



Article

Global Burden of Colistin-Resistant Bacteria: Mobilized Colistin Resistance Genes Study (1980–2018)

Mohammed Elbediwi ^{1,†}, Yan Li ^{1,†}, Narayan Paudyal ^{1,2,†} , Hang Pan ^{1,†}, Xiaoliang Li ^{1,3}, Shaohua Xie ⁴ , Andreja Rajkovic ⁵, Youjun Feng ⁶, Weihuan Fang ^{1,3}, Shelley C. Rankin ⁷ and Min Yue ^{1,3,*}

¹ Institute of Preventive Veterinary Sciences, Zhejiang University College of Animal Sciences, Hangzhou 310058, China; m.elbediwi@zju.edu.cn (M.E.); yanli3@zju.edu.cn (Y.L.); narayan.paudyal@outlook.com (N.P.); 11817015@zju.edu.cn (H.P.); xlli@zju.edu.cn (X.L.); whfang@zju.edu.cn (W.F.)

² Nepal Agricultural Research Council (NARC), Animal Health Research Division (AHRD), Kathmandu 5459, Nepal

³ Zhejiang Provincial Key Laboratory of Preventive Veterinary Medicine, Hangzhou 310058, China

⁴ Upper Gastrointestinal Surgery, Department of Molecular Medicine and Surgery, Karolinska Institute, Karolinska University Hospital, 17176 Stockholm, Sweden; shaohua.xie@ki.se

⁵ Department of Food technology, Food safety and Health, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium; andreja.rajkovic@ugent.be

⁶ Department of Medical Microbiology & Parasitology, Zhejiang University School of Medicine, Hangzhou 310058, China; fengyj@zju.edu.cn

⁷ Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104, USA; srankin@vet.upenn.edu

* Correspondence: myue@zju.edu.cn; Tel./Fax: +86-571-88982832

† These authors contributed equally to this work.

Received: 6 July 2019; Accepted: 15 October 2019; Published: 16 October 2019



Abstract: Colistin is considered to be an antimicrobial of last-resort for the treatment of multidrug-resistant Gram-negative bacterial infections. The recent global dissemination of mobilized colistin resistance (*mcr*) genes is an urgent public health threat. An accurate estimate of the global prevalence of *mcr* genes, their reservoirs and the potential pathways for human transmission are required to implement control and prevention strategies, yet such data are lacking. Publications from four English (PubMed, Scopus, the Cochrane Database of Systematic Reviews and Web of Science) and two Chinese (CNKI and WANFANG) databases published between 18 November 2015 and 30 December 2018 were identified. In this systematic review and meta-analysis, the prevalence of *mcr* genes in bacteria isolated from humans, animals, the environment and food products were investigated. A total of 974 publications were identified. 202 observational studies were included in the systematic review and 71 in the meta-analysis. *mcr* genes were reported from 47 countries across six continents and the overall average prevalence was 4.7% (0.1–9.3%). China reported the highest number of *mcr*-positive strains. Pathogenic *Escherichia coli* (54%), isolated from animals (52%) and harboring an IncI2 plasmid (34%) were the bacteria with highest prevalence of *mcr* genes. The estimated prevalence of *mcr-1* pathogenic *E. coli* was higher in food-animals than in humans and food products, which suggests a role for foodborne transmission. This study provides a comprehensive assessment of prevalence of the *mcr* gene by source, organism, genotype and type of plasmid.

Keywords: mobilized colistin resistance (*mcr*) genes; *Escherichia coli*; reservoir; food-chain; antibiotic resistance; meta-analysis

1. Introduction

The emergence of antimicrobial-resistant (AMR) bacteria has become one of the major threats to global public health, food safety and social-economic development [1–3]. Global estimates of the prevalence of bacteria that are resistant to critically important antimicrobials are required to implement control and prevention strategies, yet such data are lacking.

Carbapenem-resistant bacteria have severely challenged antimicrobial therapy. Colistin (polymyxin E) is a polycationic peptide antimicrobial that was isolated and characterized in 1949 [4]. It has been reintroduced as a “last-line” therapeutic drug, for the treatment of infections in hospitalized patients caused by carbapenem-resistant Gram-negative bacteria [5]. Before 2015, colistin resistance was perceived to be due to regulatory changes mediated by chromosomal genes (*phoPQ*, *pmrAB*, and *mgrB*) [6]. The discovery of plasmid-mediated mobilized colistin resistance (*mcr*) genes has had an obvious influence on established antibiotic therapy regimens [7]. Lack of effective antibiotics will inevitably compromise modern clinical medicine, especially the treatment of infectious diseases and surgery.

There have been an increasing number of reports on the identification of *mcr* genes in many bacterial species across the world since this gene was first characterized [8–10]. One retrospective study from China, showed the presence of the *mcr-1* gene in *E. coli* isolated from poultry in the 1980s and suggested that the emergence of the gene may be linked to the use of colistin as a growth promoter in the poultry industry [11]. Multiple *mcr* genes (*mcr-1* to -8) in addition to several *mcr-1* variants have been reported [12]. A recent study by Carroll et al. [13] described *mcr-9*, a novel *mcr* homologue in a multidrug-resistant (MDR) *Salmonella* Typhimurium strain isolated from a human patient in United States.

The *mcr* genes have been detected in a wide range of plasmid types such as IncI2, IncHI2, IncX4, IncP, IncF, and IncY. The bacteria that carry these genes have been isolated from pigs, poultry, and cattle and also from food products derived from these animals such as pork, chicken and beef, in addition to many environmental sources such as hospital sewage, rivers, and seas, [8]. Few studies have conducted an integrated analysis among animal hosts, bacterial species or genotypes, and plasmid types. Investigations on the role of the environment and/or a foodborne pathway for *mcr* dissemination are patchy and sometimes vague.

The co-occurrence of *mcr* and other resistance genes, especially those that confer resistance to carbapenem drugs [14], have already been reported. While this highlights the limitation of therapeutic choices, a recent study suggested that treatment with colistin in combination with other antibiotics is still effective against *mcr*-harboring bacteria in an animal infection model [15].

The objectives of this study were to determine (1) the global distribution of *mcr* genes; (2) potential reservoirs including the environment, animal hosts, humans, bacterial species, genotype, and plasmid type and (3) to perform an analysis on antibiotic resistance patterns in isolates obtained from the different reservoirs.

2. Methods

2.1. Search Strategy and Selection Criteria

In compliance with PRISMA recommendations [16] (Figure 1), we conducted a systematic literature review and meta-analysis study. Four English (PubMed, Scopus, the Cochrane Database of Systematic Reviews, Web of Science) and two Chinese (CNKI and WANFANG) databases were searched individually for the published papers. We focused on those published between 18 November, 2015 (first publication on *mcr* gene discovery) and 30 December, 2018. We used some keywords to search relevant publications in both English and Chinese databases. The search key words, or strings, were “*mcr* genes and polymyxins”, “prevalence of *mcr* genes”, “occurrence of *mcr* genes”, “incidence of *mcr* genes”, “*mcr-1* OR *mcr-2* OR *mcr-3* OR *mcr-4* OR *mcr-5* OR *mcr-6* OR *mcr-7* OR *mcr-8* OR variant *mcr* genes” “colistin resistance”, and “plasmid-mediated colistin resistance”.

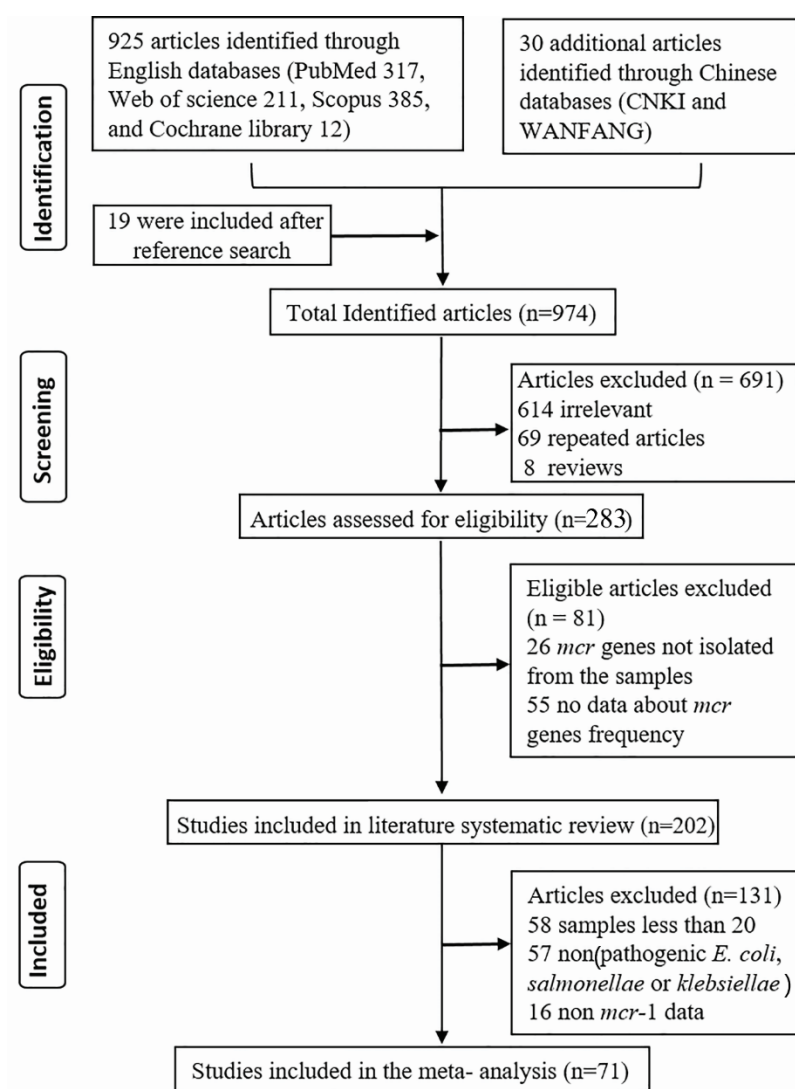


Figure 1. Flow diagram of identification and selection process included in systematic review and meta-analysis.

2.2. Study Selection

The systematic review was performed by four reviewers. Two reviewers (ME and NP) evaluated the data from English databases, and two reviewers (YL and HP) evaluated the data from Chinese databases. The search results were imported into the Endnote 7 references manager distributed by Clarivate Analytics and de-duplicated. Initial screening was done on the basis of title and abstract. Full text of the publications selected after initial screening was obtained from the library of Zhejiang University for data extraction. The relevant variables such as title, authors list, country of sampling, period of study, sampling reservoir, animal host, bacteria species and genotype, plasmid type, date of sampling and antimicrobial resistance pattern (if available) were extracted and arranged in a MS Excel spreadsheet specially formatted for the purpose.

2.3. Data Categorization

The scheme of data categorization summarized in Table 1 are shown with details in Supplemental Table S1 and Supplemental Table S2. The *mcr* variants among the various bacterial genera and species were also summarized (Table S3). The *E. coli* strains were split into two groups as pathogenic and non-pathogenic *E. coli*. Any known pathogenic serotype or variants such as O157 [17], or those

that have been reported as the causative agent of human or animal infection [18] or as a pathogenic *E. coli* (in the selected publication) were categorized into pathogenic *E. coli* group (*P. E. coli*), whereas isolates which were reported from non-clinical cases and did not cause disease were categorized as a non-pathogenic *E. coli*.

Table 1. Scheme of the data categorization.

Categories	Includes	Sub-Includes
Environmental	Sewage, river, and sea water samples	
Animal	Pig, poultry, cattle, and other animal samples	
	Poultry	Chicken, turkey, and muscovy duck
	Other animals	Migratory birds, penguins, reptiles, kelp gulls and dogs
Food	Pork, chicken meat, beef, and other foods samples	
	Other foods	Vegetables and foods from unknown sources
Human	Fecal and other samples	
	Other samples	Blood, urine, pus, sperm, abdominal and peritoneal fluids, sputum, and ascites
Harboring bacteria	Pathogenic <i>E. coli</i> (<i>P. E. coli</i>), <i>Salmonella</i> spp., <i>Klebsiella pneumoniae</i> , other bacteria	
	<i>Salmonella</i> spp.	<i>S. Typhimurium</i> , <i>S. Paratyphi B</i> , <i>S. Virchow</i> , <i>S. Rissen</i> , <i>S. Indiana</i> and <i>S. Derby</i> , <i>S. London</i>
	Other bacteria	<i>Citrobacter freundii</i> , non-pathogenic. <i>E. coli</i> , <i>Citrobacter braakii</i> , <i>Enterobacter cloacae</i> , <i>Shigella sonnei</i> , <i>Aeromonas veronii</i> , <i>Enterobacter aerogenes</i> , <i>Cronobacter sakazakii</i> , <i>Kluyvera ascorbata</i> , <i>Acinetobacter</i> , <i>Klebsiella variicola</i> , <i>Moraxella pluranimalium</i> , <i>Klebsiella oxytoca</i> , <i>Providencia alcalifaciens</i> , and <i>Raoultella ornithinolytica</i>
Plasmid types	IncI2, IncHI2, IncX4	
	Other plasmids	IncF, IncFIB, IncK, IncX, IncP, IncFII, and ColE

2.4. Data Analysis

We calculated the frequency of identification of *mcr* genes and their relationship with different reservoirs (human, animal hosts, bacteria, plasmid types) and antibiotic resistance pattern for *mcr*-positive bacteria. Pivot table function of Microsoft Excel 2016 was used to calculate the descriptive analysis (as a percentage), and the prevalence of *mcr*-harboring strains among the total strains including 95% confidence intervals (CIs), the total number of resistant isolates (number of resistant isolates/total number of positive isolates from same species) to each individual antimicrobial drug. Additionally, the sequence type (ST) of pathogenic *E. coli* (*P. E. coli*) harboring *mcr-1* as the most frequent bacteria was also calculated. Student's T-test and graphs were performed using GraphPad Prism 7. Among 202 articles that were included in the systematic review, only 134 articles mentioned the total number of strains and were included in a cumulative prevalence analysis. We excluded all the case studies, and any articles that did not mention the total number of strains.

The prevalence results are shown in percent with the 95% CI in parenthesis. The meta-analysis and Forest plotting were done using Open Meta-Analyst software. The data were analyzed in binary

random model effects at 95% confidence interval. The values of heterogeneity (I^2) [19] across the studies at 25%, 50%, and 75% were considered a slow, moderate, high heterogeneity, respectively.

3. Results

3.1. Data Selection

A total of 925 papers resulted from the initial search in English databases, 30 from Chinese databases and 19 additional English articles were included after reference search. After screening, based on the title and abstract, 691 (614 irrelevant, 69 repeated articles, and 8 reviews) were excluded. The remaining 283 publications were assessed for eligibility, and a further 80 (54 had no data about *mcr* genes and 26 publications where bacteria with *mcr* genes were not isolated from the samples) were excluded. We included 202 publications for synthesis of the systematic review. A meta-analysis of *E. coli*, *Salmonella*, and *K. pneumoniae* harboring the *mcr-1* gene was carried out with 71 publications. One hundred and thirty-one were excluded (58 papers had less than 20 samples, 57 papers were on bacteria other than pathogenic *E. coli*, *Salmonella*, and *K. pneumoniae* and 2 papers were not related to *mcr-1* data). (Figure 1). We included 134 articles in the average prevalence analysis for the countries and the burdens. Ninety-eight of these 134 articles (73.3%) used a targeted PCR method and 36 (26.7%) used a selective culture media method (Table S4). We calculated the total number of isolated strains and the total numbers of *mcr* harboring strains in the selected articles. The prevalence (%) was calculated as (total number of isolated *mcr* harboring strains/the total number of isolates in each article).

3.2. Global Dissemination of *mcr* Genes

The current global distribution of *mcr* genes, in relation to the various hosts, bacteria and plasmid types showed significant geographic segregation (Figure 2). A total of 5191 *mcr*-gene-harboring strains were reported in 202 publications. Of these, 4917 were of the *mcr-1* type while the remaining 274 were other variants. White colored countries in Figure 2 refer to countries that have not yet reported studies on *mcr* genes. *P. E. coli* (53%) among all bacteria, and animals (53%) among all hosts, were the most frequent *mcr* carriers reported globally. IncI2 (34%) plasmids were the most frequently reported plasmids.

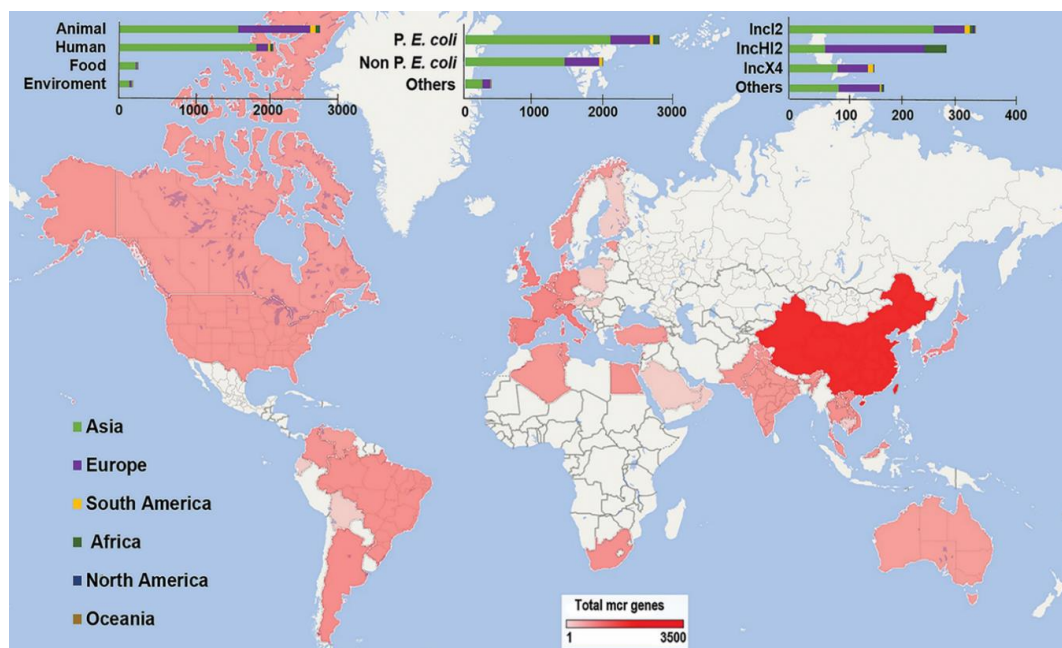


Figure 2. Global view of *mcr* genes along with the various hosts, bacteria and plasmid types. White colored countries refer to countries that have not yet reported studies on *mcr* genes.

3.3. Global Prevalence of *mcr* Genes by Sampling Source as a Reservoir

The cumulative average prevalence of *mcr* genes was 4.6% (0.1–9.3%), with the highest in environment 22% (2.8–47.8%), followed by animals 11% (0.3–22.4%), food 5.4% (0.6–11.6%), and humans 2.5% (0.1–5.1%) (Figure 3A). China shares a similar pattern with the global picture and showed an overall prevalence in the environment of 39% (8.3–88.5%); animals 14% (0.7–30%); humans 4.5% (0.2–9.3%), and food products 4.9% (0.7–10.7%). Vietnam also has a very similar pattern to China, 14.7% from animals, 4.5% in humans and 4.9% in food products (Figure S1).

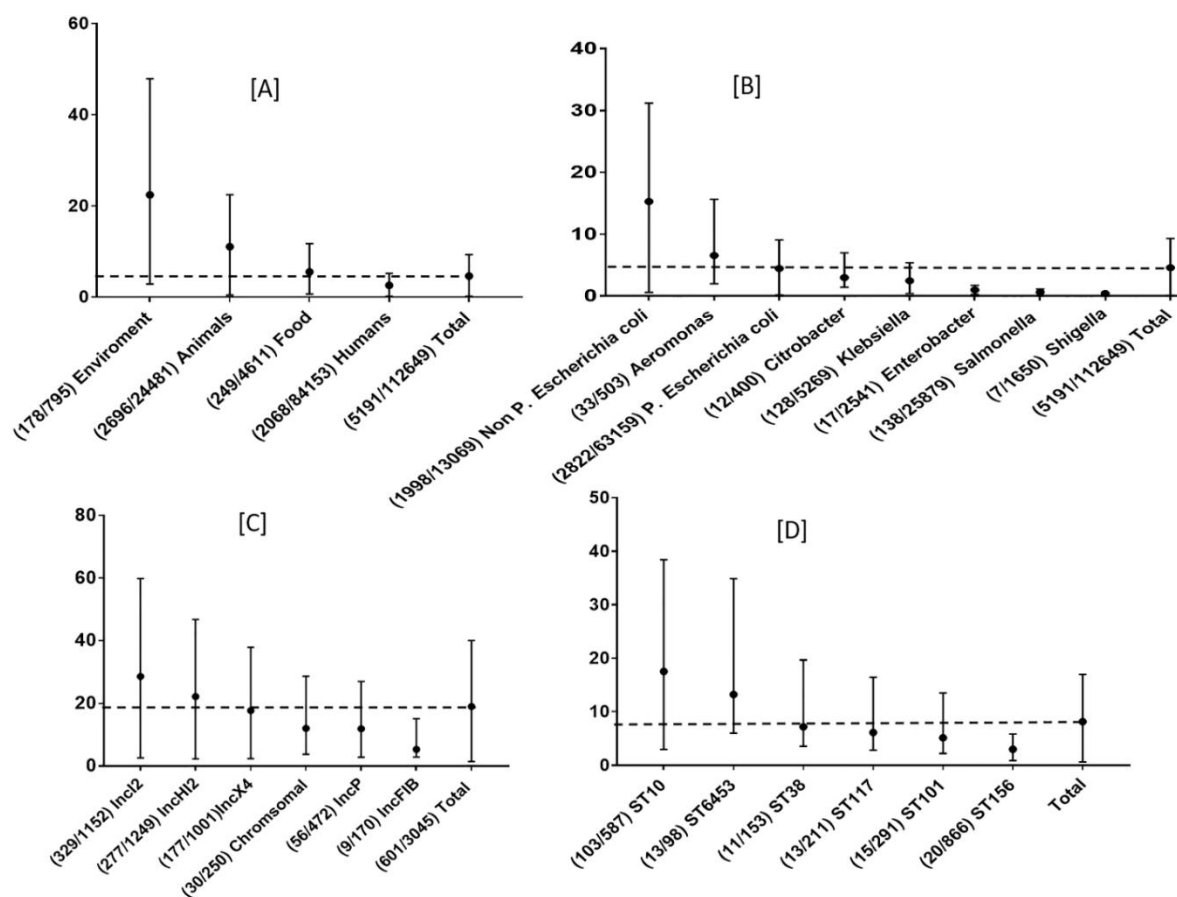


Figure 3. Prevalence of *mcr* genes from multiple sources (A), bacteria (B), plasmids (C) and pathogenic *E. coli* sequence type (ST) (D). () contain the positive strains/total strains Values in (A and B) are the prevalence of positive strains in the total number of strains, in (C), the value is the prevalence of plasmid types and in (D) *P. E. coli* harboring *mcr-1* shows the prevalence of different sequence types (ST) of the positive isolates.

3.4. Global Prevalence of *mcr* Genes by Bacterial Genera and Species

There were 15 bacterial genera, mainly of the *Enterobacteriaceae* (11/15) (Supplemental Table S2, Supplemental Table S3), in which the presence of *mcr* has been reported to date. *E. coli* (non-pathogenic and pathogenic types) followed by *Salmonella*, *Klebsiella* and *Aeromonas* were the most common bacteria from which the *mcr* genes have been isolated. Non-pathogenic *E. coli* showed the highest *mcr* prevalence of 15.2%. The isolation rate of *E. coli* varies dramatically; China leads in terms of the numbers of reports made. The *mcr-1* gene was identified in 4917 strains, 274 strains had a variant *mcr* genes.

3.5. Types of *mcr* Gene and Their Associated Reservoir

The environmental samples had the highest prevalence of strains that carried the *mcr*-gene, 22.3% (2.8–47.8), although they had the lowest positive detections (3%, $n = 178$), likely due to lack of studies

that looked at environmental sources. 2067 (39%) isolates were from human sources and the lowest prevalence 2.5% (0.1–5.1) was observed in these samples (Figures 3A and 4A). Of the isolates, 62% were from clinical patients with an infectious disease and 38% were from asymptomatic carriers. Seventy-seven percent of the isolates were from feces. Isolates from pigs (among the animals) and chicken meat (among the foods) had the highest rate of *mcr* gene detection (Figure 4A). Live animals (swine, poultry, and bovine) had a higher frequency of *mcr* genes than isolates from meat samples (pork, chicken meat and beef). Concerning the entire host community, *P. E. coli* with *mcr-1* was the most common pathogen-*mcr* association (Figure 3A, Figure 4A). IncI2 was the most prevalent plasmid type at 28.5% (2.5–95.8) as well as the most frequent type associated with poultry, human, environmental and food isolates. The IncHI2 plasmid type was associated with isolates from animals (Figure 3C, Supplemental Figure S2B). There was no significant difference between the hosts in total number of isolates and the total number of plasmids ($p > 0.05$, Student T-test).

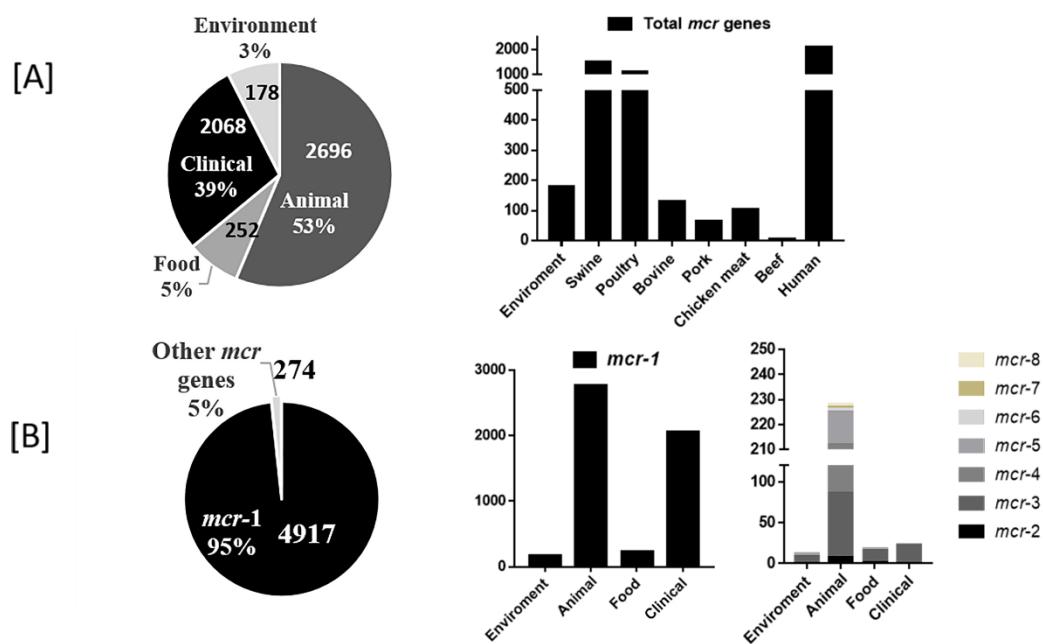


Figure 4. Frequency and distribution of *mcr* genes according to hosts and types. Hosts harboring *mcr* genes (A), *mcr* types (B).

Table 2. Antibiotic resistance of isolates that harbor *mcr* genes.

Sources	CST	TGC	GEN	CIP	AMK	CTX	CFX	TET	TMP-SMX	FOS	MEM	IMP	AMP	KAN
Human	98.69% (993)	3.39% (59)	59.55% (178)	44.09% (973)	22.60% (146)	79.61% (152)	83.72% (43)	96.43% (28)	83.08% (65)	48.62% (109)	2.76% (979)	2.76% (979)	93.33% (150)	95.00% (20)
Swine	99.37% (158)	100.00% (3)	86.67% (15)	90.91% (11)	6.06% (33)	47.06% (34)	100.00% (3)	100.00% (98)	98.98% (98)	100.00% (27)	43.64% (55)	40.38% (52)	43.75% (48)	80.65% (33)
Poultry	100.00% (125)	0.00% (38)	61.90% (21)	97.58% (124)	16.67% (18)	100.00% (55)	100.00% (3)	96.43% (56)	98.04% (51)	100.00% (54)	0.00% (3)	0.00% (3)	94.59% (74)	26.32% (19)
Bovine	100.00% (17)	0% (5)	80.00% (5)	100.00% (6)	NA	83.33% (6)	83.33% (6)	100.00% (6)	100.00% (5)	0.00% (5)	0.00% (1)	0.00% (1)	100.00% (6)	100.00% (1)
Food	97.83% (45)	100.00% (1)	33.33% (18)	69.57% (7)	100.00% (1)	81.25% (16)	100.00% (5)	84.21% (19)	87.50% (24)	100.00% (3)	0.00% (7)	0.00% (7)	81.25% (16)	40.00% (5)
Environmental	87.10% (31)	33.33% (24)	60.00% (5)	36.00% (25)	72.73% (11)	100.00% (1)	100.00% (2)	100.00% (3)	100.00% (1)	100.00% (1)	9.09% (30)	9.09% (33)	100.00% (3)	NA
Human	100.00% (36)	21.40% (7)	91.67% (12)	63.64% (11)	33.33% (6)	91.67% (12)	100.00% (6)	100.00% (4)	60.00% (5)	66.67% (3)	53.85% (13)	53.85% (13)	90.00% (10)	33.3% (6)
Animals	100.00% (13)	NA	100.00% (4)	100.00% (4)	100.00% (1)	100.00% (4)	100.00% (4)	91.67% (11)	100.00% (9)	NA	0.00% (5)	0.00% (5)	100.00% (4)	0.00% (1)
Human	100.00% (32)	NA	43.59% (39)	24.14% (29)	NA	65.52% (29)	100.00% (1)	80.65% (31)	63.16% (19)	NA	0.00% (2)	0.00% (2)	90.00% (30)	90.00% (30)
Animals	100.00% (36)	NA	96.55% (29)	97.30% (36)	96.4% (26)	87.50% (8)	0.00% (36)	100.00% (10)	100.00% (34)	96.15% (26)	0.00% (2)	0.00% (2)	100.00% (54)	100.00% (3)
Human	60.00% (5)	100.00% (1)	100.00% (3)	75.00% (4)	66.67% (3)	100.00% (1)	0.00% (2)	100.00% (2)	NA	NA	14.2% (6)	75.00% (5)	100.00% (2)	NA
Animals	96.34% (273)	0.00% (152)	31.84% (223)	76.50% (234)	1.94% (155)	93.33% (165)	99.34% (152)	96.41% (232)	NA	100.00% (3)	0.86% (233)	48.07% (231)	100.00% (71)	100.00% (3)

() contain the number of isolates. Antibiotics: CST—Colistin, TGC—Tigecycline, GEN—Gentamicin, CIP—Ciprofloxacin, AMK—Amikacin, CTX—Cefotaxime, CFX—Cefoxitin, TET—Tetracycline, TMP-SMX—Trimethoprim-sulphamethoxazole, Fos—Fosfomycin, MEM—Meropenem, IMP—Imipenem, AMP—Ampicillin, KAN—Kanamycin.

As compared to 4917 (95%) isolates that carried *mcr-1*, only 274 (5%) carried one of the other variant genes (*mcr-2* to *mcr-8*). Supplemental Figure S3 showed that *mcr-3* had a wide distribution in water, animal, food, and human isolates. *P. E. coli* had the highest frequency of *mcr* genes, except for *mcr-5* which was mainly isolated from *Salmonella* species. In addition, *mcr-7* and *mcr-8* were only reported in *K. pneumoniae* isolated from animals. IncI2 was the most common plasmid type in all hosts except animals. IncHI2, IncX4, CoIE and IncI2 plasmid types, had the highest frequency in *mcr-2*, 3, 5 and 7 respectively.

3.6. Antimicrobial Resistance in *mcr*-Positive Bacteria

The results from antimicrobial susceptibility tests were different in the various studies because of the different antimicrobial agents tested, so we included only the resistance profiles of *mcr*-harboring bacteria in our analysis. Eighty-four percent (170/202) of the articles documented the antimicrobial resistance assays, and a high occurrence of co-resistance was observed (Table 2). All water and food isolates were susceptible to the two carbapenem drugs (imipenem and meropenem). *P. E. coli* isolated from poultry and bovine were susceptible to tigecycline, unlike those from swine, which were resistant to tigecycline. Human *P. E. coli* were susceptible to tigecycline and showed low resistance (2.76%) to the carbapenem drugs. *Klebsiella pneumoniae* strains from humans were resistant to carbapenem drugs (53.85%) and none of the isolates from animals were resistant. All of the *Salmonella* isolates were susceptible to carbapenems.

3.7. Role of *mcr-1* Pathogenic *E. coli*, *Salmonella*, and *Klebsiella* in *mcr* Gene Dynamics

Because of their public health significance, we focused on pathogenic *E. coli*, *Salmonella*, and *Klebsiella*, in the meta-analysis studies. The average prevalence of *mcr-1*-harboring bacteria was calculated by a meta-analysis of 71 publications (Figures 5 and 6). The analysis showed more heterogeneity in pathogenic *E. coli* ($I^2 = 97.65\%$) with an average prevalence of 23% (20–26, $p < 0.001$) (Figure 5) than *Salmonella* ($I^2 = 80\%$) with an average prevalence of 6% (2–11, $p < 0.001$) (Figure 6A) and *Klebsiella* ($I^2 = 62\%$) with an average prevalence of 8% (3–12, $p < 0.001$) (Figure 6B). Additionally, the average prevalence of pathogenic *E. coli* in humans was 0.7% (0.5–0.9, $p < 0.001$), in animals it was 16.8% (13–20.6, $p < 0.001$) and in foods it was 7.1% (3.4–10.8, $p < 0.001$). The highest heterogeneity was among the papers that dealt with animals' isolates ($I^2 = 97.65\%$) and the lowest was in those isolates from food ($I^2 = 90.03\%$), and the humans ($I^2 = 92.83\%$) (Figure 5). The comparative analysis has not been made for humans, animals and foods of *Salmonella* and *Klebsiella* because of unavailability of their comparative data. Several studies included data on multiple hosts.

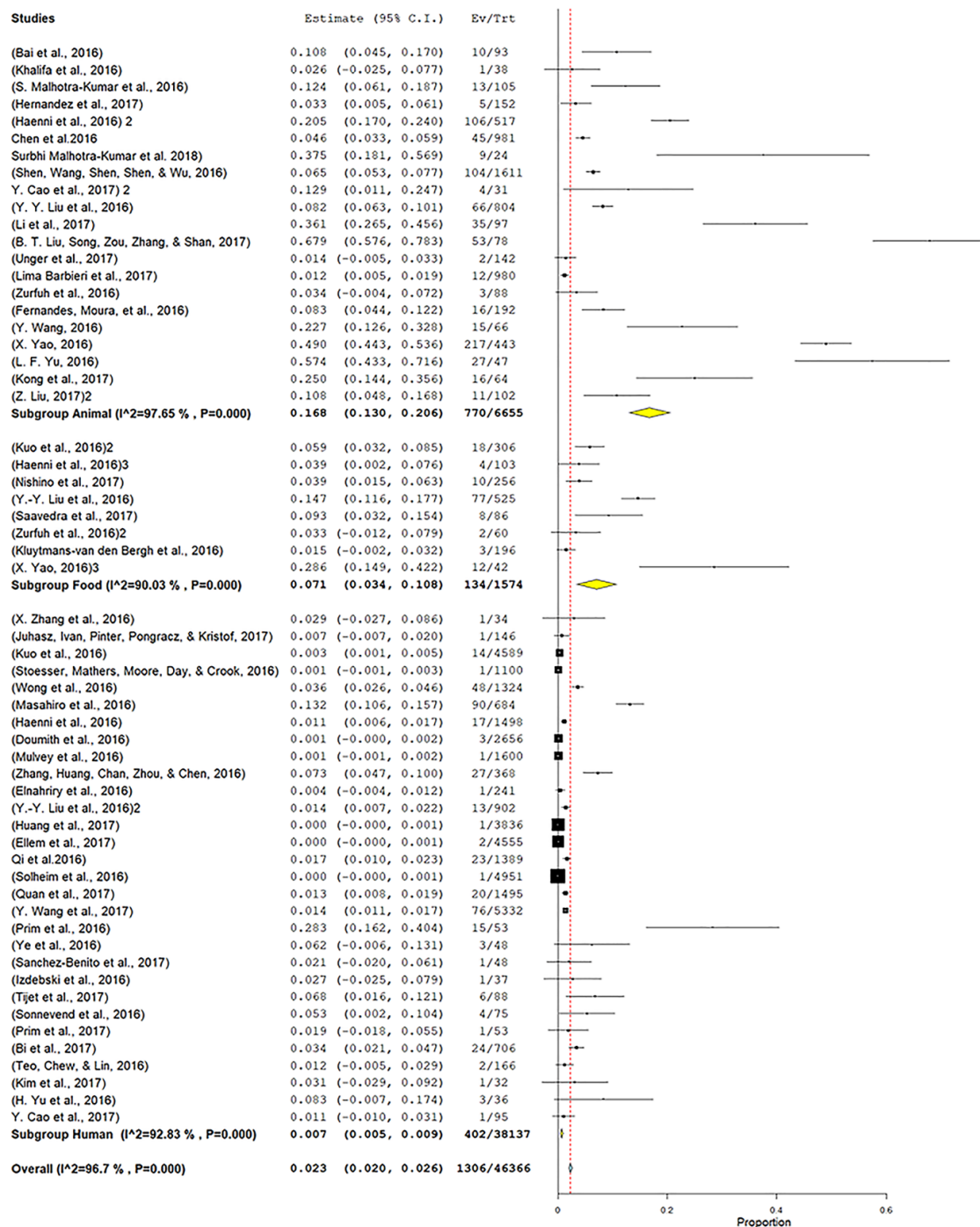


Figure 5. Prevalence of pathogenic *E. coli* carrying *mcr-1* in animals, foods and humans. X-axis is the proportion of the bacteria reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval. Studies given higher weights are indicated by larger markers. The parallelograms in yellow and the square markers in black represent the pooled point estimate for the sub-group category and individual study, respectively. The horizontal lines of the parallelograms and the square markers represent the 95% confidence interval of this combined point estimate.

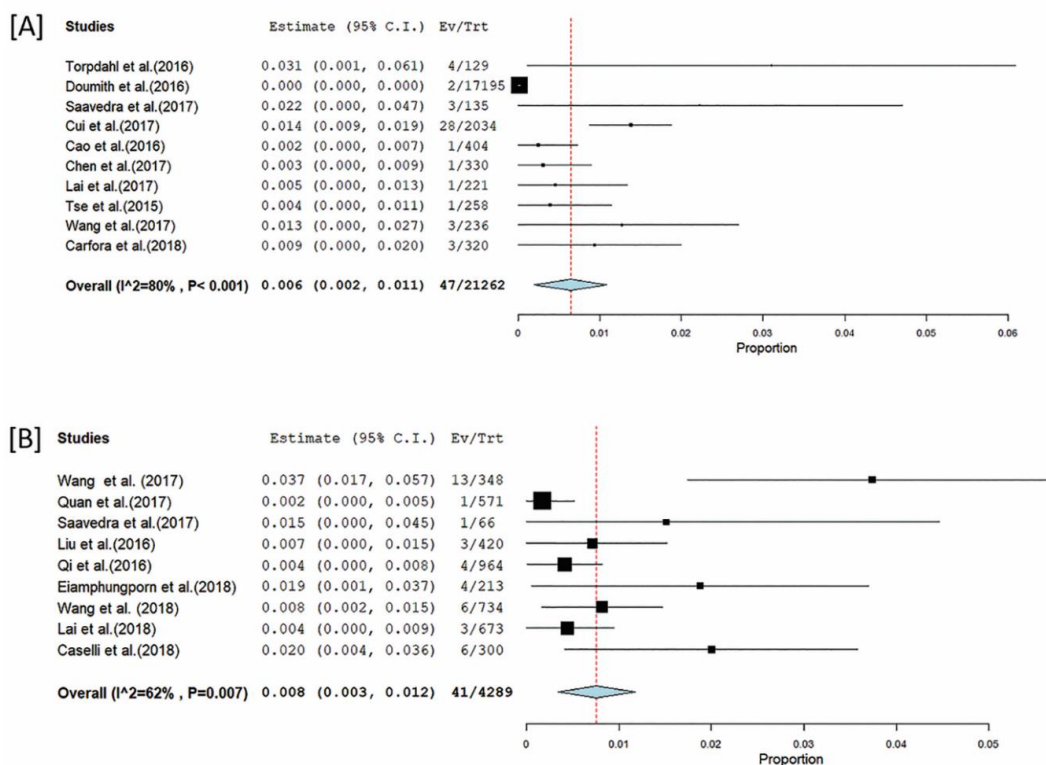


Figure 6. Prevalence of *Salmonella* (A) and *Klebsiella* (B) carrying *mcr-1*. X-axis is the proportion of the bacteria reported in individual studies as listed along the Y-axis, with the range of proportion in 95% confidence interval. Studies given higher weights are indicated by larger markers. The parallelograms in yellow and the square markers in black represent the pooled point estimate for the sub-group category and individual study, respectively. The horizontal lines of the parallelograms and the square markers represent the 95% confidence interval of this combined point estimate.

3.8. Common ST of *P. E coli* with *mcr-1*

The frequency of *P. E. coli* STs harboring *mcr-1* varied among different sources (Figure 7). ST10, the dominant ST in both animals and water, is the most common ST of those that carrying *mcr-1* globally, also has the highest prevalence 17.5% (2.9–39.4) (Figure 3D). ST116 the most abundant ST in humans, has been reported only from China (Figure 7A).

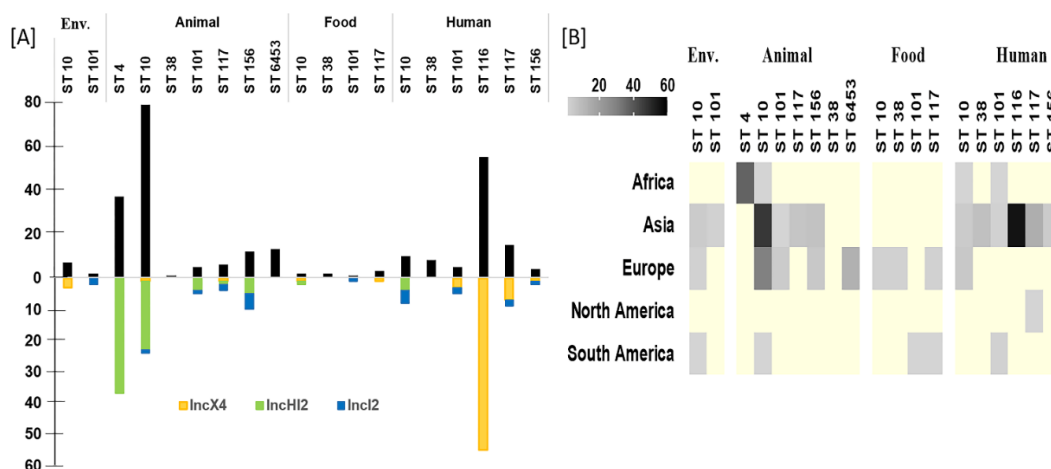


Figure 7. Role of *P. E. coli* STs in *mcr-1* gene dissemination. Particular *P. E. coli* STs that harbored *mcr-1* gene frequency (A). The global distribution of *P. E. coli* harboring *mcr-1* gene (B). Yellow cells in (B) refer to blank, Env. refers to environmental isolates. The scales refer to number of isolates.

4. Discussion

Since the first isolation of *mcr-1* in China in 2011 [7], *mcr*-harboring bacterial isolates have been reported from six continents (Asia, Europe, Africa, North America, South America, and Oceania) and over 27 bacterial species. The majority of studies have been conducted in China but *mcr*-harboring isolates were also reported from many European countries such as the UK, Spain, and Italy. The reason for the increasing reports may be due to long-term use of polymyxins in veterinary medicine in these countries. It should be noted that before 2005 there were no reports that identified the *mcr*-harboring isolates and most of the isolates currently reported to be *mcr* positive are historical isolates, dating back to as early as 1980. Similarly, global trade and travel either to countries with high or unknown prevalence (Canada [20], U.S. [21] and Japan [22]), importation of food from infected countries (Japan [23] and Tunisia [24]), and over-prescription of colistin in human medicine to treat highly resistant clinical pathogens (i.e., Argentina [25]) are also the suggested causes. To date, several other *mcr* gene variants have been identified, including *mcr-2*, -3, -4, -5, -6, -7, and -8, which share 81%, 32%, 34%, 36%, 83%, 35%, and 31% amino acid sequence identity, respectively, with *mcr-1* [26]. Recently *mcr-9* which is closely related to *mcr-3* was reported [13]. Minor variants have been reported for each of *mcr-2*, -4, and -5, whereas there are more than 10 *mcr-3* variants [26]. Among other *mcr* genes, the earliest *mcr-3* was discovered in 2005 in Germany [27]; whereas *mcr-2* (2009), -4 (2013), -5 (2011), -6 (2015), and -7 (2014) [26–29], were identified in strains collected over the past decade. This supports the hypothesis that *mcr* genes existed far earlier than first reported. Moreover, all *mcr* genes except (*mcr-6* and *mcr-9*) have been detected in samples from China. Thus far, *mcr-2* (Belgium and Spain), *mcr-3* (Brazil, Denmark, France, Germany, Japan, Spain and Thailand), *mcr-4* (Italy and Spain), *mcr-5* (Colombia, Japan, Spain and Germany), and *mcr-9* (US) [27,29–37].

A report by Biswas et al. [38] demonstrated that *mcr-3*-harboring *Salmonella* Typhimurium ST34 is highly likely to be linked with international travel. Two recent studies reported that *Moraxella* spp. a common pathogen associated with animals, but that may also be human pathogens, has been identified as a potential source of *mcr*-like genes [36,39]. A recent study described that the newly found *mcr-9* gene in *Salmonella* Typhimurium, from a clinical isolate in USA, was capable of conferring phenotypic resistance to colistin in *Enterobacteriaceae*, making it a relevant concern to public health [13]. Most of the studies where the *mcr* genes were reported included a screening approach for MDR strains (a pre-selected population of strains). Different screening methods to isolate *mcr* positive organisms were conducted in each study, the bibliography analyses may be biased by the size of the countries, missing data and their scientific intentions according to their priorities. These limitations might influence the global and individual country prevalence interpreted in current study.

4.1. Role of *mcr*-Positive Bacteria via Food-Chain Transmission

Colistin use in animal production is well-recognized as a driver for the emergence of *mcr*-positive bacteria [7]. Bacteria from animal gut and feces play an important role in persistence and transmission of bacteria that contain *mcr* genes to humans [40–44]. Bacteria with *mcr* genes have also been detected in food products [23], wastewater [45], rivers, seawater [46], and humans [47]. The estimated overall prevalence in the animal and food samples were higher than in the human samples (Figure 5, Figure 3A), and this supports the hypothesis of that the food-chain plays a role in *mcr* transmission. Additional large-scale investigations in China indicates that aquaculture could be another important reservoir for food-chain transmission of *mcr*-harboring bacteria to humans [48–51].

4.2. Role of Plasmids in *mcr* Dissemination

The *mcr* genes are generally hosted on bacterial plasmids that are highly mobile and this may accelerate the spread of resistance under selection pressure [52]. About 61% (123/202) of the articles have reported the plasmid type which our results were based on. The other articles that have not mentioned were case reports and surveys. The IncI2, IncX4 and IncHI2 are the most common plasmid

types that carry *mcr* genes. While the plasmid IncI2 is common in Asia [53], Oceania [52], North and South America [54], IncHI2 is more frequently reported in Europe [47] and Africa [24] (Figure 2). The IncI2 plasmid usually carries the partitioning genes (*yafA/yafB*), type IV pilus and shufflon, acid resistance and biofilm production, which enhance the conjugation and adherence of the bacteria [55] with this plasmid to the epithelial cells. Moreover, IncHI2 plasmids are renowned by their ability to transfer by conjugation in a wide range of temperatures [56]. IncHI2 plasmids are known to carry several resistance genes, this information shows that even if a change of antimicrobial therapy occurs during treatment, there will still be a chance to co-select for the *mcr-1* gene. Also, several genes, i.e., *relE* and *relB* (antitoxin system), *mucAB* (mutagenesis induction system), *phi* (bacteriophage inhibition), and colicin, tellurite, and heavy metals together, likely play a key role in the stability of IncHI2 plasmids [57]. The *mcr-1* gene is associated with insertion sequence IS*Apl1* which belongs to IS30 family [58]. A previous study of *mcr-1* sequences demonstrated that *mcr-1* was part of a ~2609-bp region flanked by one copy, two copies, or no copies of IS*Apl1*. Recently, Snesrud et al. [59] demonstrated that all *mcr-1* structures can be explained by loss of one, or both, copies of IS*Apl1* from an ancestral Tn6330 [60]. Transferable plasmids that harbor *mcr* genes are detected in isolates of same clonal backgrounds. The plasmids belonging to the same incompatibility group are likely to be involved in the dissemination of *mcr* via the food-chain [61].

4.3. Role of *P. E. coli* Genotype for *mcr* Dissemination

Our results show that *E. coli* ST10 (the most frequent in many continents) and ST101 (widely distributed among multiple hosts) are the major clones that harbor *mcr-1* genes. Our analysis revealed that ST10 is the dominant ST in both animals and water as mentioned earlier [62]. ST10 and ST101 belong to phylogroups A and B1. These two groups produce enzymes such as ESBLs [63], ampC [64], carbapenemase, and mBLs [65] that degrade beta-lactam antimicrobials. The presence of beta-lactamase resistance can accelerate the dissemination and persistence of these clones. ST116, with an IncX4 plasmid, is the most abundant clone in human patients, and has not yet been reported in any other hosts. The IncX4 plasmid in ST116 *E. coli* strains isolated from humans is likely the result of conjugation from ST10 strains that originated from water or food [40]. A high rate of conjugation may be enhanced by the genes essential for synthesis and assembly of the pilus, conjugative function [66,67], and *mrk* fimbria [68–70]).

4.4. Rational Antibiotic Choice for *mcr*-Carrying Bacterial Infections

Isolates that carry *mcr* genes, and show resistance to colistin, were also frequently shown to possess other antibiotic-resistant determinants [47]. Interestingly, a few studies reported that *mcr*-harboring *P. E. coli* from human [53] and pig [71] are phenotypically susceptible to colistin *in vitro*. This might be attributed to suppression of *mcr* genes [72], or unknown reasons [73]. Some reports demonstrated the effectiveness of carbapenem drugs to treat infections caused by colistin-resistant isolates (Figure 3) [46,47]. The new combination regimen of a carbapenem and tigecycline [74] has also shown effectiveness against human infections. The resistance of most human clinical *K. pneumonia* isolates to multiple antimicrobials is a serious concern [75]. *P. E. coli* from animal samples did not show any resistance to tigecycline, except the isolates from pigs. Recently, some studies have reported that several antibiotics, in combination with colistin, display growth-inhibition at levels below their corresponding clinical breakpoints such as the combination of colistin and clarithromycin [15] and colistin and amikacin [76]. Further investigation on effective antibiotic therapies, including combination therapy, is needed to treat *mcr*-carrying bacterial infection.

5. Summary

We provide a comprehensive picture of *mcr* genes from animal hosts, bacterial species, bacterial genotype, and plasmid types in 47 countries across six continents. The significant role of the food-chain and/or the environment in *mcr* gene dissemination and their relationship with other suitable vectors

(animal hosts, bacteria species, bacterial genotype, plasmid type) warrants further investigations. Co-resistance of *mcr*-positive pathogen with other antimicrobials that are critical for the treatment of drug resistant bacterial infections is an increasing concern, both in human or veterinary medicine.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-2607/7/10/461/s1>.

Author Contributions: M.E., Y.L., N.P., and H.P. collected and reviewed all the raw data from the literature search. M.E. and N.P. did the analyses for this manuscript. M.E. prepared the first draft. M.E., N.P. prepared the figures, tables and, appendix. M.Y., S.C.R., and M.E. finalized the manuscript. M.Y. and M.E. managed the project. All other authors provided data or developed models for indicators, reviewed results, initiated modelling infrastructure, or reviewed the manuscript.

Funding: This work was supported by the National Program on Key Research Project of China (2017YFC1600103; 2018YFD0500501), The Fundamental Research Funds for the Central Universities (2-2050205-18-237), Zhejiang Provincial Natural Science Foundation of China (LR19C180001), Zhejiang University of Hundred Talent Program and National Recruitment Program of Global Youth Experts.

Acknowledgments: We would like to thank Silpak Biswas for comments that greatly improved the manuscript.

Conflicts of Interest: All the authors declare no competing interests.

Data Sharing: All of our results are available for further investigation in the appendix and online.

References

1. Nolte, O. Antimicrobial Resistance in the 21st Century: A Multifaceted Challenge. *Protein Pept. Lett.* **2014**, *21*, 330–335. [[CrossRef](#)] [[PubMed](#)]
2. Fair, R.J.; Tor, Y. Antibiotics and bacterial resistance in the 21st century. *Perspect Med. Chem.* **2014**, *6*, 25–64. [[CrossRef](#)] [[PubMed](#)]
3. Theuretzbacher, U. Global antimicrobial resistance in Gram-negative pathogens and clinical need. *Curr. Opin. Microbiol.* **2017**, *39*, 106–112. [[CrossRef](#)] [[PubMed](#)]
4. Caniaux, I.; van Belkum, A.; Zambardi, G.; Poirel, L.; Gros, M.F. MCR: Modern colistin resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* **2017**, *36*, 415–420. [[CrossRef](#)] [[PubMed](#)]
5. Nation, R.L.; Garonzik, S.M.; Thamlikitkul, V.; Giamarellos-Bourboulis, E.J.; Forrest, A.; Paterson, D.L.; Li, J.; Silveira, F.P. Dosing guidance for intravenous colistin in critically-ill patients. *Clin. Infect. Dis.* **2017**, *64*, 565–571. [[CrossRef](#)] [[PubMed](#)]
6. Olaitan, A.O.; Morand, S.; Rolain, J.M. Mechanisms of polymyxin resistance: Acquired and intrinsic resistance in bacteria. *Front. Microbiol.* **2014**, *5*, 643. [[CrossRef](#)] [[PubMed](#)]
7. Liu, Y.-Y.; Wang, Y.; Walsh, T.R.; Yi, L.-X.; Zhang, R.; Spencer, J.; Doi, Y.; Tian, G.; Dong, B.; Huang, X.; et al. 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.* **2016**, *16*, 161–168. [[CrossRef](#)]
8. Jeannot, K.; Bolard, A.; Plésiat, P. Resistance to polymyxins in Gram-negative organisms. *Int. J. Antimicrob. Agents* **2017**, *49*, 526–535. [[CrossRef](#)]
9. Juhasz, E.; Ivan, M.; Pinter, E.; Pongracz, J.; Kristof, K. Colistin resistance among blood culture isolates at a tertiary care centre in Hungary. *J. Glob. Antimicrob. Resist.* **2017**, *11*, 167–170. [[CrossRef](#)]
10. Stoesser, N.; Mathers, A.J.; Moore, C.E.; Day, N.P.; Crook, D.W. Colistin resistance gene *mcr*-1 and pHNSHP45 plasmid in human isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Lancet Infect. Dis.* **2016**, *16*, 285–286. [[CrossRef](#)]
11. Shen, Z.; Wang, Y.; Shen, Y.; Shen, J.; Wu, C. Early emergence of *mcr*-1 in *Escherichia coli* from food-producing animals. *Lancet Infect. Dis.* **2016**, *16*, 293. [[CrossRef](#)]
12. Georgios, M.; Lemonia, S. Polymyxin Resistance Mechanisms: From Intrinsic Resistance to MCR Genes. *Recent Pat. Anti Infect. Drug Discov.* **2018**, *13*, 1–9. [[CrossRef](#)]
13. Carroll, L.M.; Gaballa, A.; Guldimann, C.; Sullivan, G.; Henderson, L.O.; Wiedmann, M. Identification of Novel Mobilized Colistin Resistance Gene *mcr*-9 in a Multidrug-Resistant, Colistin-Susceptible *Salmonella enterica* Serotype Typhimurium Isolate. *mBio* **2019**, *10*. [[CrossRef](#)] [[PubMed](#)]
14. Yao, X.; Doi, Y.; Zeng, L.; Lv, L.; Liu, J.H. Carbapenem-resistant and colistin-resistant *Escherichia coli* co-producing NDM-9 and MCR-1. *Lancet Infect. Dis.* **2016**, *16*, 288–289. [[CrossRef](#)]

15. MacNair, C.R.; Stokes, J.M.; Carfrae, L.A.; Fiebig-Comyn, A.A.; Coombes, B.K.; Mulvey, M.R.; Brown, E.D. Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. *Nat. Commun.* **2018**, *9*, 458. [CrossRef] [PubMed]
16. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Ann. Intern. Med.* **2009**, *151*, 264–269. [CrossRef]
17. Centers for Disease Control and Prevention (CDC); Division of Bacterial and Mycotic Diseases (DBMD). “*Escherichia coli*”. Available online: www.cdc.gov/ecoli/ (accessed on 15 March 2019).
18. Ryan, K.J.; Ray, C.G.; Ahmad, N.; Drew, W.L.; Lagunoff, M.; Pottinger, P.; Reller, L.B.; Sterling, C.R. “*Pathogenesis of Bacterial Infections*”. *Sherris Medical Microbiology*, 6th ed.; McGraw Hill: New York, NY, USA, 2014; pp. 391–406.
19. Higgins, J.; Green, S. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]*; The Cochrane Collaboration: London, UK, 2011; Available online: www.handbook.cochrane.org (accessed on 22 July 2018).
20. Payne, M.; Croxen, M.A.; Lee, T.D.; Mayson, B.; Champagne, S.; Leung, V.; Bariso, S.; Hoang, L.; Lowe, C. mcr-1-Positive Colistin-Resistant *Escherichia coli* in Traveler Returning to Canada from China. *Emerg. Infect. Dis.* **2016**, *22*, 1673–1675. [CrossRef]
21. Macesic, N.; Green, D.; Wang, Z.; Sullivan, S.B.; Shim, K.; Park, S.; Whittier, S.; Furuya, E.Y.; Gomez-Simmonds, A.; Uhlemann, A.C. Detection of mcr-1-Carrying *Escherichia coli* Causing Bloodstream Infection in a New York City Hospital: Avian Origins, Human Concerns? *Open Forum Infect. Dis.* **2017**, *4*, ofx115. [CrossRef]
22. Tada, T.; Uechi, K.; Nakasone, I.; Shimada, K.; Nakamatsu, M.; Kirikae, T.; Fujita, J. Emergence of a colistin-resistant *Escherichia coli* clinical isolate harboring mcr-1 in Japan. *Int. J. Infect. Dis.* **2017**, *63*, 21–22. [CrossRef]
23. Nishino, Y.; Shimojima, Y.; Suzuki, Y.; Ida, M.; Fukui, R.; Kuroda, S.; Hirai, A.; Sadamasu, K. Detection of the mcr-1 gene in colistin resistant *Escherichia coli* from retail meat in Japan. *Microbiol. Immunol.* **2017**, *61*, 554–557. [CrossRef]
24. Grami, R.; Mansour, W.; Mehri, W.; Bouallegue, O.; Boujaafar, N.; Madec, J.Y.; Haenni, M. Impact of food animal trade on the spread of mcr-1-mediated colistin resistance, Tunisia, July 2015. *Eurosurveillance* **2016**, *21*, 30144. [CrossRef] [PubMed]
25. Rapoport, M.; Faccone, D.; Pasteran, F.; Ceriana, P.; Albornoz, E.; Petroni, A.; Corso, A. First Description of mcr-1-Mediated Colistin Resistance in Human Infections Caused by *Escherichia coli* in Latin America. *Antimicrob. Agents Chemother.* **2016**, *60*, 4412–4413. [CrossRef] [PubMed]
26. Nang, S.C.; Li, J.; Velkov, T. The rise and spread of mcr plasmid-mediated polymyxin resistance. *Crit. Rev. Microbiol.* **2019**, *45*, 131–161. [CrossRef] [PubMed]
27. Eichhorn, I.; Feudi, C.; Wang, Y.; Kaspar, H.; Feßler, A.T.; Lübke-Becker, A.; Michael, G.B.; Shen, J.; Schwarz, S. Identification of novel variants of the colistin resistance gene mcr-3 in *Aeromonas* spp. from the national resistance monitoring programme GERM-Vet and from diagnostic submissions. *J. Antimicrob. Chemother.* **2018**, *73*, 1217–1221. [CrossRef] [PubMed]
28. Carattoli, A.; Villa, L.; Feudi, C.; Curcio, L.; Orsini, S.; Luppi, A.; Pezzotti, G.; Magistrali, C.F. Novel plasmid-mediated colistin resistance mcr-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Eurosurveillance* **2017**, *22*, 30589. [CrossRef] [PubMed]
29. Yang, Y.-Q.; Li, Y.-X.; Lei, C.-W.; Zhang, A.-Y.; Wang, H.-N. Novel plasmid-mediated colistin resistance gene mcr-7.1 in *Klebsiella pneumoniae*. *J. Antimicrob. Chemother.* **2018**, *73*, 1791–1795. [CrossRef] [PubMed]
30. Xavier, B.B.; Lammens, C.; Ruhai, R.; Kumar-Singh, S.; Butaye, P.; Goossens, H.; Malhotra-Kumar, S. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. *Eur. Commun. Dis. Bull.* **2016**, *21*. [CrossRef]
31. Borowiak, M.; Fischer, J.; Hammerl, J.A.; Hendriksen, R.S.; Szabo, I.; Malorny, B. 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.* **2017**, *72*, 3317–3324. [CrossRef]
32. Carattoli, A.; Carretto, E.; Brovarone, F.; Sarti, M.; Villa, L. Comparative analysis of an mcr-4 *Salmonella enterica* subsp. *enterica* monophasic variant of human and animal origin. *J. Antimicrob. Chemother.* **2018**, *73*, 3332–3335. [CrossRef]

33. Hernandez, M.; Iglesias, M.R.; Rodriguez-Lazaro, D.; Gallardo, A.; Quijada, N.; Miguela-Villoldo, P.; Campos, M.J.; Piriz, S.; Lopez-Orozco, G.; de Frutos, C.; et al. Co-occurrence of colistin-resistance genes *mcr-1* and *mcr-3* among multidrug-resistant *Escherichia coli* isolated from cattle, Spain, September 2015. *Eur. Commun. Dis. Bull.* **2017**, *22*. [[CrossRef](#)]
34. Litrup, E.; Kiil, K.; Hammerum, A.M.; Roer, L.; Nielsen, E.M.; Torpdahl, M. Plasmid-borne colistin resistance gene *mcr-3* in *Salmonella* isolates from human infections, Denmark, 2009–17. *Eurosurveillance* **2017**, *22*, 30587. [[CrossRef](#)] [[PubMed](#)]
35. Garcia-Graells, C.; De Keersmaecker, S.C.J.; Vanneste, K.; Pochet, B.; Vermeersch, K.; Roosens, N.; Dierick, K.; Botteldoorn, N. Detection of Plasmid-Mediated Colistin Resistance, *mcr-1* and *mcr-2* Genes, in *Salmonella* spp. Isolated from Food at Retail in Belgium from 2012 to 2015. *Foodborne Pathog. Dis.* **2017**, *15*, 114–117. [[CrossRef](#)] [[PubMed](#)]
36. Kieffer, N.; Nordmann, P.; Poirel, L. Moraxella Species as Potential Sources of MCR-Like Polymyxin Resistance Determinants. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)] [[PubMed](#)]
37. 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](#)] [[PubMed](#)]
38. Biswas, S.; Li, Y.; Elbediwi, M.; Yue, M. Emergence and Dissemination of *mcr*-Carrying Clinically Relevant *Salmonella* Typhimurium Monophasic Clone ST34. *Microorganisms* **2019**, *7*, 298. [[CrossRef](#)] [[PubMed](#)]
39. Poirel, L.; Kieffer, N.; Fernandez-Garayzabal, J.F.; Vela, A.I.; Larpin, Y.; Nordmann, P. MCR-2-mediated plasmid-borne polymyxin resistance most likely originates from *Moraxella pluranimalium*. *J. Antimicrob. Chemother.* **2017**, *72*, 2947–2949. [[CrossRef](#)] [[PubMed](#)]
40. Liu, B.T.; Song, F.J.; Zou, M.; Zhang, Q.D.; Shan, H. High Incidence of *Escherichia coli* Strains Coharboring *mcr-1* and *bla*NDM from Chickens. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)]
41. Kong, L.H.; Lei, C.W.; Ma, S.Z.; Jiang, W.; Liu, B.H.; Wang, Y.X.; Guan, R.; Men, S.; Yuan, Q.W.; Cheng, G.Y.; et al. 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.* **2017**, *61*. [[CrossRef](#)]
42. Jiang, Z.; Paudyal, N.; Xu, Y.; Deng, T.; Li, F.; Pan, H.; Peng, X.; He, Q.; Yue, M. Antibiotic Resistance Profiles of *Salmonella* Recovered From Finishing Pigs and Slaughter Facilities in Henan, China. *Front. Microbiol.* **2019**, *10*. [[CrossRef](#)]
43. Wang, X.; Biswas, S.; Paudyal, N.; Pan, H.; Li, X.; Fang, W.; Yue, M. Antibiotic Resistance in *Salmonella* Typhimurium Isolates Recovered From the Food Chain Through National Antimicrobial Resistance Monitoring System Between 1996 and 2016. *Front. Microbiol.* **2019**, *10*. [[CrossRef](#)]
44. Pan, H.; Paudyal, N.; Li, X.; Fang, W.; Yue, M. Multiple Food-Animal-Borne Route in Transmission of Antibiotic-Resistant *Salmonella* Newport to Humans. *Front. Microbiol.* **2018**, *9*, 23. [[CrossRef](#)] [[PubMed](#)]
45. Caltagirone, M.; Nucleo, E.; Spalla, M.; Zara, F.; Novazzi, F.; Marchetti, V.M.; Piazza, A.; Bitar, I.; De Cicco, M.; Paolucci, S.; et al. Occurrence of Extended Spectrum β -Lactamases, KPC-Type, and MCR-1.2-Producing Enterobacteriaceae from Wells, River Water, and Wastewater Treatment Plants in Oltrepò Pavese Area, Northern Italy. *Front. Microbiol.* **2017**, *8*. [[CrossRef](#)] [[PubMed](#)]
46. Fernandes, M.R.; Sellera, F.P.; Esposito, F.; Sabino, C.P.; Cerdeira, L.; Lincopan, N. Colistin-Resistant *mcr-1*-Positive *Escherichia coli* on Public Beaches, an Infectious Threat Emerging in Recreational Waters. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)] [[PubMed](#)]
47. Hartl, R.; Kerschner, H.; Lepuschitz, S.; Ruppitsch, W.; Allerberger, F.; Apfalter, P. Detection of the *mcr-1* Gene in a Multidrug-Resistant *Escherichia coli* Isolate from an Austrian Patient. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)]
48. Shen, Y.; Zhou, H.; Xu, J.; Wang, Y.; Zhang, Q.; Walsh, T.R.; Shao, B.; Wu, C.; Hu, Y.; Yang, L.; et al. Anthropogenic and environmental factors associated with high incidence of *mcr-1* carriage in humans across China. *Nat. Microbiol.* **2018**, *3*, 1054–1062. [[CrossRef](#)]
49. Paudyal, N.; Yue, M. Antimicrobial Resistance in the “Dark Matter”. *Clin. Infect. Dis.* **2019**, *69*, 379–380. [[CrossRef](#)]
50. Pan, H.; Zhou, X.; Chai, W.; Paudyal, N.; Li, S.; Zhou, X.; Zhou, K.; Wu, Q.; Wu, B.; Li, G.; et al. Diversified sources for human infections by *Salmonella enterica* serovar newport. *Transbound. Emerg. Dis.* **2019**, *66*, 1044–1048. [[CrossRef](#)]

51. Pan, H.; Zhou, X.; Chai, W.; Paudyal, N.; Li, S.; Zhou, X.; Zhou, K.; Wu, Q.; Wu, B.; Li, G.; et al. Cover Image. *Transbound. Emerg. Dis.* **2019**, *66*. [[CrossRef](#)]
52. Ellem, J.A.; Ginn, A.N.; Chen, S.C.; Ferguson, J.; Partridge, S.R.; Iredell, J.R. Locally Acquired mcr-1 in *Escherichia coli*, Australia, 2011 and 2013. *Emerg. Infect. Dis.* **2017**, *23*, 1160–1163. [[CrossRef](#)]
53. Wang, Y.; Tian, G.B.; Zhang, R.; Shen, Y.; Tyrrell, J.M.; Huang, X.; Zhou, H.; Lei, L.; Li, H.Y.; Doi, Y.; et al. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: An epidemiological and clinical study. *Lancet Infect. Dis.* **2017**, *17*, 390–399. [[CrossRef](#)]
54. Tijet, N.; Faccone, D.; Rapoport, M.; Seah, C.; Pasteran, F.; Ceriana, P.; Alborno, E.; Corso, A.; Petroni, A.; Melano, R.G. Molecular characteristics of mcr-1-carrying plasmids and new mcr-1 variant recovered from polyclonal clinical *Escherichia coli* from Argentina and Canada. *PLoS ONE* **2017**, *12*, e0180347. [[CrossRef](#)] [[PubMed](#)]
55. Lv, L.; Partridge, S.R.; He, L.; Zeng, Z.; He, D.; Ye, J.; Liu, J.-H. Genetic Characterization of IncI2 Plasmids Carrying blaCTX-M-55 Spreading in both Pets and Food Animals in China. *Antimicrob. Agents Chemother.* **2013**, *57*, 2824–2827. [[CrossRef](#)] [[PubMed](#)]
56. Tato, M.; Coque, T.M.; Baquero, F.; Canton, R. Dispersal of carbapenemase blaVIM-1 gene associated with different Tn402 variants, mercury transposons, and conjugative plasmids in Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **2010**, *54*, 320–327. [[CrossRef](#)] [[PubMed](#)]
57. Johnson, T.J.; Wannemuehler, Y.M.; Scaccianoce, J.A.; Johnson, S.J.; Nolan, L.K. Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring among extraintestinal pathogenic *Escherichia coli* isolates. *Antimicrob. Agents Chemother.* **2006**, *50*, 3929–3933. [[CrossRef](#)]
58. Poirel, L.; Kieffer, N.; Nordmann, P. In Vitro Study of ISApI1-Mediated Mobilization of the Colistin Resistance Gene mcr-1. *Antimicrob. Agents Chemother.* **2017**, *61*. [[CrossRef](#)]
59. Snesrud, E.; He, S.; Chandler, M.; Dekker, J.P.; Hickman, A.B.; McGann, P.; Dyda, F. A Model for Transposition of the Colistin Resistance Gene mcr-1 by ISApI1. *Antimicrob. Agents Chemother.* **2016**, *60*, 6973–6976. [[CrossRef](#)]
60. Snesrud, E.; McGann, P.; Chandler, M. The Birth and Demise of the ISApI1-mcr-1-ISApI1 Composite Transposon: The Vehicle for Transferable Colistin Resistance. *mBio* **2018**, *9*. [[CrossRef](#)]
61. Zurfluh, K.; Nüesch-Inderbinen, M.; Klumpp, J.; Poirel, L.; Nordmann, P.; Stephan, R. Key features of mcr-1-bearing plasmids from *Escherichia coli* isolated from humans and food. *Antimicrob. Resist. Infect. Control* **2017**, *6*, 91. [[CrossRef](#)]
62. Aibinu, I.; Odugbemi, T.; Koenig, W.; Ghebremedhin, B. Sequence Type ST131 and ST10 Complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. *Clin. Microbiol. Infect.* **2012**, *18*, E49–E51. [[CrossRef](#)]
63. Oteo, J.; Diestra, K.; Juan, C.; Bautista, V.; Novais, Â.; Pérez-Vázquez, M.; Moyá, B.; Miró, E.; Coque, T.M.; Oliver, A.; et al. Extended-spectrum β -lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2. *Int. J. Antimicrob. Agents* **2009**, *34*, 173–176. [[CrossRef](#)]
64. Guillouzouic, A.; Caroff, N.; Dauvergne, S.; Lepelletier, D.; Perrin Guyomard, A.; Kempf, I.; Reynaud, A.; Corvec, S. MLST typing of *Escherichia coli* isolates overproducing AmpC beta-lactamase. *J. Antimicrob. Chemother.* **2009**, *63*, 1290–1292. [[CrossRef](#)] [[PubMed](#)]
65. Chakraborty, A.; Saralaya, V.; Adhikari, P.; Shenoy, S.; Baliga, S.; Hegde, A. Characterization of *Escherichia coli* Phylogenetic Groups Associated with Extraintestinal Infections in South Indian Population. *Ann. Med Health Sci. Res.* **2015**, *5*, 241–246. [[CrossRef](#)] [[PubMed](#)]
66. Nunez, B.; Avila, P.; de la Cruz, F. Genes involved in conjugative DNA processing of plasmid R6K. *Mol. Microbiol.* **1997**, *24*, 1157–1168. [[CrossRef](#)] [[PubMed](#)]
67. Nair, M.K.M.; De Masi, L.; Yue, M.; Galván, E.M.; Chen, H.; Wang, F.; Schifferli, D.M. Adhesive Properties of YapV and Paralogous Autotransporter Proteins of *Yersinia pestis*. *Infect. Immun.* **2015**, *83*, 1809. [[CrossRef](#)] [[PubMed](#)]
68. Johnson, T.J.; Bielak, E.M.; Fortini, D.; Hansen, L.H.; Hasman, H.; Debroy, C.; Nolan, L.K.; Carattoli, A. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. *Plasmid* **2012**, *68*, 43–50. [[CrossRef](#)] [[PubMed](#)]

69. Hayden, H.S.; Matamouros, S.; Hager, K.R.; Brittnacher, M.J.; Rohmer, L.; Radey, M.C.; Weiss, E.J.; Kim, K.B.; Jacobs, M.A.; Sims-Day, E.H.; et al. Genomic Analysis of *Salmonella enterica* Serovar Typhimurium Characterizes Strain Diversity for Recent, U.S. Salmonellosis Cases and Identifies Mutations Linked to Loss of Fitness under Nitrosative and Oxidative Stress. *mBio* **2016**, *7*, e00154-16. [[CrossRef](#)]
70. Yue, M.; Han, X.; Masi, L.D.; Zhu, C.; Ma, X.; Zhang, J.; Wu, R.; Schmieder, R.; Kaushik, R.S.; Fraser, G.P.; et al. Allelic variation contributes to bacterial host specificity. *Nat. Commun.* **2015**, *6*, 8754. [[CrossRef](#)]
71. Fernandes, M.R.; Moura, Q.; Sartori, L.; Silva, K.C.; Cunha, M.P.; Esposito, F.; Lopes, R.; Otutumi, L.K.; Goncalves, D.D.; Dropa, M.; et al. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the mcr-1 gene. *Eur. Commun. Dis. Bull.* **2016**, *21*. [[CrossRef](#)]
72. Pham Thanh, D.; Thanh Tuyen, H.; Nguyen Thi Nguyen, T.; Chung The, H.; Wick, R.R.; Thwaites, G.E.; Baker, S.; Holt, K.E. Inducible colistin resistance via a disrupted plasmid-borne mcr-1 gene in a 2008 Vietnamese *Shigella sonnei* isolate. *J. Antimicrob. Chemother.* **2016**, *71*, 2314–2317. [[CrossRef](#)]
73. Liassine, N.; Assouvie, L.; Descombes, M.-C.; Tendon, V.D.; Kieffer, N.; Poirel, L.; Nordmann, P. Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract Enterobacteriaceae in Switzerland. *Int. J. Infect. Dis.* **2016**, *51*, 4–5. [[CrossRef](#)]
74. Quan, J.; Li, X.; Chen, Y.; Jiang, Y.; Zhou, Z.; Zhang, H.; Sun, L.; Ruan, Z.; Feng, Y.; Akova, M.; et al. Prevalence of mcr-1 in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: A multicentre longitudinal study. *Lancet Infect. Dis.* **2017**, *17*, 400–410. [[CrossRef](#)]
75. Di Pilato, V.; Arena, F.; Tascini, C.; Cannatelli, A.; Henrici De Angelis, L.; Fortunato, S.; Giani, T.; Menichetti, F.; Rossolini, G.M. mcr-1.2, a New mcr Variant Carried on a Transferable Plasmid from a Colistin-Resistant KPC Carbapenemase-Producing *Klebsiella pneumoniae* Strain of Sequence Type 512. *Antimicrob. Agents Chemother.* **2016**, *60*, 5612–5615. [[CrossRef](#)] [[PubMed](#)]
76. Tao, M.-T.; Yang, R.-S.; Liao, X.-P.; Liu, Y.-H.; Zhou, Y.-F.; Sun, J.; Feng, Y. Increased activity of colistin in combination with amikacin against *Escherichia coli* co-producing NDM-5 and MCR-1. *J. Antimicrob. Chemother.* **2017**, *72*, 1723–1730. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).