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Current scenario of plasmid-mediated colistin resistance in Latin America

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Abstract   Colistin resistance can occur by chromosomal mutations and by acquisition of plasmid-carrying determinants, mainly *mcr-1*. In the recent years, we have observed the outburst of this resistance gene in our region. Due to the risk of the rapid dissemination of *mcr-1*, this finding has worried and alerted different actors from the health field and has become one of the most prolific topics. Our review compiles available reports of well-documented *mcr-1*-positive strains of *Enterobacteriaceae*, obtained from different samples in Argentina and other countries of Latin America. Furthermore, it addresses the association of *mcr-1* with ESBL resistance markers and outlines the platforms involved in their dissemination.
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Escenario actual de la resistencia a la colistina mediada por plásmidos en América Latina

Resumen   La resistencia a la colistina puede ocurrir por mutaciones cromosómicas o por la adquisición de determinantes localizados en plásmidos, el principal es *mcr-1*. En los últimos años hemos observado la explosiva aparición de este gen de resistencia en nuestra región. Debido al riesgo que implica la rápida diseminación de *mcr-1*, este hallazgo ha preocupado y alertado a los diferentes actores del área de la salud, y se ha convertido en uno de los temas de

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investigación más importantes. La presente revisión compila los informes de aislamientos portadores de *mcr-1* debidamente documentados en *Enterobacteriaceae*, obtenidos de diferentes muestras en Argentina y otros países de América Latina. Además, aborda la asociación de *mcr-1* con otros marcadores de resistencia, como las BLEE, y describe las plataformas involucradas en su diseminación.

Polymyxins (colistin and polymyxin B) are polycationic peptides, which act by targeting the negative charges in bacterial lipopolysaccharide (LPS), the complex lipoglycan that makes up most of the gram-negative outer membrane. Colistin (Polymyxin E) is one of the few cationic antimicrobial peptides commercialized in both human and veterinary medicine. Since its introduction in the 1950s, colistin (COL) has been used mainly as a topical treatment in human medicine owing to its nephrotoxicity and neurotoxicity when given systemically. There are two commercially available forms: colistin sulphate (CS) and colistin methanesulphonate sodium (CMS), the latter being less toxic than colistin when administered parenterally and consequently being the most used both in parenteral and inhalational formulations. In aqueous solutions, CMS undergoes *in vivo* conversion[9]. On the other hand, CS is the only form of COL approved in pig production in some countries for the control of *Enterobacteriaceae* infections, particularly for those caused by *Escherichia coli*. However, COL has been massively used at sub-inhibitory concentrations as growth promoters for decades to improve weight gain and therefore to maximize feed conversion efficiency in animal production[22]. Noteworthy, the last survey conducted by the World Organization for Animal Health (OIE) in the year 2012 revealed that only 10 countries reported the use of COL as growth promoter[42]. Interestingly, in the late 2000s and after decades of COL use in swine, several studies began to report a significant COL resistance rate in *Enterobacteriaceae*[1,4,23,33,49,55]. The increasing incidence of infections caused by multidrug resistant gram-negative bacteria, especially the ones caused by carbapenemase-producing bacilli in the last years, has led to the use of polymyxins as one of the last resort treatments[49].

Until very recently, COL resistance occurred by chromosomal mutations only, and therefore, this resistance phenotype was spread exclusively *via* clonal transmission of resistant isolates. However, two years ago Liu et al. reported the emergence of a plasmid-mediated colistin resistance determinant, *mcr-1*[39]. Due to the risk of its rapid dissemination, this finding has worried and alerted different actors from the health field, becoming one of the most prolific topics.

Acquired resistance to colistin

Acquired resistance to colistin in *Enterobacteriaceae* was described many years ago[45,64], but in the last years colistin-resistant *K. pneumoniae* isolates were more frequently reported worldwide[10,11,32,52], among others. In Latin America there are reports from Brazil, Ecuador, Venezuela and Colombia[18,27,50,56,58].

Acquired resistance to COL in *Enterobacteriaceae* was first documented in Argentina in the last years together with the emergence of KPC-producing isolates, probably derived from the use of this antimicrobial agent in the therapy of infections by these multidrug-resistant *K. pneumoniae* isolates. This resistance is believed to have occurred by selection of resistant subpopulations in the epidemic clone belonging to ST258. However, it has also been observed in non KPC-producing *K. pneumoniae* isolates probably as a result of clonal spread[43]. The last report of the PAHO (Panamerican Health Organization) from the year 2015 informed a COL resistance rate of 10.3% in *K. pneumoniae* isolates from hospitalized patients in Argentina, whereas in *Escherichia coli* and *Enterobacter cloacae* the percentage only reached 2.1%[51]. Similar results were obtained at the University Hospital José de San Martín, where COL resistance in *K. pneumoniae* in the year 2010 was 2% and 3%, 3.6%, 3.8%, 7%, 12% and 8%, respectively, in the subsequent years (unpublished results). With regard to *E. coli* isolates, COL resistance emerged in the last two years, where an important increase from 0.2% to 1.3% was observed, mostly as a result of plasmid-mediated resistance[44]. On the other hand, acquired resistance to COL remains very low in *Acinetobacter baumannii* (0.2%) and *Pseudomonas aeruginosa* (2.5%) isolates[51].

Chromosomal resistance: different pathways

Bacteria often use different strategies to protect themselves from exposure to cationic antimicrobial peptides such as colistin. Such strategies include modifications of their lipopolysaccharides (LPSs), which are involved in the initial step of interaction between polymyxins and bacteria[8,47,48]. These alterations can be achieved by several covalent changes in lipid A (also called endotoxin molecule) of LPS. Among them, the cationic substitution of the phosphate group by the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (PEtN) are the most common LPS modifications. L-Ara4N modification is more effective than PEtN modification because the decrease in the net charge leading to colistin resistance is more effective. The synthesis and transfer of L-Ara4N and PEtN is mediated by an operon regulated by the activation of two-component systems (TCSs). PmrA/PmrB is the main

system contributing to polymyxin resistance (by environmental stimuli, and specific mutations within the TCS), although in some gram-negative species is known to occur *via* PhoP/PhoQ[8,48].

Other modifications associated to chromosomal polymyxin resistance have been described, such as the reduction of acyl groups mediated by an *lpxR*-like deacylation and hydroxylation of lipid A[48]. Moreover, it was reported that *K. pneumoniae* uses alternative strategies such as participation of an efflux pump or capsule formation[48].

The plasmid-mediated resistance gene *mcr-1*

In 2015, the emergence of a plasmid-mediated colistin resistance marker, named *mcr-1*, was reported in China[39]. Since then, numerous reports of the *mcr-1* gene have been documented in *Enterobacteriaceae* cultured from food, animals, environment and humans worldwide, most of them detected in over 20 countries within 3 months of its first report[8].

The *mcr-1* gene encodes a PEtN transferase leading to the addition of PEtN to the lipid A. An *in silico* analysis of the amino acid sequence of this protein revealed that it is strongly related to enzymes present in gram-negative bacteria, some of which are intrinsically resistant to polymyxin[8,19,48,52]. This gene was detected mainly in *E. coli* and to a lesser extent, in other bacteria such as *K. pneumoniae*. To date, several variants of *mcr-1* have been reported in the literature (*mcr-1.2* to *mcr-1.8*, and *mcr-1.10*)[2,20,40,66,69] or are deposited in Genbank (variants *mcr-1.9*, *mcr-1.11*, *mcr-1.12*, and *mcr-1.13*). These variants have one amino acid change each and exhibit a similar effect on colistin resistance compared with *mcr-1*.

Very recently, four additional *mcr* determinants have been reported, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5*. The *mcr-2* gene was identified in an *E. coli* isolated from pigs and cattle in Belgium[68]. This marker was also recovered from human samples in Switzerland[37]. Phylogenetic analysis of *mcr-2*, which was also described as a PEtN transferase, showed 80.6% amino acid identity with *mcr-1* and provided strong evidence that this protein may have originated from *Moraxella catarrhalis*[68]. The *mcr-3* gene was found in *E. coli* isolated from pigs in Malaysia, in a clinical sample of *K. pneumoniae* isolated from Thailand, and a clinical sample of *Salmonella* Typhimurium from the United States[70]. Sequence analysis showed that *mcr-3* is closely related to PEtN from *Aeromonas* spp. The *mcr-4* gene was found in *Salmonella* Typhimurium and *E. coli* from human and food-producing animals in Europe[13]. MCR-4 has 34.0%, 35.0% and 49.0% amino acid sequence identity to MCR-1, MCR-2 and MCR-3, respectively. It has been proposed that *mcr-4* may have emerged from a *Shewanella* species, a bacterium commonly present in aquatic niches. Lastly, *mcr-5* was initially found in 14 *Salmonella* Paratyphi B dTa+ isolates[12]. MCR-5 shares 36.11% protein identity with MCR-1, 35.29% with MCR-2, 34.72% with MCR-3 and 33.71% with MCR-4. Sequence analysis revealed that *mcr-5* might have been transferred from the environmental rod *Cupriavidus gilardii*.

These genes have completely changed the scenario of colistin resistance since they have become a potential threat to public health. In Argentina and other countries of Latin
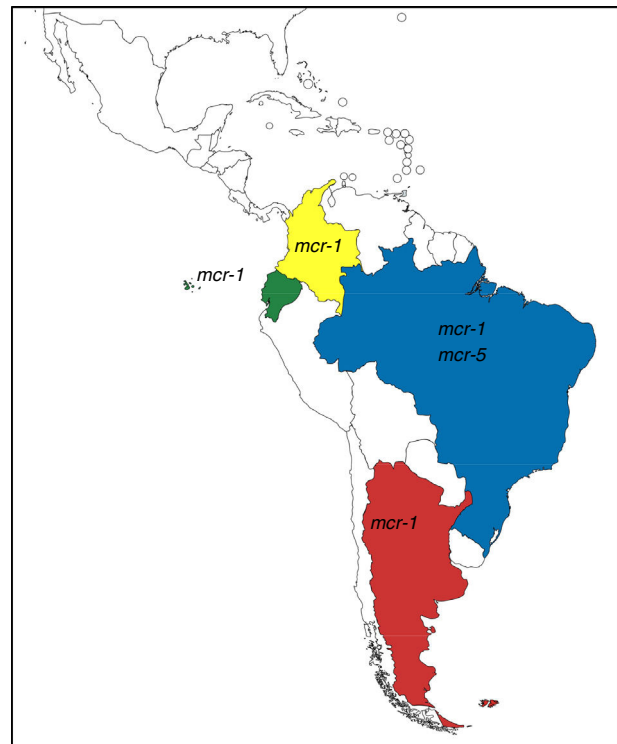

Figure 1 *mcr* distribution in Latin America.

America, *mcr-1* occurrence is currently low, however, it is possible to observe its rapid increase (Table 1). Moreover, *mcr-5* has been recently found in a colistin-resistant *E. coli* isolate from Brazil (NZ_PDMU00000000) (Fig. 1).

Furthermore, there is a clear association between this determinant and the different genes encoding β-lactamases, particularly those from the *bla*$_{CTX-M}$ family. It is worth mentioning that *mcr-1* can be found in isolates collected not only from human and animal clinical samples but also from different sources such as pigs, wild birds, penguins, poultry production and environmental niches (Table 1). Among these isolates, *E. coli* was the most common species reported in Brazil and Argentina.

The coexistence of *mcr-1* and carbapenem resistance determinants (such as KPC-2, NDM and IMP-8 carbapenemases) in human samples is of great concern (Table 1). These findings highlight that *Enterobacteriaceae* isolates carrying both *mcr-1* and carbapenemase-encoding genes may emerge as a serious threat to antimicrobial therapy.

Genetic environment and dissemination of the *mcr* gene

Previous studies have shown that *mcr* genes are commonly located in self-transferable plasmids, which are responsible for inter-species dissemination worldwide. Plasmid incompatibility groups IncI2, HI1, HI2, P, X1/X2, X3/X4, X4, FI, FIP and FII have been reported as carriers of the different *mcr* variants[12,14,18,23,26,34,39–41,52,58,68,70]. Furthermore, a hybrid plasmid, identified as IncI2/IncFIB also harbored this

Table 1 *mcr-1*-positive isolates reported in Latin America

Species (n)	Origin	Year	Country	Associated resistance (n)	COL MIC (μg/ml)	Reference
E. coli (16)	Chicken and pig	2012–2013	Brazil	CTX-M-8 (6), CTX-M-15 (2), CTX-M-1 (1), CMY-2 (1)	1–16 (MIC$_{50}$ = 8)[a]	27,28
E. coli (9)	Human (blood, urine, abdomen, bone, abscess)	2012–2016	Argentina	CTX-M-14 (2), CTX-M-2 (1), CTX-M-15 (1)	8–16 (MIC$_{90}$ = 8)[b]	53
E. coli (10)	Human	2016	Argentina	No data[h]	No data[h]	53
E. coli (1)	Human (diabetic foot)	2016	Brazil	CTX-M-8	>4[c]	26
E. coli (6)	Human (urine)	2014–2016	Argentina	ESBL, KPC	4–16[d]	14,44
E. coli (1)	Human (peritoneal fluid)	2016	Ecuador	CTX-M-55	8[a]	50
E. coli (31)	Healthy poultry	2013–2016	Argentina	CTX-M-2, CTX-M-14, CMY-2, *qnrB*, *qnrS*	4–32 (MIC$_{50}$ = 8)[e]	21,22
E. coli (1)	Human (urine, neurogenic bladder)	2016	Argentina	CTX-M-type	8[f]	5
E. coli (1)	Dog	2014	Argentina	CTX-M-2	8[e]	57
E. coli (1)	Human (rectal swab)	2016	Argentina	IMP-8	8[f]	24
E. coli (2)	Human (urine)	2016	Argentina	CTX-M-2 (1)	4–8[e,f]	30
E. coli (7), *K. pneumoniae* (1)	Human (urine, abdominal, skin)	2016	Argentina	ESBL (1)	>4[d]	46
E. coli (83), *K. pneumoniae* (1), *Citrobacter amalonaticus* (1)	Human (mainly urine, blood, abdominal fluid)	2012–2016	Argentina	CTX-M-type (36), pAmpC (4), PER (1), SHV (1), KPC (1), NDM (2)[g]	MIC$_{50}$ = 8; MIC$_{90}$ = 8[b]	25,54
E. coli (5)	Human (urine, abdominal fluid)	2016	Argentina	ESBL (2)	≥4 (MIC$_{90}$ = 4)[d]	7
Salmonella Typhimurium (3), *E. coli* (8), *K. pneumoniae* (1)	Human (fecal matter, urine, vaginal discharge, blood, abscess)	2013–2016	Colombia	No data[h]	>4[a]	59,60
E. coli (8)	Food (chicken meat)	2016	Brazil	CTX-M-2 (4), CTX-M-8 (2), CMY-2 (1)	2–8[e]	41
E. coli ([h])	Environment and avian (penguin, seawater)	2012–2016	Brazil	No data[h]	No data[h]	29
E. coli (6)	Human (mainly urine, blood, peritoneal fluid, bone)	2016	Brazil	No data[h]	4–8[f]	31
E. coli (61)	Healthy poultry	2015–2016	Brazil	No data[h]	>8[a]	6
E. coli (5)	Wild birds (Kelp gulls)	2012	Argentina	CTX-M-2 (1), CTX-M-14 (4)	4–8[e]	36
E. coli (1)	Penguin	2013	Brazil	CTX-M-1	8[a]	61
E. coli (1)	Human (rectal swab)	2014	Brazil	KPC-2	4[e]	16
K. pneumoniae (1)	Human (rectal swab)	2014	Brazil	KPC-2, CTX-M-15	4[e]	17
E. coli (2)	Human	2015	Brazil	KPC-2 (1), CTX-M-2 (1)	4–8[f]	15
K. pneumoniae (1)	Human	2016	Brazil	KPC-2	16[e]	3

Susceptibility to colistin was evaluated by: (a) unspecified method, (b) agar dilution, (c) MicroScan system (Beckman Coulter), (d) Phoenix (Becton, Dickinson), (e) microdilution method, (f) Vitek-2, (g) NDM was detected in *C. amalonaticus*, (h) no data described in the Abstract.

resistance gene[67]. In South America only a few reports identified the plasmids responsible for *mcr* spread. To date, only plasmids from incompatibility groups X4 and I2 carry the *mcr* gene in our region[3,21,41,66]. An initial analysis of the plasmid backbone suggests that these elements are highly similar to those reported in China, Europe or North America.

A common feature of *mcr-1* variants is their genetic environment. This gene is commonly adjacent to the *pap2* gene. Both genes can be found either surrounded by two copies of insertion sequence IS*Apl1*, yielding a composite transposon, downstream to a single copy of IS*Apl1* or in the absence of this element[62]. It has been proposed that the presence or absence of this IS correlates with the adaptation of *mcr-1* to a new host. A composite transposon indicates a recent acquisition of this marker whereas the absence of IS*Apl1* or the presence of a single copy suggests that it has been already adapted. Since all three genetic environments have been found in different plasmids, it has been hypothesized that independent recombination events have led to the adaptation of the *mcr-pap2* segment to specific incompatibility groups, which are in turn responsible for dissemination of polymyxin resistance. However, a recent report demonstrated that the composite transposon Tn*6330*, which has two IS*Apl1* surrounding *mcr-1*, can translocate to other plasmids[35]. Therefore, the possibility of a wider dissemination to plasmids from other incompatibility groups should be considered. It is worth noting that a study on IS*Apl1* function showed that when this sequence is active it is detrimental to the host, which leads to its loss[63]. Indirectly, this phenomenon might control the dissemination of *mcr-1*.

In 2016 Xavier et al. identified the first *mcr-2* gene in an IncX4 plasmid from bovine and porcine *E. coli*[68]. Further analysis showed that *mcr-2* is located at the same site than *mcr-1* when found in the IncX4 plasmid. Despite this fact, *mcr-2* was found surrounded by two copies of the insertion sequence IS*1595* allowing this DNA to generate circular intermediates[65]. This finding reveals the potentiality of *mcr-2* to disseminate to other plasmids and therefore, to other organisms. More recently, novel *mcr* determinants have been described. The *mcr-3* gene has been found in plasmids from the IncHI2 group harbored in *Enterobactericeae* as well as in the chromosome of *Aeromonas veronii*[38,70]. When located in plasmids, *mcr-3* is adjacent to the truncated transposon element TnAs2[70], a mobile element probably responsible for its spread. On the other hand, *mcr-4* and *mcr-5* genes were found in small non-self-transferable plasmids from the ColE family[12,13]. Furthermore, the *mcr-5* gene is located in a Tn*3*-family transposon.

Concluding remarks

The data summarized herein show the rapid spread of *mcr-1* in Latin America. To the best of our knowledge, *mcr-2*, *mcr-3* and *mcr-4* determinants have not been found in our region yet.

The *mcr-1* marker has been found in a wide variety of ecological niches, which represents a troublesome scenario for public health. In the absence of new antimicrobial agents against multidrug-resistant gram-negative pathogens, the effect of plasmid-mediated colistin resistance on human health should not be underestimated.

There is an urgent need for surveillance and molecular epidemiological studies in both human and veterinary medicine addressing the dissemination of *mcr-1* among bacterial isolates. The fact that most *mcr* genes were found in other regions raises the question of whether a unique variant is locally disseminated or if the other variants were accurately sought.

Furthermore, we suggest a re-evaluation of the antimicrobial agents used in animal feeding in order to reduce the potential risk to pandrug resistance.

Conflict of interest

The authors declare that they have no conflicts of interest.

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