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Focused Commentary On The Colistin Resistant Enterobacteriaceae

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ABSTRACT

The continuing emergence of drug-resistant bacteria could lead human mortality due to a common infection and could result in a post-antibiotic era. The incidence of colistin-resistant Enterobacteriaceae (CoRE) has been increasing annually since extended-spectrum beta-lactamases (ESBLs) were detected in 1983 and carbapenemase-resistant Enterobacteriaceae (CRE) were reported in 1999. CoRE incidence has been increasing because of selective pressure, since colistin is used as a reserve drug for CRE. Pandrug resistance is generally observed for CoRE, which possess the ESBL or carbapenem resistance genes in the chromosome or plasmid. The Clinical and Laboratory Standards Institute criteria or breakpoint need to be established and colistin usage should be restricted. The mechanisms of colistin resistance are known to involve *PhoPQ* and *PmrAB*; however, another gene,*mgrB*, is known to be more important and was recently found to play a major role. Transferable plasmid-mediated colistin resistance was detected by Liu in 2016 in China. CoRE is postulated to be transferred to humans from animals by selective pressure of colistin, which is added to animal feed; because, the strains isolated from humans and animals had the same sequence type. Global spread is suggested to occur via the transfer of animal products or by travelers. A strategy to screen for the transfer of colistin-resistant strains and carbapenem-resistant strains is important. In addition, commercial media or kits should be developed to rapidly detect the *mcr-1* gene.

Keywords: Enterobacteriaceae, drug-resistant bacteria, colistin resistance.

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INTRODUCTION

The antimicrobial resistance mechanisms of bacteria have evolved through several decades, and antimicrobial-resistant strains have continuously been replaced with more resistant and more toxic strains (1). The medical community experiences a great shock whenever a new antibiotic-resistant strain emerges. We have been fighting against multidrug-resistant bacteria for several decades, although the prevalence rate differs between nations and locations, and there are endemic areas with specific strains. Recently, Gram-positive and -negative superbacteria have been identified that are panresistant to all antibiotic drugs (2). The panresistant Gram-negative strains emerged after 2010 and have rapidly increased in recent years. This scenario hints toward the emergence of a 'post-antibiotic era', in which even common infections such as the common cold or simple skin infections eventually lead to human death.

ESBL and CRE

In 1983, extended-spectrum beta-lactamase (ESBL) was first identified, which is resistant to all extended-spectrum cephalosporins (3), and this was shocking news. The prevalence of ESBL has been increasing rapidly since its discovery. Now, most Gram-negative pathogens are ESBL strains, showing a prevalence of 93.54% in India, an ESBL endemic country, in 2018 (4).

In the 1990's, carbapenem-resistant Enterobacteriaceae (CRE) first appeared, which is resistant to all carbapenems. CRE harbor transferable carbapenemases such as KPC, NDM, or OXA-48, and most CRE are *Klebsiella pneumoniae* species (5, 6). CRE has been the most important issue in the clinical microbiology field in the last decade, with multidrug-resistant characteristics of CRE and epidemic KPC-producing *K. pneumoniae* strains disseminated globally with sequence type (ST) 258 (7). The CRE strains have spread rapidly and CRE prevalence rates of more than 20–30% are reported in most countries (8). The CRE prevalence rate was 89% in China, a CRE endemic country, in 2018 (9).

The epidemiology of Colistin-Resistant Enterobacteriaceae

The types of polymyxin used clinically include polymyxin B and polymyxin E, also known as colistin. Colistin was initially found in the 1940's, but was soon replaced with aminoglycosides because of adverse side effects like nephrotoxicity or neurotoxicity. After the 1990's, colistin has returned as a last-resort drug for treating CRE (10).

At last, colistin resistance emerged in 2010 in Italy, and outbreaks of colistin-resistant KPC-producing *K. pneumoniae* are continuously reported. In 2013 in South Africa, oral colistin use for gut decontamination of OXA-48 CRE led to the occurrence of colistin-resistant *K. pneumoniae* (CoRKP) (11).

Colistin-resistant Enterobacteriaceae have been rapidly increasing due to selective pressure. The emergence of colistin resistance is clinically very important because colistin is the last-line drug to treat CRE, which is generally panresistant(12).

Italy, China, and Greece, which are CRE endemic countries, documented a sharp increase in colistin resistance in recent years (13).

The incidence of CoRKP increased 3-fold from 2010 to 2014 due to selective pressure in Italy (14). Another Italian report showed that CoRKP accounted for 49.7% of CoRKP bloodstream infections from 2012 to 2013 (15).

It is crucial to note that the colistin-resistant strain was isolated very soon after the emergence of the CRE strain. The incidence of colistin-resistant Enterobacteriaceae (CoRE) is increasing more rapidly than that of CRE.

More importantly, CoRE, which are capable of directly colonizing the human gut, exhibit acquired resistance immediately, instead of colistin-susceptible strains' acquisition of a resistance gene by selective pressure, which takes a long time.

As seen in a report from Spain that showed colistin resistance without previous history of colistin usage, colistin resistance can occur from direct colonization without selective pressure by previous colistin usage (16). An Italian report also showed that most CoRKP bloodstream infections occur without a previous history of colistin usage (15) Therefore, colistin resistance should be tested in specimens from colistin-treated patients and in patients without a previous history of colistin usage.

Fortunately, the prevalence of CoRE isolates is still low; for example, authors found a 0.7% CoRE prevalence rate out of all clinical isolates of Enterobacteriaceae (17). As a preventive measure, restricted colistin use, upgraded screening surveillance programs, and the potentiation of antibiotic stewardship are required. If a CoRE strain is isolated in the healthcare setting, isolation precautions should be taken and strict infection control should be maintained (18). Because the microbiological definition of colistin resistance is not yet determined and the CLSI criteria and breakpoint are not yet established, guidelines should be set through conducting studies (19).

The known Colistin Resistance mechanism of CoRE

Colistin, which is a cationic cyclic lipodecapeptide, acts by replacing a divalent cation from the phosphate group of lipopolysaccharides in the cell wall, thereby changing cell wall permeability (20).

The chromosomal PmrA/PmrB and PhoP/PhoQ component system promotes colistin resistance, modifying lipid A by adding an L-Ara4N moiety to lipid A, which confers a negative charge to lipopolysaccharides. MALDI-TOF analysis of outer membrane proteins in a colistin-susceptible strain and a colistin-resistant strain reveals a prominent difference in protein peaks, supporting the above-mentioned colistin resistance mechanism (21).

According to the EUCAST guideline, the breakpoints of colistin resistance are documented as >2 mg/L for Enterobacteriaceae, Pseudomonas, and Acinetobacter species (22). According to the CLSI guideline, the breakpoint of colistin resistance is >2 mg/L in Pseudomonas or Acinetobacter and no breakpoint has been set for Enterobacteriaceae (23). The CLSI and EUCAST joint recommendation suggested the ISO 20776 standard broth microdilution method (22). The British Society for Antimicrobial Chemotherapy (BSAC) recommends 4 mg/L as a colistin resistance breakpoint of Enterobacteriaceae (22).

MgrB mutation

In a recent study, the newly discovered *mgrB* (PhoPQ negative regulator) mutation is highlighted as the more dominant colistin-resistance mechanism than *PmrA/PmrB* or *PhoP/PhoQ* mutations in CoRKP (24).

This mutation, especially an insertion of *mgrB* that upregulates PhoPQ level, results in a high level of colistin resistance (25). It is now believed that the *mgrB* alteration is a more common colistin resistance mechanism in the CoRKP species than other chromosomal mutations, and many molecular studies with whole genome sequencing (WGS) provided evidence of this.

Population analysis of CoRKP showed a heteroresistant profile and the colistin-resistant subpopulation showed the presence of the *mgrB* mutation (26). To investigate the molecular structure of 13 multidrug resistant CoRKP, whole genome sequencing (WGS) was performed and revealed *mgrB* mutation in all 13 isolates, suggesting *mgrB* mutation is the major cause of colistin resistance in CoRKP (27). In other reports, WGS of KPC-producing CoRKP also revealed the *mgrB* mutation (28).

In *mgrB*-mutated strains, the wild type *mgrB* gene restored colistin susceptibility in complementation experiments (29), suggesting that *mgrB* mutations cause colistin resistance. Moreover, in one experiment, when colistin was added to the wild type *mgrB* isolates *in vivo*, *mgrB* alterations emerged. Therefore, it is thought that the resistance mechanism that occurs upon selective pressure in most CoRKP isolates is the *mgrB* mutation (30). Therefore, it is thought that the resistance mechanism of CoRKP with a previous history of colistin usage is *mgrB* mutation.

mgrB mutation is thought to occur irrespective of sequence type because various sequence types have been revealed. If an isolate has ST258, the global carbapenemase sequence type, colistin resistance could

disseminate worldwide, and many ST258 CoRKP outbreaks are currently documented globally (**Table 1**).

Table 1. The characteristics of strains harbouring mgrB alteration.

Species	N	mgrB	Clonality	MIC	ST	AD	mgrB mu	Country	Ref
K. pneu	47	12	unrelated	8-24	ST258	KPC-2 KPC-3 CTX-M-2 CTX-M-15	IS5-like ISKpn13 ISKpn14 IS10R	France	66
K. pneu	20	15	unrelated	32-128	ND	ND	IS5-like IS1-like	Iran	67
K. pneu	5	5	related	8-24	ST258	KPC2	IS5	Hungary	68
K. pneu	25	23	related	8-32	ND	NDM PhoQ	early stop codon	UK	69
K. pneu	29	22	related	>8	ST512 (CC258)	KPC PhoP PmrA PmrB	ISL3 IS5 deletion	Italy	70
K. pneu	66	35	related	8-32	ST258 ST512	KPC-1 KPC-3 SXT, TGC GEN etc	IS5-like IS1F-like ISKpn14 deletion point mut	Italy	71

Abbreviations; N; number, MIC; minimal inhibitory concentration, ST; sequence type, AD; antimicrobial determinant, mgrB mu; mgrB mutation type, Ref; reference, ND; not determined

Clonal dissemination

CoRE can be disseminated by both clonal spread and non-clonal spread. Many clonal outbreaks of *mgrB*-mutated *K. pneumonia* have been reported. A well-known example of clonal spread is the global dissemination of *K. pneumonia* ST258 strains. In one report, most CoRKP isolates harbored mgrB mutations, and all the isolates with an mgrB mutation showed ST258, a global CRE sequence type. From a clinical microbiological point of view, it is very important that all CoRKP with *mgrB* mutations showed a global sequence type.

The ST258 CoRKP epidemic clone has been disseminating worldwide in the past 2–3 years and has been reported in dozens of countries (31). However, various sequence types among mgrB-altered *K. pneumoniae* isolates are also reported, suggesting that they are clonally unrelated. This means that the mechanism is due to selective pressure. Non-clonal spread occurs mostly by mobile genetic elements like plasmids, transposons, and other mechanisms. An example of nonclonal dissemination is *mcr-1*-harboring *E. coli* strains (described below).

Antimicrobial determinants

mgrB-altered *K. pneumonia* strains usually contain several additional antimicrobial determinants, showing multidrug or pandrug resistance. In one report, WGS revealed 11 additional antimicrobial determinants in addition to *mgrB* in CoRKP(28). In many reports, the important antimicrobial determinants of *mgrB*-mutated CoRKP are KPC, CTX-M-15, OXA-48, or NDM-1 (27). Fluoroquinolone resistance genes or aminoglycoside resistance genes are also described, generally providing the CoRKP strain with panresistance.

Mcr-1

Surprisingly, the mobilized colistin resistance-1 (*mcr-1*) gene, which is mediated by a transferable plasmid, was discovered in colistin-resistant *E. coli* strains in China by Liu and colleagues in 2016 (32, 33). Phylogeneticaly, the *mcr-1* gene is closely related to that of the Neisseria spp. (34), inferring that the *mcr-1* gene originated from Neisseria spp. Traditionally, colistin resistance has been known to occur by chromosomal mutation, and the discovery of a plasmid-mediated resistance mechanism is shocking because colistin is the last-line antimicrobial against MDR strains. In one experiment, an *E. coli* isolate with a MIC of 0.25 µg/ml exhibited an elevated MIC of 4 µg/ml when conjugated with the *mcr-1* gene (29). Moreover, the *mcr-1* gene was successfully transferred to another strain in a conjugation experiment (35).

mcr-1 is a phosphoethanolamine transferase that modifies lipopolysaccharides by adding phosphoethanolamine to lipid A, which confers colistin resistance.

Next-generation sequencing analysis of plasmids from colistin-resistant *E. coli* strains revealed the presence of the *mcr-1* gene in the plasmids. In addition, the presence of an ISApl-1 mobile element near the *mcr-1* gene was confirmed (36).

In many studies, a sharply increasing rate of *mcr-1* detection is observed, as we have seen the spread of the *mcr-1* gene to more than 30 countries in five continents with significant infections such as blood stream infections in less than one year (37).

The *mcr-1*-containing strains are found in various sequence types, various plasmids, and they have various antimicrobial resistance determinants as shown by WGS analyses (38). No clonal relationship is observed. These findings highlight the plasmid-mediated horizontal transfer mechanism of *mcr-1*, with the possibility of continued evolution and dynamic transmission (39). (**Table 2**).

Table 2. The characteristics of strains harbouring mcr-1 gene.

Species	N	mcr-1	clonality	MIC	ST	AD	Plasmid	ISApl1	Country	Ref
E. coli	20	20	unrelated	3-8	various	PhoQ PmrB	IncHI2 IncI2 IncP	19	global	52
E. coli	1	1	ND	4	ST457	StrA blaCTX-M-55 aac(3)-IVa sul2	IncF IncN	0	USA	72
E. coli	1	1	ND	2-4	ND	ND	IncI2	1	S. Africa	73
E. coli	1	1	ND	2-4	ND	ND	IncI2	1	China	73
E. coli	1	1	ND	2-4	ND	ND	IncFII	1	Belgium	73

Abbreviations; N; number, MIC; minimal inhibitory concentration, ST; sequence type, AD; antimicrobial determinant, Ref; reference, ND; not determined

Even though *mcr-1* was discovered in 2016 in China, *mcr-1* is thought to have been present undetected in animals and in the environment for several decades, and was first detected in 2016. Shen documented the *mcr-1* gene in chickens in the 1980's, and the *mcr-1* gene was detected in a female calf in Europe in 2005 and in humans in Vietnam in 2008. The *mcr-1* gene was documented in the NCBI databasein the 1980's, suggesting the selection of *mcr-1* gene by colistin usage, which has long been colonized in the human gut (40).

The surveillance of MCR-1

The highly transmissible *mcr-1* strains spread globally, similar to the CRE strains; therefore, surveillance screening should be performed. Many efforts have been made to strengthen colistin resistance surveillance since the mcr-1 was discovered in 2016; for example, the US decided to perform the colistin E-test on every ESBL isolate, resulting in the detection of *mcr-1* in one *E. coli* isolate in Pennsylvania (41).

Fortunately, the *mcr-1*-containing strains, mostly *E. coli* strains, are described to colonize the human gut at a prevalence rate of less than 1% (42), suggesting that the prevalence rate is still low. However, 21% of ESBL *E. coli* was described as an *mcr-1* strain in France (43), supporting the urgent need for a surveillance study and the underestimated *mcr-1* prevalence rate.

A number of antibiotic resistance databases have been developed such as ARDB, CARD, ResFinder, CBMAR, and others based on bioinformatics tools (44). In the Netherlands, the *mcr-1* gene was added to the antibiotic surveillance database of ResFinder.

The MIC of MCR-1

Interestingly, in a Chinese report, the colistin-resistant *E. coli* strains were all *mcr-1*-positive, yet only some of the *K. pneumoniae* strains were *mcr-1*-positive (45). These findings suggest the main resistance mechanism of *E. coli* is plasmid-mediated *mcr-1*, and that of *K. pneumoniae* is chromosomal *mgrB* mutation. The mean MIC_{90} of colistin-resistant *E. coli* was found to be 8 mg/L, and that of *K. pneumonia* was 128 mg/L.

These findings suggest that the mcr-1 mechanism confers low-level resistance and *mgrB* mutation provides high-level resistance (46). Many review articles say that the strains with *mgrB* mutations have higher MIC values compared with the strains with *mcr-1* (47). Moreover, because *mcr-1*-positive strains exhibit low resistance, we can observe colistin-susceptible *mcr-1*-positive strains with MIC values ≤2 mg/L that are capable of silent dissemination. We could detect more strains if the breakpoint was reduced to ≥1 mg/L from ≥2mg/L.

Mobile elements and plasmids in mcr-1 strains

Several WGS analyses to elucidate genomic structure of mcr-1 strains revealed the presence of an ISApl-1 insertion sequence near the mcr-1 gene (48). For example, 22 out of 77 NCBI sequences of *mcr-1*-carrying *E. coli* strains revealed the ISApl-1 association (49). The current hypothesis is that ISApl-1 acts to transfer the *mcr-1* gene (49, 50).

The *mcr-1*gene is known to spread mainly to *E. coli* species, and additionally to *K. pneumoniae* or other Enterobacteriaceae species. The *mcr-1* variants from *mcr-2* to *mcr-6* have also been identified in *E. coli* species or other Enterobacteriaceae species (51). However, we do not know why *mcr-1*-containing strains are mostly *E. coli* strains. The genomic structure of *E. coli* species might be acceptable for ISApl-1 mobilization.

The reports about *E. coli* and *K. pneumonia* species harboring the *mcr-1* gene show that all *E. coli* species harboring the *mcr-1* gene are associated with the ISAPl-1 element, however no *Klebsiella* species harboring the *mcr-1* gene were associated with the ISAPl-1 element (52).

Regarding *mcr-1*-carrying plasmids, more than eight kinds of plasmids are reported, such as pHNSHP45 in China and Taiwan (9); a similar one in Japan; IncX4 in China and Brazil; IncX4, IncI2, IncHI2, and IncK2 in Switzerland; IncF1 in China; and IncP in Belgium (53).

Several *mcr-1*-carrying plasmids can be found in one strain (54). The fact that various plasmids are found to contain the *mcr-1* gene suggests the horizontal transfer characteristic of the *mcr-1* gene.

Since the IncX4 plasmid found in China and Brazil has been described on different continents, in different species, and in different sequence types, this suggests a highly transmissible plasmid characteristic, resulting in intercontinental spread (55). IncI2 and IncHI2 exhibit high transference.

The mechanisms of CoRE dissemination

Increasing colistin resistance is certainly understandable in countries that use colistin heavily, due to selective pressure, but resistance has also developed in countries where colistin is not heavily used. The widely disseminated colistin resistance in Europe is thought to be due to the heavy colistin use in animal feed, even if colistin is not extensively used to treat patients in Europe (37).

The total consumption of colistin increased by 50% from 2010 to 2014 in Europe, and the consumption was 600 times greater in food and animal feed than in humans (37).

According to a Chinese report, CoRE colonization was less than 1% in humans, compared with 20% in animals. These findings indicate the evolution of a colistin-resistant strain in human that originated from animal strains (12). The spread of colistin resistance could be prevented by restricting colistin use in animal feed (12).

Interestingly, the Chinese *mcr-1* allele sequence was 100% correlated with Belgian animals'*mcr-1* allele, suggesting the Chinese*mcr-1* allele originated from an animal strain (56).

In addition, the resistance could be disseminated to the global marke by transporting pig or chicken meat. A traveler could be the origin of *mcr-1* spread, as a traveler not harboring *mcr-1* acquired the *mcr-1* gene through travel (37). The transfer from a European traveler to South America was noted between continents (57). In addition, European travelers acquired colistin resistance after they traveled to Peru, Bolivia, Thailand, China, Vietnam, Cambodia, or Laos (58).

Diagnostic method

Many studies have investigated diagnostic methods to detect colistin resistance, and the CLSI and the EUCAST recommend the broth microdilution method for the detection of CoRE.

Comparing the accuracies of Vitek2, Sensititre, and E-test with reference to broth microdilution revealed accuracies of 93% in Vitek2, 89% in Sensititre, and 75% in E-test for essential agreement. Vitek2 or Sensititre areuseful for general laboratories, but the E-test is less accurate (59).

Because the *mcr-1* gene is spreading rapidly, anew screening method is urgently needed to prevent the rapid dissemination of *mcr-1* strains. By using the characteristics of EDTA to inhibit *mcr-1*, *mcr-1* could be detected in 2–3 hours. In detail, the colistin-EDTA combination revealed different inhibition zones and MICs compared with those of colistin alone. A 3-mm narrow disk diffusion and 4-fold decreased MIC were observed when incubated with EDTA (60).

The modified rapid polymyxin Nordmann Poirel test (MPNP) is a simple method to identify the *mcr-1* strain in 2 hours by using the color change of phenol red with high sensitivity and specificity (61). Although requiring further improvements, a universal culture media detecting colistin resistance in 2–3 hours has been developed. For example, Nordmann reported in-house superpolymyxin media containing colistin at 3.5 mg/L, which screens for colistin resistance (62).

Our understanding of the *mcr-1* genomic structure has increased due to marked upgradesin next-generation sequencing (NGS) technology, and many documents about CoRE genomic structure and antimicrobial characteristics using WGS have been described. The limitation of NGS is that we cannot exclude other resistance mechanisms using the NGS technology, even if a resistance gene is not found.

Treatment

Because colistin is the last-resort drug against CRE, infections with colistin-resistant Enterobacteriaceae have no therapeutic options. A few reports suggest that ceftazidime-avibactam, imipenem-relebactam, meropenem-RPX7009, eravacycline, or plazomycin can be administered to treat CoRE (63). Other reports say that the antimicrobial combination therapy with colistin is effective; especially, a number of papers reported that colistin-rifampin combination showed additive or synergistic effects (64). Marjan and colleagues developed ceragenin that exhibits a similar structure to that of endogenous antimicrobial peptide (AMP), which is effective

against colistin-resistant bacteria. The advantage of ceragenin is that it is capable of being mass-produced and is not degraded by proteases (65). Tigecycline is occasionally reported as effective against CoRE.

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