Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

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Summary

Background **Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.**

Methods **The *mcr-1* gene in *E coli* strain SHP45 was identified by whole plasmid sequencing and subcloning. MCR-1 mechanistic studies were done with sequence comparisons, homology modelling, and electrospray ionisation mass spectrometry. The prevalence of *mcr-1* was investigated in *E coli* and *Klebsiella pneumoniae* strains collected from five provinces between April, 2011, and November, 2014. The ability of MCR-1 to confer polymyxin resistance in vivo was examined in a murine thigh model.**

Findings **Polymyxin resistance was shown to be singularly due to the plasmid-mediated *mcr-1* gene. The plasmid carrying *mcr-1* was mobilised to an *E coli* recipient at a frequency of 10^{-1} to 10^{-3} cells per recipient cell by conjugation, and maintained in *K pneumoniae* and *Pseudomonas aeruginosa*. In an in-vivo model, production of MCR-1 negated the efficacy of colistin. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression in *E coli* resulting in the addition of phosphoethanolamine to lipid A. We observed *mcr-1* carriage in *E coli* isolates collected from 78 (15%) of 523 samples of raw meat and 166 (21%) of 804 animals during 2011–14, and 16 (1%) of 1322 samples from inpatients with infection.**

Interpretation **The emergence of MCR-1 heralds the breach of the last group of antibiotics, polymyxins, by plasmid-mediated resistance. Although currently confined to China, MCR-1 is likely to emulate other global resistance mechanisms such as NDM-1. Our findings emphasise the urgent need for coordinated global action in the fight against pan-drug-resistant Gram-negative bacteria.**

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Introduction

Antimicrobial resistance is now recognised as one of the most serious global threats to human health in the 21st century. There is now evidence of political traction, with endorsements of statements by the WHO and US Centers for Disease Control and Prevention describing a global crisis and an impending catastrophe of a return to the pre-antibiotic era.[1–4] These serious concerns have been catalysed by the rapid increase in carbapenemase-producing Enterobacteriaceae expressing enzymes such as KPC-2 (*Klebsiella pneumoniae* carbapenemase-2) and NDM-1 (New Delhi metallo-β-lactamase-1).[5,6] For serious infections caused by carbapenemase-producing Enterobacteriaceae, the treatment options are restricted and invariably rely on tigecycline and colistin—either singularly or in combination with other antibiotics.[7,8] Thus the global increase in carbapenemase-producing Enterobacteriaceae has resulted in increased use of colistin with the inevitable risk of emerging resistance.[9] This delicate balance between clinical necessity and prevention of resistance is further compromised by agricultural use of human antibiotics, where some countries have actively used colistin in animal production.[10]

Colistin belongs to the family of polymyxins, cationic polypeptides, with broad-spectrum activity against Gram-negative bacteria, including most species of the family Enterobacteriaceae. The two polymyxins currently in clinical use are polymyxin B and polymyxin E (colistin), which differ only by one aminoacid from each other and have comparable biological activity. The mechanism of resistance to polymyxins is modification of lipid A, resulting in reduction of polymyxin affinity. Until now, all reported polymyxin resistance mechanisms are chromosomally mediated, and involve modulation of two component regulatory systems (eg, *pmrAB*, *phoPQ*, and its negative regulator *mgrB* in the case of *K pneumoniae*) leading to modification of lipid A with moieties such as

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Research in context

Evidence before this study

On Aug 15, 2015, we searched PubMed with the terms "*E coli* and colistin resistance", "*Klebsiella pneumoniae* and colistin resistance", "*Klebsiella* and colistin resistance", "China and colistin", and "plasmid mediated colistin resistance" for reports published between Jan 1, 2000, and Aug 15, 2015, with no language restrictions. Our search identified no results of relevance to this study. We also searched with the terms "*E coli* and colistin resistance" and "*Klebsiella* and colistin resistance" and found no reports of plasmid-mediated colistin resistance, novel mechanisms of colistin resistance, and in-vivo resistance.

We monitored the prevalence of antimicrobial resistance of *Escherichia coli* from food animals annually and found an increase of colistin resistance in recent years. From the published literature, we know that no plasmid-mediated colistin resistance mechanism has been reported.

Added value of this study

This study reports data for the following: the first report of plasmid-mediated colistin resistance (designated *mcr-1*), the proportion of *mcr-1*-positive samples in animals and human beings, rapid dissemination of *mcr-1* between Gram-negative strains, in-vivo colistin resistance mediated by *mcr-1*, MCR-1 modification of lipid A and mediating colistin resistance, structural modelling on MCR-1, and sequencing of a *mcr-1*-positive plasmid.

Implications of all the available evidence

The emergence of *mcr-1* heralds the breach of the last group of antibiotics, polymyxins, by plasmid-mediated resistance. Although currently confined to China, *mcr-1* is likely to spread further. Further surveillance and molecular epidemiological studies on the distribution and dissemination of *mcr-1* are urgently required, along with the re-evaluation of the use of polymyxins in animals. Our findings highlight the urgent need for coordinated global action in the fight against extensively-resistant and pan-resistant Gram-negative bacteria.

phosphoethanolamine or 4-amino-4-arabinose, or in rare instances total loss of the lipopolysaccharide.[11–13] Thus far, the polymyxins remain one of the last classes of antibiotics in which resistance is not known to spread from cell to cell (ie, plasmid mediated).

We report the first case of a plasmid-mediated colistin resistance mechanism, designated MCR-1. We describe its putative structure, mechanism of action, and its emergence in Enterobacteriaceae from animal and human isolates, and provide evidence for the spread of *mcr-1* from the veterinary sector to human beings.

Methods

Strains

In our routine surveillance project on antimicrobial resistance in commensal *E coli* from food animals, we noted a major increase of colistin resistance in China in recent years. We suspected that the recent rapid increase of colistin resistance might be plasmid mediated because polymyxin resistance is difficult to generate in *E coli* strains by chromosomal mutations. Thus, one *E coli* strain (designated SHP45, minimum inhibitory concentration [MIC] of colistin of 8 mg/L and of polymyxin B of 4 mg/L) was randomly selected for conjugation experiment. Susceptibility testing to a wide range of antibiotics is routinely undertaken and polymyxin susceptibility testing is done via agar dilution method. SHP45 was recovered from an intensive pig farm (Shanghai, China) in July, 2013, and showed resistance to most classes of antibiotics apart from the carbapenems. *pmrAB*, *phoPQ*, and *mgrB* genes of SHP45 were amplified and sequenced with the primers listed in the appendix (p 1).

See Online for appendix

Clinical isolates (902 *E coli* and 420 *K pneumoniae*) were collected from two tertiary hospitals in Guangdong and Zhejiang provinces (figure 1). Although these isolates were retrospectively screened and patient data are not included in this study, ethical approval was nonetheless sought and obtained. Details of sites of infection for *E coli* and *K pneumoniae* isolates are shown in the appendix (p 2). These isolates were screened for the presence of *mcr-1* by PCR with the primers CLR5-F (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-R (5′-CTTGGTCGGTCTGTA GGG-3′) and amplicons were subsequently sequenced. Samples from pigs at slaughter were collected from two slaughterhouses processing pigs from different intensive farms located in Guangdong, Guangxi, Hunan, and Jiangxi provinces during 2012–14. No more than five rectal swab samples were collected from the same pig farm. Retail meat (pork and chicken) samples were randomly collected from 30 open markets and 27 supermarkets located in seven regions of Guangzhou (figure 1) during 2011–14. Only one isolate was collected from each animal or retail meat sample. These isolates were all randomly collected without any selection criteria. 804 isolates from pigs at slaughter and 523 isolates from raw meat were screened to understand the spread of *mrc-1* in food animals and food.

Porcine *E coli* strain SHP45, *E coli* C600, *Pseudomonas aeruginosa* HE26, *E coli* W3110, *E coli* E11 (ST131), *K pneumoniae* MPC11, and *K pneumoniae* 1202 (ST11) were chosen for the conjugation, transformation, and stability studies. *E coli* 363R (wild-type, *mcr-1* positive) was chosen for the in-vivo murine model because it came from an inpatient and we were simulating human colistin dosing in this model. Additionally, we were able to cure the *mcr-1* plasmid from 363R, to create 363S, and thereby any difference that was noted to be due to colistin dosing in the model was due to the presence of

the plasmid only. All genetic modification of organisms described in this report is covered by the genetically modified organisms licence held at China Agricultural University and was done in the BSL-2 laboratory approved by Beijing Institute of Animal Health Supervision. All experimental proceduress followed biosafety procedures and were approved by Beijing Institute of Animal Health Supervision. All bacteria were grown in Mueller Hinton broth supplemented with antimicrobial agents as appropriate.

Procedures

Molecular biology and antimicrobial susceptibility testing

The transfer frequency of polymyxin resistance was investigated by conjugation experiments with streptomycin-resistant *E coli* C600 as the recipient strain. Transconjugants were selected on MacConkey agar plates supplemented with colistin (2 mg/L) and streptomycin (2000 mg/L). Transfer frequencies were calculated as the number of transconjugants obtained per recipient. The polymyxin resistance plasmid, designated pHNSHP45, was extracted from the transconjugant and used to transform polymyxin-susceptible strains from different species—namely, *E coli* E11 (ST131), *K pneumoniae* MPC11, *K pneumoniae* 1202 (ST11), and *P aeruginosa* FE26 by electroporation and selection with 2 mg/L of colistin. *E coli* 363R (wild-type, *mcr-1* positive) was cured to an *mcr-1*-negative genotype (363S) by passing in subminimum inhibitory concentrations of novobicin and screened for the presence of *mcr-1* as previously described.[5] Plasmid stability was assessed in daily serial passages of culture without antibiotic and the culture daily analysed for colistin resistance and confirmed by presence of *mcr-1* with DNA probing. Plasmid analysis was carried out by nuclease digestion and pulsed-field gel electrophoresis as previously described,[5] and subsequently probed with the *mcr-1* DNA fragment. Susceptibility testing was done by agar dilution on various antibiotics and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (version 5.0).[14]

Plasmid sequencing and identification of plasmid-mediated colistin resistance determinant

Plasmid pHNSHP45 was extracted from the *E coli* transconjugant with the Qiagen Midi kit (Qiagen, Hilden, Germany) and sequenced (Mi Seq, Illumina, San Diego, CA, USA), producing 400-bp paired-end reads (Majorbio Company, Shanghai, China). A draft assembly of the plasmid was made with GS De Novo Assembler (Brandford, CT, USA), which produced a single contig. Gene prediction and annotation were done with Glimmer 3.02 and BLAST. IncI2 plasmid pHN1122-1[15] (GenBank accession number JN797501) was used as the reference plasmid for annotation. To confirm the role of the putative polymyxin resistance gene, a roughly 2000-bp DNA fragment, including the putative polymyxin resistance gene, designated *mcr-1,* and its flanking sequence were ligated into a cloning vector pUC18 yielding pUC18-*mcr-1*. pUC18-*mcr-1* was then used to transform *E coli* W3110 by electroporation.

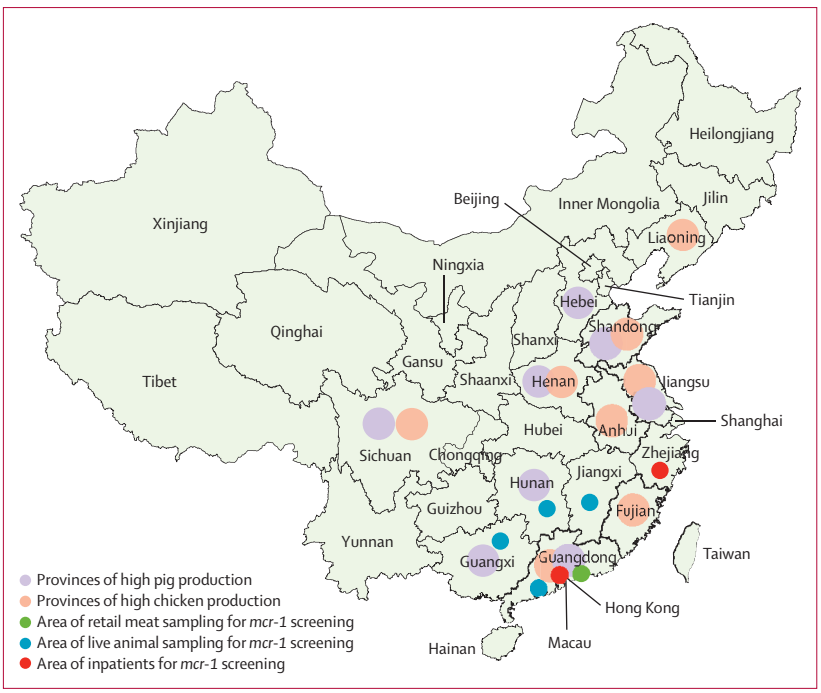

Figure 1: **Map of China**

Analysis of lipid A by mass spectrometry and protein modelling

Lipid A was isolated from the *E coli* transformants by the modified Bligh-Dyer method as previously described.[16] Extracted lipid A was dissolved in chloroform/methanol (4:1) and subjected to electrospray ionisation mass spectrometry (MALDI SYNAPT Q- TOF MS, Water Corp, Milford, MA, USA) in the negative ion mode. Data acquisition and analysis were done with MassLynx V4.1 software (Water Corp, Milford, MA, USA).

A homology model of the complete MCR-1 protein was constructed with the i-Tasser server (appendix p 5).[17] The presence of transmembrane regions was investigated with transmembrane predictions and transmembrane helices Markov model.[18,19]

For more on **i-Tasser** see http://zhanglab.ccmb.med.umich.edu/I-TASSER/

In-vivo analysis on the contribution of MCR-1 to colistin resistance

These studies were done in BALB/c mice and colistin dosing delivered in a similar manner as previously described. Experimental details are fully described in the appendix (p 3).

For more on **Glimmer** see http://www.cbcb.umd.edu/software/glimmer/

For more on **BLAST** see http://blast.ncbi.nlm.nih.gov/Blast.cgi

Role of the funding source

The funder had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to

all the data in the study and had final responsibility for the decision to submit for publication.

Results

The index *mcr-1*-positive *E coli* SHP45 was used for initial plasmid transfer experiments. pHNSHP45 was successfully transferred from *E coli* SHP45 to *E coli* C600 conferring a minimum inhibitory concentration of colistin of 8 mg/L (table 1). To identify the gene responsible for polymyxin resistance, pHNSHP45 was completely sequenced, which revealed a plasmid 64 015 bp in size with an average GC content of 43·0%. pHNSHP45 contains 60 predicted open reading frames (figure 2) and possesses a typical IncI2-type backbone (57 624 bp) encoding replication, horizontal transfer, maintenance, and stability functions (figure 2). In addition, a 1626-bp open reading frame with 49% GC content that was named *mcr-1* was found located downstream of an insertion sequence (figure 2). The nucleotide sequence of pHNSHP45 has been submitted to GenBank with accession number KP347127.

The ability of pHNSHP45 (*mcr-1*) to transfer colistin resistance between relevant species and genera was assessed by conjugation and transformation. Experiments with *E coli* strains showed that polymyxin resistance could be successfully transferred from *E coli* SHP45 to *E coli* C600 at a frequency of 10^{-1} to 10^{-3} cells per recipient cell by conjugation, with the minimum inhibitory concentrations for colistin and polymyxin B for the transconjugant increasing eight-fold to 16-fold compared with the untransformed control (table 1). No other antimicrobial resistance was co-transferred with pHNSHP45 to the recipient strain. pHNSHP45 could be transferred via transformation, but not conjugation, into *K pneumoniae* 1202 (ST11) producing KPC-2, *E coli* E11 (ST131) producing KPC-2, *K pneumoniae* MPC11, and *P aeruginosa* strain HE26, giving 8-fold to 16-fold increases in colistin's minimum inhibitory concentrations (table 1). The stability of pHNSHP45 was examined in its native *E coli* host SHP45, and *E coli* C600 (pNHSHP45), with and without the presence of colistin. After 14 days of passaging either with or without colistin (2 mg/L), the plasmid was stable in both *E coli* C600 (pNHSHP45) and in strain *E coli* SHP45 (appendix p 6). For the transformant of *E coli* W3110 that harboured pUC18-*mcr-1* carrying a 1949-bp fragment from pHNSHP45, a 4-fold increase in the minimum inhibitory concentrations of colistin and polymyxin B was noted compared with those of the host strains (table 1). All these data show that the *mcr-1* product confers colistin resistance upon host bacteria.

To investigate the level of dissemination of *mcr-1*, a retrospective study on a collection of *E coli* from pigs at slaughter and retail meats was undertaken, and we noted that the proportion of positive samples increased from year to year (table 2). *mcr-1*-positive *E coli* and *K pneumoniae* were also obtained from patients, but with lower proportion of positive samples (table 2). In 1322 samples from inpatients presenting with infections, we detected *mcr-1*-positive Enterobacteriaceae in 16 cases (four from urine, three from sputum, three from drainage fluid, three from ascetic fluid, two from bile, and one from wound). The proportion of *mcr-1* positive samples from different origins varied from 0 to 5·9% (appendix p 2). These data show that *mcr-1* positive Enterobacteriaceae are already established as a cause of infection in human beings. We are undertaking a comprehensive study to ascertain the prevalence of *mcr-1*-positive Enterobacteriaceae in patients admitted to hospital and to determine the risk factors for infections and clinical outcomes.

The deduced aminoacid sequence of the *mcr-1* gene product, MCR-1, aligned closely with phosphoethanolamine transferases (EptA) found in *Paenibacillus sophorae* (63% identity, GenBank accession number WP_036596266.1), *Enhydrobacter aerosaccus* (63%; WP_007116571), *Moraxella catarrhalis* (59%; WP_003672704), and *Dichelobacter nodosus* (54%; WP_012030864; appendix p 7).[20] The dendrogram (con-

	Origin	Polymyxin E (colistin)	Polymyxin B
Escherichia coli SHP45 (*mcr-1*)	Pig	8·0	4·0
E coli C600	..	0·5	0·5
E coli C600+ pHNSHP45 *mcr-1*)	Transconjugant	8·0	4·0
E coli E11 (ST131, KPC-2-producer)	Human	0·5	0·5
E coli E11 (ST131, KPC-2-producer) + pHNSHP45 (*mcr-1*)	Transformant	4·0	2·0
Klebsiella pneumoniae MPC11	Human	0·5	0·5
K pneumoniae MPC11 + pHNSHP45 (*mcr-1*)	Transformant	8·0	4·0
K pneumoniae 1202 (ST11, KPC-2-producer)	Human	0·5	0·5
K pneumoniae 1202 (ST11, KPC-2-producer) + pHNSHP45 (*mcr-1*)	Transformant	4·0	4·0
Pseudomonas aeruginosa HE26	Human	0·5	0·5
P aeruginosa HE26 + pHNSHP45(*mcr-1*)	Transformant	8·0	4·0
E coli W3110+ pUC18	Laboratory strain	0·5	0·5
E coli W3110 + pUC18-*mcr-1*	Transformant	2·0	2·0

Table 1: **Minimum inhibitory concentration (mg/L) for parental strain, transformants, and transconjugant**

structed with MEGA 6.0.6 software package) suggested an evolutionary relationship between these phosphoethanolamine transferases and MCR-1 (appendix p 8). The evolutionary relationship between MCR-1 and the phosphoethanolamine transferase of polymyxin-producing bacteria, *Paenibacillus* spp, indicated a potential intergeneric transfer of the gene from the chromosome of unknown polymyxin-producing bacteria to *E coli*.

Preliminary analysis of the MCR-1 protein sequence indicated possession of an integral membrane-bound portion, with hydropathy plots predicting five transmembrane α-helices in the N-terminal 200 aminoacids of the sequence (figure 3A)[21] in accordance with previous descriptions of members of this protein family. Homology modelling of the complete protein structure with the i-Tasser homology modelling server identified the soluble portions of two phosphoethanolamine transferases, LptA from *Neisseria meningitidis* and EptC from *Campylobacter jejuni*, as the closest relatives of known structure (Protein Data Bank IDs 4KAY and 4TNO; 41% and 40% sequence identities with MCR-1, respectively; appendix p 9). Models for MCR-1 produced by i-Tasser vary in their N-terminal (putative transmembrane) regions but are in close agreement with one another and with the overall folds of these two structures in the C-terminal domain (figure 3B). These structural models, together with sequence comparisons (above) also showed that key residues identified as likely to be important to the catalytic activity of LptA and EptC are conserved in MCR-1 (appendix p 5).[21,22] Thus, the MCR-1 sequence and predicted structure are consistent with a membrane-anchored enzyme with likely phosphoethanolamine transferase activity and confirmed by lipid A analysis as described in the appendix (p 10).

The question as to whether MCR-1 can mediate colistin resistance in vivo was addressed by using *E coli* strain 363 carrying *mcr-1* (363R) and the same strain cured of *mcr-1* (363S) in a murine thigh infection model simulating human colistin dosing (figure 4). At 10^6 colony forming units, carriage of 363S was reduced by more than three-log orders of magnitude over a 72-h period, compared with just over one-log order for the same strain carrying *mcr-1*. These results indicate that the presence of *mcr-1* indeed affords in-vivo protection against colistin (figure 4).

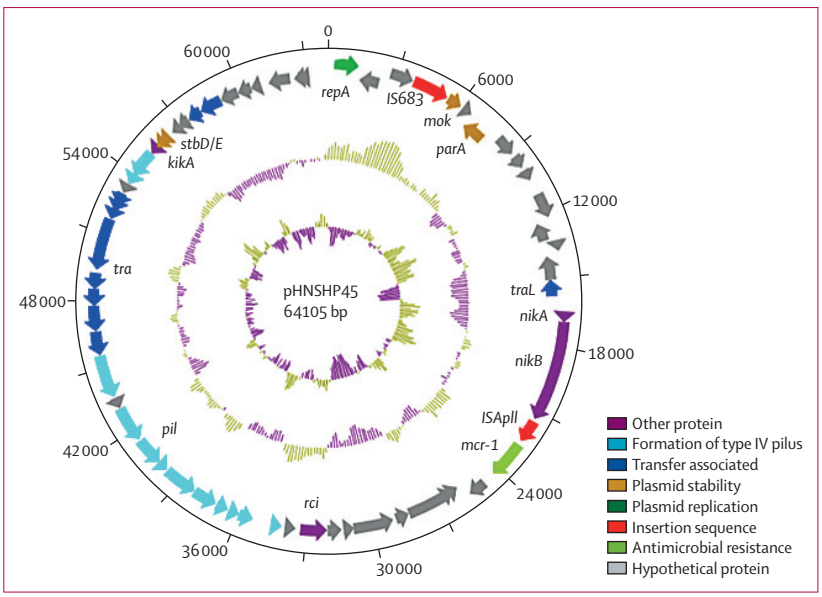

Figure 2: **Structure of plasmid pHNSHP45 carrying *mcr-1* from *Escherichia coli* strain SHP45**

	Year	Positive isolates (%)/number of isolates
Escherichia coli		
Pigs at slaughter	All	166 (20·6%)/804
Pigs at slaughter	2012	31 (14·4%)/216
Pigs at slaughter	2013	68 (25·4%)/268
Pigs at slaughter	2014	67 (20·9%)/320
Retail meat	All	78 (14·9%)/523
Chicken	2011	10 (4·9%)/206
Pork	2011	3 (6·3%)/48
Chicken	2013	4 (25·0%)/16
Pork	2013	11 (22·9%)/48
Chicken	2014	21 (28·0%)/75
Pork	2014	29 (22·3%)/130
Inpatient	2014	13 (1·4%)/902
Klebsiella pneumoniae		
Inpatient	2014	3 (0·7%)/420

Table 2: **Prevalence of colistin resistance gene *mcr-1* by origin**

Discussion

Until now, colistin resistance has occurred via chromosomal mutations and, although clonal outbreaks have been reported, the resistance is often unstable, imposes a fitness cost upon the bacterium and is incapable of spreading to other bacteria.[7] The rapid dissemination of previous resistance mechanisms (eg, NDM-1) indicates that, with the advent of transmissible colistin resistance, progression of Enterobacteriaceae from extensive drug resistance to pan-drug resistance is inevitable and will ultimately become global.[5] In this context the emergence of transmissible, plasmid-mediated colistin resistance in the form of MCR-1 is a finding of global significance.

It is disconcerting that the *mcr-1*-containing plasmid, pHNSHP45, has a very high in-vitro transfer rate between *E coli* strains (10^{-1} to 10^{-3}). Moreover, pHNSHP45 is capable of transfer into epidemic strains of Enterobacteriaceae, such as *E coli* ST131 and *K pneumoniae* ST11, as well as into *P aeruginosa*, suggesting that *mcr-1* is likely to spread rapidly into key human pathogens. Preliminary plasmid stability data suggests that pHNSHP45 is stable in both transconjugants and its parent host strain, even without the selective pressure of polymyxins (appendix p 6). Extrapolating these data to the broader environment would suggest that *mcr-1* plasmids will be maintained

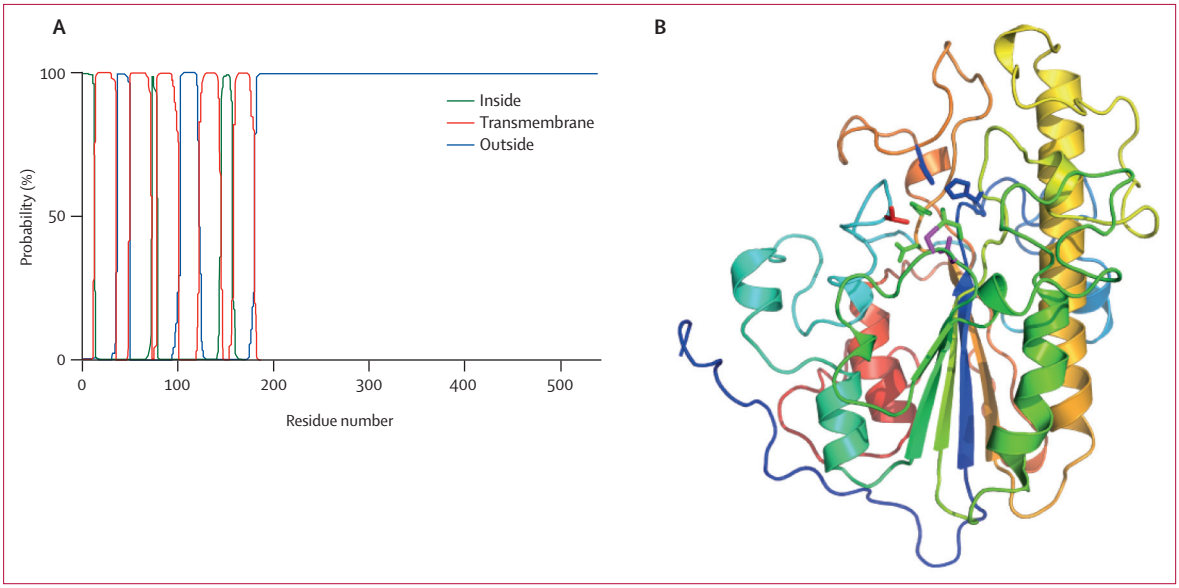

Figure 3: **Hydropathy plot predicting five transmembrane α-helices in the N-terminal 200 aminoacids of MCR-1 (A) and i-Tasser homology modelling analysis of MCR-1 based on models from LptA (*Neisseria meningitidis*; Protein Data Bank ID 4KAY) and EptC (*Campylobacter jejuni*; Protein Data Bank ID 4TNO; B)**

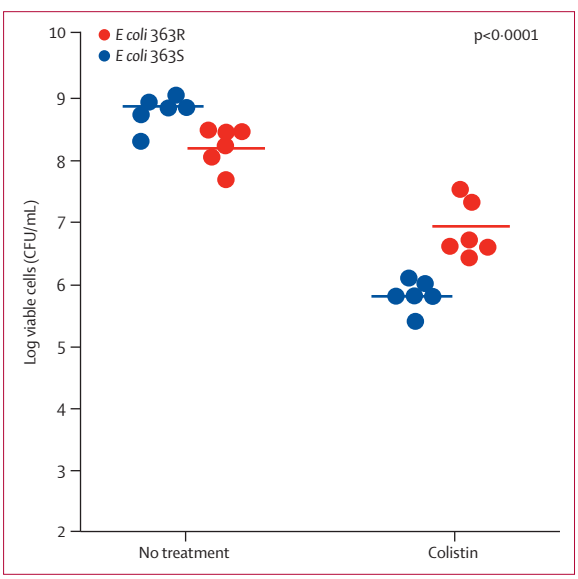

Figure 4: **In-vivo effects of colistin treatment (7·5 mg/kg of colistin sulfate per 12 h) in a murine thigh model showing 10^6 CFU infection with *Escherichia coli* with *mcr*-1 (363R, red circles) and without *mcr*-1 (363S, blue circles)**

p value calculated by a two-sample *t* test for the log difference in CFUs between 363S and 363R after treatment was also indicated. CFU=colony forming unit.

in Enterobacteriaceae populations regardless of selection pressure, and that this will facilitate their spread into human populations.

The high prevalence of *mcr-1* in *E coli* isolates of animal and retail meat origins was surprising, and suggested that *mcr-1* might already be widespread in food animals in south China. However, the preliminary data shown for raw meat is likely to be unrepresentative in view of the small sampling size, and caution must be shown when extrapolating these data. Nevertheless, it is notable that these data contrast with the relatively low proportion of *mcr-1*-positive isolates of human origin. In view of the difference between the proportion of positive samples in animals and human beings, it is likely that MCR-1-mediated colistin resistance originated in animals and subsequently spread to people (table 2). Although rarely used in human treatment, colistin still remains a valid option for carbapenemase-producing Enterobacteriaceae infections, and when it has been occasionally used in China, has produced efficacious results.[23,24] Although the levels of maximum inhibitory concentrations of polymyxin conferred by MCR-1 are not very high (4–8 mg/L), in an in-vivo infection model, MCR-1 provided adequate protection from colistin (figure 4). Thus, acquisition of *mcr-1* by carbapenemase-producing Enterobacteriaceae strains has the potential to make them truly pan-drug resistant and the resulting infections untreatable.

During the writing of this report, we noted that five *E coli* DNA contigs containing *mcr-1*-like genes from Malaysia have been recently submitted to the European Molecular Biology Laboratory (GenBank accession number JWKG01000081.1, JWKF01000084.1, JUJZ01000081.1). Although no additional information is available, the possibility that *mcr-1*-positive *E coli* have spread outside China and into other countries in southeastern Asia is deeply concerning.

The potential for *mcr-1* to become a global concern will then depend on several factors: the continued use of colistin in the veterinary sector providing selective

pressure—both in and outside China; the stability of the *mcr-1*-positive plasmids and their ability to transfer to human pathogenic *E coli* strains—eg, ST131; and the population dynamics across China's borders.

China is the world's largest poultry and pig producer, and in 2014 produced 17·5 million tonnes and 56·7 million tonnes, respectively.[25] Most of the production is for domestic consumption with about 10% for export.[26] The global market value of veterinary drugs increased from US$8·7 billion in 1992 to $20·1 billion in 2010, and in 2018 is anticipated to reach $43 billion.[27–29] China is also one of the world's highest users of colistin in agriculture.[29] Driven largely by China, the global demand for colistin in agriculture is expected to reach 11 942 tonnes per annum by the end of 2015 (with associated revenues of $229·5 million), rising to 16 500 tonnes by the year 2021, at an average annual growth rate of 4·75%.[29] Of the top ten largest producers of colistin for veterinary use, one is Indian, one is Danish, and eight are Chinese. Asia (including China) makes up 73·1% of colistin production with 28·7% for export including to Europe.[29] In 2015, the European Union and North America imported 480 tonnes and 700 tonnes, respectively, of colistin from China.[29] Colistin sulphate (together with other antibiotics) has also been used in farmed fish diets where it has been shown to improve health and promote growth.[29,30] This increasingly heavy use of colistin could have resulted in high selective pressure in the veterinary environment and led to the acquisition of *mcr-1* by *E coli*. We anticipate that the amount of colistin used in animal feed is likely to provide survival advantage for MCR-1-producer bacterial populations over colistin-sensitive bacterial populations. This usage pattern can also explain the apparently discrepant prevalence of *mcr-1* between animal and human *E coli* isolates.

Although in its 2012 World Health Organization Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) report the WHO concluded that colistin should be listed under those antibiotics of critical importance, it is regrettable that in the 2014 Global Report on Surveillance, the WHO did not to list any colistin-resistant bacteria as part of their "selected bacteria of international concern".[3,31] It has been suggested that all countries should adopt the risk management options recently established by the Food and Agriculture Organization of the United Nations and Codex Alimentarius Commission to curtail the spread of antimicrobial resistance in agriculture.[32,33]

In the absence of new agents effective against resistant Gram-negative pathogens, the effect on human health by mobile colistin resistance cannot be underestimated. It is imperative that surveillance and molecular epidemiological studies on the distribution and dissemination of *mcr-1* among Gram-negative bacteria in both human and veterinary medicine are initiated, along with re-evaluation of the use of polymyxins in animals.

Contributors

J-HL and JSh designed the study. Y-YL, YW, L-XY, RZ, GT, BD, L-FY, DG, HR, XC, LL, DH, HZ, and JSp collected the data. J-HL, Y-YL, TRW, JSh, YW, L-XY, XH, LL, JSp, YD, and ZL analysed and interpreted the data. J-HL, TRW, and YD wrote the report. All authors reviewed, revised, and approved the final report.

Declaration of interests

We declare no competing interests.

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