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REVIEW

Polymyxin E–Resistant Gram-Negative Bacteria in Tunisia and Neighboring Countries: Are There Commonalities?

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Abstract: The current global dissemination of polymyxin E resistance constitutes a real public health threat because of the restricted therapeutic options. This review provides a comprehensive assessment of the epidemiology of polymyxin E–resistant bacteria, with special reference to colistin-resistant Gram-negative bacteria in Tunisia and neighboring countries, based on available published data to January 2020. We aimed to determine their prevalence by species and origin, shedding light on the different genes involved and illustrating their genetic support, genetic environment, and geographic distribution. We found that colistin resistance varies considerably among countries. A majority of the research has focused on Algeria (13 of 32), followed by Tunisia (nine of 32), Egypt (nine of 32), and Libya (one of 32). All these reports showed that colistin-resistant Gram-negative bacteria were dramatically disseminated in these countries, as well as in African wildlife. Moreover, high prevalence of these isolates was recorded from various sources (humans, animals, food products, and natural environments). Colistin resistance was mainly reported among *Enterobacteriaceae*, particularly *Klebsiella pneumoniae* and *Escherichia coli*. It was associated with chromosomal mutations and plasmid-mediated genes (*mcr*). Four *mcr* variants (*mcr1*, *mcr2*, *mcr3*, and *mcr8*), mobilized by several plasmid types (IncHI2, IncP, IncFIB, and IncI2), were detected in these countries and were responsible for their rapid spread. Countrywide dissemination of high-risk clones was also observed, including *E. coli* ST10 and *K. pneumoniae* ST101 and ST11. Intensified efforts to raise awareness of antibiotic use and legalization thereon are required in order to monitor and minimize the spread of multidrug-resistant bacteria.

Keywords: Gram-negative bacteria, polymyxin E resistance, chromosomal mutations, *mcr* genes, plasmids

Introduction

Colistin is an ancient antibiotic known also as polymyxin E, it was discovered in 1949 and introduced to human medicine in the 1950s. It has bactericidal activity against most Gram-negative bacteria by targeting lipopolysaccharides in the outer membrane.[1] In the early 1980s, it was abandoned and replaced by other antibiotics because of its neurotoxicity and nephrotoxicity.[1] However, the global spread of multidrug-resistant bacterial infections in the 21st century led to the revival of colistin in clinical practice.[1,2] Indeed, it is considered one of the last-resort antibiotics used for the treatment of severe infections, caused in particular by carbapenem-resistant Gram-negative bacteria.[3,4] Several studies have revised and evaluated

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the colistin dosing regimen, as well as the best way to use and minimize its toxicity, sos as to ensure its safety and efficacy.[5–8] Colistin consumption has been continuously increasing since 2000 worldwide, including low-income countries.[9]

The use of colistin has not been restricted only to human medicine. It has also been used in veterinary medicine for decades, even for the prevention of microbial infections.[10] Unfortunately, in several regions, such as China, India, and Africa, it has been used without strict regulation in animal feed as a growth promoter, eg, for livestock, poultry production, and aquaculture.[10–13] Excessive use of colistin has led to the emergence and spread of colistin-resistant Gram-negative bacteria, constituting an emerging phenomenon and a real threat to public health.[14] High rates of colistin resistant Gram-negative species, especially *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, have been recorded throughout the globe. They were isolated from different origins notably animals, food products of animal origin, humans, and natural environments.[14,15] The dissemination of these isolates constitutes a real concern and a potential risk to human health. In fact, they can be transmitted to healthy humans through the food chain.[15] Facing this problem, several countries have banned the use of or tightened polices on colistin use in animal husbandry to reduce and monitor this crisis.[12] Some species of *Proteus*, *Providencia*, *Morganella*, *Neisseria*, and *Serratia* are naturally resistant to colistin.[16] These species should not be ignored, because they could cause severe infections with limited treatment options.

Generally, bacteria acquire resistance to colistin via mutations of chromosomal genes, eg, *mgrB,* and some operons and regulators, such as *pmrABC*, leading to lipopolysaccharide modifications.[17] The plasmid-mediated colistin gene *mcr1* was reported for the first time in 2015 in China.[18] Since then, it has disseminated dramatically around the world and become the most commonly reported mechanism implicated in colistin resistance.[14] The emergence of this gene is considered an alarming problem, because it contributes to the rapid dissemination of colistin-resistant isolates by horizontal gene transfer. So far, ten MCR variants have been identified, taking into consideration a heterogenous geographic distribution.[12,14]

This review summarizes the epidemiology of colistin-resistant bacteria isolated from different origins in Tunisia and neighboring countries and determines their prevalence based on available published data to January 2020. We also review the genes involved in colistin resistance, illustrating their genetic support, genetic environment as well as geographic distribution.

Prevalence and Origins of Colistin-Resistant Isolates

All studies that had investigated the dissemination of colistin-resistant Gram-negative bacteria in Tunisia and neighboring countries — Algeria, Libya, Egypt, Morocco, and Mauritania — to January 2020 are summarized in Table 1. Algeria had the most published papers (n=13), followed by Tunisia (n=9), Egypt (n=9), and Libya (n=1). However, there were no published data from Morocco or Mauritania (Figure 1). The lack of such data does not mean an absence of colistin-resistant isolates in these two countries, but could be explained by the absence of surveillance programs and diagnostic microbiology laboratories. Furthermore, a Moroccan study showed that colistin was one of the most used antibiotics in broiler production and overdosed in most administrations.[19]

As shown in Figure 2A, the highest prevalence of colistin-resistant Gram-negative bacteria was recorded in Tunisia (58.09%), followed by Egypt (24.76%), Algeria (14.29%), and Libya (2.86%). The distribution of colistin-resistant isolates per origin showed a higher prevalence in animals than humans, suggesting the major role of animals in the dissemination of colistin resistance (Figure 2B). In sum, 59.52% of colistin-resistant bacteria were isolated from animals (chickens, bovids, camels, Barbary macaques, and wild birds), and 20.48% and 2.86% were isolated from clinical specimens and healthy humans, respectively (Figure 2B, and Table 1). The detection of colistin-resistant isolates in healthy humans has recently been reported in Egypt, which is of concern.[15] These findings could be explained by the transmission of colistin resistance via the food chain, which deepens the antibiotic-resistance crisis and its challenges.[15] Colistin-resistant isolates were detected in foodstuffs (10%), such as raw bovine milk, Karish cheese, raw chicken meat, and beef sausage. Colistin-resistant bacteria were also observed in agricultural soil and manure (3.81%), as well as water sources (3.33%, Figure 2B and Table 1).

In the main, four colistin-resistant species were identified in these four countries. Colistin resistance was frequently observed among *Enterobacteriaceae*, with *Escherichia coli* (n=154) dominant, followed by

Table 1 Characteristics of polymyxin E–resistant Gram-negative bacteria detected in Tunisia and neighboring countries to January 2020

	Colistin-resistance mechanisms	Species	Sequence type	Origins	Locations	Isolation years	Genetic support	References
Tunisia	Mutated *pmrB* gene	*A. baumannii* (n=1)	ST281/ ST641	Clinical specimen	University Hospital of Sousse	2012	Chromosome	[22]
	Mutated *mgrB* gene	*K. pneumoniae* (n=13)	ST11 ST15 ST101 ST147 ST392	Clinical specimen	University Hospital of Sousse	2012–2016	Chromosome	[21]
	mcr1	*E. coli* (n=2)	ST2179	Fecal samples of Healthy chickens	Industrial egg-laying hen farm (north)	2013	IncP ISA*pl*	[43]
	mcr1	*E. coli* (n=37)	-	Fecal samples of healthy chickens imported from France	Three Tunisian farms	2015	IncHI2	[53]
	Mutated *mgrB* gene	*K. pneumoniae* (n=7)	ST147 ST101	Clinical specimen	Tahar Sfar Hospital (Mahdia)	2015–2016	Chromosome	[23]
	mcr1	*E. coli* (n=5)	ST10 ST57 ST69 ST349 ST1011	Cloacal swabs of healthy chickens	Northern poultry farms	2016	IncHI2	[24]
	mcr1	*E. coli* (n=1)	ST162	Fecal sample of camels	Southern butchery farm	2016–2018	IncHI2	[25]
	mcr1	*E. coli* (n=4)	ST1642 ST10	Bovine fecal samples Raw bovine milk sample	Northern bovine farm Northern market	2017–2018	Plasmid	[26]
	mcr1	*E. coli* (n=52)	ST10 ST57 ST69 ST117 ST162 ST398 ST2220 ST5416 ST5686	Chicken fecal samples Raw chicken-meat samples	Northern chicken farms Free/controlled outlets (north)	2018	Plasmid	[27]

(*Continued*)

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Table 1 (Continued).

	Colistin-resistance mechanisms	Species	Sequence type	Origins	Locations	Isolation years	Genetic support	References
Algeria	*mcr1*	*E. coli* (n=1)	ST405	Clinical specimen	University Hospital of Sidi Belabess	2011	Plasmid	[28]
	mcr1	*E. coli* (n=1)	ST405	Clinical specimen	University Hospital of Oran	2011	IncFIB	[44]
	Mutated *pmrB* gene	*A. baumannii* (n=1)	ST2	Clinical specimen	University Hospital of Béni-Messous (Algiers)	2013–2014	Chromosome	[29]
	mcr1	*E. coli* (n=1)	-	Chicken fecal samples	-	2015	Plasmid	[31]
	mcr1	*E. coli* (n=8)	ST48	Chicken fecal samples	Chicken farms (Algiers/Blida)	2016	Plasmid	[54]
	mcr1	*E. coli* (n=1)	ST405	Stool sample of Barbary macaques	Toudja forest, Bejaia	2016	Plasmid	[32]
	mcr1	*E. coli* (n=2)	ST23 ST115	Seawater	Algiers coast	2016	IncI2 IncHI2A	[34, 45]
	mcr1	*E. coli* (n=1)	-	Clinical specimen	Sétif Hospital	-	Plasmid	[55]
	Mutated *mgrB* gene (n=1), mutated *pmrA* gene (n=2), mutated *pmrB* gene (n=3)	*K. pneumoniae* (n=3)	ST101	Clinical specimens	Annaba University Hospital	2016	Chromosome	[30]
	mcr1	*E. coli* (n=6)	ST10 ST345 ST405	Agricultural soils Manure	Farmlands in Oran	2016–2018	Plasmids	[33]
	mcr3	*E. coli* (n=2)	ST155	Agricultural soils				
	Mutated *mgrB* gene	*K. pneumoniae* (n=1)	ST2620	Clinical specimens	Ibn Rochd Hospital of Annaba	2017	Chromosome	[56]
	Mutated *pmrB* gene	*K. pneumoniae* (n=1)	ST3242					
	mcr8	*K. pneumoniae* (n=1)	ST336	Clinical specimen	Sétif Hospital	2018	Plasmid	[57]
Libya	Mutated *mgrB* gene	*K. pneumoniae* (n=6)	ST101	Clinical specimens	Tripoli Medical Centre	2014–2015	Chromosome	[41]

Egypt	*mcr1*	*E. coli* (n=4)	-	Lesions of chicken with colibacillosis	-	2010	Plasmid	[36]
	mcr1	*E. coli* (n=1)	ST10	Cow suffering from subclinical mastitis	-	2014	Plasmid	[38]
	mcr1	*E. coli* (n=1)	ST1011	Clinical specimen	Cairo city hospital	2015	Plasmid	[58]
	Mutated *pmrCAB* genes	*A. baumannii* (n=2)	-	Clinical specimens	El-Kasr El-Aini Hospital	2015	Chromosome	[48]
	mcr1	*E. coli* (n=5)	-	Cloacal swabs from healthy broilers	Broiler farms in northern Egypt	2016	Plasmid	[37]
	mcr1	*E. coli* (n=1)	ST69	Karish cheese	-	2016–2017	IncHI2 ISA*pl*	[40]
	mcr1	*E. coli* (n=1)	-	Clinical specimens	National Cancer Institute, Cairo	2016–2017	Plasmid	[59]
		K. pneumoniae (n=1)	ST11					
	Mutated *mgrB* gene (n=1)	*K. pneumoniae* (n=1)	ST1399				Chromosome	
	mcr1	*E. coli* (n=12) *K. pneumoniae* (n=5) *P. aeruginosa* (n=6)	-	Wild-bird fecal samples Water Human stool samples	Five governorates (Giza, Cairo, El-Sharkia, El-Ismailia, and Port-said)	2017–2018	Plasmid	[15]
	mcr2	*E. coli* (n=3) *K. pneumoniae* (n=4) *P. aeruginosa* (n=1)						
	mcr1^{+}, *mcr2*	*E. coli* (n=1) *K. pneumoniae* (n=2)						
	mcr1	*E. coli* (n=1)	ST101	Beef-sausage sample	-	-	IncI2	[39]

Note: -, not determined.

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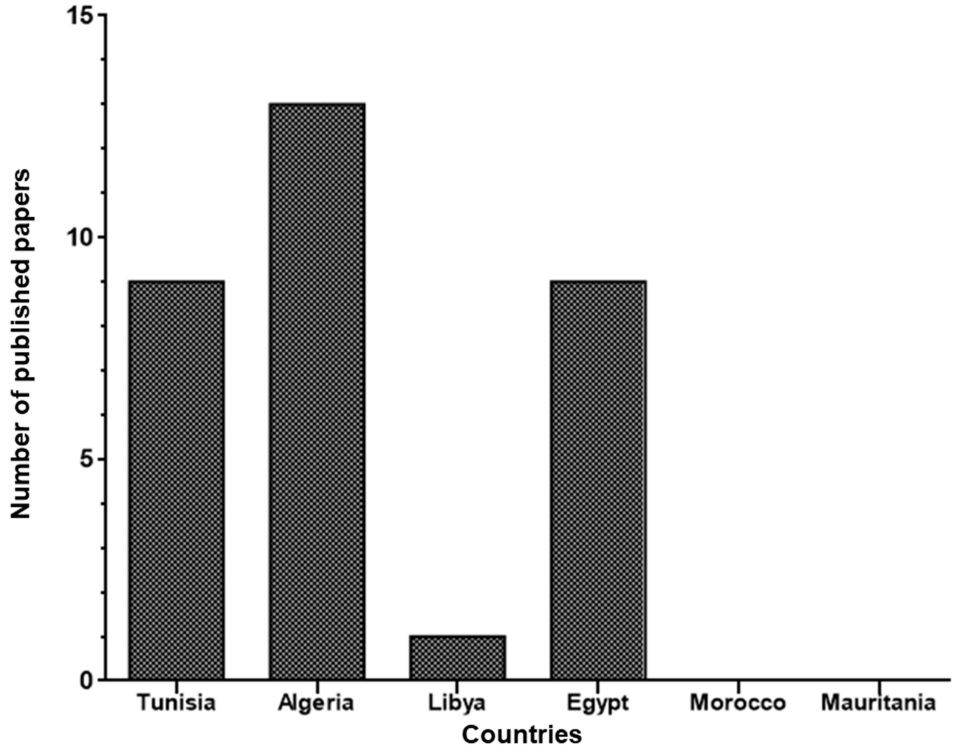


Figure 1 Papers reporting colistin resistance in each country to January 2020.

Klebsiella pneumoniae (n=45). However, few colistin-resistant isolates belonging to the nonfermentative Gram-negative bacteria *Acinetobacter baumannii* (n=4) and *Pseudomonas aeruginosa* (n=7) were recorded (Tables 1 and 2). We also noticed that colistin-resistant *K. pneumoniae* and *A. baumannii* were isolated mainly

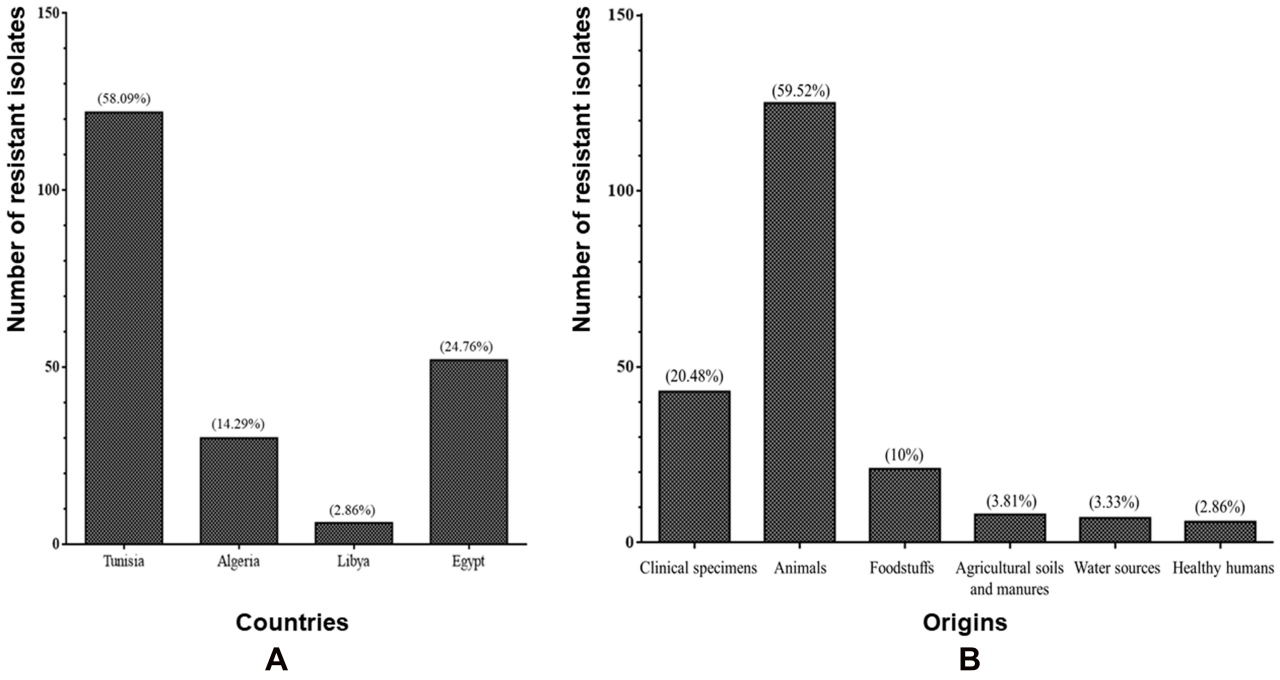


Figure 2 (**A**) Distribution of colistin resistant isolates per country and (**B**) per origin.

from clinical specimens. Obviously, when the number of samples increased, the number of resistant isolates did also.

Generally, these species constituted the main cause of the most worrisome infections occurring in health-care settings and the community.[20] In Tunisia, colistin-resistant *K. pneumoniae* and *A. baumannii* isolates were recovered from clinical specimens of patients hospitalized in coastal cities.[21–23] Colistin-resistant *E. coli* isolates were detected among chickens and bovids on northern farms, in addition to camels on a southern farm.[24–26] As described in Table 1, colistin-resistant *E. coli* was also detected in food of animal origin.[26,27]

On the other hand, Algerian studies reported the isolation of these three species from clinical specimens of patients hospitalized in different governorates of the country.[28–30] However, only *E. coli* isolates were detected in other sources, including animals,[31,32] agricultural soil and manure,[33] in addition to seawater.[34]

The detection of colistin-resistant *E. coli* in Barbary macaques in the north of Algeria constituted the first report of such isolates in the wildlife of Africa.[32] A second African study describing the isolation of colistin-resistant isolates in wild animals has recently been published in Egypt. It showed the detection of three colistin-resistant species — *E. coli*, *K. pneumoniae*, and *P. aeruginosa* — among wild birds.[15] Colistin-resistant *P. aeruginosa* isolates of other origins were not detected in Egypt nor in the other countries included in this review (Table 1). The presence of colistin-resistant Gram-negative bacteria in the wildlife samples is a real concern. In fact, wildlife plays a major role in the global dissemination of multidrug-resistant bacteria, including colistin-resistant isolates. As such, wild birds are considered reservoirs and vectors for the transmission of antibiotic-resistant -genes.[35] Moreover, the health situation in Egypt has become more critical since the discovery of colistin-resistant *E. coli* and *K. pneumoniae* in healthy humans and water.[15] Egyptian studies have also described the detection of colistin-resistant *E. coli*, *K. pneumoniae*, and *A. baumannii* in clinical specimens collected from hospitalized patients. Colistin-resistant *E. coli* isolates have been recovered from chickens,[36,37] bovids,[38] and food of animal origin.[39,40] To date, only one study has reported the detection of colistin-resistant isolates in Libya. These isolates were recovered from clinical specimens of hospitalized patients in Tripoli and were identified as *K. pneumoniae*.[41]

All these reports confirmed the dissemination of colistin-resistant Gram-negative bacteria in Tunisia and neighboring countries, with high prevalence of various origin. The question is whether there are commonalities or even links among the detected colistin-resistant isolates at genetic and clonal levels. This is elucidated upon in the two next sections.

Colistin-Resistance Genes and Genetic Environments

As shown in Tables 1 and 2, several colistin-resistance genes were detected, showing a heterogeneous distribution among countries and species. *mcr* was observed more often than chromosomal mutations. Indeed, we noted the detection of four *mcr* variants — *mcr1*, *mcr2*, *mcr3*, and *mcr8* — with of *mcr1* dominant (75.48%). Obviously, this observation is limited to the published reports included in our study and does not reflect the real scenery of colistin resistance, because of the lack of reporting in several governorates in these countries. More epidemiological studies are required to clarify the health situation and get closer to reality.

These four *mcr* genes are distantly related, as shown by the phylogenetic tree of the nine variants of *mcr* genes in Figure 3. This phylogenetic tree was performed using the Clustal Omega program (https://www.ebi.ac.uk/services) after collecting the nucleotide sequences from the GenBank database (https://www.ncbi.nlm.nih.gov), and distances were calculated using the neighbor-joining algorithm (Figure 3).

mcr1 was predominant among *E. coli* isolates, showing high prevalence in Tunisia (63.125%), followed by Egypt (23.75%), and Algeria (13.125%). The emergence of *mcr1* among *K. pneumoniae* and *P. aeruginosa* isolates was observed only in Egypt (Table 2). Likewise, the detection of *mcr2* and the coexpression of *mcr1* and *mcr2* were described only in Egypt among *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.[15] The variants *mcr3* and *mcr8* were described only in Algeria among *E. coli* and *K. pneumoniae*, respectively, as demonstrated in Table 2. As such, *mcr* genes constitute the main mechanism conferring resistance to colistin among *E. coli* isolates. However, these genes were rarely detected among *K. pneumoniae* (n=13) and *P. aeruginosa* (n=7).

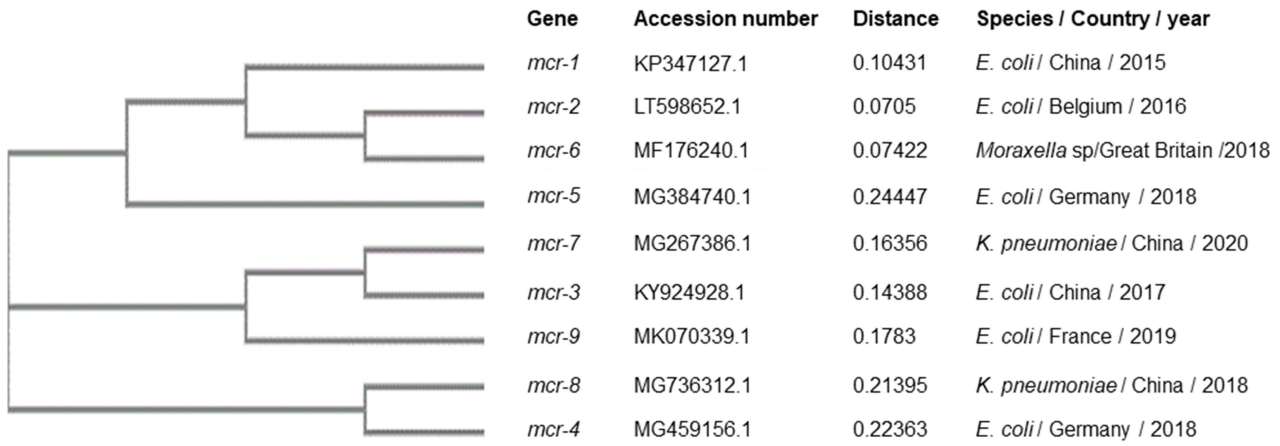
Table 2 Distribution of colistin-resistance genes per species and country

	Species	Mutated chromosomal genes				Plasmid-mediated genes				
		mgrB	*pmrA*	*pmrB*	*pmrCAB*	*mcr1*	*mcr2*	*mcr1 +, mcr2*	*mcr3*	*mcr8*
Tunisia										
	E. coli	-	-	-	-	101	-	-	-	-
	K. pneumoniae	20	-	-	-	-	-	-	-	-
	A. baumannii	-	-	1	-	-	-	-	-	-
	Total	20 (71.43%)	-	1 (16.67%)	-	101 (63.125%)	-	-	-	-
Algeria										
	E. coli	-	-	-	-	21	-	-	2	-
	K. pneumoniae	1	2	4	-	-	-	-	-	1
	A. baumannii	-	-	1	-	-	-	-	-	-
	Total	1 (3.57%)	2 (100%)	5 (83.33%)	-	21 (13.125%)	-	-	2 (100%)	1 (100%)
Libya										
	K. pneumoniae	6	-	-	-	-	-	-	-	-
	Total	6 (21.43%)	-	-	-	-	-	-	-	-
Egypt										
	E. coli	-	-	-	-	26	3	1	-	-
	K. pneumoniae	1	-	-	-	6	4	2	-	-
	A. baumannii	-	-	-	2	-	-	-	-	-
	P. aeruginosa	-	-	-	-	6	1	-	-	-
	Total	1 (3.57%)	-	-	2 (100%)	38 (23.75%)	8 (100%)	3 (100%)	-	-
Total		28 (13.21%)	2 (0.94%)	6 (2.83%)	2 (0.94%)	160 (75.48%)	8 (3.77%)	3 (1.42%)	2 (0.94%)	1 (0.47%)

mcr is characterized by a variety of plasmid types or even associated with transposons, ensuring its rapid spread worldwide.[42] The present study showed that *mcr1* was carried mainly by the IncHI2 replicon in Tunisia (Table 1). To our knowledge, the IncP plasmid type harboring *mcr1* has been described in only one report in this country. This was detected in *E. coli* isolated from fecal samples of healthy chickens.[43] However, in Algeria, *mcr1* was detected in various plasmid groups, noting IncFIB, IncI2, and IncHI2A.[34,44,45] Interestingly, the two plasmid types harboring *mcr1*, IncHI2 and IncI2, were also identified among *E. coli* isolated from food of animal origin in Egypt.[39,40] A recent study showed that plasmids belonging to three incompatibility groups — IncI2, IncX4, and IncHI2 — are involved in the global dissemination of *mcr* genes.[46] The IncI2 plasmid is commonly reported in Asia and America, and the IncHI2 plasmid frequently reported in Europe and Africa.[46] This gene is also associated with a transposable element, namely the insertion sequence IS*Apl*.[40,43]

Colistin-resistant *A. baumannii* was rarely isolated in these countries: there was a clear absence of *mcr* genes among these isolates (Table 2). In contrast, dissemination of colistin-resistant *A. baumannii* harboring plasmid-encoded *mcr* genes has recently been described in Spain, China, and Iraq.[14,47] We found that colistin resistance among the detected *A. baumannii* clinical isolates was usually due to mutated chromosomal genes, including *pmrB* and the *pmrCAB* operon (Table 1). The mutated *pmrB* gene leads to colistin resistance by alteration of lipid A, which is a component of lipopolysaccharide. This mechanism has been detected in Tunisia and Algeria.[22,29] Also, mutations in the *pmrCAB* operon have been identified in Egypt.[48]

Colistin resistance through mutations of chromosomal genes was also observed in *K. pneumoniae* isolates, with



Gene	Accession number	Distance	Species / Country / year
mcr-1	KP347127.1	0.10431	*E. coli* / China / 2015
mcr-2	LT598652.1	0.0705	*E. coli* / Belgium / 2016
mcr-6	MF176240.1	0.07422	*Moraxella* sp/Great Britain /2018
mcr-5	MG384740.1	0.24447	*E. coli* / Germany / 2018
mcr-7	MG267386.1	0.16356	*K. pneumoniae* / China / 2020
mcr-3	KY924928.1	0.14388	*E. coli* / China / 2017
mcr-9	MK070339.1	0.1783	*E. coli* / France / 2019
mcr-8	MG736312.1	0.21395	*K. pneumoniae* / China / 2018
mcr-4	MG459156.1	0.22363	*E. coli* / Germany / 2018

Figure 3 Phylogenetic tree of the nine variants of *mcr* genes generated using the Clustal Omega program (https://www.ebi.ac.uk/services). Distances were calculated using the neighbor-joining algorithm.

mutated *pmrA* and *pmrB* detected in clinical specimens in Algeria. However, mutated *mgrB* constituted the main mechanism conferring resistance to colistin among *K. pneumoniae* in Tunisia and Libya. This mechanism has rarely been described in Algeria and Egypt (Tables 1 and 2). This species constitutes the main reservoir of colistin-resistance genes, including both acquired and mutated ones.

Sequence Types

Several colistin-resistant bacterial clones were disseminated among the countries included in the present study, as shown in Table 1. Thirteen distinct sequence types of colistin-resistant *E. coli* were detected in Tunisia, with four clones predominant: ST10, ST162, ST57, and ST69. Colistin-resistant isolates assigned to ST10 originated from chickens and bovids, whereas ST162 isolates were detected among chickens and camels. ST57 and ST69 were isolated from chickens. The other disseminated clones originated from chickens also: ST2179, ST349, ST1011, ST1642, ST117, ST398, ST2220, ST5416, and ST5686.

Seven sequence types of colistin-resistant *E. coli* were identified in Algeria, with ST405 the most abundant. This clone was isolated from different origins, including clinical specimens, Barbary macaques, and agricultural soil and manure. Regarding the latter, three other colistin-resistant *E. coli* clones were detected — ST10, ST345, and ST155. In addition, two *E. coli* clones, ST23 and ST115, were identified in seawater. However, only one colistin-resistant *E. coli* clone (ST48) was isolated from chickens in this country (Table 1).

There were four major colistin-resistant *E. coli* clones in Egypt: ST10, ST1011, ST69, and ST101. ST10 was also recorded in Tunisia and Algeria, while ST1011 and ST69 were detected in Tunisia from different origins (Table 1). A recent report recorded the dominance of *E. coli* ST10 in animal and water. This clone is considered the most common ST of those harboring *mcr1* worldwide.[46]

Many colistin-resistant *K. pneumoniae* belonging to different sequence types were detected in Tunisia (ST11, ST15, ST101, ST147, and ST392), Algeria (ST101, ST2620, ST3242, and ST336), Egypt (ST11 and ST1399), and Libya (ST101), and were mainly isolated from clinical specimens (Table 1). It is worth noting that colistin-resistant *K. pneumoniae* ST101 was disseminated in three neighboring countries — Tunisia, Algeria, and Libya. Infections caused by such clones have been described in Italy and Serbia.[49] We also noticed the spread of colistin resistant ST11 isolates in both Tunisia and Egypt. These two sequence types belonged to the same clonal complex (CC11) and were considered high-risk clones in terms of morbidity and mortality rates.[49] The isolates assigned to CC11 usually show a multidrug-resistance profile and also harbor numerous antibiotic-resistance genes, especially those coding for ESBLs and carbapenemases, thus restricting therapeutic options.[50] Countrywide dissemination of the multidrug-resistant *K. pneumoniae* ST147 and ST15, particularly carbapenemase producers, has been reported in Mediterranean countries, including Tunisia.[4,51]

Regarding the uncommon colistin-resistant *A. baumannii*, we noted the detection of two circulated clones — ST641 in Tunisia and the international clone

ST2 in Algeria — according to Pasteur's scheme.[22,29] However, it should be noted that other sequence types might have emerged, despite the lack of sequence-type identification in the Egyptian report.[48]

Impact of High-Throughput Sequencing in the Detection of Colistin-Resistance Genes

The seriousness of the antibiotic-resistance crisis has prompted researchers to develop sophisticated methods to facilitate the determination of antibiotic-resistance mechanisms in a rapid and more precise manner. Herein, we are talking about whole-genome sequencing using high-throughput technologies, which have achieved significant progress. This method could be considered a scientific revolution in molecular biology, including the antibiotic-resistance field. It is becoming a potent tool of choice to identify resistance genes, especially those implicated in colistin resistance.[52] This method seems to be a key to the revelation of new colistin-resistance mechanisms by detection of unknown mutations of chromosomal genes and new variants of *mcr* genes,[52] and also leads to precise and in-depth research using bioinformatic analysis to determinate the genetic environment of the detected genes and their genetic support.[52] Several reports using whole-genome sequencing to investigate the colistin-resistance mechanism, have been performed in Tunisia,[21,22] Algeria,[45] and Egypt.[39,40] It is very important to note the use of this method in low-income countries, such as Tunisia, Algeria, and Egypt. This step is an indicator of the scientific progress in these countries and proves the significance of efforts of researchers to improve the quality of the health-care system in their countries, despite the difficult financial conditions.

Conclusion

Colistin is considered a beacon in the darkness of the antibiotic-resistance challenge.[12] It is used as the last-resort antibiotic for the treatment of multidrug-resistant Gram-negative bacterial infections. However, extensive use has led to the emergence and dissemination of colistin resistant Gram-negative bacteria, which constitute a real threat to public health. The flexibility of the bacterial genome and its ability to adapt and resist colistin has prompted researchers to find other solutions.

As described in this review, colistin resistant Gram-negative bacteria were disseminated in Tunisia and neighboring countries, showing high prevalence with different origins (humans, animals, food products, and natural environments). The detection of colistin-resistant isolates in the African wildlife is a real concern. Both plasmid-mediated genes and chromosomal mutations were involved in colistin resistance. We found that colistin resistance in *K. pneumoniae* was caused mainly by chromosomal mutations. However, it was often caused by *mcr* in *E. coli*. Colistin resistance varies considerably among countries, and there is countrywide dissemination of several clones. These findings emphasize the seriousness of antibiotic resistance in these countries, requiring urgent interventions to monitor and control the situation before it becomes worse, in order to avoid pandrug resistance and a therapeutic impasse. Preserving human health and natural wealth is everyone's responsibility. When crises intensify, every small detail can carry a great meaning and achieve a great goal.

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