Changes in colistin resistance and *mcr-1* abundance in *Escherichia coli* of animal and human origins following the ban of colistin-positive additives in China: an epidemiological comparative study

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Summary

Background Following the discovery and emergence of the plasmid-mediated colistin resistance gene, *mcr-1*, the Chinese government formally banned colistin as an animal growth promoter on April 30, 2017. Herein, we report patterns in colistin resistance and *mcr-1* abundance in *Escherichia coli* from animals and humans between 2015 and 2019, to evaluate the effects of the colistin withdrawal.

Methods We did an epidemiology comparative study to investigate: annual production and sales of colistin in agriculture across mainland China according to data from the China Veterinary Drug Association from 2015 to 2018; the prevalence of colistin-resistant *E coli* (CREC) in pigs and chickens in 23 Chinese provinces and municipalities as reported in the China Surveillance on Antimicrobial Resistance of Animal Origin database from Jan 1, 2015, to Dec 31, 2016, and Jan 1, 2017, to Dec 31, 2018; the presence of residual colistin and *mcr-1* in faeces from 118 animal farms (60 pig, 29 chicken, and 29 cattle) across four provinces over July 1, 2017, to August 31, 2017, and July 1, 2018 to August 31, 2018; the prevalence of *mcr-1*-positive *E coli* (MCRPEC) carriage in healthy individuals attending routine hospital examinations across 24 provinces and municipalities from June 1 to July 30, 2019, comparing with equivalent 2016 data (June 1 to September 30) from our previous study in the same hospitals; and the patterns in CREC prevalence among hospital *E coli* infections across 26 provinces and municipalities from Jan 1, 2015, to Dec 31, 2016, and Jan 1, 2018, to Dec 31, 2019, reported on the China Antimicrobial Surveillance Network.

Findings After the ban on colistin as a growth promoter, marked reductions were observed in the production (27 170 tonnes in 2015 *vs* 2497 tonnes in 2018) and sale (US$71·5 million in 2015 *vs* US$8·0 million in 2018) of colistin sulfate premix. Across 118 farms in four provinces, mean colistin residue concentration was 191·1 μg/kg (SD 934·1) in 2017 versus 7·5 μg/kg (50·0) in 2018 (p<0·0001), and the median relative abundance of *mcr-1* per 16S RNA was 0·0009 [IQR 0·0001–0·0059] in 2017 versus 0·0002 [0·0000–0·0020] in 2018 (p=0·0001). Across 23 provinces and municipalities, CREC was identified in pig faeces in 1153 (34·0%) of 3396 samples in 2015–16 versus 142 (5·1%) of 2781 in 2017–18 (p<0·0001); and in chickens in 474 (18·1%) of 2614 samples in 2015–16 versus 143 (5·0%) of 2887 in 2017–18 (p<0·0001). In hospitals across 24 provincial capital cities and municipalities, human carriage of MCRPEC was identified in 644 (14·3%) of 4498 samples in 2016 versus 357 (6·3%) of 5657 in 2019 (p<0·0001). Clinical CREC infections in 26 provinces and municipalities comprised 1059 (1·7%) of 62 737 *E coli* infections in 2015–16 versus 794 (1·3%) of 59 385 in 2018–19 (p<0·0001).

Interpretation The colistin withdrawal policy and the decreasing use of colistin in agriculture have had a significant effect on reducing colistin resistance in both animals and humans in China. However, continuous colistin monitoring is essential, in particular to act as an early warning system for colistin stewardship in Chinese hospitals.

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Introduction

Although colistin has been widely used as an animal feed additive for more than half a century,[1] plasmid-mediated colistin resistance determinants did not emerge until 2015, when the first mobile colistin resistance gene, *mcr-1*, was identified in isolates from animals and humans in China.[2] Within 4 years of its discovery, *mcr-1*-positive bacteria have been reported in animals, meat products, humans (both faecal carriage and infections), and the environment in more than 50 countries across

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See **Online** for appendix 2

Research in context

Evidence before this study

On Nov 7, 2019, we searched PubMed with the terms "colistin and ban", "colistin and withdrawal", "colistin and restriction", and "colistin and cessation", for reports published in any language between Jan 1, 2016, and Oct 31, 2019. The date of Jan 1, 2016 was chosen as this was the first published report of the plasmid-mediated colistin resistance gene, *mcr-1*, and coincided with the timing of the first discussions within China on banning colistin as a growth promoter. We found four reports (two in the UK and two in China) on the consequences of reducing use of colistin on pig farms (a single farm in three reports and five farms in the other report). All these reports were limited in sample number but observed an elimination or reduction of *mcr-1* abundance and colistin resistance on farms after the withdrawal or restriction of colistin use. We did not find any study examining the efficacy of colistin withdrawal policies at the national level, nor any study examining the effects on poultry or pig populations, or on human colonisation and infections with colistin-resistant *Escherichia coli*. We also found no study examining the overall implications of this policy change on animal, human, and environmental sectors under a "One Health" perspective.

Added value of this study

To the best our knowledge, this study presents the first national-level analysis of the patterns in colistin resistance in *E coli* of animal origin between 2015–18, and the first biological assessment of the patterns in residual colistin and *mcr-1* abundance on pig, chicken, and cattle farms from four Chinese provinces. Furthermore, we present the first multiprovince study on the patterns in *mcr-1* and colistin resistance in *E coli* from human faecal flora and clinical samples over 2015–19. We also correlate findings with annual production and sales of colistin in Chinese agriculture.

Implications of all the available evidence

The discovery of *mcr-1* in China and its national implications to both animals and humans prompted the Chinese government to officially announce its withdrawal as an agricultural growth promoter on April 30, 2017. Our study indicates that, in the short term, the withdrawal of colistin from animal feed in China has significantly reduced colistin resistance and the prevalence of *mcr-1* in both the animal and human sectors. As colistin is a last resort antibiotic to treat crucial Gram-negative infections, and carbapenem resistance continues to increase worldwide, our study has global implications regarding the use of colistin in agriculture and reignites the debate on separating antibiotic use between humans and animals.

six continents.[3,4] Furthermore, an additional eight distinct mobile colistin resistance genes, *mcr-2* to *mcr-9*, have been identified.[5]

Since 2016, WHO has listed colistin as one of the crucially important antibiotics;[6] however, the global spread of *mcr-1* genes amongst bacteria from both animals and humans has contributed to the global policy discussions regarding the use of colistin.[7–9] In 2016, the European Medicines Agency updated the risk level of colistin resistance from low to high.[10] Many countries have approved the withdrawal of colistin as a feed additive in animals, including Brazil (November, 2016), Thailand (February, 2017), China (April, 2017), Japan (July, 2018), Malaysia (January, 2019), Argentina (February, 2019), and India (July, 2019).[11–14] In addition, global colistin sulphate production decreased from 13 746 tonnes in 2016 to 4292 tonnes in 2019.[15] However, poultry and swine industries still account for 96% of total colistin sulphate use worldwide,[15] with farms continuing to use colistin as a preventive measure and for individual treatment of livestock.

Despite the implementation of policies restricting the use of colistin in agriculture, the implications of these changes and their subsequent effects are largely unknown. According to announcement number 2428 issued by the Chinese Ministry of Agriculture and Rural Affairs, colistin sulfate premix was prohibited as a growth promoter in mainland China on April 30, 2017. However, colistin remains available by prescription for treatment of disease and metaphylaxis in animals.[11] Herein, 2 years after the implementation of China's colistin withdrawal policy, and 2 years after the initiation of polymyxin B and colistin therapy in humans in mainland China (in February, 2017),[11] we evaluate: annual production and the sale of colistin in agriculture; residual colistin and *mcr-1* abundance in faeces from representative animal farms; and the prevalence of colistin resistance, and characterisation of *mcr-1*-positive *Escherichia coli* (MCRPEC) isolated from animals and humans between 2015 and 2019.

Methods

Study design

We did an epidemiological comparative study using five independent approaches to assess the effects of the colistin policy change in China (figure 1). The design of each approach was as follows.

We captured data on annual colistin sulfate premix production and sales across mainland China between 2015 and 2018, to compare usage over 2015–16 (before colistin withdrawal) and 2017–18 (after colistin withdrawal).

We acquired prevalence data on colistin-resistant *E coli* (CREC) from pigs and chickens in 23 Chinese provinces and municipalities (appendix 2 pp 1–2) from Jan 1, 2015, to Dec 31, 2016, and Jan 1, 2017, to Dec 31, 2018, and analysed the difference in prevalence before and after the policy change.

We investigated concentrations of residual colistin and the relative abundance of *mcr-1* on 118 animal farms

from four Chinese provinces from July 1, 2017, to August 31, 2017 (3 months post-withdrawal of colistin) and July 1, 2018 to August 31, 2018 (15 months post-withdrawal).

We investigated the prevalence of MCRPEC carriage by screening healthy individuals in 24 Chinese hospitals (appendix 2 pp 8–9) across 24 provinces and municipalities between June 1 and July 30, 2019, and compared these data with our previous 2016 dataset from the same hospitals.[11]

We compared patterns in the prevalence of CREC among human cases of *E coli* infection recorded in hospitals across 26 provinces and municipalities from Jan 1, 2015, to Dec 31, 2016, and Jan 1, 2018, to Dec 31, 2019, to understand whether the policy change had increased colistin resistance in Chinese tertiary health institutions.

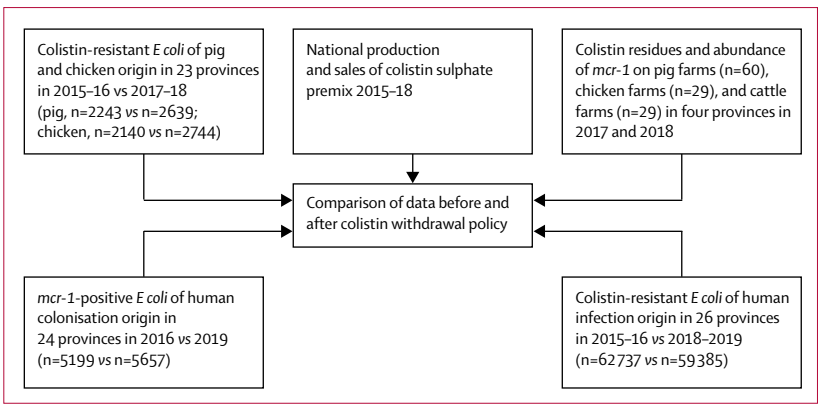

Figure 1: **Data included in the present study**
E coli=Escherichia coli.

Data sources and sample collection

Data for each of our five approaches were acquired from the following sources.

Data on colistin sulfate premix production and sales in Chinese agriculture in 2015–18 were acquired from the China Veterinary Drug Association.

Data on the prevalence of CREC were collected from the China Surveillance on Antimicrobial Resistance of Animal Origin (CSARAO) database , hosted by the China Institute of Veterinary Drug Control. The Provincial Institutes of Veterinary Drug Control are the major institutions that collected the samples and data for the CSARAO database. To collect these data, all samples (cloacal swabs from chicken farms, rectal swabs from pig farms, and cecal samples from abattoirs) were transported in Cary-Blair transport medium (Landbridge Technology, Beijing, China), then inoculated on MacConkey plates (Landbridge Technology) at 37°C for 20 h. Suspected *E coli* were isolated from each plate and the species confirmed by ATB Expression or Vitek 2 Compact (bioMérieux, Lyons, France), MALDI-TOF mass spectrometry (Bruker Daltonik, Bremen, Germany), or 16S rRNA sequencing. All *E coli* were subjected to antimicrobial susceptibility testing for colistin resistance by broth dilution according to the Clinical and Laboratory Standards Institute approach.[16] Because of the varying sampling timepoints (some locations began monitoring colistin resistance in 2016 after the discovery of *mcr-1* while others were less rigorous), we searched the CSARAO database from 23 provinces and municipalities across two timeframes: 2015–16, before implementation of colistin withdrawal, and 2017–18, after implementation of colistin withdrawal.

To investigate residual colistin and *mcr-1* abundance, we chose four domestic provinces located in four distinct regions of mainland China: Liaoning (northeast), Shaanxi (northwest), Guizhou (southwest), and Hunan (central; appendix 2 p 46). Each province harbours different agricultural animals and levels of farming,[17] and thus seemed representative of Chinese animal farms. Farms were selected with consideration of the types of domestic animals, herd size, and geographic distribution of farms. In total we collected faecal samples from 118 farms of chickens (n=29), pigs (n=60), and cattle (n=29) over our designated periods between July and August in 2017 and 2018. Colistin has never been approved for use in cattle in China and therefore cattle farms were included as a negative control. Five faecal samples were collected from each farm according to a quadrilateral approach (one sample from the centre and four samples from each corner of the farm), and total DNA was extracted in triplicate from the combined samples from each farm for detection of the relative abundance of *mcr-1*. Total DNA was extracted from faeces using a HiPure Soil DNA kit (Magen Biotech, Guangzhou, China) according to the manufacturers' instructions. All details of provinces, animals, and farm numbers are listed in appendix 2 (pp 3–7).

Prevalence of MCRPEC carriage was assessed in rectal swabs of healthy individuals, collected across 24 provinces and municipalities and collated by The Second Affiliated Hospital of Zhejiang University (Hangzhou, China). A total of 24 hospitals from 24 provincial capital cities and municipalities, corresponding to our previous study,[11] were recruited into this study. Hospitals were asked to enrol participants over a 5-day period from June 1 to July 30, 2019. We included healthy individuals attending their routine physical examination, during which stool samples were collected. The inclusion criteria were individuals who were considered generally healthy and consented to the study without defined age restrictions. We excluded neonates, pregnant women, individuals with gastroenteritis, individuals presenting with chronic illnesses, such as cancer, or obvious clinical disease symptoms, and individuals who had taken antibiotics within the past 3 months. Ethical permission for this study was agreed on April 19, 2019, by the ethics committee of Zhejiang University. Because of uneven economic and social development in different provinces in China, the number of individuals attending physical examinations during the 5-day period varied substantially across different

For the **China Veterinary Drug Association (in Chinese)** see http://www.cvda.org.cn/index.html

For the **CSARAO database (in Chinese)** see http://125.124.115.227:8088/sy/

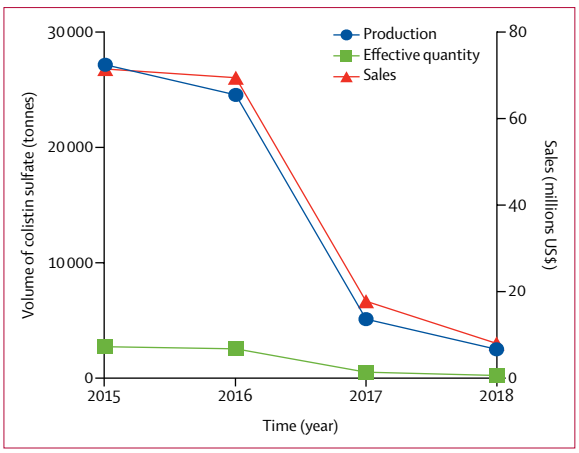

Figure 2: **Production and sales of colistin sulfate premix in China in 2015–18**

locations. Therefore, the number of samples from each location varied. All samples were subjected to an enrichment method for screening of the *mcr-1* gene as our previous study described.[11] Colonies on agar were confirmed for *mcr-1* by PCR, and the species were identified by MALDI-TOF mass spectrometry (Bruker Daltonik). The equivalent dataset from 2016 were extracted from our previous study and represented the period from June 1, to Sept 30, 2016.[11]

For **CHINET** see www.chinets.com

Prevalence data on clinical CREC infections were acquired from the China Antimicrobial Surveillance Network (CHINET), hosted by Huashan Hospital, Fudan University (Shanghai, China). All CHINET data were obtained from cooperative hospitals across 26 provinces and municipalities by identical protocols. *E coli* isolates were from clinical samples from patients with suspected infections. Isolates collected from specimens for research purposes were excluded from this study. To avoid duplication, only one isolate from the same species was collected per patient based on personal barcodes. Antimicrobial susceptibility testing was done following the guidelines recommended by the Clinical and Laboratory Standards Institute.[16] Ethical permission for this study was agreed on Nov 24, 2015, before the assessment period, by the ethics committee of Huashan Hospital of Fudan University.

Quantification of residual colistin

We quantified residual colistin from faecal samples as previously described,[18] with minor modifications. All samples were prepared in duplicate with 1 g aliquots of homogenised sample. Trichloroacetic acid in acetonitrile (30:70, v/v) was added to each sample and vortexed for 1 min. Samples were vigorously mixed for 5 min, adjusted to pH 9·0, and stored at –80°C for 20 min. After adjusting to 1 mL by the addition of water, 50 µL formic acid was added to each sample and thoroughly mixed. Colistin A and colistin B, the major components that account for more than 85% of total colistins within colistin sulfate premix,[19] served as markers of colistin residues and were measured with ultra-high performance liquid chromatography-tandem mass spectrometry (Waters, Milford, MA, USA). The recovery rates of spiked samples, calculated by matrix-matched calibration, were in the range of 80–102%. The limit of quantification (LOQ) for both colistin A and colistin B was 10 µg/kg, with the concentration of colistin calculated as the sum of colistin A and B. For samples in which colistin could not be detected, we assigned a value of 0,[20] and a pseudo-number of 1 was added to all colistin concentrations before being converted to log scale.[21]

Relative abundance of *mcr-1*

We detected the relative abundance of *mcr-1* by quantitative RT-PCR analysis using the 16S rRNA gene as a control for normalisation and previously described primers.[22,23] Assays were done with the QuantStudio 7 Flex Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The reaction components and conditions were as described previously.[22] The cutoff cycle threshold value for 16S rRNA was 26 and for *mcr-1* was 31.[22,23] A pseudocount of 10^{-9} was added to all relative abundance values before conversion to log scale.[21] Diagrams of colistin residue and mcr-1 abundance were generated with the ggplot2 in R software (version 3.6.1) with medians and IQRs. When the majority of data were lower than the LOQ, mean values are reported.

Sequence and plasmid types of MCRPEC

We sequenced MCRPEC strains collected in this study using the Illumina platform. We also downloaded all available whole-genome sequences of other MCRPEC strains isolated in China from NCBI (as of Nov 11, 2019). The procedure for genome sequencing, assembly, and detection of sequence and plasmid types has been described in our previous study,[11] and all MCRPEC profiles are listed in appendix 2 (pp 11–45).

Statistical analysis

Comparison of prevalence between two time points was tested by χ^2 analysis or Fisher's exact test; if the minimum theoretical frequency was less than 1 or sample number was less than 40, we used Fisher's exact test. We used the Mann-Whitney U test to evaluate the significance of differences between the 2017 and 2018 data on residual colistin and relative abundance of *mcr-1*. A p value of less than 0·05 was considered to indicate statistical significance. All statistical analyses were done in R software and SPSS (version 25.0).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

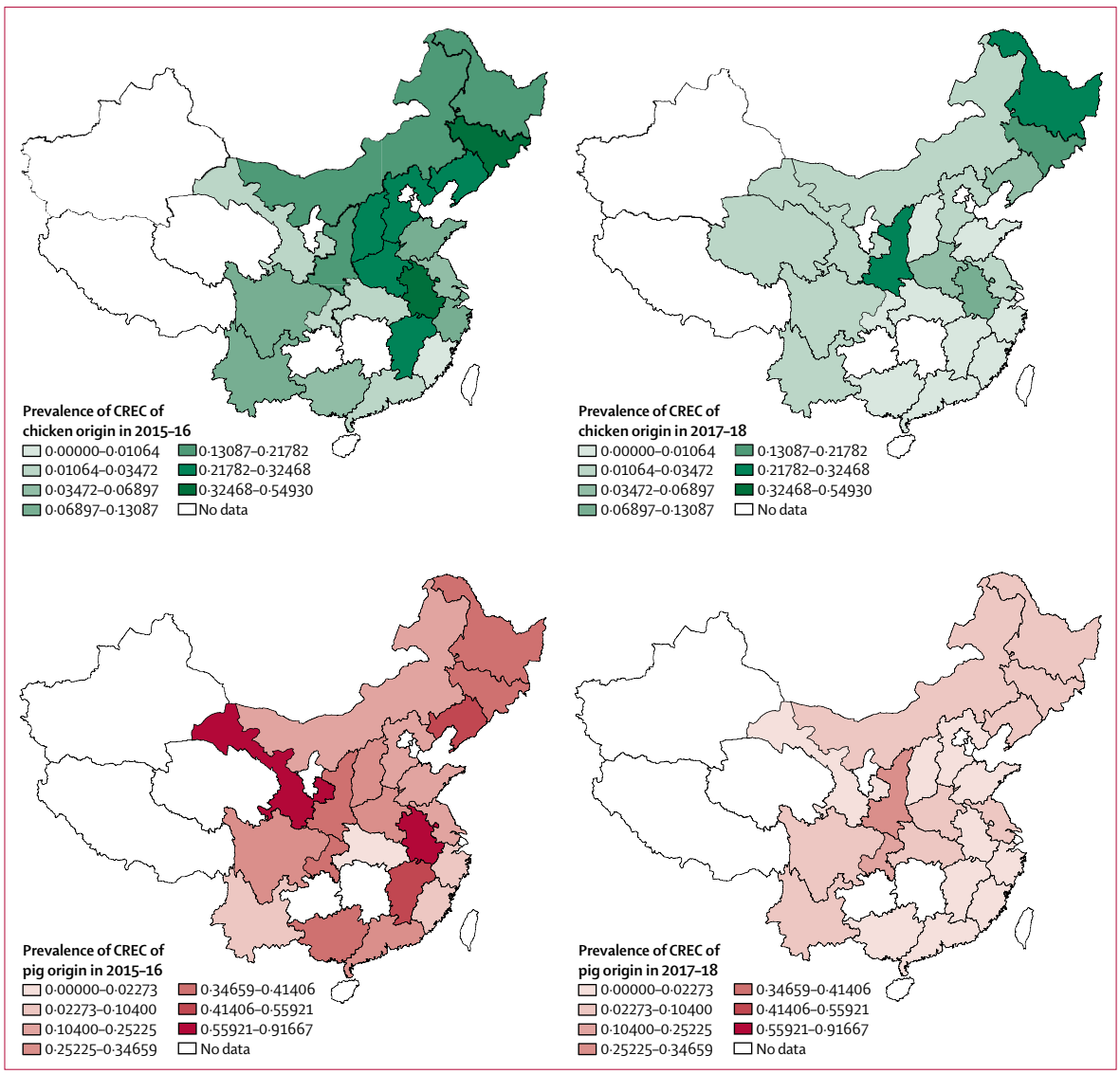

Figure 3: **Prevalence of animal-derived CREC in 23 provinces in China in 2015–16 and 2017–18**
Prevalence is presented as fraction of CREC out of all samples in the CSARAO database from each location during the specified period. Prevalence ranges were set according to the Jenks natural breaks classification method. CREC=colistin-resistant *Escherichia coli*.

Results

To assess the effect of the colistin policy change in China, we surveyed the production of colistin sulfate premix across mainland China from 2015 to 2018. The production of colistin sulfate premix decreased each year, from 27 170 tonnes in 2015 to 2497 tonnes in 2018, with the most substantial decrease occurring between 2016 and 2017 (24 563 tonnes to 5112 tonnes; figure 2 and appendix 2 p 1). Corresponding production of the premix active component (colistin) decreased from 2736 tonnes in 2015 to 234 tonnes in 2018, and sales of colistin sulfate premix also decreased from US$71·5 million to US$8·0 million (figure 2 and appendix 2 p 1).

We analysed data on the prevalence of CREC for the years 2015–16 and 2017–18 as reported in the CSARAO. The prevalence of CREC of pig and chicken origin in 2015–16 was significantly higher than that in 2017–18 in most of the 23 selected provinces and municipalities across China (figure 3 and appendix 2 pp 1–2). Overall prevalence of CREC across the 23 locations showed a similar pattern; in pigs, prevalence decreased, from 1153 positive samples (34·0%) of 3396 total samples in 2015–16, to 142 (5·1%) of 2781 in 2017–18 ($p<0·0001$); and in chickens, from 474 positive samples (18·1%) of 2614 total samples in 2015–16, to 143 (5·0%) of 2887 in 2017–18 ($p<0·0001$; appendix 2 pp 1–2). Provinces with no significant difference in CREC prevalence in pigs were Hubei, Inner Mongolia, Shaanxi, Yunnan, and Zhejiang, and provinces with no significant difference in CREC prevalence in chickens were Chongqing, Fujian,

Gansu, Heilongjiang, Hubei, Jiangsu, and Shaanxi. A high prevalence of CREC persisted in pigs from Shaanxi after colistin withdrawal, and prevalence of CREC among chickens in Heilongjiang and Shaanxi increased after colistin withdrawal, although the differences were not significant. CREC were not recovered from pigs in five provinces and chickens in nine provinces after colistin withdrawal (figure 3 and appendix 2 pp 1–2).

We quantified colistin residue concentrations in faeces samples collected from 118 farms across four provinces between July and August in 2017 and 2018. Across all farms, mean colistin residue concentrations were 191·1 μg/kg (SD 934·1) in 2017 (3 months after colistin withdrawal) versus 7·5 μg/kg (50·0) in 2018 (15 months after withdrawal; p<0·0001). With respect to species, residual colistin concentrations detected in 2018 were significantly lower than those detected in 2017 in samples from chickens and pigs (figure 4). As expected, no residual colistin was detected in samples from cattle farms at either timepoint, as our negative control (figure 4B and appendix 2 pp 3–7). The residual colistin concentrations were lower than the limit of quantification in samples from 47 (78·3%) of 60 pig farms and 17 (58·6%) of 29 chicken farms at the 2017 sampling point, and in samples from 57 (95·0%) pig farms and 27 (93·1%) chicken farms at the 2018 sampling point. Residual colistin concentrations in samples from ten (16·7%) pig farms and 12 (41·4%) chicken farms in 2018 were lower than those in 2017, among which, concentrations in samples from ten pig farms and ten chicken farms were lower than the limit of quantification in 2018. Residual colistin in samples from one chicken farm in Liaoning province markedly decreased, from 9121 μg/kg in 2017 to 0 μg/kg in 2018. Overall, no significant difference was observed in Guizhou and Hunan between 2017 and 2018, while colistin residue concentration was significantly decreased in 2018 in Liaoning and Shaanxi (figure 4C and appendix 2 pp 3–7). We also analysed the relative abundance of *mcr-1* with respect to species and province and found that overall, the relative abundance of *mcr-1* in faecal samples from all 118 animal farms was significantly lower at the 2018 sampling point (median 0·0002 [IQR 0·0000–0·0020]) than at the 2017 sampling point (median 0·0009 [0·0001–0·0059]; p=0·0001; figure 4D and appendix 2 pp 3–7). When examined by animal species, an overall decrease in the relative abundance of *mcr-1* in faeces was observed in cattle, pigs, and chickens (figure 4E), with a similar pattern observed in samples from Guizhou, Liaoning, and Shaanxi (figure 4F). Of the farms showing lower residual colistin in 2018 versus 2017, the relative abundance of *mcr-1* on six (60·0%)of the ten pig farms and ten (83·3%) of the 12 chicken farms was lower in 2018 than in 2017, thus correlating with the residual colistin data.

We assessed the prevalence of MCRPEC in human faecal samples and the prevalence of CREC isolated from human infections. To examine MCRPEC colonisation, we collected 5657 human faecal samples from hospitals across 24 provincial capital cities and municipalities throughout June and July, 2019, and compared prevalence of MCRPEC with equivalent data from 2016.[11] In 2019, the highest prevalence of MCRPEC carriage was 29 positive samples (19·5%) of 149 total samples (Henan province), which was markedly lower than the highest prevalence (18 [32·7%] of 55 samples; Liaoning province) observed in 2016. The overall prevalence across all locations was significantly decreased, from 644 positive samples (14·3%) of 4498 total samples in 2016, to 357 (6·3%) of 5657 in 2019 (p<0·0001; figure 5A and appendix 2 pp 8–9). Overall, the prevalence of MCRPEC carriage decreased in 19 of 24 locations between 2016 and 2019, and was significantly lower in 2019 across 14 locations. In addition, no MCRPEC carriage was detected in two locations in 2019. An increase in MCRPEC carriage from 2016 to 2019 was observed in Henan, Jilin, Shanxi, Shaanxi, and Ningxia, although the difference was significant only in Henan (figure 5A and appendix 2 pp 8–9).

We acquired CREC prevalence data from CHINET. Data were obtained across 26 provinces and municipalities, and prevalence was compared between 2015–16 and 2018–19. Overall, the prevalence of CREC among clinical strains of *E coli* in China showed a significant decrease between 2015–16 (1059 cases [1·7%] of 62737 total infections) and 2018–19 (794 [1·3%] of 59385; p<0·0001; figure 5B and appendix 2 p 10). Ten locations, particularly Heilongjiang, showed a significant decrease in CREC in 2018–19; nine showed no significant difference; and 12 showed an increased prevalence of CREC in 2018–19 compared with 2015–2016, with seven provinces showing significant increases (figure 5B and appendix 2 p 10).

Based on expected decreases in CREC and MCRPEC in animals and humans, we analysed the sequence types of MCRPEC after the colistin withdrawal policy to examine any shifts in ST clades. We collected 866 MCRPEC strains, comprising 617 isolates (118 from pigs, 126 from chickens, and 373 from humans) from before the policy implementation (2015–17) and 249 isolates (50 from pigs, 70 from chickens, and 129 humans) from after the policy change (2017–19; figure 6 and appendix 2 pp 11–45). Overall, the ST clades most common to humans across both timepoints were ST10 (62 [64·6%] of 96 isolates), ST206 (23 [67·6%] of 34), ST48 (18 [52·9%] of 34), ST101 (17 [63·0%] of 27), ST2599 (20 [90·9%] of 22), ST155 (12 [63·2%] of 19), ST58 (eight [88.9%] of nine), ST410 (eight [88.9%] of nine), ST216 (seven [100%]), and ST453 (six [100%]). ST10 was also the predominant clade across both timepoints (pre-withdrawal of colistin, 75 [12·2%] of 617 isolates; post-withdrawal of colistin, 21 [8·4%] of 249); however, the proportion of human isolates identified as ST10 decreased from 53 (70·7%) of 75 isolates in 2015–17, to nine (42·9%) of 21 in 2017–19. In pigs, the predominant clades across both timepoints were ST744 (21 [63·6%] of 33 isolates), ST1716 (17 [94·4%] of 18),

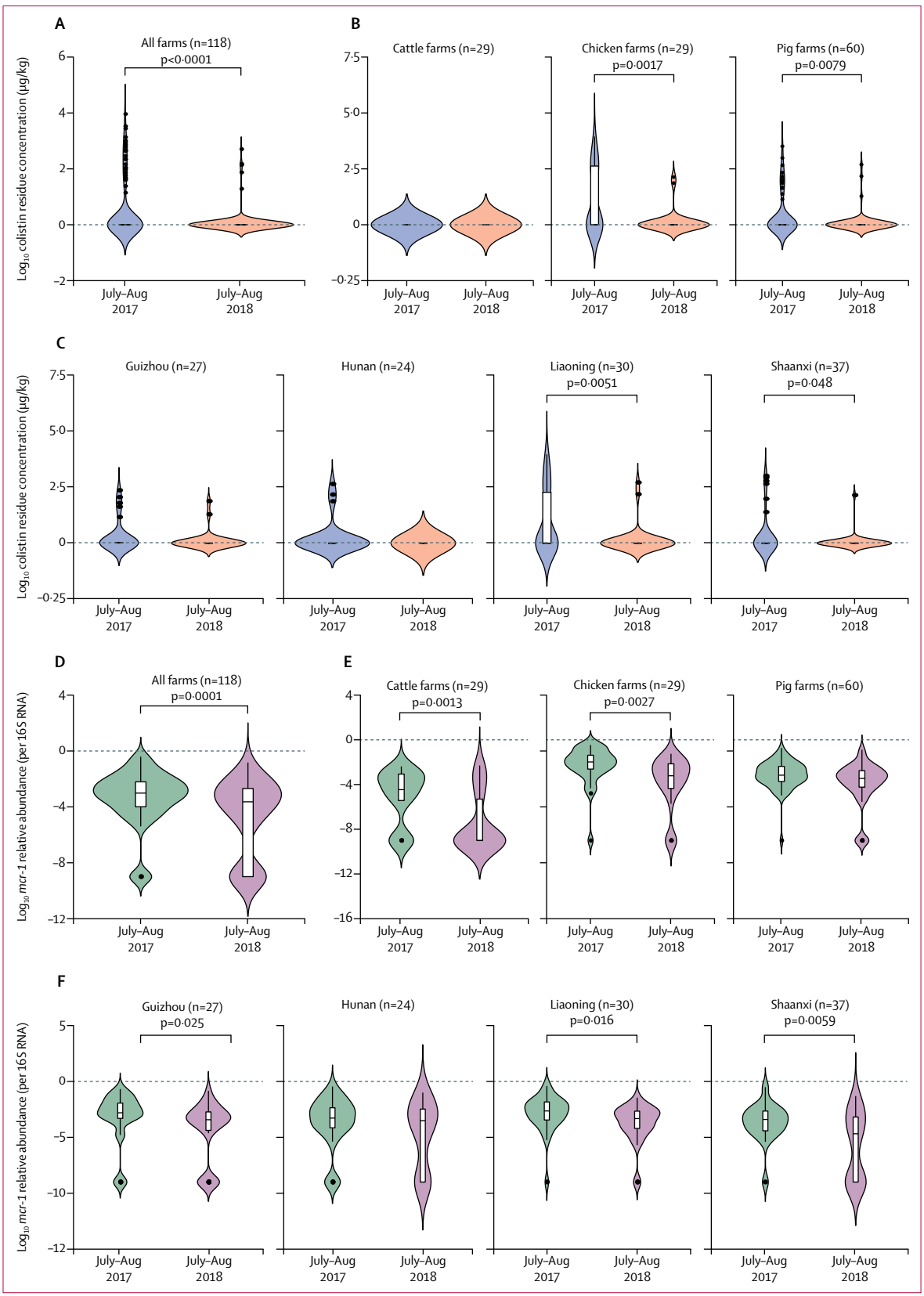

Figure 4: **Residual colistin concentrations (A–C) and relative abundance of *mcr-1* (D–F) in animal faeces between 2017 (3 months after colistin withdrawal) and 2018 (15 months after colistin withdrawal) by farm type and province**
Violin boxplots show the distribution of data around the median and IQR. Whiskers denote the range of points within the first quartile minus 1·5 × the IQR and within the third quartile plus 1·5 times the IQR. p values lower than the threshold for significance (<0·05) are shown. Boxplots are absent for datasets with more than 75% of values lower than the limit of quantification, resulting in a median and IQR of 0.

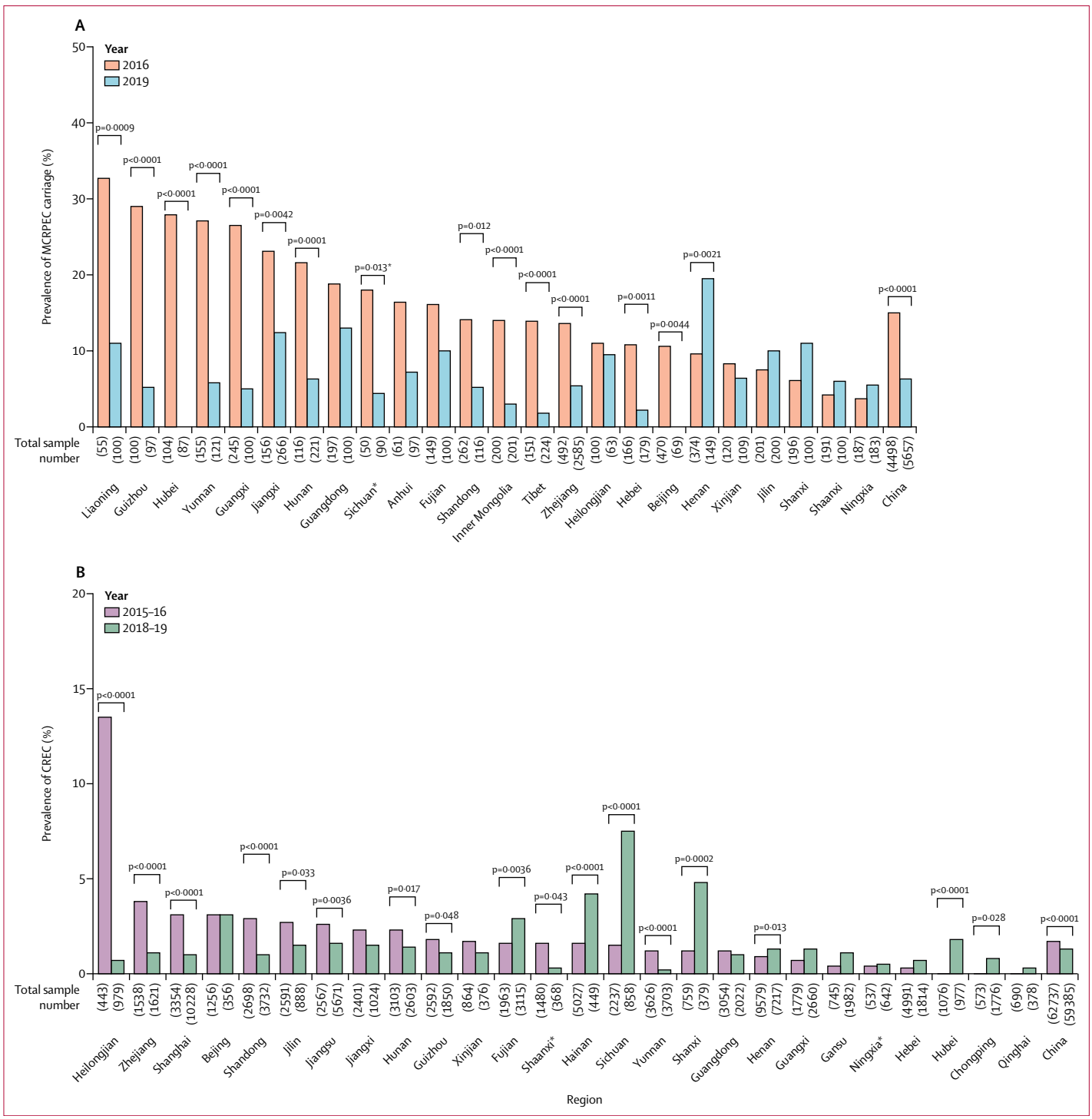

Figure 5: **Prevalence of MCRPEC and CREC in humans**
(A) MCRPEC colonisation samples from 24 provinces and municipalities in China in 2016 and 2019. (B) Clinical CREC strains from 26 Chinese provinces and municipalities in 2015–16 and 2018–19. All data and p values are provided in appendix 2 (pp 8–11). MCRPEC=*mcr-1*-positive *Escherichia coli*. CREC=colistin-resistant *Escherichia coli*. *Data compared by Fisher's exact test (all other data compared by χ^2 analysis).

and ST4429 (eight [100%]), and only in chickens were ST6716 (six isolates) and ST2732 (eight) identified. ST156 was not identified in pigs before colistin withdrawal, whereas, six (46·2%) of 13 ST156 isolates were identified in pigs after colistin withdrawal. This clade was associated with humans before colistin withdrawal (eight [29·6%] of 27 ST156 isolates) but not after the policy change. ST1716 (18 isolates), ST2705 (13), ST224 (ten), ST4429 (eight, all in pigs), and ST216 (seven, all in humans) were only present before colistin withdrawal, and ST6716 isolates (six, all in chickens) emerged after colistin withdrawal (figure 6 and appendix 2 pp 11–45). Regarding plasmid type, the percentage of IncI2-type plasmid significantly increased after colistin withdrawal (157 [25·4%] of 617 isolates in 2017–19 *vs* 86 [34·5%] of 249 in 2015–17; p=0·0070; appendix 2 pp 11–45).

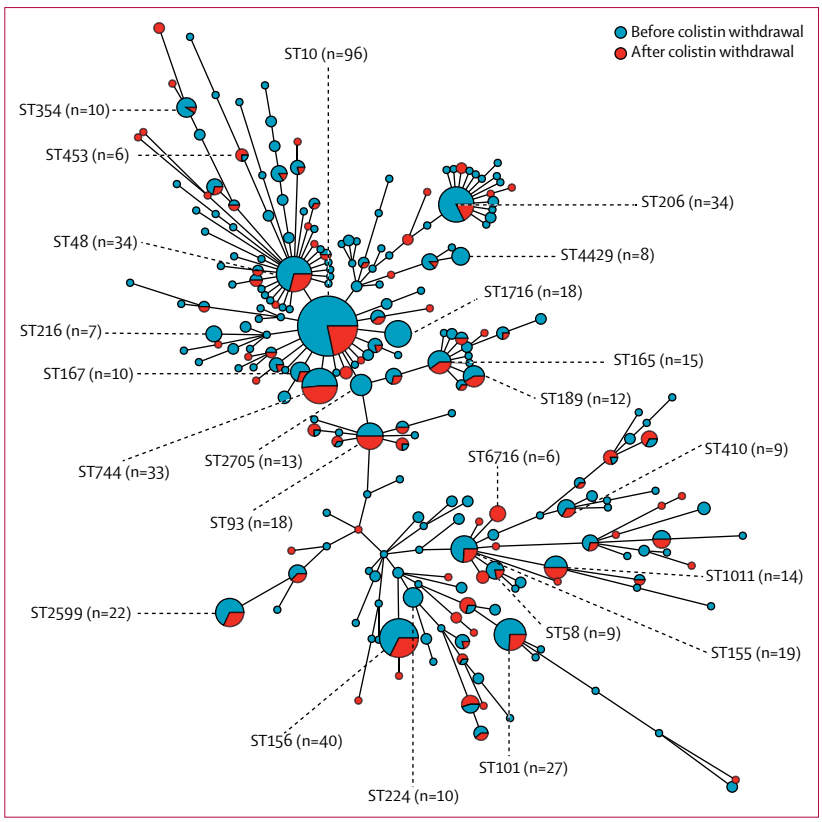

Figure 6: **Minimum spanning tree of *mcr-1*-positive *Escherichia coli* STs as determined by multilocus sequence typing**
The size of the nodes reflects the number of isolates contained within that particular clade. ST=sequence type.

Discussion

Our data provide evidence that the withdrawal of colistin as an animal growth promoter in China has had a significant and positive impact on reducing colistin residues and CREC in animals, and MCRPEC carriage and CREC infections in humans. Specifically, we identified significant reductions in colistin residue concentrations and *mcr-1* abundance in animal faeces, a reduction in CREC of animal origin, and reductions in the presence of MCRPEC isolates derived from human *E coli* colonisation samples and CREC isolates derived from human *E coli* infections. These initial findings could be attributable to two factors. First, the substantial decrease in sales (and therefore consumption) of colistin in animal husbandry as a direct consequence of the colistin withdrawal policy in April, 2017; and second, the acquisition and expression of *mcr-1* has a significant fitness cost to bacterial hosts such as *E coli* and *Klebsiella pneumoniae*.[24,25]

Ever since the approval of antibiotics for use in agriculture, with the inevitable development of resistance, a debate has been ongoing between clinical and agricultural sectors as to whom is responsible for the rise in multidrug-resistant and extensively drug-resistant infections in humans. The rapid emergence of colistin resistance in China was attributed to the use of colistin in growth promotion, given its minimal use in human medicine. Our data affirm this notion and show that withdrawing colistin as a feed additive has significantly reduced the prevalence of MCRPEC and CREC in humans, and therefore clinical colistin resistance rates. On a Portuguese pig farm, a significant decrease in CREC presence in faeces, from 98 (98·0%) of 100 isolates in 2016 to 14 (27·5%) of 51 in 2018, was also observed after a ban on colistin use.[26] These findings mirror the effects of other antibiotics being withdrawn from animal feeds—namely, avoparcin (banned in 1997)[27] and tylosin (banned in 1995).[28] However, two decades later, we are seeing an increase in carbapenem resistance, rapid spread of colistin resistance, and most recently, emergence of mobile tigecycline resistance.[29–31] Therefore, the withdrawal of colistin as a growth promoter is a crucial step in preserving this essential antibiotic for human medicine. In 2019, apramycin, a veterinary antibiotic[32] exclusively and increasingly used in agriculture in Australia, Belgium, France, Germany, Ireland, Italy, the UK, and the USA, has been approved for phase 1 clinical trials,[33] and thus the debate between agricultural and clinical sectors continues, with little clarity or leadership. As antibiotics are essential for both human and veterinary medicine, the potentially long-term challenge will be to discriminate and achieve an improved balance of the drugs used in animals and humans.

Our data indicates a cause and effect relationship resulting from China's policy change (appendix 2 p 47), and although this prompts some optimism, the ability of bacteria to adapt and evolve continues to engage and challenge various stakeholders.[34] Our MCRPEC analysis indicates that the percentage of IncI2-type plasmid significantly increased after the colistin withdrawal. IncI2 plasmids possess other resistance genes on the same mobile element as *mcr*, such as bla_{CTX-M},[35] and thus the plasmids might be positively selected with the use of β-lactam antibiotics such as amoxicillin, which is still widely used in animals. Furthermore, new *mcr* homologues

are emerging, such as *mcr-3.1* and *mcr-3.5*, which do not pose such a high fitness cost and in the short-to-medium term might replace *mcr-1*.[36] Therefore, vigilant monitoring and resistance surveillance is in hospitals.

We acknowledge that our study has several limitations. Our investigation blends four datasets that were largely independent. In our analysis of residual colistin and *mcr-1* abundance in animals, our sampling timepoints on representative farms were 3 months and 15 months after colistin withdrawal. Unfortunately, because of when we planned this study, we did not have data from before the withdrawal of colistin. Additionally, only one *mcr* variant, *mcr-1*, was investigated in this study. Although our studies and others confirm *mcr-1* as the overwhelming predominant variant, minor *mcr* homologues might exist and confer a level of resistance.[37,38] We were also unable to obtain timely data on animal-derived food products and environmental samples as we have reported previously.[39] Notwithstanding, our colistin residue data might reflect amounts observed in canals and rivers and in wastewater. In terms of policy implementation, the withdrawal of colistin from food additives is predicated on the compliance of the livestock industry, which is difficult to monitor particularly in small rural areas. We observed an increase in CREC or MCRPEC in animals and humans in a minority of locations after the colistin ban. The use of polymyxins for therapeutic use in large animals (pigs) and its increasing use in humans might mitigate the reduction of colistin resistance in some places. We also acknowledge the ambiguous interpretation between use of an antibiotic as a growth promoter and use as a metaphylactic to collectively protect animals from disease outbreaks.

In the interests of food safety and public health, a strict cessation policy (number 194) issued by the Chinese Ministry of Agriculture and Rural Affairs announced that all antimicrobials were to be prohibited as growth promoters from July 1, 2020,[40] with the exception of traditional Chinese medicines. This momentous step should be broadly welcomed; however, many other countries need to accelerate their national action plans and policies on antibiotics used as growth promoters.[41] The response by India to withdrawal colistin as a growth promoter in 2019[14] is encouraging. However, in view of the growing global population with an increasing demand for meat, an urgent and serious global discussion is warranted on how to preserve colistin (and other key classes of antibiotics) for future generations. Such a discussion should address how to implement definitive and enforced policies that separate classes of drugs to be used in humans and those to be used in animals.

Contributors

JS, YaW, TRW, and SX designed the study. CX, RZ, YC, YS, FH, DL, JL, YG, XX, JJ, XW, YF, LY, JW, JL, CC, DY, JC, RF, YoW, YL, KL, HC, MZ, LL, JT, YQ, ZS, SW, XY, CW, and SX collected the data. YS, CX, YaW, RZ, YC, FH, SX, TRW, and JS analysed and interpreted the data. YaW, TRW, YS, and CX wrote the manuscript. All authors reviewed, revised, and approved the final report.

Declaration of interests

We declare no competing interests.

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