



Ecology of *bla*_{NDM} and *mcr-1*

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Antibiotic resistance is one of the most worrying threats to global public health today. Numerous common infections are becoming harder, and even impossible, to treat due to the loss of effectiveness of these molecules against resistant bacteria. This is a complex scenario involving multiple elements, and must therefore be confronted with a multidisciplinary approach (1).

Carbapenem-resistant enterobacteriaceae (CRE) are not susceptible to one of the last line antibiotics, carbapenems (2). Consequently, these resistant bacteria are included in the critical group of WHO Priority Pathogen List for Research and Discovery of New Antibiotics (3). New Delhi metallo- β -lactamase (NDM) variants are considered a particularly hazardous resistance mechanism because of its wide range of activity against β -lactams, including carbapenems, and its high dissemination capacity (4). However, despite the worldwide spread of these genes, the scarce use of carbapenems in veterinary medicine, together with the low prevalence of CRE from animal sources, suggests that the dissemination of this resistance mechanism occurs in a human context (5).

Many of CRE are multidrug-resistant (MDR) organisms, since they tend to accumulate additional genetic determinants that confer resistance to other classes of antibiotics, even other last resort molecules, increasing the morbidity and mortality of the infections caused by these organisms (6). Polymyxins, and specifically colistin due to its clinical importance, is one of these last line antibiotics against MDR Gram negative bacteria, and were included in the Critically Important Antimicrobials group (7).

Resistance to colistin has been usually associated to chromosomal mutations, with vertical transmission

and slow evolution (8). However, since the discovery of *mcr-1*, the first colistin resistance mechanism able to be mobilized between bacteria, the effectiveness of this antibiotic is at risk (9). Due to the increasing spread of *mcr-1*-positive Enterobacteriaceae (MCRPE) detected in numerous countries and from diverse sources, colistin was moved to the Highest Priority group of the Critically Important Antimicrobials list (10). Moreover, colistin has been commonly used in veterinary medicine, but not in human infections, where its renal toxicity lead to a limited prescription. For that reason, the possible transmission of *mcr-1* from animal origin has been suggested, although there are not conclusive studies (11).

Considering these facts, the association of *mcr-1* with *bla*_{NDM} in CRE constitutes an alarming situation for both human and animal health, regarding the drastically reduction of available effective treatments against infections caused by these bacteria (12).

Surveillance of these two relevant resistance mechanisms and the elements involved in its dissemination is essential to introduce accurate control measures and reduce their impact in public health. This surveillance must be done under a 'One Health' approach, including samples from human, animal, both companion and production animals, food and environment (13).

The work carried out by Wang *et al.* is a perfect example of application of this approach to characterize the whole scenario of *bla*_{NDM} and *mcr-1* in Chinese poultry production, considering multiple pieces of the puzzle: hatchery and commercial farms (including chicken, wild bird, dog, farmer, flies and sewage samples), a slaughterhouse and supermarkets (14).

In this work, all the CRE isolated, except 14 *E. coli*, were carriers of a *bla*_{NDM} variant, bringing to light the high prevalence (33.2%) of this resistance mechanism to carbapenems in Chinese farming. The 14 CREC that were not *bla*_{NDM}-positive, were neither positive for any known carbapenemase gene, and the underlying resistance mechanism should be deciphered. Among the 161 carbapenem-resistant *Escherichia coli* (CREC), the most frequent variant was *bla*_{NDM-5}, whose high prevalence in Chinese patients has been previously described (15). Interestingly, *bla*_{NDM} genes were not detected in samples from hatchery farms, indicating that the acquisition of CREC by the chicken takes place in a later phase in the production process. On the other hand, the highest occurrence of CREC and MCRPE was identified in commercial farms, which coincides with other studies, remarking the importance of this chicken production phase as a critical stage in the acquisition of these bacteria, probably due to the antibiotic pressure to which they are subjected (16). Moreover, 37 (23%) CREC were also MCRPE, showing the high coexistence of these two dangerous resistance determinants.

In addition to the isolation of CRE and MCRPE, the study included the *bla*_{NDM} and *mcr-1* gene detection directly from the samples. Comparing these results with those obtained from the CREC and MCRPE isolation, the detection rates of the two genes were much higher in the direct screening, increasing a 52% in the case of *bla*_{NDM} in a slaughterhouse and 62.5% regarding *mcr-1* detected in supermarket samples. Supported by this approach, Wang *et al.* coined the term 'phantom resistome' referring the resistance determinants that are present in a sample but are not able to be identified by phenotypic testing of the isolates. This 'phantom resistome' constitutes an important reservoir and can be part of the origin of dissemination of these resistance mechanisms, as it has been demonstrated in previous studies where the resistance gene from susceptible bacteria was successfully transferred conferring resistance to the recipient (17). Additionally, the detection of *bla*_{NDM} and *mcr-1* in this 'phantom resistome' remains stable at extremely elevated levels throughout time, which indicates that this reservoir is a constant risk to gene dissemination and specific control measures must be taken in order to mitigate and reduce its spread.

In-depth analysis of the presence of *bla*_{NDM} and *mcr-1* in different elements involved in one of the farms evaluated revealed very high rates of these genes in dogs, wild birds, farmers and flies, bringing to light that resistance gene

dissemination is not a confined phenomenon that takes place in the chicken and their products, but all the elements linked to the production chain. Regarding specifically the presence of CREC in flies, the rates decrease as the distance from the chicken house increased, showing that the physical location is a focal point and the flies a vehicle responsible for gene propagation. Studies of this kind provide data about resistance gene flow, the interconnected elements and the processes between them, which is highly valuable, especially for the food chain (18).

*bla*_{NDM} and *mcr-1* were not located in the same plasmid in any case, which, attending to the high rates of occurrence of both resistance mechanisms, reflects the remarkable success in the dissemination of these genes independently. This fact is supported by the elevated conjugation frequencies obtained, reaching 3.2×10^{-1} and 5.0×10^{-3} cells per donor cell in the case of *bla*_{NDM}- and *mcr-1*-carrier plasmids, respectively. Furthermore, the co-transfer of both *bla*_{NDM} and *mcr-1*-carrier plasmids was observed in 5 strains, demonstrating the relatively high frequency (13.5%) of this threatening event, which confers resistance to many clinically important molecules. The effective association of resistance genes and the plasmids involved in its dissemination must be analysed in order to control their spread at all levels (19).

Regarding the antimicrobial susceptibility testing results, a high proportion of CREC were resistant to other important antibiotics apart from carbapenems and colistin, such as gentamicin (79.5%) or ciprofloxacin (99.4%), which emphasizes the multi-drug resistance profile that this kind of bacteria usually shows. However, all of them were susceptible to tigecycline, the only remaining effective antibiotic in many *bla*_{NDM}- and *mcr-1*-carrier strains (2).

To totally characterize the genomic organization of CREC, and therefore the structures involved in the mobilization and dissemination of *bla*_{NDM} and *mcr-1*, whole-genome sequencing (WGS) was performed by Wang *et al.* This approach reveals the association between the different elements and assigns the antimicrobial resistance profiles to the surveillance of these prevalent MDR clones (20). Moreover, the inclusion of carbapenem-susceptible *E. coli* in the WGS analysis makes it possible to compare between these strains and the CREC, which determines the molecular features that influence in the possession of these resistance mechanisms.

The most frequent sequence type (ST) identified in *bla*_{NDM}-carriers *E. coli* (minus *mcr-1*) was ST101, which has been frequently related to NDM-producer *E. coli*

worldwide, supporting the notion of a successful association between this resistance mechanism and this specific ST (21). In addition, ST156 *E. coli* was detected in diverse sources, from chicken farms (including chickens, dogs, flies and wild birds), the slaughterhouse and supermarkets, showing the broad distribution of this sequence profile in the Chinese chicken production chain. This exact ST has been described in a *bla*_{NDM-5}-carrier *E. coli*, the most prevalent *bla*_{NDM} variant detected by Wang *et al.*, that also harboured *mcr-1*, from a duck in China (22). These findings suggest the tight link between these two resistance genes and ST156, which seems to be the main responsible genotype involved in the spread of *bla*_{NDM-5} and *mcr-1* in the poultry production context. Furthermore, *E. coli* belonging to ST156 presented a high commonality based on core-genome single nucleotide polymorphism (SNP) analysis, strongly confirming the implication of this clone in the dissemination of *bla*_{NDM} and, also, *mcr-1*, being in this group the majority of *bla*_{NDM} and *mcr-1* co-carrier *E. coli* isolates. Other global *bla*_{NDM}-carrier CREC from human infections were incorporated in the comparison and showed the same genotypes than those identified in the analysis performed by Yang Wang *et al.* This demonstrates the clonal relationship between NDM-producer *E. coli* worldwide, even connecting chicken production with human disease.

*bla*_{NDM}-carrying mobile genetic elements (MGEs) of the 161 *bla*_{NDM}-positive CREC were clustered in three main types (I, II and III) that were well characterized in the study. These three types are present in different STs isolated from diverse sources, suggesting that there is not a specific link between these *bla*_{NDM}-associated MGEs and the STs identified. However, the type II was present in more than the half of *bla*_{NDM}-positive CREC (52.17%), belonging to 15 different STs and from all kind of samples collected, and being the most prevalent MGE connected to this gene. Interestingly, the most prevalent STs among *bla*_{NDM}-positive *E. coli*, ST101 and ST156, were the only two STs in which the three MGE types were detected. This indicates a special capacity of these two STs to be associated with diverse MGE responsible for this resistance gene dissemination. Additionally, the most linked MGEs related to ST156 and found in most kind of samples were type I and III, pointing out that these MGEs-ST complex can constitute high-risk combinations for the dissemination of *bla*_{NDM} in the chicken production chain.

According to the genetic context of *mcr-1*, the analysed *E. coli* were classified into four types (A, B, C and D). Type A was the most prevalent genetic structure associated to

mcr-1 (88.64%), sharing a high identity with the *mcr-1*-adjacent elements in the pHNSHP45 characterized by Liu *et al.* (9) However, this structure does not contain *IS**Apl1*, a mobilization component that has been frequently linked to *mcr-1* (23). Type A genetic context is present in all MCRPE analysed belonging to ST156, which are from different kind of samples recovered throughout Chinese chicken production chain. These finding alerts about the spread capacity of this resistance gene associated to this genetic structure and contained in this specific ST. Remarkably, the type C genetic environment showed the integration of *mcr-1* in the chromosome, including the *IS**Apl1* element. This event has been described before and supports the idea that *IS**Apl1* is one of the main causes implicated in the global dissemination of *mcr-1* (24).

Altogether, the results from Wang *et al.* reveal the presence of *bla*_{NDM} integrated in a type III MGE that is found in numerous ST156 *E. coli* that are disseminated in diverse elements of the Chinese chicken production chain. Furthermore, most of these ST156 *E. coli* also harbour the *mcr-1* gene, making this resistance determinants-genetic contexts- ST association a very worrying concern that menaces the clinical effectiveness of most last resort antibiotics.

Commercial farms were one of the main critical points in the dissemination of *bla*_{NDM} and *mcr-1* in the Chinese poultry industry, where chicken and dogs are the major sources of these genes, but also flies suppose a focus for *mcr-1* dissemination. The potential role of flies in the dissemination of these resistance genes is a particularly alarming issue, since it constitutes a link between a broad range of elements of the process, including the environment, as it has been shown previously (25). Beyond this local spread, the detection of *bla*_{NDM} and *mcr-1* in wild birds with migratory behaviour to long distance destinies implies the possibility of dissemination of these resistance mechanisms to completely new areas, including new elements in this transmission chain, making it a problem on a larger scale (26).

From commercial farms, the levels of *bla*_{NDM} and *mcr-1* keep relatively constant in the subsequent stages of chicken production chain, reaching supermarket products. This fact exposes the relevance of this kind of studies to look into resistance gene dissemination routes and the involved elements. The application of adequate control measures at diverse levels based on this knowledge can reduce and prevent the spread of these resistance mechanisms, safeguarding the clinical effectiveness of last resort

antibiotics such as carbapenems and colistin (27).

At present, carbapenems are included in the Critically Important Antimicrobials list, but not in the Highest Priority group due to the limited transmission of CRE from non-human sources (10). Considering the results observed by Wang *et al.*, the inclusion of carbapenems in this category should be evaluated, since these resistant bacteria probably have a human origin, but the animal and environmental components of food chain are sources of dissemination of MDR bacteria that can reach the human context, being a serious global concern for Public Health.

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