *Antimicrob. Agents. Chemother.* **2010**, *54*, 1623–1626. [CrossRef]
22. Carattoli, A.; Seiffert, S.N.; Schwendener, S.; Perreten, V.; Endimiani, A. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. *PLoS ONE.* **2015**, *10*, e0123063. [CrossRef]
23. Zajacova, Z.S.; Konstantinova, L.; Alexa, P. Detection of virulence factors of *Escherichia coli* focused on prevalence of EAST1 toxin in stool of diarrheic and non-diarrheic piglets and presence of adhesion involving virulence factors in *astA* positive strains. *Vet. Microbiol.* **2012**, *154*, 369–375. [CrossRef] [PubMed]
24. Madoroba, E.; Van Driessche, E.; De Greve, H.; Mast, J.; Ncube, I.; Read, J.; Beeckmans, S. Prevalence of Enterotoxigenic *E. coli* virulence genes from scouring piglets in Zimbabwe. *Tropl. Anim. Health. Prod.* **2009**, *41*, 1539–1547. [CrossRef] [PubMed]