Anthropogenic and environmental factors associated with high incidence of *mcr-1*carriage in humans across China

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ABSTRACT

MCR-1-positve *Escherichia coli* (MCRPEC) have been reported in humans worldwide; however, thus far, their prevalence is low and potential sources for human *mcr-1* carriage have not yet been identified. Here, we analyse a nationwide epidemiological dataset on MCRPEC in humans throughout China and assess the factors associated with MCRPEC carriage using natural and national anthropogenic data. We identified 774 non-duplicate MCRPEC isolates from 774 stool samples collected from 5,159 healthy individuals in 30 provinces and municipalities in 2016, with a prevalence of MCRPEC ranging from 3.7 to 32.7% (average: 15.0%)—substantially higher than previously reported. MCRPEC carriage was associated with provincial regions, the production of sheep and freshwater aquaculture, annual consumption of total meat, pork and mutton, and daily intake of aquaculture products. MCRPEC was significantly more prevalent in provinces with higher aquaculture industries. Whole-genome sequencing analysis revealed that the MCRPEC isolates were clustered into four distinct lineages, two of which were dominant and harboured most of the MCRPEC isolates. The high prevalence of MCRPEC in the community poses a substantial risk for colistin usage in clinical practice and suggests the need for intestinal screening of *mcr-1* carriers in intensive care units in Chinese hospitals. Furthermore, our data suggest that aquaculture is a significant reservoir of *mcr-1*.

Since the discovery of the plasmid-mediated colistin resistance determinant MCR-1 in Enterobacteriaceae in 2015, it has been reported globally in over 50 countries on 5 continents within 2

years[1,2]. So far, nine *mcr-1* variants (*mcr-1.2* to *mcr-1.10*)[3,4,5,6] have been reported. Additionally, *mcr* has been identified in extensively drug resistant Enterobacteriaceae isolates carrying plasmid-borne carbapenemase and ESBL genes[7]. Given that polymixins are a last-resort treatment for extensively drug resistant bacterial infections, the spread of *mcr* across pathogenic bacterial clones is likely to pose a serious public health crisis. In July 2016, the European Medicines Agency updated the risk level of colistin resistance transfer from low to high. In November 2016, the Ministry of Agriculture of China (article number 2428) withdrew colistin as a feed additive and growth promoter, and this was officially enforced in April 2017[8]. In February 2017, the Department of Livestock Development in Thailand also banned the use of colistin as a feed additive. Despite these regulatory edicts, colistin is still widely used in animal production and will continue to be used in farms either metaphylactically or for treating individual animals.

Unlike the high detection rates of MCR-positive Enterobacteriaceae (MCRPE) in animals[7,9], in human populations, MCRPE has been reported to be substantially lower. Among *Escherichia coli* isolates associated with human infections, 1.3–1.7% have been found to be positive for *mcr-1* [2,10,11], while only 0.2–0.7% of clinical *K. pneumoniae* isolates have been found to harbour *mcr-1* [1,2,10,11]. For *E. coli* isolates derived from human colonization, the prevalence of *mcr-1*-positve *E. coli* (MCRPEC) varies between inpatients (0.4–2.9%)[2,12] and healthy individuals (0.7–6.2%)[2,13,14]. Most of these studies focused on MCRPE from clinical samples in inpatients, while the studies on MCRPE were from healthy individuals and of a small sample size involving one or two hospitals[2,14]. Recently, we reported the widespread distribution of MCRPEC among Chinese farms and proposed the term 'phantom resistome'—a phenomenon where *mcr-1* rates are considerably higher in DNA extracted from samples than isolates grown on media containing colistin[15]. A recent study examining the acquisition of MCRPE when travelling to India used an enrichment method and showed higher rates than non-enrichment methods, which may, in part, explain the 'phantom resistome'[16].

Our previous MCRPEC data showed that carriage among humans had a weak but positive association with living distance from commercial animal (pig and poultry) farms, but is not associated with diet preference (vegetarian versus non-vegetarian)[2]. These findings suggest a possible link of MCRPEC colonization and carriage between humans and animals, but this link remains unproven due to the limited sampling size and narrow geographic scope of previous studies[2,12]. Accordingly, we have determined the prevalence and molecular epidemiology of MCRPEC carriage in healthy individuals across 30 (out of 34) provinces and municipalities in China using an enrichment method to capture a larger sector of the 'phantom resistome' and a truer prevalence of human carriage. Furthermore, given the potential spread of MCRPEC in Chinese communities, we analysed the anthropogenic and

environmental factors associated with human MCRPEC carriage. In addition, as *mcr* genes may have originated in aquatic environments[17,18], the 30 Chinese provinces and municipalities were further divided into low, medium and high areas of aquaculture industrial intensity[19], to access the impact of aquaculture-associated anthropogenic factors on MCRPEC carriage.

RESULTS

Distribution of *mcr-1*

Our inclusion and exclusion criteria for subjects, and criteria for processing of samples. From 1 June to 30 September 2016, we collected 5,159 rectal swabs from healthy individuals across 30 provinces and municipalities in China. Zhejiang and Beijing contributed the largest numbers of samples (both >470). Sichuan province and Shanghai contributed the smallest (both ≤50). The ratio of males to females in this study was 1.2:1. The age of subjects ranged from 12 to 89 years and 83.8% were between 21 and 50 years old (Table 1). A total of 774 faecal samples were positive for the *mcr-1* gene (15.0%), and MCRPEC (774 non-duplicate isolates) from each sample were further characterized. Liaoning province displayed the highest *mcr-1* prevalence of 32.7% (95% confidence interval (CI): 20.7–46.7%), while Ningxia province showed the lowest prevalence of 3.7% (95% CI: 1.5–7.6%). The mean positive prevalence of *mcr-1* carriages was 15.0% (95% CI: 14.0–16.0%) among the 30 provinces and municipalities (Figure 1).

Correlation of *mcr-1* carriages with environmental and anthropogenic factors

Given that previous studies have suggested that MCRPEC started and spread within Chinese farming communities, we analysed the potential association of *mcr-1* carriage with behavioural and non-behavioural factors, including environmental and anthropogenic factors. When analysed by univariable analysis, 18 of 27 associations were shown to be significant ($P \leq 0.05$; Table 1). No observable difference was seen between males and females (odds ratio (OR) = 1.0, 95% CI: 0.9–1.2). The odds of MCRPEC for ages 81 to 90 (OR = 3.9, 95% CI: 1.1–14.5) were significantly higher than for other age brackets, but accounted for 0.2% of the samples. After removing highly correlated variables, 13 remained, 7 of which were significantly ($P < 0.05$) associated with *mcr-1* positivity by multivariable logistic analysis (Table 2). In this model, geographical zones with low aquaculture industry had significantly lower odds (OR = 0.5, 95% CI: 0.3–0.7) of *mcr-1* positivity compared with those with higher aquaculture activity. Populations with lower levels (≤100 g d^{-1}) of aquatic food intake also had significantly lower odds (OR = 0.6, 95% CI: 0.5–0.7) of *mcr-1* positivity. Unsurprisingly, the consumption of meat—especially pork and mutton—was significantly associated with higher *mcr-1* prevalence (Table 2). The Hosmer–Lemeshow 'goodness-of-fit test' ($\chi^2 = 0.6$, d.f. = 8, $P = 1 > 0.05$)

indicated that the model was a robust fit for the data. The estimated area under the receiver operating characteristic curve was 0.65, indicating good predictability.

Minimum inhibitory concentration profiles of MCRPEC

We tested the antimicrobial susceptibility of all MCRPEC isolates from the 30 provinces and municipalities to 9 clinical antibiotics (cefepime, amikacin, piperacillin/tazobactam, ceftazidime, ticarcillin/clavulanic acid, ciprofloxacin, imipenem, colistin and cefoperazone/sulbactam). In general, MCRPEC was more commonly resistant to ticarcillin/clavulanic acid (57.88%) and ciprofloxacin (38.89%) than the other antimicrobial agents, and only a small number of *mcr-1*-positive isolates were resistant to amikacin (1.16%), imipenem (1.29%) and cefoperazone/sulbactam (2.32%). Significant differences in the minimum inhibitory concentration profiles among the three aquaculture conditional areas were only found for cefepime ($P = 0.002$), ceftazidime ($P = 0.018$) and ciprofloxacin ($P = 0.011$).

Relationship of the MCRPEC isolates determined by whole-genome sequencing

The core-genome-based phylogenetic analysis, together with Bayesian analysis of the population structure (BAPS) was employed to define lineages within the 287 sequenced MCRPEC isolates from 30 provinces and municipalities across China. Genomic data from an additional 16 clinical MCRPEC isolates from Asia, Europe, and North and South America were added for comparison. The BAPS analysis revealed four distinct lineages among the 287 MCRPEC isolates (Figure 3). The main lineages, L3 ($n = 78$) and L4 ($n = 183$), comprised MCRPEC isolates from 29 (except Xinjiang province) and 30 provinces, respectively. The additional 16 international MCRPEC isolates fell into lineages L1 ($n = 3$), L3 ($n = 7$) and L4 ($n = 6$), and displayed high similarity to the isolates analysed in this study. There was no obvious correlation between lineages and provinces. Notably, the isolates from the low, medium and high aquaculture areas correlated within lineages L3 and L4, in which three highly homologous sublineages, S1, S2 and S3, harboured 19, 13 and 17 MCRPEC isolates from 15, 9 and 10 provinces, respectively. Two isolates, each from the USA and the Netherlands, also fell into cluster S2 (Figure 3).

Distribution of antibiotic-resistance and virulence-associated genes among MCRPEC isolates

Antibiotic resistance genes (ARGs) and virulence-associated genes (VAGs) in the 287 MCRPEC isolates were determined from whole-genome sequences. A heat map showing the percentages of both ARGs and VAGs for each province or municipality was constructed (Fig. 4). No obvious association of ARGs or VAGs was found between provinces. Noticeably, most isolates belonged to phylogenetic group A and only a few isolates belonged to phylogroups B2 and D, but phylogroup B2 and D isolates

harboured a larger number of VAGs than those from groups A and B1. ARGs of the isolates from groups B2 and D were not strongly associated with VAGs of isolates from the same groups (Fig. 4). For VAGs, the *mcr-1* gene co-existed more with *hcpA*/*B*/*C* (which encodes the haemorrhagic pilus of *E. coli*), *ecpA*(common pilus), *hlyE*/*clyA* (haemolysin/cytolysin A), *espL*/*R*/*X* (type III secretion system), *fimH* (type I fimbriae) and *ibeB*/*C*, whereas VAGs of pathogenic *E. coli*, such as ETEC, EAEC, EIEC, EPEC, EHEC, DAEC and so on, were rarely found in MCRPEC isolates from our study. In addition, resistance (*floR*) to florfenicol—a veterinary drug—was commonly associated with *mcr-1*. Associations of tetracycline ARGs (*tetA* and *tetR*), plasmid-mediated quinolone-resistance genes (*oqxA/B*), the β-lactamase ARG (*bla* $_{\text{TEM}}$) and some aminoglycoside ARGs (*aadA*, *strA/B* and *aph*(3')-*Ia*) with *mcr-1* were higher than with other ARGs; namely, other β-lactamase ARGs (*bla* $_{\text{CTX}}$, *bla* $_{\text{OXA}}$, *bla* $_{\text{CMY}}$, *bla* $_{\text{SHV}}$ and so on) and carbapenem ARGs (*bla* $_{\text{KPC}}$ and *bla* $_{\text{NDM}}$) (Figure 4).

Diversity of MCRPEC isolates and plasmid Inc types

MLST (multi-locus sequence type) groups of MCRPEC were assessed using minimum spanning trees for all 287 isolates from the 30 provinces and municipalities, which were divided into three sectors according to distinct aquaculture activities. The 287 MCRPEC strains were separated into 135 MLST clades showing marked diversity across each province. ST10 was the dominant clade, containing 46 (16.0%) MCRPEC strains, while other MLST clades, including ST48, ST206, ST93, ST101, ST1684 and ST216, were also widely distributed across low, medium and high regions of aquaculture industry. ST410 ($n = 5$) and ST155 ($n = 9$) were not identified in the medium-to-high regions of aquaculture activity, while ST189 ($n = 5$) and ST58 ($n = 5$) were not present in low areas of aquaculture. Furthermore, *mcr-1* was identified in plasmids containing a variety of different Inc (incompatible) types; that is, IncX4-type ($n = 83$, 28.9%), IncI2-type ($n = 60$, 21.0%), IncHI2-type ($n = 23$, 8.0%), IncP-type ($n = 8$, 2.8%), IncY-type ($n = 8$, 2.8%), IncF-type ($n = 1$) and syncretic plasmids ($n = 9$, 3.1%).

Distinct prevalence of MCRPEC isolates of human and chicken origin

Given the significant correlation between MCRPEC carriage in humans and the animal food chain, as shown above, we attempted to further analyse the association between human MCRPEC isolates and other MCRPEC isolates of animal origin using whole-genome phylogenetic analysis. However, in the National Center for Biotechnology Information (NCBI) database, we only found 88 whole genomes of MCRPEC isolates from Chinese chickens in previous studies[15,20] (all sequences are available in NCBI under BioProject accession numbers PRJNA349231 and PRJNA417344). Unfortunately, there are no MCRPEC whole-genome sequences available for swine, sheep, cattle or fish from China. Although

MLST diversity was also found in MCRPEC isolates from chickens, minimum spanning tree analysis showed distinct clustering between human and chicken isolates. ST10 was also the dominant clade, with 46 and 5 MCRPEC isolates from humans and chickens, respectively. However, ST10 MCRPEC isolates were more common in human carriage than in chickens (16.0 versus 5.7%, $P = 0.013$). In contrast, ST156 was more prevalent in chicken carriage than from human intestines (23.9 versus 1.0%, $P < 0.0001$). Interestingly, several sequence-type clades, each containing five or more isolates, were only observed in human carriage, such as ST155, ST58, ST189, ST410, ST216, ST2705 and ST1684. Core-genome phylogenetic analysis supported the commonality of ST156 clades among disparate origins, provinces and even countries. ST345 and ST101 also overlapped for MCRPEC isolates from humans and chickens.

DISCUSSION

A key finding of this study is the high prevalence of MCRPEC carriage as human normal flora (15.0%), which is considerably higher than was previously reported (0.7–6.2%)[2,13,14]. Our results suggest that the true prevalence of MCRPEC was previously underestimated, probably as a consequence of directly screening for *mcr-1*-positive Enterobacteriaceae without an enrichment step. In contrast with previous studies, we first determined the presence of *mcr-1* by PCR, and then isolated MCRPEC with an enrichment step using selective media with colistin, which resulted in a significant increase in MCRPEC detection. The higher MCRPEC prevalence will have major clinical implications as nosocomial outbreaks of MCRPEC are likely to occur if appropriate screening in Chinese intensive care units, high-dependency units, and transparent and haematology units is not enforced. Although few isolates were from phylogenetic group B2, patients presenting with significant co-morbidities will be at risk of infection and there is the high possibility of *mcr-1* transfer in vivo into B2 pathogens. Given that polymyxin B has recently been approved for use in human medicine by the China Food and Drug Administration (5 January 2017) further studies are urgently needed to assess the impact of MCRPEC carriage prevalence on endogenous infections and treatment failure.

Using microbiological and anthropogenic data, we identified possible associations with human MCRPEC carriage. The positive correlation between human MCRPEC carriage and the consumption of total meat, pork and sheep is in accordance with the notion that the spread of *mcr-1* probably occurred from farm animals to humans[1,9]. However, the identification of aquatic food as a correlated factor of MCRPEC was unexpected. Although colistin is not officially used in Chinese aquaculture, the aquatic environment is likely to be contaminated by colistin through contamination from farms as the drug is very poorly absorbed after oral administration in pigs and poultry[21], and can be excreted in

high-levels with *mcr-1*/MCRPEC from the animal faeces[22]. Consequently, when manure is used for irrigation in agriculture, to feed farmed fish or directly discharged into the aquatic environment it will pollute rivers and lakes with MCRPEC. The high stability of colistin in water[23] will further exacerbate the persistence and dissemination of MCRPEC in the aquatic environment by providing a selective pressure. Aquatic food produce may also be contaminated by MCRPEC, which is supported by the daily intake of aquatic food products as a correlated factor for MCRPEC carriage.

The high diversity of sequence-type clades of MCRPEC across China further confirms the wide distribution of *mcr-1*-carrying *E. coli* and that colistin resistance is mediated more by plasmids than dominant bacterial clones. The unique main sequence-type clusters in humans (ST10) and chickens (ST156) implied a different colonized MCRPEC and transmission route from each origin, but this finding might be restricted by the limited animal sampling size and locations in the two previous studies[15,20]. However, all MCRPEC isolates were restricted to a limited number of distinct *E. coli* lineages; the 375 examined isolates (287 from humans and 88 from chickens) can be placed into 4 broad lineages (Figure 3). This limited diversity contrasts with the transmission of the *bla* $_{NDM}$ gene, which was found in eight distinct *E. coli* lineages from a single poultry production chain in one province (Shandong)[15]. These data suggest that the host spectrum of *mcr-1*-carrying plasmids is considerably narrower than *bla* $_{NDM}$-carrying plasmids, which would also suggest why *mcr-1* is mainly found in *E. coli* and has yet to be reported outside Enterobacteriaceae. The predominant association of *mcr-1* with narrow-host types of plasmids; for example, IncI2, IncHI2 and IncX4[7], is also likely to contribute to the limited number of clusters of MCRPEC isolates in China and worldwide.

Although this is the largest and most comprehensive analysis of its type, we acknowledge several limitations in this study. First, for the sampling, we only utilized one hospital per province, which may not be fully congruent with the socioeconomic parameters representative of each province. Second, total meat consumption consisted of all meat types, not just pork; however, pork accounted for 62.4% of the annual consumption of total meat in China[24]. Third, the possible transmission of MCRPEC to humans through aquatic food was only identified by correlation analysis, and has not been proven by detailed epidemiological data. However, it has been hypothesized that *mcr-1* originated from an aquacultural environment[17,18], and several recent studies have reported the widespread distribution of the waterborne *mcr-1* gene in Asia, Europe and South America[7,25,26]. Fourth, all human MCRPEC isolates analysed in this study were collected in 2016; however, most of the environmental and anthropogenic data were from 2015, although we would anticipate little variation. Lastly, we collected 774 MCRPEC isolates from 30 provinces and municipalities, but only provide complete genomic data for 37.1% of the isolates ($n = 287$).

Despite these limitations, our study provides new evidence on a China-wide scale that foodborne transmission of MCRPEC is a major correlated factor for human carriage. We would suggest that the methods applied in this study be fully adopted to understand the true prevalence of MCRPEC not just in China, but globally. It is hoped that the newly released polices on the withdrawal of colistin as a feed addictive, in combination with prudent colistin usage as a disease treatment in farm animals, might reduce the risk of MCRPEC in humans. We have shown that aquatic food production - a previously unknown *mcr-1* source—is significantly associated with human MCRPEC carriage, highlighting the need for a broader one-health approach to AMR studies. Hitherto, the primary focus of *mcr* surveillance programmes has been pig and poultry farms; thus, in China, Southeast Asia and South America, regions of freshwater and marine food produce should be incorporated into future surveillance programmes[25]. The clinical impact of the high MCRPEC carriage in healthy people remains unknown and should be examined further, not least to inform current Chinese hospital infection control programmes and screening programmes supporting high-risk Chinese patients.

METHODS

Subject enrolment

We undertook a cross-sectional study from 1 June to 30 September 2016 to investigate the prevalence of MCRPEC isolates from humans in China. A total of 30 hospitals from 30 provincial capital cities were recruited to the study, which covered all Chinese provinces and municipalities except Chongqing, Hong Kong, Macao and Taiwan. Hospital staff were asked to enrol subjects over a five-day period. We included healthy individuals attending routine physical examinations, during which stool samples were collected. The inclusion criteria were subjects who were considered generally healthy and consented to the study. Exclusion criteria included neonates, pregnancy, gastroenteritis and subjects presenting with chronic illnesses; for example, cancer. Vegetarians were also excluded, in keeping with data from previous studies[2]. Ethical permission for the study was agreed on 22 February 2016 by the Zhejiang University ethics committee under the project DETER-XDR-China. A brief survey of the selected individuals was conducted regarding meat consumption and antibiotics used in the past three months. Individual consent forms were translated into Mandarin and consent was obtained for all healthy individuals. Due to uneven economic and social development in different provinces in China, the numbers of individuals attending physical examinations during a five-day period varied significantly between different provinces. Therefore, the numbers of samples from each province varied too.

Sample collection

All colistin-resistant isolates were recovered from rectal swabs following three steps. First, stool samples were cultured in lysogeny broth (Luqiao) and DNA was extracted from the enriched broth using the boiling method. Second, the broth suspension of *mcr-1*-positive samples was enriched using Enterobacteriaceae enrichment broth (Luqiao) and enrichments were inoculated onto MacConkey agar (Luqiao) plates containing 2 mg l^{-1} colistin. Third, colonies on selected agar were confirmed for the *mcr-1* gene by PCR, and the species were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonik)[27].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of all non-duplicated MCRPEC isolates to nine clinical antimicrobial agents (cefepime, amikacin, piperacillin/tazobactam, ceftazidime, ticarcillin/clavulanic acid, ciprofloxacin, imipenem, colistin and cefoperazone/sulbactam), all of which are often prescribed to patients as part of the Chinese national formulary, was undertaken via the broth microdilution method using ATCC25922 as a quality control strain. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing clinical breakpoints (version 6.0) and the Clinical and Laboratory Standards Institute document M100-S25[28].

Molecular analysis

We selected 287 MCRPEC isolates from all 30 provinces and municipalities, and sequenced 10 from each (if available). Genomic DNA of the selected isolates was extracted using the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. Indexed DNA libraries were constructed using KAPA HyperPrep Kit Illumina platforms (Roche) with standard protocols, and were sequenced on an Illumina HiSeq X Ten platform with the 150-base pair paired-end strategy (Annoroad). The draft genomes were assembled using SPAdes version 3.9.0 [29], and MLST typing, ARGs and VAGs were identified by using the SRST2 toolkit version 0.2[30]. A minimum spanning tree of all sequence types was generated using BioNumerics version 7.0 (Applied Maths) using the BURST algorithm for related sequence types between different backgrounds. All assembled genomes were used for core-genome alignments to produce a phylogenetic tree using the Harvest package version 1.1.2 [31], and the corresponding features of each isolate were visualized using the online tool iTOL 3[32]. The lineages of the phylogenetic tree were defined using BAPS version 6.0 [33]. The *mcr-1*-carrying contigs generated by Illumina sequencing were examined for Inc types using PlasmidFinder version 1.3[34]. The heat map was generated using R 3.3.2 (R Foundation for Statistical Computing) using the

gplots 3.0.1 package (https://cran.r-project.org/web/packages/gplots/). All whole-genome sequencing data have been deposited in the NCBI database BioProject: PRJNA400107.

Data collection and statistical analysis

Natural (annual precipitation in the capital city of each province) and anthropogenic data for the 30 provinces and municipalities in China were collected from the National Bureau of Statistics of China, China Agriculture Yearbook and National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. The six anthropogenic factors included gross domestic product, population (number and density), pollutants (smoke dust, household garbage and industry discharge), animal production (farm animals and freshwater aquaculture, mainly including fish and shrimp), annual animal-derived food consumption and daily animal-derived food intake. Most of the data were from 2015, but the latest population data were taken from the sixth population census of China in 2010 and the latest data of daily animal-derived food intake were collected from 2010–2012. Data were organized in Excel 2016 (Microsoft). Descriptive analysis on percentages and prevalences (together with 95% CIs) were performed using functions provided in Excel 2016 (Microsoft). Univariable analysis conducted using SPSS version 22 (IBM) was adopted to select variables with $P \leq 0.05$. Significant variables ($P \leq 0.05$) were subsequently assessed for collinearity via Cramer's coefficient phi (Φ). If a pair of variables was highly correlated ($\Phi > 0.8$), the more biologically plausible variable was kept for the multivariable logistic analysis. Significant variables in the univariable analyses were kept for the multivariable analysis. Multivariable logistic regression models using a forward stepwise process were then adopted for controlling confounders. Variables with $P < 0.05$ were kept in the final model. The goodness of fit to the logistic regression model was tested using the Hosmer–Lemeshow goodness-of-fit test. Subsequently, a receiver operating characteristic curve was used to test the predictive ability of the mode.

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Figure 1

MCRPEC prevalence among 30 provinces and municipalities in China.

The sample size is listed in parentheses after the name of each province. Error bars represent 95% CIs. The dashed line indicates the average prevalence of mcr-1-positive carriages in China. The mean and median prevalence are 16.40 and 15.11%, respectively, across China.

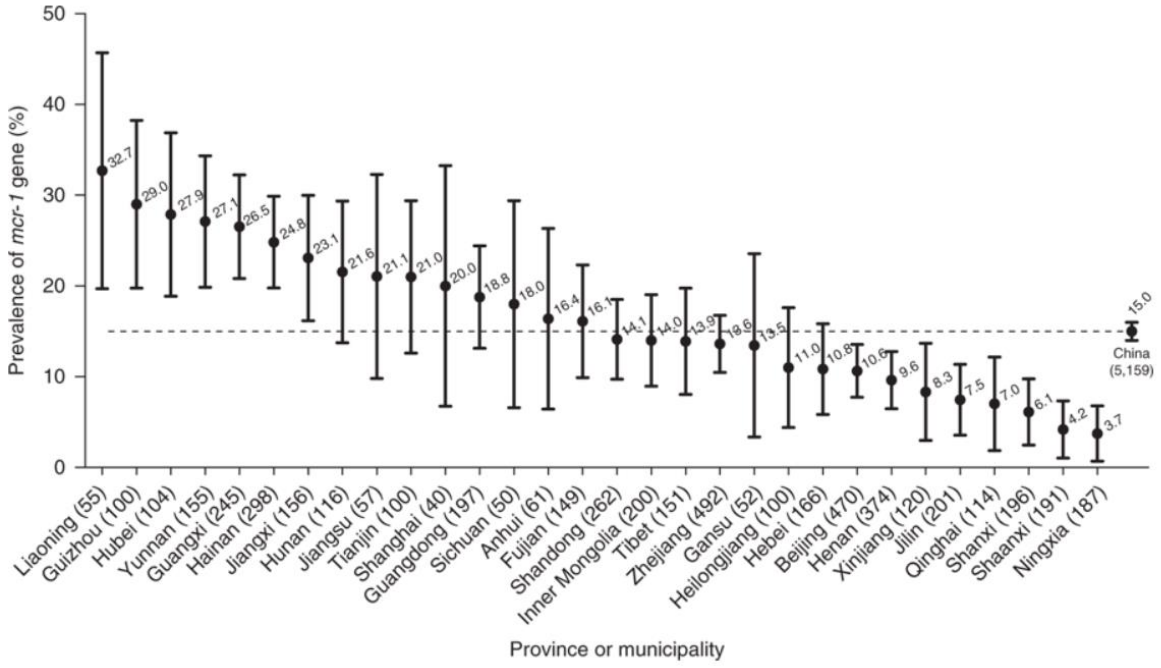

Figure 2:

Mapped MCRPEC prevalence, precipitation and freshwater aquaculture production, and intake of animal products per province.

a–d, Prevalence of MCRPEC isolates (a), precipitation and zoning of distinct aquaculture conditions (b), freshwater aquaculture production (c) and daily dietary intake of animal products (d) among 30 provinces and municipalities in China. In a, the black dots indicate the locations of province capital cities and sample collection sites. Mean intakes of aquatic products, total meat and eggs were 120.72, 107.32 and 24.44 g d–1, respectively, across China. Median intakes of aquatic products, total meat and eggs were 64.68, 112.89 and 24.95 g d–1, respectively, across China.

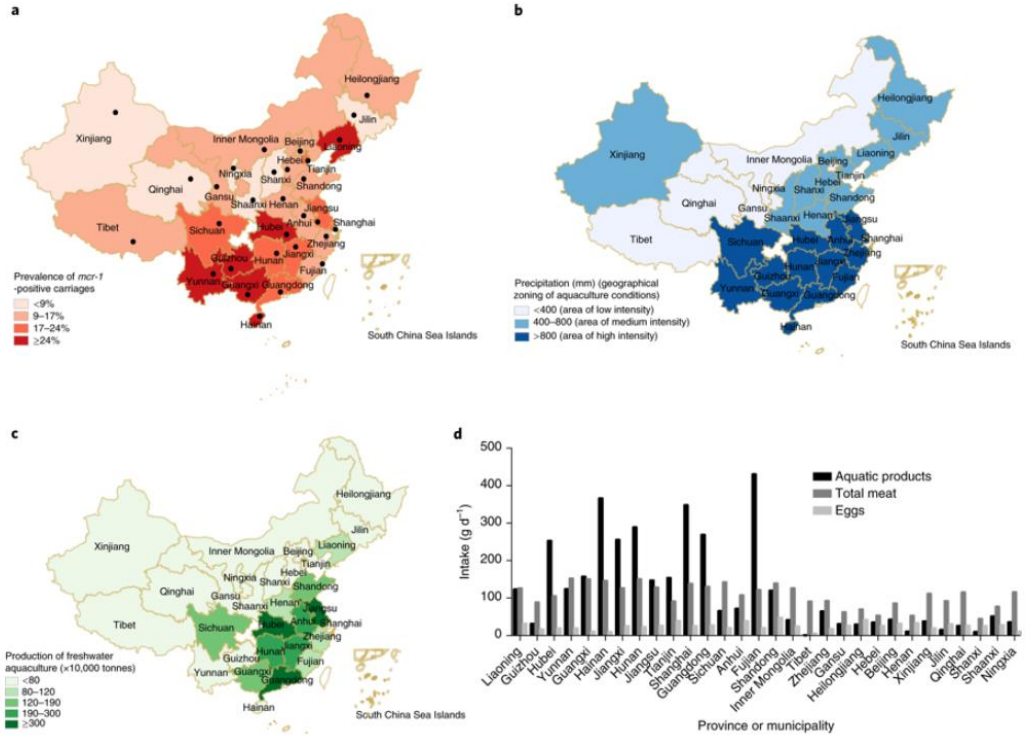

Figure 3

Genetic relationships between the MCRPEC isolates.

Phylogenic tree of 287 MCRPEC isolates from the 30 provinces in China and 16 human-origin isolates from other countries. Each isolate is labelled with its province or country and sequence type. Isolates from the 14, 11 and 5 provinces or municipalities within the low, medium and high aquaculture regions are marked in red, black or green, respectively. Lineage 1, 2, 3 and 4 branches are coloured blue, purple, red and green, respectively. Red stars represent the 16 isolates from other countries

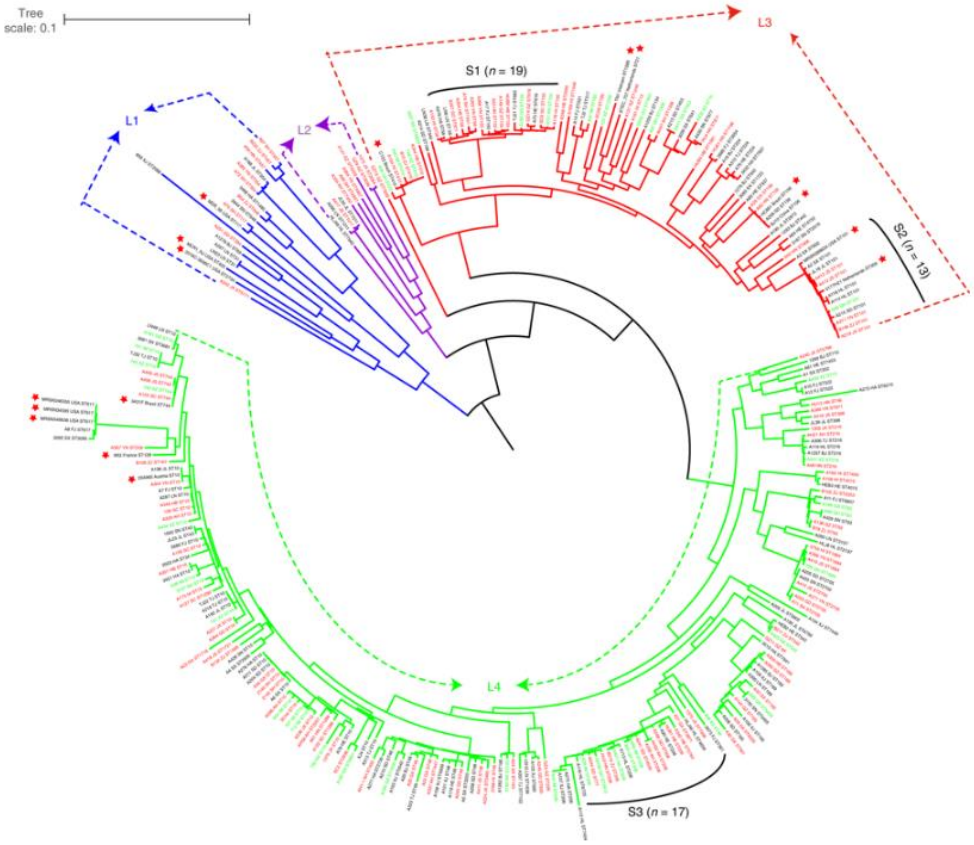

Figure 4

Distribution of phylogenetic group, Inc type, ARGs and VAGs among MCRPEC isolates from 30 provinces and municipalities in China.

The colour of each box represents the percentage of the corresponding item among sequenced isolates in the corresponding province.

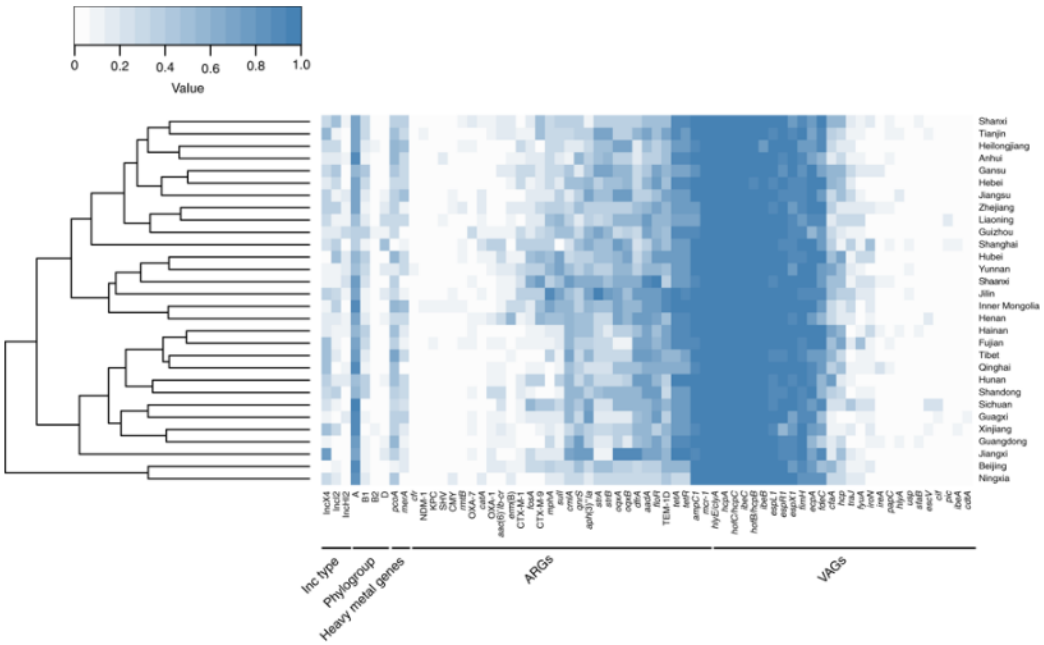

Table 1

Descriptive and univariable analysis of the variables of interest

Variable	**Category**	**Percentage**	**Prevalence, % (95% CI) (*n* = 5,159)**	**OR (95% CI)**	***P***
Total samples in study		100	15.0 (14.0–16.0)	NA	NA
Gender					0.899
	Male	53.9	15.1 (13.8–16.4)	1.0 (0.9–1.2)	
	Female	46.1	14.9 (13.5–16.4)	1.0	
Age[a]					0.048
	10–20	3.5	12.7 (8.2–18.5)	1.0	
	21–30	26.2	13.8 (12.0–15.7)	1.1 (0.7–1.7)	
	31–40	36.4	15.3 (13.7–17.0)	1.2 (0.8–2.0)	
	41–50	21.2	15.1 (13.0–17.3)	1.2 (0.8–1.9)	
	51–60	7.6	15.7 (12.3–19.7)	1.3 (0.8–2.1)	
	61–70	3.6	18.1 (12.9–24.3)	1.5 (0.9–2.7)	
	70–80	1.2	19.7 (10.6–31.8)	1.7 (0.8–3.6)	
	81–90	0.2	36.4 (10.9–69.2)	3.9 (1.1–14.5)	
Geographical zoning of aquaculture conditions[a]					<0.001
	Low	13.7	10.1 (8.0–12.6)	1.0	
	Medium	43.3	10.6 (9.3–11.9)	1.1 (0.8–1.4)	
	High	43.0	21.0 (19.4–22.8)	2.4 (1.8–3.1)	
Annual freshwater aquaculture production (10,000 tonnes)[a,b]					<0.001
	≤100 × 10,000	66.3	13.1 (12.0–14.3)	1.0	

Variable	**Category**	**Percentage**	**Prevalence, % (95% CI) (*n* = 5,159)**	**OR (95% CI)**	***P***
	>100 × 10,000	33.7	18.8 (17.0–20.7)	1.5 (1.3–1.8)	
Annual aquatic product consumption (kg person^{-1})[a,c]					<0.001
	≤10	54.8	11.0 (9.9–12.1)	1.0	
	>10	45.2	19.9 (18.3–21.5)	2.0 (1.7–2.3)	
Daily aquatic product intake (g d^{-1})[a,d]					<0.001
	≤100	62.5	10.7 (9.7–11.8)	1.0	
	>100	37.5	22.1 (20.3–24.0)	2.4 (2.0–2.8)	
Gross domestic product (100 million RMB)[c]					0.985
Population number (10,000)[a,e]					0.040
Population density (10,000 km^{-2})[e]					0.580
Pollutants (10,000 tonnes)[c]			Smoke dust[a]		0.002
			Household garbage		0.208
			Chemical oxygen demand		0.213
			Ammonia nitrogen[a]		0.018
			Total nitrogen		0.908
			Total phosphorus		0.275
Annual animal production (10,000 tonnes)[f]			Pig[a]		<0.001
			Chicken[a]		<0.001
			Cattle		0.906
			Sheep[a]		0.001
Annual animal-derived food consumption (kg person^{-1})[c]			Meat[a]		<0.001

Variable	**Category**	**Percentage**	**Prevalence, % (95% CI) (*n* = 5,159)**	**OR (95% CI)**	***P***
			Pork[a]		<0.001
			Beef		0.300
			Mutton[a]		<0.001
			Chicken meat[a]		<0.001
			Egg[a]		<0.001
Daily animal-derived food intake (g d^{-1})[d]			Total meat[a]		<0.001
			Egg[a]		<0.001

Table 2

Multivariable logistic regression analysis of factors associated with mcr-1 positivity (n = 5,159)

Variable	**Category**	**OR (95% CI)**	***P***
Geographical zoning of aquaculture conditions	Low[a]	0.5 (0.3–0.7)	0.002
	Medium[a]	0.7 (0.5–0.9)	0.016
	High[a]	1.0	0.005
Daily aquatic product intake (g d^{-1})[a]	≤100[a]	0.6 (0.5–0.7)	<0.001
	>100[a]	1.0	<0.001
Total population[a]			0.003
Annual animal production (10,000 tonnes)		Sheep[a]	<0.001
Annual animal-derived food consumption (kg $person^{-1}$)		Meat[a]	<0.001
		Pork[a]	0.014
		Mutton[a]	0.003

[a]Significant variable in multivariable logistic regression analysis ($P < 0.05$).