Extended-spectrum β-lactamase, carbapenemase, and the *mcr-1* gene: Is there a historical link?

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The recent discovery of a plasmid-mediated *mcr-1* gene encoding for colistin resistance in *Escherichia coli* and *Klebsiella pneumoniae* from animals, food, and humans in China [1] has initiated the global research of this plasmid in different hosts and different Gram-negative bacteria (GNB) [2].

The *mcr-1* gene has been identified in five continents from bacteria isolated from several origins, including animals, food, the environment, and humans [3, 4]. Several studies, conducted mostly in animals, have reported the identification of the *mcr-1* gene among Extended-Spectrum β-Lactamases (ESBL) producing *E. coli* [5-8]. In a retrospective study, Shen and collaborators reported the identification of the *mcr-1* gene in three *E. coli* strains from chickens in China isolated in the 1980s [9]. To the best of our knowledge, this is the oldest identification of the *mcr-1* gene reported in scientific literature.

Is it possible there is a simultaneous coexistence between ESBL, carbapenemase enzymes, and the *mcr-1* gene?

Historical events concerning the discovery and emergence of plasmid-mediated colistin-resistant bacteria as well as ESBL and carbapenemase genes are traced in Figure 1. Colistin was discovered in 1949 and became available for clinical use in the 1960s for the treatment of GNB [10]. Colistin use was very restricted between 1970 and the late 1990s in humans due to its reported nephrotoxicity and the development of less-toxic antimicrobial agents. However no restriction was reported on colistin use in veterinary medicine during this period [3].

Extended-spectrum (or third-generation) cephalosporins (e.g., cefotaxime, ceftriaxone, ceftazidime) were introduced into clinical use in the early 1980s [11]. These β-lactam antibiotics were regarded as a major advance in the treatment of infection caused by β-lactamase-producing bacteria [12]. However, the emergence of resistance against these antibiotics was observed, with the first report on plasmid-encoded β-lactamase enzymes capable of hydrolyzing the extended-

spectrum cephalosporins in *K. pneumoniae* published in 1983 [11]. This seems to correspond to the first identification of the *mcr-1* gene in *E. coli*, according to Shen and collaborators [9], which indicates a temporal concurrence between the first identification of ESBL enzymes and that of the *mcr-1* gene.

In 1985, the first carbapenems (imipenem) were marketed for the treatment of infections caused by *Enterobacteriaceae*, particularly those producing ESBLs [13, 14]. After a decade of practical use of carbapenems, a strain carrying the plasmid *K. pneumoniae* carbapenemase (KPC-1) was first observed in North Carolina in 1996 before progressively appearing worldwide [15].

The presence of ESBL and carbapenemase genes in the same bacterial strains was reported for the first time in *Klebsiella spp.* collected from October 2006 to November 2007 by the Emory University Hospital Microbiology Laboratory, Atlanta, GA, USA [16]. In this study, authors reported the presence of an ESBL in 19 of 26 (73%) of the KPC isolates [16]. Knowing the technical challenges in identifying ESBL and carbapenemase genes among resistant bacterial strains [17], it is difficult to affirm the absence of these genes before its first description.

The emergence of multidrug-resistant (MDR) GNB and the lack of new antimicrobial agents occurred concurrently with a resurgence of interest in colistin use in human medicine starting in the late 1990s [10].

The first identification of a co-localization of *mcr-1* and ESBL genes on a unique plasmid dates back to 2005 [6]. From 2006 to 2014, Haenni and collaborators reported an increase of the proportion of *mcr-1* genes among ESBL-producing *E. coli* in French calves, from 4.76% to 21.28% in 2006 and 2014 respectively [8]. In these two old bacterial collections, the *mcr-1* gene was detected in ESBL producing isolates likely because these previously identified ESBL isolates or sequences were available in the laboratories, which was not the case for non-ESBL isolates [6, 8]. This may have resulted in the preferential detection of the *mcr-1* gene in these identified

ESBL isolates; non-ESBL isolates in existence could not be tested because they were not available in laboratories [7]. The oldest collection of *E. coli* strains harboring the *mcr-1* gene was collected in China between 1970 and 2014, however we have no information if these isolates are ESBL producing bacteria or not [9].

The prevalence of the *mcr-1* gene among ESBL producing isolates from farm animals was not statistically higher than that found in ESBL-positive *E coli* isolates from humans [7, 18]. In 2009, the New Delhi metallo-beta-lactamase-1 (NDM-1) was discovered – a novel broad-spectrum carbapenemase with the ability to inactivate all β-lactams except aztreonam and with the characteristic of not being inhibited by clavulanic acid [19]. Since 2009, there have been two studies, the first carried out in China [9] and the second in Japan [20], that have both reported a significant increase in *mcr-1* gene prevalence in *E. coli* strains obtained from food animals. This finding was explained by the increased use of colistin in animal production in these two countries over the last few years. The sudden and permanent increase of the *mcr-1* gene over time presents a striking similarity to the increase in the numbers of β-lactamase enzymes identified globally, as previously presented by Davies [21]. More recently, two *E. coli* strains harboring *mcr-1* and carbapenemase genes were isolated from the urine samples of two patients in the United States. The first strain was harboring *mcr-1* and $bla_{\text{CTX-M}}$ genes [22] and the second strain was harboring *mcr-1* and $bla_{\text{NDM-5}}$ genes [23]. In China, two *E. coli* strains coproducing MCR-1 and NDM-1, were recovered from two patients with bloodstream infections [24]. MCR-1 producing *E. coli* coproducing either ESBL, AmpC (CMY-2) cephalosporinase, or NDM-9 enzymes were also isolated from chicken meat [7, 25]. However, in the absence of therapeutic historical data in these studies, it is difficult to determine whether β-lactam or colistin use had greater involvement in the exacerbation of ESBL and carbapenemase enzyme spread. Interestingly, Haenni and collaborators showed an increasing prevalence of the *mcr-1* gene in ESBL isolates from French

calves in spite of a decrease in colistin use in animal husbandry in France [8]. Likewise in Brazil, the *mcr-1* gene was identified at a prevalence of 3 % in *E. coli* strains in poultry that had not been exposed to polymyxin at any point in their lives (around 40 days) [26].

Moreover, in countries where colistin is not approved for veterinary use, such as the United States, it is difficult to accuse animal productions of being responsible for colistin resistance transfer to humans. Even in Europe, studies could not confirm a causal link between animals and humans regarding colistin resistance transfer [7].

Some studies reported that the prevalence of the *mcr-1* gene is more significant in ESBL positive isolates compared to non-ESBL ones [8]. However, given that the identification of ESBL and/or carbapenemase genes in bacteria harboring the *mcr-1* gene was not performed in over 50% of the scientific studies [27], it is difficult to establish a link between ESBL positive or negative isolates and the prevalence of the *mcr-1* gene identified worldwide. Several studies have reported that the prevalence of the *mcr-1* gene was more significant in ESBL positive isolates compared to carbapenemase positive ones [27, 28].

We believe that a historical link has existed between *mcr-1,* ESBL, and carbapenemase genes since the 1980s, however this historical evidence requires confirmation through the identification of the *mcr-1* gene present in several old collections of ESBL-positive strains to trace the kinetics over time between ESBL, carbapenemase, and *mcr-1* genes.

It is reasonable to consider that the use of broad-spectrum cephalosporins or other β-lactam antibiotics in either veterinary or human medicine may have led to colistin resistance. This fact might explain the identification of the *mcr-1* gene in patients in countries where colistin is not approved for farm animals, such as the United States. Moreover, some studies raised the possibility of acquiring ESBL, carbapenemase, and *mcr-1* genes following a stay in endemic

countries and a subsequent human transmission of these genes [29], which might be the case in the United States and others countries.

The re-evaluation of colistin use in livestock, as initiated by several regulatory agencies such as the European Medicines Agency (EMA), needs an overall approach that includes not only colistin use reduction but also the reduction of all antibiotic use, especially those of critical importance for human health.

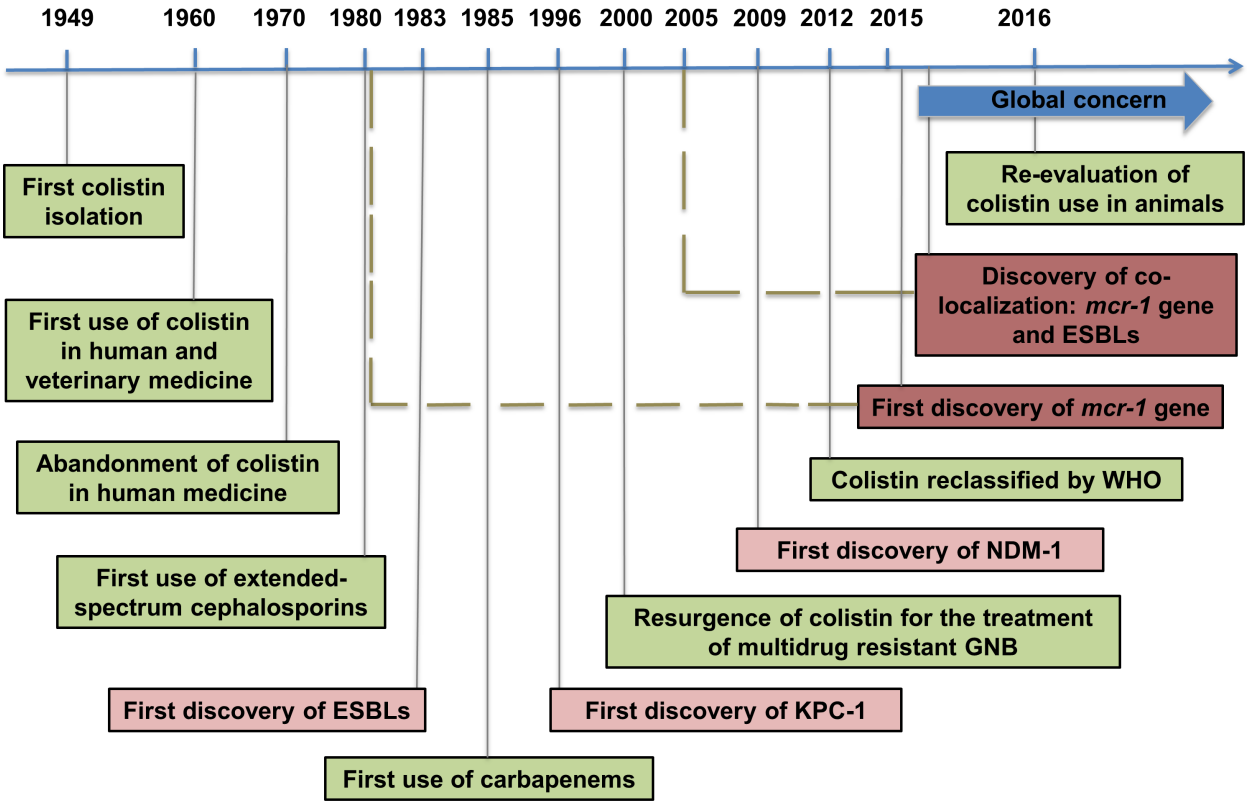

Fig. 1. Schematic illustration of some historical events that combine ESBL and carbapenemase enzyme identification with colistin resistance *mcr-1* gene emergence. ESBL: Extended-Spectrum β-Lactamases. **KPC-1**: *Klebsiella pneumoniae* carbapenemase-1. **GNB**:

Gram-negative bacteria. **NDM-1**: New Delhi metallo-beta-lactamase-1. **WHO**: World Health Organization. Dashed lines indicate a retrospective study.

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Competing interests

The authors declare that they have no competing interests.

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