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Commentary

Colistin resistance, beyond the current knowledge

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Colistin is an antibiotic that has recently been 'rediscovered' in human medicine due to the problems encountered in the treatment of multidrug-resistant Gram-negative bacteria. Its use was largely limited to food-producing animals, mainly pigs, for the treatment of intestinal infections. However, other uses have been reported across the world. While colistin has not been used in the US, it is used as a therapeutic agent in food-producing animals in Europe, and also as a livestock growth-promoter in several Asian countries.

Until very recently, there was no transferable colistin resistance known and few researchers were interested in studying colistin resistance in general. Only after the first discovery of the *mcr*-1 gene [1], did people get interested and many manuscripts were published, generating slightly random and difficult to place data often on already sequenced bacteria. To date, the epidemiology of *mcr*-mediated resistance remains largely unclear and new well-structured studies need to be performed to gain a better view on the epidemiology of this resistance, including mutation-mediated resistance. Since most studies focus now on *mcr*-mediated resistance, mutation-mediated resistance has been neglected. However, its importance in the spread of colistin resistance should not be underestimated.

The diversity of *mcr*-genes is being increasingly appreciated due to ongoing discoveries. *mcr*-resistance genes have been found all over the world, including countries with zero to marginal use of colistin. To date, eight different *mcr*-genes have been discovered, of which *mcr*-8 has been reported only very recently [2]. They have been associated with humans, and a wide variety of animals, including flies [3, 4]. The prevalence of the different genes differs substantially when using culture or non-culture techniques. Furthermore, each of these *mcr*-genes has multiple variants [3, 4, 5, 6]. Taken together, this indicates that *mcr*-related colistin resistance mechanisms have been around for a while, but have only been discovered recently. This was probably because colistin was, until recently, only used in veterinary medicine. In this context there were no clinical resistance problems detected as the veterinary clinical labs were mainly using the disk diffusion test that is not suitable for detecting colistin resistance in a reliable way [7]. To date, colistin resistance has been mainly been detected in commensal bacteria and few studies have been looking in animal pathogens. In 2010 there was, however, a manuscript showing a sharp increase in resistance in pathogenic *E. coli* from animals [7]. Unfortunately, at that time no further investigations were performed to understand this increase.

In a study recently published in *EBioMedicine* [8], the investigators looked specifically at *mcr-3* prevalence in pigs using PCR, and found big variation between farms. Few studies have focused on the prevalence of a specific *mcr* gene by PCR, but those that used a non-culture method found a higher prevalence and larger variety of *mcr*-genes in a wide variety of animal and human samples. Methodological differences also account for differences in results and this should be taken into account when performing prevalence studies. It is clear that in order to fully understand the epidemiology of *mcr*-related colistin resistance, we need well standardized studies on prevalence, variants, location (plasmids) and clonal lineages of *mcr* genes in different parts of the world including those countries in which colistin is only marginally used.

The question remains where do these genes come from? There are clear indications that they have been mobilized from certain bacterial species, and the presence of insertion sequences as shown in this study is indicative of that [8]. It is also clear that the presence of insertion sequences have led to the fact that *mcr*-genes can be found on different plasmids, though more studies are necessary to determine on which plasmids these genes have spread. When these events happened is also not clear, but given the wide variety of *mcr*-variants, it could have been long ago, or alternatively, it could be that these genes have a high evolution rate. Structure-activity relationships and molecular interactions may bring insight regarding possible evolutionary capabilities, though it currently remains unclear what the drivers for this evolution are. The genes may be adapting to the codon usage of the new host. It is clear by this study of Xu et al., as well as previous studies performed by this group, that we can talk now about a functional and mechanistic similarity between the different *mcr*-endoded lipid A phosphoenolamine transferases. Similar studies on the newly discovered *mcr* genes are necessary to confirm this notion.

Colistin and polymyxin B are both cationic polypeptide antibiotics and are related to the cationic peptides of the innate immunity (eg. defensins, manganins). A relationship between the susceptibility to these cationic peptides and polymyxines has been demonstrated before, however, this was mutation-mediated resistance [9]. Whether *mcr*-mediated resistance also lowers susceptibility of cationic peptides of

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the innate immunity still needs to be investigated. This resemblance may be one of the reasons why colistin resistance was so hard to arise. If bacteria acquiring colistin resistance also became resistant to the innate immune system, these bacteria could become very dangerous pathogens. There are certainly more studies required to elucidate how these lipid A phosphoenolamine transferases operate and what their effect is on the virulence of bacteria. To the best of our knowledge, such studies have not been performed with *mcr*-positive strains, and are warranted.

In conclusion, we are still unaware of the true spread and variability of *mcr*-related as well as mutation-mediated polymyxin resistance. Well-designed studies are necessary to explore this. We are also still unaware of all of the secondary effects of this resistance regarding the viability and virulence of colistin-resistant bacteria. Further studies are necessary.

Disclosure

None.

Conflict of interest

The authors declare no conflicts of interest.

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