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How many *mcr-1*-harbouring bacteria were spreading geographically?

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Abstract

Colistin is widely used as an antibiotic of last resort for treating infections caused by multidrug-resistant gram-negative bacteria such as carbapenemase-producing *Enterobacteriaceae*. Recently, the emergence of plasmid-mediated (horizontally-transferable) colistin resistance *(mcr-1*) has become a great challenge to global public health. The *mcr-1* gene was detected in ESBL (Extended Spectrum β-Lactamase)-producing and/or carbapenemase-producing *Enterobacteriaceae*. Therefore, there is a huge risk of the emergence of pan-drug-resistant gram-negative bacteria. In this paper we discuss the epidemiological analyses of *mcr-1* positive *Enterobacteriaceae* and structural analyses of PmrC that was recently identified as a protein associated with colistin resistance.

Keywords: Plasmid-mediated colistin resistance, *Mcr-1*, Epidemiology, Dissemination, PmrC.

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Introduction

If antibiotic resistant pathogens remain unchecked, it is estimated that by 2050 the global mortality attributed to antibiotic-resistant bacterial infections will soar to 10 million, at a cost of over $100 trillion (http://amr-review.org/). The spread of carbapenemase-producing *Enterobacteriaceae* is a significant threat to public health. For serious infections caused by carbapenemase-producing *Enterobacteriaceae*, the treatment options are restricted and invariably rely on tigecycline and colistin [1]. Therefore, the global increase in carbapenemase-producing *Enterobacteriaceae* has resulted in increased use of colistin with the inevitable risk of emerging resistance. Colistin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. However, a plasmid-mediated (horizontally-transferable) colistin resistance (*mcr-1*) gene was recently reported in China [1] and subsequently detected in Asia (Vietnam, Laos, Thailand, Cambodia, Malaysia, Singapore, Taiwan and Japan), Europe (The Netherlands, Germany, Belgium, Switzerland, France, Denmark, United Kingdom, Spain, Italy, Sweden and Portugal), Africa (Algeria, Egypt, South Africa and Tunisia), and America (Canada, Argentina and Brazil) (Figure 1) [2-47]. We also found two *Escherichia coli* isolates harbouring *mcr-1* gene (GenBank accession no. KU886144 from a human being in Ecuador and GenBank accession no. KU743383 from a pig slurry in Estonia) in the public NCBI database. To investigate how many *mcr-1* positive *Enterobacteriaceae* have been spreading globally, we analysed all these findings that were searched from the following databases PubMed, Medline, Embase, NCBI, and Google Scholar as of 4th May 2016. The analysis results showed the following important aspects: (i) the *mcr-1*-harboring bacteria had spread to most continents; (ii) four further studies are needed to fight against plasmid-mediated (horizontally-transferable) colistin resistance, particularly in pan-drug-resistant gram-negative bacteria.

Recent global dissemination of mcr-1-harbouring Enterobacteriaceae

As of 4th May 2016, 863 *mcr-1* positive *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae, Salmonella enterica, Enterobacter aerogenes, Enterobacter cloacae*, human gut microorganisms and so on) were detected globally and *mcr-1*-harbouring bacteria have mainly spread in Asia (n=550) and Europe (n=224) (Figure 1) [1-42]. Because in most studies *mcr-1* carriers have been identified from random sample collections, the reliable prevalence of *mcr-1* positive isolates is not known. In America, only 29 isolates (or 60 in Africa) were detected in animal and/or human being [11,19,21,43-47].

550 (63.7%) of 863 *mcr-1* positive *Enterobacteriaceae* were detected in Asia [1,9,13,15-17,20,21,27-32,35-41] [26.0% in

Europe [2-8,10,12,14,17,22-26,33,34,42], 7.0% in Africa [11,21,43,44,46] and 3.3% in America [19,45,47]. *mcr-1* positive isolates were distributed to adjacent countries in Asia (or Europe), suggesting the easy spread by a potential travel dissemination pathway as well as a possible dissemination pathway through international trade of foods.

Of note, the spread between Asia and Europe might be allowed by a potential travel dissemination pathway (from China, Vietnam, Laos, Thailand and Cambodia to Netherlands), a potential dissemination pathway by international trade of food (fresh vegetables; from Thailand and Vietnam to Switzerland) or a potential dissemination pathway speculated by the sequence comparison of the isolated plasmids (from China to Portugal) (Figure 1) [4,33,42]. The *mcr-1* dissemination between Europe and North Africa might be allowed by a potential travel dissemination pathway (from Tunisia to Netherlands) or a potential dissemination pathway through international trade of foods (chickens, from France to Tunisia) (Figure 1) [4,11].

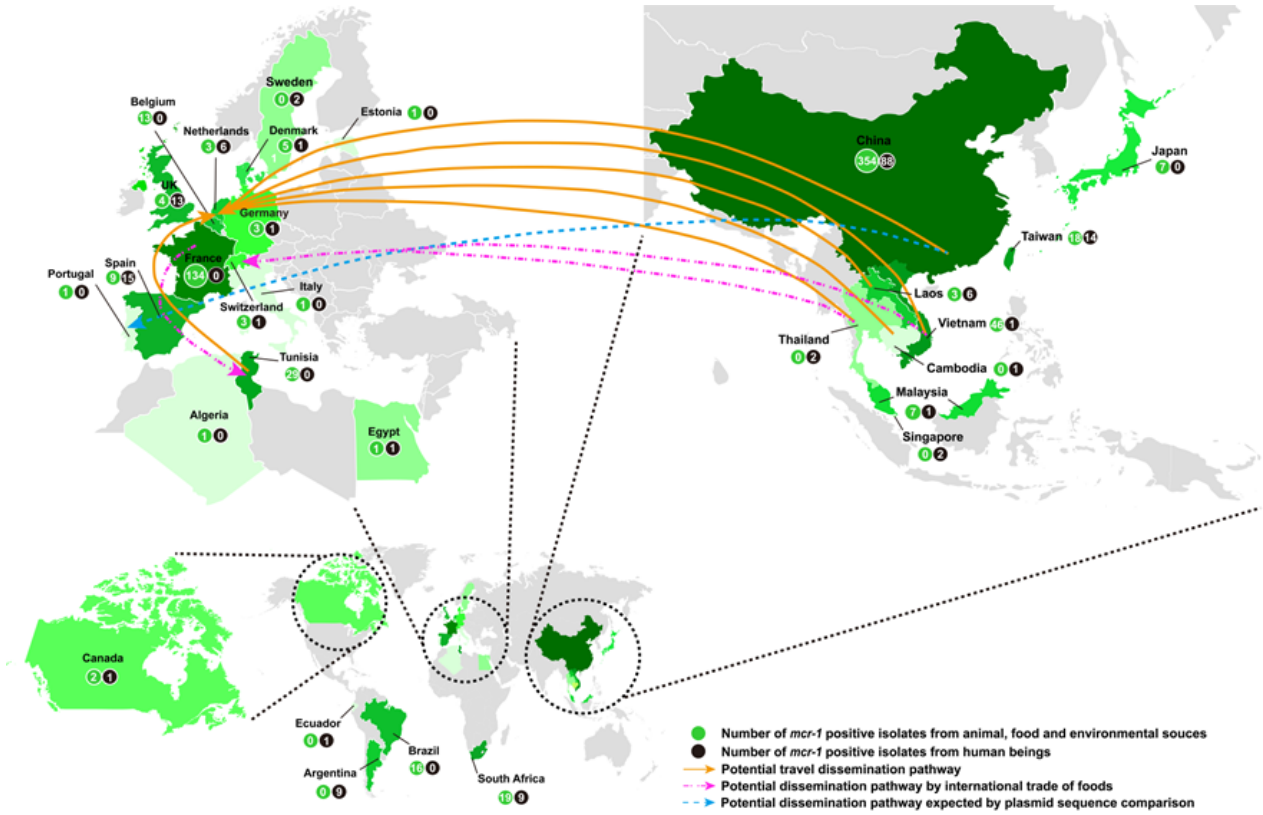

Figure 1. *Epidemiological features of mcr-1-harbouring Enterobacteriaceae (as of 4th May 2016). The size of circle is not proportional to the amount of mcr-1 positive isolates. The deeper color means the more amounts of mcr-1 positive isolates.*

Scope for further studies

First, *mcr-1* positive isolates from human beings were detected after the dissemination of New Delhi Metallo-β-lactamase-1 (NDM-1) positive *Enterobacteriaceae* that were susceptible to tigecycline and colistin. The limitation of colistin use in treatment of infection via ESBL-producing/*mcr-1*-harbouring gram-negative bacteria [4,7,10-12,14,19,29,30,35,38] or carbapenemase-producing/*mcr-1*-harbouring Gram-negative bacteria [9,24,35] may increase tigecycline use and then the possibility of emergence of tigecycline resistance mechanisms other than an efflux pump. To prevent the emergence of a pan-drug resistance in gram-negative bacteria, the continued monitoring of colistin and/or tigecycline resistance and their underlining mechanisms in human, animal, food and environmental sources have to be required.

Second, 162 (18.8%) of 863 *mcr-1* positive *Enterobacteriaceae* were investigated about plasmids associated with *mcr-1* gene and harboured a low variety of plasmids (IncI2, IncHI1, IncHI2, IncP, IncFI and IncX4) [4,9-13,18,19,23,24,30,32,33,35,38,41]. To clarify the diversity of the plasmid backbones spreading *mcr-1* gene within the remaining 701 isolates or isolates detected in the future, additional studies about the plasmids (or the mobile elements) are needed. Like chromosomal localization of the commonly plasmid-borne *qnrB* (plasmid-mediated quinolone resistance) genes, the chromosomal location of *mcr-1* gene associated with a mobile element such as *ISApl1* may be observed in the near future. In addition to the plasmid itself, the mobile element alone can represent the transfer mechanism of *mcr-1* into *Enterobacteriaceae*.

Finally, *mcr-1* gene was not detected in 803 (48.2%) of 1,666 colistin resistant *Enterobacteriaceae* [1-44,46,47], suggesting that they might potentially harbour other colistin-resistant mechanism (probably, new groups of MCR) or even novel *mcr-1* alleles. The pmrC gene was recently identified as a gene

associated with colistin resistance by our group [48]. Homology modelling of *MCR-1* and PmrC were performed by an automated homology modelling approach using LptA (PDB ID 4KAY) and EptC (PDB ID 4TN0) as templates in Swiss-Model (http://swissmodel.expasy.org/) program. Although PmrC showed 29% amino acid sequence identity to the *MCR-1* sequence, the Root Mean Square Deviation (RMSD) of the C_α trace between PmrC and *MCR-1* models was 1.0 Å, supporting that PmrC structure was quite similar to *MCR-1* structure as described in the supplementary appendix (Figure S1, available as supplementary material). Structure-based alignment of PmrC, *MCR-1*, LptA and EptC revealed that key residues identified as to be important to the catalytic activity of LptA and EptC are conserved in PmrC and *MCR-1* (Figure S2, available as Supplementary Material). Taken together, the PmrC predicted structure is consistent with a lipid A phosphoethanolamine transferase, which was functionally confirmed by lipid A analysis as previously described [48]. Therefore, the PmrC may probably be another group of MCR. However, further studies are needed to confirm the hypothesis that PmrC may be a new group of MCR in addition to *MCR-1* group and continuously monitor another group of MCR.

Supplementary Data

Figures S1 and S2 are available as Supplementary Materials.

Conflict of Interests

The authors declare that they have no competing interests.

Acknowledgments

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