Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study

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Summary

Background

The *mcr-1* gene confers transferable colistin resistance. *mcr-1*-positive Enterobacteriaceae (MCRPE) have attracted substantial medical, media, and political attention; however, so far studies have not addressed their clinical impact. Herein, we report the prevalence of MCRPE in human infections and carriage, clinical associations of *mcr-1*-positive *Escherichia coli* (MCRPEC) infection, and risk factors for MCRPEC carriage.

Methods

We undertook this study at two hospitals in Zhejiang and Guangdong, China. We did a retrospective cross-sectional assessment of prevalence of MCRPE infection from isolates of Gram-negative bacteria collected at the hospitals from 2007 to 2015 (prevalence study). We did a retrospective case-control study of risk factors for infection and mortality after infection, using all MCRPEC from infection isolates and a random sample of *mcr-1*-negative *E coli* infections from the retrospective collection between 2012 and 2015 (infection study). We also did a prospective case-control study to assess risk factors for carriage of MCRPEC in rectal swabs from inpatients with MCRPEC and *mcr-1* negative at the hospitals and collected between May and December, 2015, compared with *mcr-1*-negative isolates from rectal swabs of inpatients (colonisation study). Strains were analysed for antibiotic resistance, plasmid typing, and transfer analysis, and strain relatedness.

Findings

We identified 21 621 non-duplicate isolates of Enterobacteriaceae, *Acinetobacter* spp, and *Pseudomonas aeruginosa* from 18 698 inpatients and 2923 healthy volunteers. Of 17 498 isolates

associated with infection, *mcr-1* was detected in 76 (1%) of 5332 *E coli*isolates, 13 (<1%) of 348 *Klebsiella pneumoniae*, one (<1%) of 890 *Enterobacter cloacae*, and one (1%) of 162 *Enterobacter aerogenes*. For the infection study, we included 76 *mcr-1*-positive clinical *E coli* isolates and 508 *mcr-1*-negative isolates. Overall, MCRPEC infection was associated with male sex (209 [41%] *vs* 47 [63%], adjusted p=0·011), immunosuppression (30 [6%] *vs* 11 [15%], adjusted p=0·011), and antibiotic use, particularly carbapenems (45 [9%] *vs* 18 [24%], adjusted p=0·002) and fluoroquinolones (95 [19%] *vs* 23 [30%], adjusted p=0·017), before hospital admission. For the colonisation study, we screened 2923 rectal swabs from healthy volunteers, of which 19 were MCRPEC, and 1200 rectal swabs from patients, of which 35 were MCRPEC. Antibiotic use before hospital admission (p<0·0001) was associated with MCRPEC carriage in 35 patients compared with 378 patients with mcr-1-negative *E coli* colonisation, whereas living next to a farm was associated with mcr-1-negative *E coli* colonisation (p=0·03, univariate test). *mcr-1* could be transferred between bacteria at high frequencies (10^{-1} to 10^{-3}), and plasmid types and MCRPEC multi-locus sequence types (MLSTs) were more variable in Guangdong than in Zhejiang and included the human pathogen ST131. MCRPEC also included 17 unreported ST clades.

Introduction

The relentless increase in multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria is worrying and has led to several global initiatives to unify national and international agenda to combat MDR and XDR infections.[1, 2, 3, 4] Global awareness was precipitated by the rapid dissemination of carbapenem resistance mechanisms such as NDM-1, KPC, and OXA-48/181, and the realisation of the small number of antibiotics left to treat serious infections, such as colistin.[5, 6, 7, 8] Until recently, colistin resistance was reported to be mediated by chromosomal mutations and possibly imposed a fitness cost to the organism.[9, 10] Resistance to colistin is common in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* but rare in *Escherichia coli*, and because of the complex mechanism involving lipopolysaccharide structures, the thought that this mechanism might become transferable was readily dismissed.[11]

However, we reported the gene encoding the first transferable colistin resistance mechanism, termed *mcr-1*, in Enterobacteriaceae of both food-producing animal and human origins.[12] Colistin has not yet been approved for use in human beings in China, but it has been used in animals as a therapeutic drug and feed additive since the early 1980s. Thus, we speculated that the emergence and spread of *mcr-1* probably occurred first in animals and spread to human beings.[12] Colistin will soon be made available in China for use in human beings for the treatment of infections caused by MDR Gram-negative pathogens, such as carbapenem-resistant Enterobacteriaceae (CRE). However, the coexistence of *mcr-1* and carbapenem resistance genes, such as $bla_{\text{NDM-5/9}}$ and $bla_{\text{KPC-2}}$, identified in *E coli* from human infections and chicken meat, is of great concern, because the occurrence of the *mcr-1* gene in CRE would seriously compromise treatment options not only in China but also globally.[13]

mcr-1-positive Enterobacteriaceae (MCRPE) have been reported across southeast Asia, north Africa, Europe, and North America. Hitherto, most of the bacteria carrying *mcr-1* in human infections have been retrieved from historical studies with *mcr-1* identified retrospectively from whole genome sequence data.[14, 15] Furthermore, very few clinical reports have shown the effect of MCRPE on patient outcomes and these reports had very small sample sizes.[16]

Although colistin is not yet used in clinical practice in China, it is commonly used as the last-line antibiotic in many countries, sometimes even for empirical treatment.[8] In view of the increased reliance on colistin in the treatment of CRE and the intercontinental dissemination of *mcr-1*, the effect of the emergence of MCRPE in the clinical setting needs to be urgently addressed. We ascertained the prevalence of MCRPE, and the clinical associations and outcomes of *mcr-1*-positive *E coli* (MCRPEC) infections in patients in hospital from two provinces, Zhejiang and Guangdong. We also determine the prevalence of MCRPEC colonisation of patients in hospital and healthy volunteers and examine risk factors for carriage among patients. We have applied multi-locus sequence typing (MLST) analysis comparing MCRPEC and non-MCRPEC causing infections.

Methods

Study design and materials

We undertook this epidemiological and clinical study at two geographically remote tertiary care hospitals in Zhejiang and Guangdong, China. Each hospital contains more than 3000 beds and serves mainly Hangzhou (catchment of 9 million people) and Guangzhou (catchment of 13·5 million people), the capital cities, as well as a wider catchment area for neighbouring cities in each province.

First, we did a retrospective cross-sectional study to assess the prevalence and clinical characteristics of MCRPE (prevalence study). We included non-duplicate clinical isolates of Enterobacteriaceae, *Acinetobacter* spp, and *Pseudomonas aeruginosa* from inpatients collected between Jan 1, 2007, and April 30, 2015 (Fig 1), as part of a study of β-lactam resistance. For the prevalence study, we included patients who had been admitted for 2 or more days at any units of the two hospitals and who had a sample taken more than 48 h after admission. We excluded patients with all forms of gastroenteritis and those with active bleeding from the rectum or anal fissures. The source of infection was determined as pneumonia, urinary tract infection, surgical site infection, intra-abdominal infection, line-related infection, or bacteraemia as defined by the US Centers for Disease Control and Prevention. [17]

Second, we did a retrospective case-control study to assess risk factors for infection and 30-day mortality after infection with MCRPEC (infection study; Fig 1). We included all MCRPEC identified in the prevalence study, and a sample of *mcr-1*-negative *E coli* infection (ten times the number of positive isolates) selected by a blinded random draw done by the investigators. For the random selection, we assigned a continuing number, which did not indicate any medical information, for all inpatients, followed with putting all the numbers into an electronic pool in a computer, and then selecting the numbers with a computer mouse. We included *mcr-1*-negative isolates collected between Jan 1, 2012, and April 30, 2015, because no *mcr-1*-positive isolates were collected during 2007–11.

We also did a prospective case-control study to assess risk factors for carriage of MCRPEC (colonisation study; Fig 1). We included isolates from rectal swabs collected at the hospitals and rectal swabs from healthy volunteers attending primary care practices for routine annual physical examinations, collected from May 1, to Dec 31, 2015, assessed using clinical microbiology. For the random selection, we used the method described previously for the infection study. We compared risk factors for MCRPEC carriage (*mcr-1*-positive cases from inpatients) with *mcr-1*-negative *E coli* isolates from inpatients selected by a blinded random draw (12 times the number of positive isolates). For the colonisation study, we included isolates from rectal samples collected within 24 h of admission to hospital for the inpatients who were admitted for more than 2 days, and samples collected within 12 h of physical examination for the healthy volunteers. We excluded neonates, pregnant women, patients or healthy volunteers with gastroenteritis, and patients with gastrointestinal cancer, gastrointestinal surgery, peptic ulcer, gastrointestinal bleeding, inflammatory bowel disease (eg, Crohn's disease, ulcerative colitis), intestinal polyps, intestinal fistula, anal fistula, or anal fissure.

Ethical approval was given by The Second Affiliated Hospital of Zhejiang University and Sun Yat-Sen University Zhong Shan School of Medicine. Individual consent forms were translated into Mandarin and consent was obtained for all inpatients and healthy volunteers either face to face or by phone. We excluded patients from whom we could not obtain consent (eg, patient had moved or had died). All participants held the right to withdraw from the study at any stage.

Procedures

For analysis of risk factors during infection, we procured data from electronic medical records on patient demographics, underlying medical conditions, site of infection, intensive care unit (ICU) admission, presence of immunosuppressive factors (eg, AIDS, use of corticosteroids for more than 7 days), antibiotic use within 3 months before hospital admission or in hospital before isolation of pathogen, and clinical outcomes at 30 days.

For the analysis of risk factors of colonisation study, we obtained patient demographics, ICU admission, residence, proximity to commercial animal farms, education and income, diets and drinking water, and antibiotic use before and during hospital admission from medical records and a survey questionnaire. To reduce the potential recall bias on the risk factor of proximity to commercial animal farms, we checked the location of home addresses with Google maps and Baidu maps to confirm proximity to farms.

We initially identified all isolates from the retrospective collection (Fig 1) using Columbia blood agar (Oxoid, Hampshire, UK) with 5% sheep blood (Luqiao, Beijing, China), and identified species by MALDI-TOF mass spectrometry (BrukerDaltonik GmbH, Bremen, Germany). Where the species could not be well interpreted by MALDI-TOF, we applied 16S rDNA sequencing. We screened isolates from rectal samples for the colonisation study by inoculating on Columbia blood agar. Only one suspected Enterobacteriaceae isolate was analysed per sample. To determine presence of MCRPE from rectal samples and all clinical isolates we assessed growth on media containing 2 mg/L of colistin. All isolates were subsequently screened for *mcr-1* by PCR and sequencing.[12]

We determined the plasmid or chromosomal location of *mcr-1* gene in all MCRPEC isolates from both clinical and carriage isolates using the method previously described.[12] We investigated the frequency of transfer of *mcr-1*-carrying plasmid in both the infection and colonisation *E coli* isolates by conjugation experiments using streptomycin-resistant *E coli* C600 or rifampicin-resistant *E coli*EC600 as the recipient strains, and transfer frequencies were calculated as the number of transconjugants per recipient. Antimicrobial susceptibility testing of all MCRPEC, as well as the *mcr-1*-negative *E coli* isolates used in the infection and colonisation studies, was done by agar dilution and interpreted according to European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (version 6.0), and the Clinical and Laboratory Standards Institute document M100-S25.[18]

Whole cell DNA of all MCRPEC was prepared with the Wizard Genomic DNA Purification Kit (Promega, Beijing, China), submitted to whole genome sequencing,[18] and a draft assembly of the sequences was done with the CLC Genomics Workbench 8.5 (CLC Bio, Aarhus, Denmark). MLST, Mob typing, and Inc typing of *mcr-1*-positive isolates of both wild-type and transconjugants were done by searching the assembled contigs of the recognised chromosomal or plasmid sequences, and by specific PCR assays as needed. Sequence types (STs) and corresponding MLST gene allele profiles were entered into BioNumerics (Applied Maths, Belgium). The BURST algorithm was used to relate STs between various criteria of geographical location and clinical history.

Statistical analysis

Clinical data were collected and collated into specifically designed databases within Microsoft Excel 2013 and Minitab17 statistical software. Databases were password protected throughout the course of the study. We calculated risk factors for *mcr-1*-positive *E coli*infection and colonisation using a two-stage approach. We used univariate statistical tests to assess key risk factors for potential association. Those significant at the 10% level were entered into multivariable logistic regression using IBM SPSS software. Separate models were fitted for overall antibiotic use and exploration of the effect of individual classes of antibiotics. Given the categorical nature of the variables, binary data were tested by χ^2 analysis or Fisher's exact test. Continuous data were tested with the Mann-Whitney *U* test.

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

Results

Prevalence of *mcr-1*

For the prevalence study, we analysed 17 498 isolates from 18 698 inpatients, including 10 688 Enterobacteriaceae, 3549 *Acinetobacter* spp, and 3261 *P aeruginosa* (Fig 1). We detected *mcr-1* in 76 (1%) of 5332 *E coli*, 13 (<1%) of 3480 *K pneumoniae*, one (<1%) of 890 *E cloacae*, and one (1%) of 162 *Enterobacter aerogenes*. Over the sampling period, we noted a general increase in the prevalence of *mcr-1*-positive *E coli* in both hospitals. A non-linear increase in *mcr-1*- positive *K pneumoniae* was also noted.

For the colonisation study, we analysed rectal swabs of 1200 inpatients and 2923 healthy volunteers, from which we identified 4123 Enterobacteriaceae. 35 (3%) of 1199 *E coli* isolates from inpatients were positive for *mcr-1*, and one of one *E cloacae* isolate from inpatients was positive; 19 (1%) of 2923 *E coli* isolates from healthy volunteers were positive for *mcr-1*.

Minimum inhibitory concentration (MIC) profiles of MCRPEC and *mcr-1*-negative *E coli* isolates

In the infection study, resistance was significantly more common in E coli isolates with *mcr-1* (n=76) than in those without *mcr-1*(n=508) for most antimicrobial drugs tested, including not only colistin and polymyxin B, but also amoxicillin plus clavulanic acid, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, ertapenem, fosfomycin, nitrofurantoin, and ciprofloxacin (Table 1).

There were large differences in MIC_{50} between MCRPEC isolates from inpatients with infections (n=76) and those from rectal swabs in healthy people (n=19), with differences of more than 32-fold for ampicillin, 512-fold for cefotaxime, 64-fold for ceftazidime, 64-fold for cefepime, and 64-fold for ciprofloxacin. However, the MIC_{50} values from clinical *E coli* isolates from inpatients with infections (n=76) were similar to the isolates from rectal swabs from inpatients (n=35), except for ceftazidime, cefepime, and ciprofloxacin, which were eight-fold, 32-fold and 16-fold higher, respectively.

Coexistence of *mcr-1* and carbapenem resistance

Among all 146 MCRPE (91 from the retrospective collection and 55 from the colonisation collection), five *E coli* isolates from clinical samples were carbapenem resistant: three from Zhejiang and two from Guangdong. All five isolates were resistant to ertapenem, with MICs of 4 mg/L (n=2), 8 mg/L (n=2), and 64 mg/L (n=1), whereas only one isolate (ZJ487 from an intra-abdominal fluid sample in Zhejiang) was resistant to imipenem (>256 mg/L) and had intermediate resistance to meropenem (4 mg/L); the other four isolates were susceptible to both imipenem and meropenem. Four of these five inpatients with CRE recovered after treatment, with only one inpatient from Guangdong without improvement. Screening for carbapenem resistance genes among the five carbapenem-resistant *E coli* harbouring *mcr-1* showed that two isolates, the ZJ487 with ST90 from intra-abdominal fluid of an inpatient from Zhejiang and GD50 with ST744 from a faecal sample of a healthy volunteer from Guangdong, were bla_{NDM-1}-positive. Further analysis showed that bla_{NDM-1} and *mcr-1* were located on the chromosome and on plasmids of these

isolates, respectively. In addition to *bla*$_{NDM-1}$, isolate ZJ487 carried *bla*$_{TEM-1}$, *bla*$_{SHV-12}$, *bla*$_{CTX-M-55}$, and *bla*$_{OXA-1}$ and isolate GD50 carried *bla*$_{TEM-1}$, *bla*$_{SHV-12}$, *bla*$_{CTX-M-14}$, and *bla*$_{CTX-M-15}$. The other three isolates were negative for carbapenemase genes but were extended-spectrum β-lactamase-positive (eg, *bla*$_{CTX-M-15}$ in two isolates and *bla*$_{CTX-M-55}$ in another).

Risk factors

For the infection study, we collected clinical data from 76 *mcr-1*-positive isolates and randomly selected 760 *mcr-1*-negative clinical *E coli* isolates (of which 252 were excluded because of incomplete data, leaving 508) from 4609 *E coli* infection cases. 30-day mortality was 7·3% for *mcr-1*-negative infections and 11·8% for *mcr-1*-positive infections. Being male, immunosuppressed, or having received antibiotics in the past 3 months were significant risk factors for *mcr-1*-positive infection (Table 2). Use of carbapenems and fluoroquinolones in the previous 3 months was particularly associated with increased risk. Patient age was not associated with *mcr-1*-positivity (Table 2). We saw no association with either *mcr-1*-positive or *mcr-1*-negative infections in patients with neutropenia or undergoing artificial lung ventilation (Table 2). With only two regions (Guangdong and Zhejiang), we analysed region as a fixed effect and it was no longer significant in the multivariable model (Table 2).

In the colonisation study, we identified 35 MCRPEC isolates from rectal swabs of inpatients. We randomly selected 420 *mcr-1*-negative isolates from inpatients for comparison, of which 42 were excluded because of incomplete data, leaving 378 isolates of *mcr-1*-negative *E coli* (Fig 1; Table 3). Antibiotic use before hospital admission was a major risk factor for colonisation by *mcr-1*-positive *E coli* (unadjusted p=0·0008; adjusted p<0·0001). Living in proximity to livestock was a risk factor for colonisation by *mcr-1*-negative *E coli* in the unadjusted analysis (p=0·03) but not in the adjusted analysis (p=0·995; Table 3).

In addition, we analysed all associations and outcomes for both *mcr-1*-negative *E coli* and MCRPEC comparing Guangdong with Zhejiang. Significant differences were noted in several social aspects between the two regions, including living place, education, income, diet, and drinking water.

High frequency of transfer of *mcr-1* carrying plasmids

S1-pulsed field gel electrophoresis and Southern blotting of 130 MCRPEC (76 from the infection study and 54 [one isolate had lost the plasmid during this stage of the analysis and could not be included in these data] from the colonisation study) showed that *mcr-1* was located on plasmids with sizes ranging from 32 kb to 400 kb in 123 isolates, and on the chromosome in seven isolates Among the 123 isolates carrying *mcr-1* on plasmids, *mcr-1* was successfully transferred to *E coli* EC600 or C600 on 42 (34%) occasions. Frequency of transfer differed substantially, ranging from 10^{-1} to 10^{-10}, while 29 (69%) of these 42 isolates had high conjugation rates Two main markers (Inc and Mob) of *mcr-1*-carrying plasmids in the transconjugants were characterised. IncX4 and IncI2 were the most prevalent Inc types identified in 13 and 14 transconjugants, respectively. Other Inc types included F (n=7), K (n=6), FIB

(n=6), I1-1γ (n=6), N (n=4), and FIIS (n=1). 11 transconjugants were non-typeable using the current Inc classification. Mob typing showed that P51 and F12 were found in 11 and nine transconjugants, respectively. Both H121 and Qu were found in one transconjugant, but 24 transconjugants could not be typed using the Mob system.

Diversity of MCRPEC strains

We assessed clonality and clades of both *mcr-1*-negative and *mcr-1*-positive *E coli* strains from the infection study (Fig 1) as minimum spanning trees for Guangdong (Fig 2A) and Zhejiang (Fig 2B). Both the number and diversity of strains enrolled from Guangdong were greater than those from Zhejiang, with Guangdong having 21 ST clades representing five or more *E coli* compared with eight STs from Zhejiang. From these clades, *mcr-1*-positive *E coli* were found in ST602, ST101, ST410, ST95, ST1193, ST354, ST156, ST10, ST117, ST405, ST131, and ST457 from Guangdong compared with ST410, ST617, ST10, ST648, and ST354 form Zhejiang (Fig 2). Overall, our data suggest substantial horizontal dissemination of *mcr-1* through *E coli* populations; *mcr-1* was found in 30 (26%) of 116 ST clades from Guangdong and 25 (34%) of 73 ST clades from Zhejiang. Many of these ST clades were large, including ST131, ST95, ST1193, and ST405 from Guangdong. *mcr-1* was not found in substantial *E coli* clades such as ST69, ST648, and ST38. By contrast with Guangdong, *mcr-1* was not identified in the clades ST131, ST1193, or ST405 in Zhejiang. Across all tested samples, we discovered isolates in 17 new distinct STs carrying *mcr-1*.

The clades identified among the carriage isolates (both hospital and community) from the colonisation study were diverse from both Zhejiang and Guangdong with some commonality in the clades isolated from clinical samples—eg, ST10, ST156, ST117, and ST131 (Fig 3). However, ST48, ST4014, and ST58 were not isolated clinically (Fig 3).

Discussion

MCRPE gained global attention after our finding of the mobile colistin resistance mechanism MCR-1.[12] Following our report of emergence of MCR-1 in both animals and human beings, we undertook a comprehensive study on the prevalence, clinical association, and carriage of MCRPE in human beings. Analysis of the different associations and outcomes from all infections (*mcr-1*-negative and MCRPEC) showed one unexpected result. There was a gender bias (p=0·011) in whether the infection was *mcr-1*positive with a male:female ratio of 41:59 for *mcr-1* negative Enterobacteriaceae and 63:37 for MCRPEC infections. Unsurprisingly, previous antibiotic exposure was strongly associated with the presence of *mcr-1*-positive infection. In view of the potential of *mcr-1*to coexist with other resistance genes on the same plasmids, coselection of *mcr-1* with other antibiotics, regardless of the use of colistin in human beings in China, is a likely scenario. Our first study of MCRPE showed close association with animals and husbandry practices and thus we anticipated a positive association with close proximity to farms.[12] Counterintuitively, however, we observed a weak association for MCRPEC carriage with living distantly from farms.

MLST analysis shows the extreme divergence of *E coli* strains carrying *mcr-1* not only between Zhejiang and Guangdong, but also within each hospital. MCRPEC could be divided into 30 different clades from Guangdong and 25 from Zhejiang, in most cases these involved only one representative isolate. Of these *mcr-1*-positive clades, only ST10, ST410, and ST354 were common to both regions. The diversity of strains carrying *mcr-1* is further shown by the fact that 17 isolates belonged to new or unreported MLST groups. The high level of heterogeneity is supported by our data showing high transmission rates of *mcr-1*-carrying plasmids into laboratory recipients (transconjugants), with the dominant Inc type being IncX4 that has been previously reported in $bla_{\text{CTX-M}}$-carrying *E coli*from both human beings and food animals in Hong Kong, China.[19] 66% of isolates carrying plasmid-borne *mcr-1* could not be transferred into recipient *E coli* isolates, although increased transfer frequencies were observed in some other *E coli* isolates from this study. Although Guangdong and Zhejiang have common *mcr-1*-positive ST groups, these *E coli* clades have different plasmid backbones, suggesting independent acquisition of *mcr-1* by these STs.

E coli ST156 belongs to phylogenetic group A and is generally not considered to be highly virulent, although a recent study from Spain would suggest otherwise.[20] ST156 has been previously reported from China and associated with MDR and XDR phenotypes, but mainly from poultry.[21, 22] *E coli* ST101 is widely regarded as having environmental lineage and being associated with water and sewerage, and also poultry; however, it has been associated with carbapenem resistance and XDR clinical outbreaks.[22, 23] To the best of our knowledge, this study is the first report of *E coli* ST101 in mainland China. *E coli* ST95 is now thought to be more pathogenic than are ST156 and ST101 and considered a human and avian pathogen.[24] *E coli* ST10 has only previously been reported from animals in China and thus this is the first report of ST10 from human infections in China.[25] ST131 has long been recognised as the virulent global epidemic strain associated with human disease and also displaying MDR and XDR phenotypes.[26] Until now, the only ST131 isolate reported carrying *mcr-1* was from chicken meat

imported into Denmark.[27] In this study, we describe three ST131 *mcr-1*-positive isolates from Guangdong that would suggest colistin resistance might be associated with poorer clinical outcome than other STs. Chinese reports on patient infections suggest that *E coli* ST131 is prevalent[28] and thus, the probability of *mcr-1*plasmids further being disseminated among these pathogenic *E coli* seem highly likely. However, in this study, the patients infected with *mcr-1*-positive *E coli* ST131 causing skin and soft tissue, bloodstream, and urinary tract infections recovered, suggesting that in this limited sample *mcr-1*-positive *E coli* ST131 strains were not pathogenic. Some clades only found in carriage isolates—eg, ST48, ST4014, and ST58—were not isolated clinically, suggesting they could be less likely to cause disease.

We acknowledge several limitations in our study. First, although we noted significant increases of *mcr-1*-harbouring clinical isolates during 2012–15 compared with 2007–11, these were from retrospective data taken from samples in 2012–15. The possibility of loss of *mcr-1*-carrying plasmids during storage cannot be excluded, and so the prevalence of *mcr-1* and numbers of colistin resistance phenotypes from these retrospectively collected isolates might be underestimated. Second, we only included 378 (33%) of 1164 *mcr-1*-negative colonisation isolates and 508 (11%) of 4533 *mcr-1*- negative infection isolates for MLST. Analysis of more isolates for MLST would provide a more compelling database and a greater case for strain relatedness between isolates. Third, we have noted differences between Guangdong and Zhejiang provinces, but cannot further speculate as to why these differences have occurred without more in-depth observations and analysis and they might not be generalisable across other provinces and all of China. Last, the risk factors that were reported by the patients and the volunteers might be confounded by recall bias.

Although there have been many reports of carbapenem resistance in Chinese hospitals, most MCRPEC were carbapenem-susceptible; however, in this study, resistance to ciprofloxacin, cefotaxime, and cefepime was especially common. These results suggest that MCRPEC were able to recruit other resistance genes and become multiply drug-resistant, which could raise difficulties in the treatment of patients with clinical infection due to MCRPEC. Despite the fact that almost all MCRPEC were colistin-resistant, clinical colistin resistance in China is somewhat of an enigma because colistin is not generally used in human beings. The first report of *mcr-1* in China resulted in substantial policy discussions and has encouraged the withdrawal of colistin.[29] In part, this report is likely to advocate the use of colistin in Chinese patients and its subsequent withdrawal from the farming sector. Accordingly, this study provides a useful baseline of colistin resistance to monitor the effect of its introduction and provide predictive outcomes on treatment failures.

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Table 1
Minimum inhibitory concentration profiles of clinical infection-derived *Escherichia coli* with or without *mcr-1*

	***mcr-1*-negative *E coli* (n=508)**	***mcr-1*-positive *E coli* (n=76)**	**p value**
Colistin	53/455 (10%)	74/2 (97%)	<0·0001
Polymyxin B	117/391 (23%)	74/2 (97%)	<0·0001
Tigecycline	24/467 (5%)	1/71 (1%)	0·178
Ampicillin	442/66 (87%)	72/4 (95%)	0·053
Amoxicillin plus clavulanic acid	125/383 (25%)	45/31 (59%)	0·0005
Cefotaxime	292/200 (58%)	64/10 (84%)	0·0005
Ceftazidime	172/260 (34%)	41/24 (54%)	0·0008
Cefepime	185/219 (36%)	43/21 (57%)	0·001
Gentamicin	227/277 (45%)	46/25 (61%)	0·002
Amikacin	12/490 (2%)	14/60 (18%)	<0·0001
Ertapenem	12/490 (2%)	5/70 (7%)	0·041
Imipenem	4/504 (1%)	1/75 (1%)	0·641
Meropenem	1/501 (<1%)	0/75	0·699
Fosfomycin	182/326 (36%)	45/31 (59%)	0·0008
Nitrofurantoin	11/497 (2%)	7/69 (9%)	0·0009
Ciprofloxacin	313/175 (62%)	62/11 (82%)	0·0005

Data are number resistant/number sensitive (% of resistance rates). p value for comparisons of the resistance rates of *mcr-1*-positive and *mcr-1*-negative groups.

Table 2

Risk factors and associated outcomes of *mcr-1*-positive *Escherichia coli* infection

	***mcr-1*-negative *E coli* (n=508)**	***mcr-1*-positive *E coli* (n=76)**	**Unadjusted p value ***	**Adjusted p value †**	**Adjusted p value ‡**
Region			0·006	0·208	0·044
Guangdong	348 (90%)	40 (10%)			
Zhejiang	160 (82%)	36 (18%)			
Age (years)	54·7 (18·17)	56·2 (17·85)	0·485		
Sex (male)	209 (41%)	47 (63%)	001	0·010	0·011
Site of infection			0·074	0·513	0·513
Respiratory	54 (11%)	16 (21%)			
Bloodstream	73 (14%)	8 (11%)			
Skin and soft tissue	37 (7%)	8 (11%)			
Intra-abdominal	141 (28%)	22 (29%)			
Urinary tract	199 (39%)	22 (29%)			
Bone or joint	0	0			
CNS	4 (1%)	0			
Admitted to ward (intensive care unit)	38 (8%)	8 (11%)	0·358		
Comorbidities and risk factors					
Immunosuppression	30 (6%)	11 (15%)	0·006	0·011	0·011
Diabetes	80 (16%)	9 (12%)	0·377	..	..
Neutropenia	11 (2%)	1 (1%)	1·000	..	..
Artificial lung ventilation	18 (4%)	2 (3%)	1·000	..	..
Vascular catheter	131 (26%)	22 (29%)	0·559	..	..
Abdominal or pelvic catheter	40 (8%)	2 (3%)	0·099	0·096	0·114
Urinary catheter	128 (25%)	24 (32%)	0·237	..	..
Antibiotic use in the past 3 months	405 (80%)	74 (97%)	0·0009	0·008	..
Type of antibiotic used in the past 3 months					
Penicillin and β-lactamase inhibitor combination	102 (20%)	23 (30%)	0·043		0·559
Cephalosporin (narrow)	20 (4%)	4 (5%)	0·538		..
Cephalosporin (broad)	127 (25%)	23 (30%)	0·327		..
Carbapenem	45 (9%)	18 (24%)	0·0001		0·002
Aminoglycosides	21 (4%)	3 (4%)	1·000		..
Fluoroquinolones	95 (19%)	23 (30%)	0·019		0·017

Data are mean (SD) or n (%). *Univariate tests: everything significant at 10% level selected for multivariable models. †Model including: region, site of infection, sex, immunosuppression, abdominal or pelvic catheter, and antibiotic use in the past 3 months. ‡Model including: region, site of infection, sex, immunosuppression, abdominal or pelvic catheter, and use in the past 3 months of penicillins and β-lacatamase inhibitors, carbapenems, and fluoroquinolones

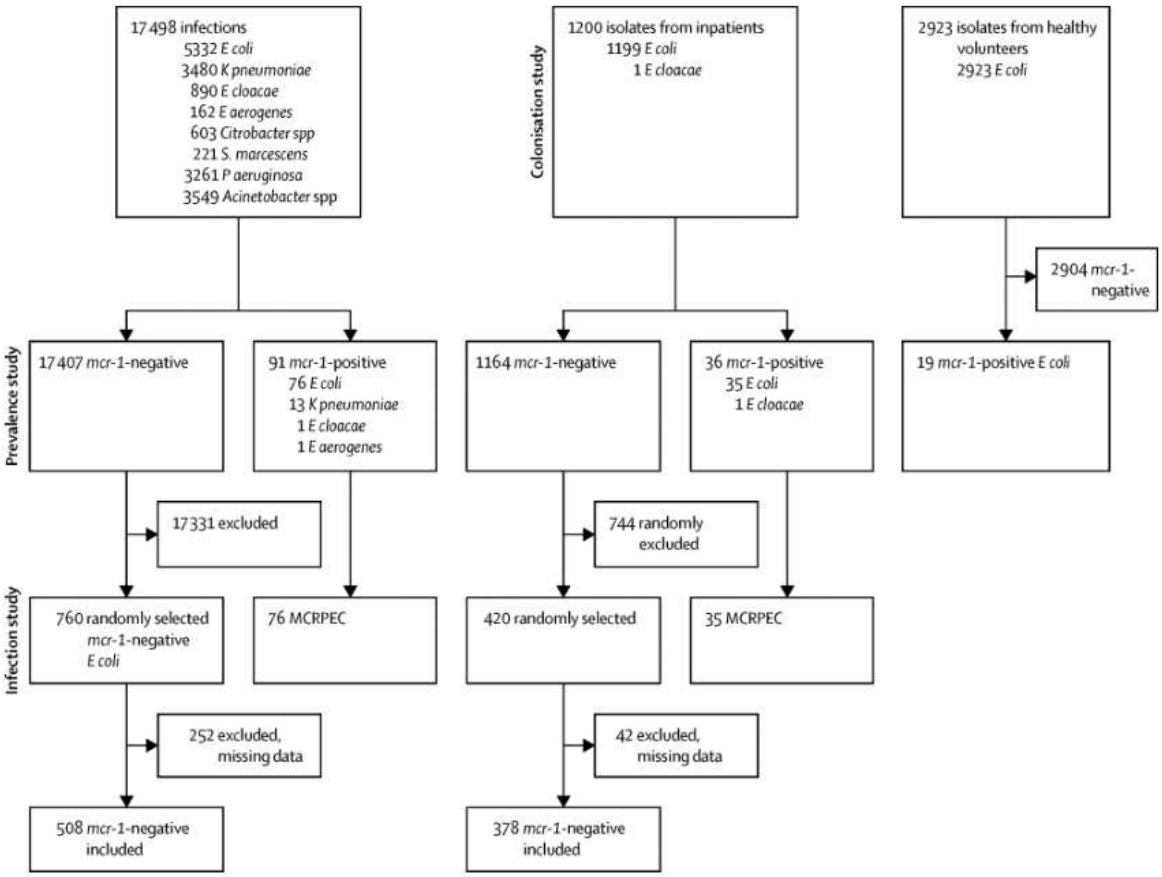
Table 3
Risk factors associated with *mcr-1*-positive *Escherichia coli* colonisation in patients in hospital

	***mcr-1*-negative *E coli* (n=378)**	***mcr-1*-positive *E coli* (n=35)**	**Unadjusted p value ***	**Adjusted p value †**
Region			0·19	
Guangdong	128 (34%)	8 (23%)		
Zhejiang	250 (66%)	27 (77%)		
Age (years)	60 (10–82)	55 (27–89)	0·92	
Sex				
Male	197 (52%)	21 (60%)	0·37	
Female	181 (48%)	14 (40%)	..	
Admitting ward				
Non-intensive care unit	332 (88%)	31 (89%)	0·89	..
Intensive care unit	46 (12%)	4 (11%)	..	..
Living place				
City/county	216 (57%)	17 (49%)	0·33	..
Village	162 (43%)	18 (51%)	..	..
Proximity to commercial animal farm				
<1 km	142 (38%)	10 (29%)	0·03	0·995
1–10 km	62 (16%)	12 (34%)	..	..
>10 km	174 (46%)	13 (37%)	..	..
Education				
None/primary	166 (43·9%)	22 (63%)	0·09	0·052
Secondary	147 (38·9%)	9 (26%)	..	..
Tertiary	65 (17·2%)	4 (11%)	..	..
Income class				
Low	166 (44%)	20 (57%)	0·13	..
Middle/high	212 (56%)	15 (43%)	..	..
Diets				
Vegetarian	11 (3%)	0 (0%)	0·61	..
Non-vegetarian	367 (97%)	35 (100%)	..	..
Antibiotic use before hospital stay				
Yes	88 (23%)	20 (57%)	0·0008	<0·0001
No	243 (64%)	15 (43%)	..	..
Not sure	47 (12%)	0	..	..
Antibiotic use during hospital stay ‡				
Yes	318 (84%)	32 (91%)	0·25	..
No	60 (16%)	3 (9%)	..	..
Drinking water				
Municipal	316 (84%)	30 (86%)	0·75	..
Non-municipal (vendor, well, or other)	62 (16%)	5 (14%)	..	..

Data are median (IQR) or n (%). *Univariate tests. All those testing at p<0·05 were deemed significant. All those testing at a 10% significance level were selected for multivariable analysis. †Multivariable model includes proximity to commercial animal farms, education, and antibiotic use before hospital admission. ‡Before *E coli* isolation.

Figure 1

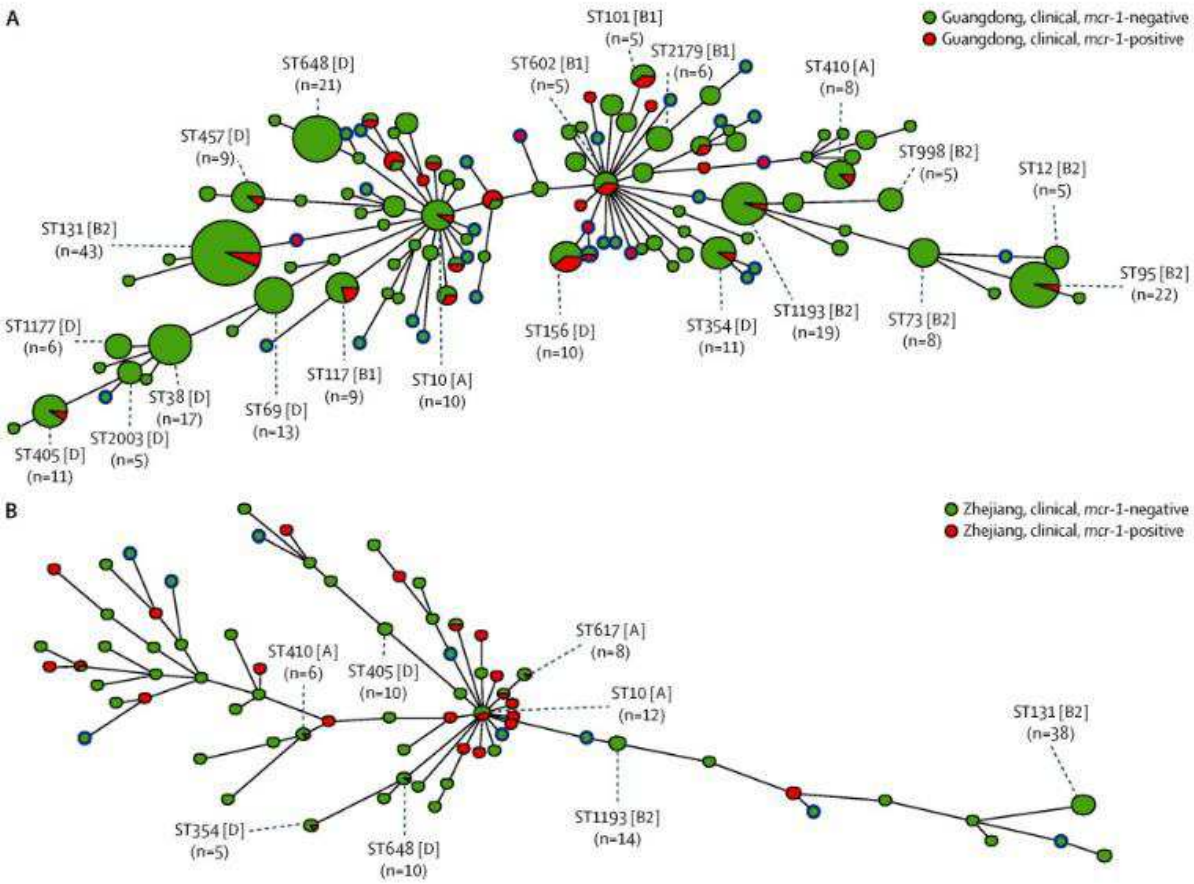
Flowchart diagram of samples collection in two hospitals in Zhejiang and Guangdong provinces



P aeruginosa=Pseudomonas aeruginosa. E coli=Escherichia coli. K pneumoniae=Klebsiella pneumoniae. E cloacae=Enterobacter cloacae. E aerogenes=Enterobacter aerogenes. S marcescens=Serratia marcescens. MCRPEC=*mcr-1*-positive *Escherichia coli.* .

Figure 2

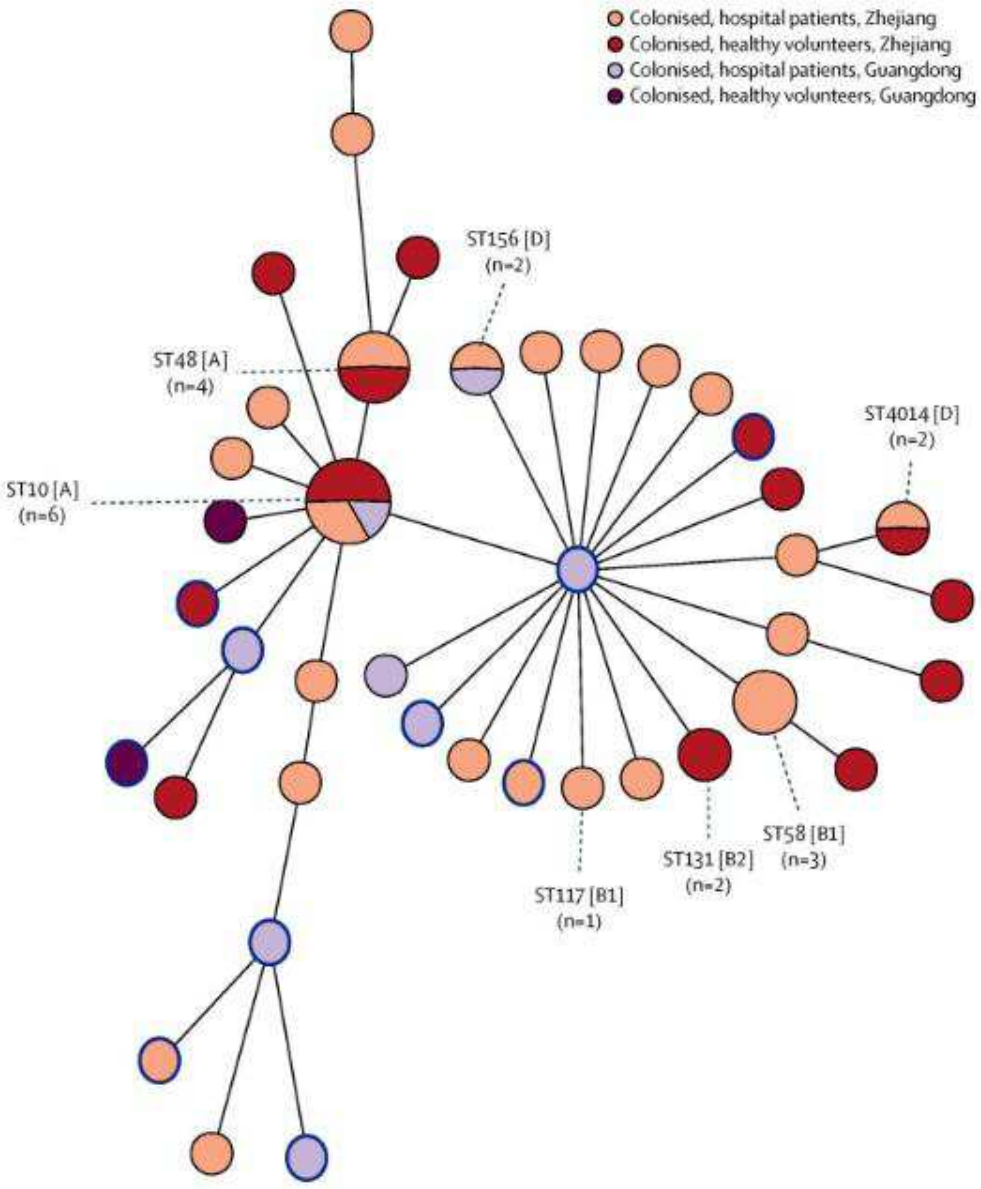
Minimum spanning trees of *mcr-1*-positive *Escherichia coli* by MLST type and gene allele profile for Guangdong (A) and Zhejiang (B)



Each node within the tree represents a single ST. The size of the nodes is proportional to the number of isolates represented by said node. Length of branches between each node is proportional to the number of different alleles (out of seven MLST genes) that differ between two linked nodes/ST. Selected nodes are labelled with corresponding ST, phylogenetic group, and number of isolates represented. New STs identified are in blue. MLST=multi-locus sequence typing. ST=sequence type

Figure 3

Minimum spanning tree of *mcr-1*-positive *Escherichia coli* by MLST type and gene allele from colonised patients from hospitals and colonised healthy volunteers



Each node within the tree represents a single ST. The size of the nodes is proportional to the number of isolates represented by said node. Length of branches between each node represents the number of different alleles (out of seven MLST genes) that differ between two linked nodes/ST. Selected nodes are labelled with corresponding ST, phylogenetic group, and number of isolates represented. New STs identified with the blue border. MLST=multilocus sequence typing. ST=sequence type.