Prospective study on human fecal carriage of *Enterobacteriaceae* possessing *mcr-1* and *mcr-2* genes in a regional hospital in Hong Kong

Wai-Sing Chan, Chun-Hang Au, Dona N. Ho, Tsun-Leung Chan, Edmond Shiu-Kwan Ma and Bone Siu-Fai Tang*

**Abstract**

**Background:** Human fecal carriage of *Enterobacteriaceae* possessing mobilized colistin resistance genes (*mcr-1* and *mcr-2*) remains obscure in Hong Kong. As part of routine surveillance on emerging antibiotic resistance, we conducted a prospective study on this topic in a regional hospital in Hong Kong.

**Methods:** From October 31 to November 25, 2016, all fecal specimens submitted for routine analysis were included in this surveillance study. These comprised 672 consecutive routine fecal specimens collected from 616 individuals. Fecal specimens were screened for colistin-resistant *Enterobacteriaceae* by culture-based method, and the presence of *mcr-1* and *mcr-2* genes in resistant isolates was identified by polymerase chain reaction and Sanger sequencing. Whole genome sequencing (WGS) of *mcr-1*-possessing *Escherichia coli* strains was facilitated using Illumina® MiSeq® followed by sequence analysis with appropriate bioinformatics tools.

**Results:** Fourteen *mcr-1*-positive *E. coli* strains were isolated from 14 separate individuals (2.08% of total fecal specimens), with 9 of them being asymptomatic, healthy clients coming for health assessment. No *mcr-2*-possessing *Enterobacteriaceae* was identified. Colistin minimum inhibitory concentrations of these *mcr-1*-positive isolates ranged from 2 to 4 μg/mL. All these isolates were susceptible to carbapenems with 2 being extended spectrum β-lactamase producers. WGS data revealed that these isolates belonged to at least 12 different sequence types (STs) and possessed diversified plasmid replicons, virulence and acquired antibiotic resistance genes. Further study on an *E. coli* ST201 strain (Pasteur scheme) revealed coexistence of 47,818-bp IncP-1 and 33,309-bp IncX4 types of *mcr-1* plasmids, which was a combination of stability and high transmissibility.

**Conclusions:** To the best of our knowledge, this is the first study on human fecal carriage of *Enterobacteriaceae* possessing *mcr-1* and *mcr-2* genes in Hong Kong. Our data further revealed asymptomatic carriage of *mcr-1*-possessing *Enterobacteriaceae* by both patients and healthy individuals. This is alarming considering wide diversity and high transmissibility of *mcr-1* plasmids, which potentially facilitate emergence of pan-drug-resistant bacteria in future infection. This also highlights the importance of surveillance on emerging antibiotic resistance, especially for patients under intensive care.

**Keywords:** Colistin resistance, Fecal carriage, Hong Kong, *mcr-1* and *mcr-2*
Background
Recent reports on plasmid-mediated colistin resistance of Enterobacteriaceae from research groups worldwide have heralded concerns about emergence of ‘superbugs’ which are resistant to this last resort of treatment [1–3]. With broad range of compatible plasmids and bacterial hosts [4], mobilized colistin resistance gene (mcr-1) has successfully disseminated globally. The presence of mcr-1-carrying Enterobacteriaceae in food animals worldwide [1, 5, 6] further facilitates transmission of mcr-1 to human. Escherichia coli has been the major bacterial host of mcr-1 gene, and recent study has unraveled significant geographical clustering with regional spread of IncH12 and IncI2 types of mcr-1 plasmids in Europe and Asia, respectively [7].

In Hong Kong, Wong and coworkers reported a mcr-1 positive rate of 0.4% among 1,324 Enterobacteriaceae isolates from patients, which included 2 asymptomatic carriers [8]. At present, no prevalence data on human fecal carriage of mcr-1- and mcr-2-possessing Enterobacteriaceae has been documented in our region. As a routine surveillance on emerging antibiotic resistance, we conducted a prospective study on this topic in a regional hospital in Hong Kong.

Methods
Collection of human fecal specimens
The goal of this surveillance study was to have a gross picture on (1) fecal carriage of mcr-1- and mcr-2-harbor ing Enterobacteriaceae among patients/clients using our service, (2) characteristics of the carriers and (3) phenotypic and molecular traits of the positive strains. This information is important for infection control and risk assessment. From October 31 to November 25, 2016, all fecal specimens submitted for routine analysis were included in this surveillance study. These comprised 672 consecutive routine fecal specimens collected from 616 individuals, including 79 fecal specimens from 67 outpatients, 171 fecal specimens from 144 inpatients and 422 fecal specimens from 418 asymptomatic clients coming for health assessment. Forty four out of 144 inpatients had been screened for vancomycin-resistant enterococci (VRE), which is routinely performed for inpatients who have been hospitalized in/outside Hong Kong, underwent VRE screening in other hospitals, undergone surgical operation overseas or staying in a nursing home within past 6 months. After routine testing, leftover fecal specimens were used for surveillance screening.

Screening of colistin-resistant Enterobacteriaceae in fecal specimens
The concept of Blackburn and coworkers’ method for screening carbapenem-resistant bacteria was adopted [9], with some modifications. Briefly, fecal specimens with approximate sizes of 10 μL inoculation loop were homogenized in 500 μL sterile, double-deionized water and centrifuged at 1,500 rpm for 30 s. An aliquot of 10 μL supernatant from each sample was inoculated on chromID® CPS® Elite agar (bioMérieux, Marcy, l’Etoile, France) and a piece of polymyxin B disc (PB300, Oxoid, Basingstoke, Hampshire, England) was applied on the area of inoculation, followed by overnight incubation at 35 °C in ambient air. Bacterial growth around polymyxin B disc, namely inside the inhibition zone of approximately 11 mm in diameter (taking the Clinical and Laboratory Standards Institute (CLSI) guidelines’ interpretative criteria of polymyxin B for Pseudomonas aeruginosa as reference), was subjected to polymerase chain reaction (PCR) targeting mcr-1 and mcr-2 genes.

Colonial PCR targeting mcr-1 and mcr-2 genes
Polymyxin B-resistant bacterial colonies from each sample were homogenized in 20 μL sterile, double-deionized water. A 1 μL aliquot of this suspension was added to PCR cocktail comprising 20 μL Platinum® PCR Supermix (Invitrogen, Carlsbad, CA) and 1 μL of each 10 μM primer (mcr-1 forward primer, 5′-GTGCTGAGCA-TACTTCTGTG-3′ [10] and reverse primer, 5′-CCCAAAACCAATGATACGCA-3′ [8], corresponding to amplicon size of 398 bp; mcr-2 forward primer, 5′-TGTTGTCTTGCTGGATTGA-3′ and reverse primer, 5′-AGATGTATTGTGGCTGCAGTCG-3′ [2], corresponding to amplicon size of 567 bp). PCR was performed using GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA) with initial cell lysis and denaturation at 95 °C for 10 min, three-step cycling for 40 times (95 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s) and final extension at 72 °C for 5 min. The amplicons were electrophoresized in 2% agarose gel.

Bacterial identification of PCR-positive isolates
PCR-positive colony suspensions were subcultured on CPS agar and incubated at 35 °C overnight in ambient air. Recovered bacterial colonies were identified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS, Microflex LT, Bruker Daltonik, Bremen, Germany). Briefly, minute amount of bacterial colony was smeared on target plate and covered with 1 μL of 10 mg/mL matrix solution (α-cyano-4-hydroxycinnamic acid, Bruker Daltonik, Bremen, Germany). The dried samples were subjected to MS measurement after successful calibration with Bacterial Test Standard (Bruker Daltonik, Bremen, Germany). Spectra were obtained with an accelerating voltage of 20 kV in linear mode with m/z range of 2,000 to 20,000 Da, followed by analysis with MALDI Biotyper version 3.0 and reference library version 3.1.2.0 (Bruker Daltonik, Bremen, Germany).
Antibiotic susceptibility testing of PCR-positive isolates

Antibiotic susceptibilities were determined using Kirby-Bauer disk diffusion method with reference to CLSI guidelines. Minimum inhibitory concentration (MIC) of colistin was determined using broth microdilution (BMD) (SensiT®test Colistin, Liofilchem® Diagnostics, L’Aquila, Italy). Susceptibility results of VITEK® 2 AST cards (AST-N338, bioMérieux, Marcy, l’Etoile, France) and polymyxin B Etest strips (bioMérieux, Marcy, l’Etoile, France) served as additional reference.

Sanger sequencing of full mcr-1 genes

Total nucleic acid of PCR-positive isolates was extracted using NucliSENS® easyMag® automated system (bioMérieux, Marcy, l’Etoile, France) and full mcr-1 genes were amplified using primers published by Ye and coworkers [10]. Amplicons were purified enzymatically with ExoSAP-IT® (Affymetrix, Santa Clara, CA), followed by cycle sequencing using BigDye® Terminator v.1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and purification of sequencing products using BigDye® XTerminator® Purification Kit (Applied Biosystems, Foster City, CA, USA). Purified sequencing products were analyzed using 3130xl Genetic Analyzer/3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Whole genome sequencing (WGS) of mcr-1-possessing E. coli strains

Briefly, DNA concentrations of total nucleic acid extracts were measured using Quant-it™ PicoGreen® dsDNA Assay Kit via Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, CA). DNA concentrations were adjusted to 0.2 ng/μL, followed by tagmentation and limited-cycle amplification using Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, USA). Indexed libraries were purified using Agencourt® AMPure® XP (Beckman Coulter, Beverly, MA, USA) and analyzed by 2 × 300 bp paired-end sequencing on MiSeq platform using MiSeq v3 Reagent Kit (Illumina, San Diego, CA, USA).

Bioinformatics analysis of WGS data

WGS short reads were assembled into contigs using SPAdes version 3.10.1 [11]. E. coli sequence types (STs) were determined using MLST databases of The University of Warwick [12] and PubMLST [13]. Acquired resistance genes were identified using ResFinder 2.1 [14]. Virulence genes were detected using VirulenceFinder 1.5 [15]. Alignment of contigs to appropriate reference sequences was performed using Mauve version 2.4.0 [16] followed by post-assembly gap filling by PCR and Sanger sequencing. Prokka version 1.11 [17] was used for plasmid annotation with manual editing. Plasmid replicons and insertion sequences were identified using PlasmidFinder 1.3 [18] and IS finder [19]. Plasmid genetic maps were generated using SnapGene® Viewer 4.1. Sequence comparisons between plasmids were generated using BLAST Ring Image Generator (BRIG) [20].

Results and Discussion

Fecal carriage of mcr-1-/mcr-2-possessing Enterobacteriaceae

Seventy nine out of 672 fecal specimens revealed bacterial growth inside the inhibition zone of polymyxin B disc and subjected to colony PCR, with 14 specimens being mcr-1-positive (2.08%) and none being mcr-2-positive. Results of MALDI-TOF MS showed that all 14 isolates were E. coli (top identification scores ranged from 2.271 to 2.526) which were consistent with the appearance of bacterial colonies on CPS agars (red-colored). These E. coli strains were isolated from 14 separate individuals including 3 inpatients, 2 outpatients and 9 asymptomatic clients coming for health assessment, with age from 1 to 58 years (Table 1). Regarding available clinical/epidemiological information of these mcr-1 carriers, Subject 1 and 10 had received antibiotic treatment other than colistin prior to fecal specimen collection. For others, none had been prescribed with antibiotics in our hospital. We could not access any information on antibiotic usage outside our hospital. Subject 3 had been hospitalized in another hospital within 6 months prior to admission. None of these mcr-1-possessing E. coli strains caused diseases in their human hosts. In general, no apparent correlation was observed between mcr-1 fecal carriage and the available clinical/epidemiological information.

The fecal carriage data further revealed asymptomatic carriage of mcr-1-possessing Enterobacteriaceae by both patients and healthy individuals. The mcr-1 positive rate estimated in this study (2.08%) was much higher than that reported by another local research group (0.4%) [8], which implies the prevalence was higher than expected in Hong Kong. This difference in mcr-1 positive rate might be explained by different screening targets adopted, with the study by Wong and coworkers focusing on clinical isolates whereas our study screening fecal specimens. As fecal specimens normally contain numerous strains of Enterobacteriaceae per sample, the chance of isolating mcr-1-harboring strains might be increased. Compared with human fecal carriage data of other regions, the mcr-1 positive rate estimated in this study was lower than that of mainland China (4.9 - 6.2%) [21, 22] and higher than that of several European countries (Switzerland, 0% [23]; Western France, 0% [24]; The Netherlands, 0.35% [25]). This is not surprising considering high prevalence of mcr-1-harboring Enterobacteriaee among livestock animals in mainland China [26], which is a major supplier of food animals in Hong Kong.
On the other hand, no *mcr-2*-carrying *Enterobacteriaceae* was isolated. In another large-scale study in Japan, *mcr-2* gene was not detected among 9,306 *E. coli* strains isolated from healthy animals [27]. Considering these prevalence data together with reports of zero human fecal carriage from various European countries [23–25], it appears that *mcr-2*-carrying *Enterobacteriaceae* has not been disseminated globally at present.

### Antibiotic susceptibility of *mcr-1*-positive isolates

Antibiotic susceptibility results are summarized in Table 1. Colistin MICs ranged from 2 to 4 μg/mL by BMD and 2 to ≥16 μg/mL by VITEK® 2 AST. The range of polymyxin B MICs was 4 to 12 μg/mL by Etest. The *mcr-1*-positive isolates displayed different patterns of susceptibility to a panel of 19 antibiotics for *Enterobacteriaceae*. With exception of Subject 8, all other isolates were intermediate or resistant to at least 1 antimicrobial agent, including penicillins (ampicillin, 71.4%), β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanate, 50%; piperacillin/tazobactam, 14.3%; ticarcillin-clavulanate, 21.4%), cepham (cefepeime, 14.3%; cefotaxime, 14.3%; ceftazidine, 14.3%; cefuroxime, 57.1%; cephalothin, 64.3%), aminoglycosides (amikacin, 28.6%; gentamicin, 21.4%; tobramycin, 28.6%), tetracyclines (minocycline, 35.7%), quinolones (ciprofloxacin, 35.7%; levofloxacin, 35.7%; levofloxacin, 28.6%) and folate pathway inhibitors (trimethoprim/sulfamethoxazole, 78.6%). All these isolates were susceptible to carbapenems with 2 being extended-spectrum β-lactamase (ESBL) producers.

The range of colistin MICs in this study was 2 to 4 μg/mL by BMD, which appeared to be narrower than that of *mcr-1*-positive *E. coli* isolates causing human diseases (4 to 16 μg/mL by BMD) reported by various research groups [28–31]. Colistin MICs determined by VITEK® 2 AST were consistent with that of BMD for 35.7% of the isolates, whereas MICs were 2 to ≥4-fold higher than that of BMD for the remainder. Albeit underestimation of colistin MIC was not observed in this study, the suitability of using VITEK® 2 AST for colistin MIC determination awaits further evaluation due to its high very major error rate (36%) revealed by a recent study [32]. In addition, as 21.4% of the isolates displayed colistin MICs lower than the resistant breakpoint (>2 μg/mL) published by The European Committee on Antimicrobial Susceptibility Testing (EUCAST), we echo

| Subject | Age/Year | Subject type | ESBL producer | Carbapenem susceptible | PB MIC (μg/mL) | PE MIC (μg/mL) | PE MIC (μg/mL) | Non-susceptible antibiotics (based on CLSI guidelines) |
|---------|----------|--------------|---------------|------------------------|----------------|----------------|----------------|--------------------------------------------------|
| 1       | 45       | OP           | No            | Yes                    | 8              | 4              | 8              | AMR, CF, SXTR                                      |
| 2       | 54       | HA           | No            | Yes                    | 8              | 2              | 8              | SXTR                                             |
| 3       | 42       | IP           | No            | Yes                    | 8              | 4              | 8              | CFR, MTR, SXTR                                    |
| 4       | 54       | HA           | No            | Yes                    | 12             | 4              | 8              | AMR, AMC, AN, MIR, SXTR                           |
| 5       | 4        | IP           | No            | Yes                    | 4              | 4              | 4              | AMR, AMC, AN, CF, CIP, CN, CXMR, LEV, SXT, TIM, TOBR, TZIP |
| 6       | 45       | HA           | No            | Yes                    | 8              | 4              | 8              | AMR, AMC, CIP, CN, CXMR, LEV, SXT, TIM, TOBR      |
| 7       | 43       | HA           | No            | Yes                    | 8              | 4              | 4              | AMR, AMC, CIP, CN, CXMR, LEV, MIR, SXTR, TIM, TZIP |
| 8       | 31       | HA           | No            | Yes                    | 12             | 4              | ≥16            | All susceptible                                   |
| 9       | 52       | HA           | No            | Yes                    | 4              | 2              | 4              | CFR, CXMR                                        |
| 10f     | 1        | IP           | No            | Yes                    | 6              | 4              | 8              | AMR, AN, CF, CXMR, MIR, TOBR                      |
| 11      | 58       | HA           | No            | Yes                    | 8              | 4              | 8              | AMR, SXT                                           |
| 12      | 7        | OP           | Yes           | Yes                    | 12             | 4              | 4              | AMR, AMC, CAZ, CF, CIP, CTX, CXMR, FEP, SXT       |
| 13      | 49       | HA           | Yes           | Yes                    | 8              | 4              | 4              | AMR, AMC, AN, CAZ, CF, CIP, CN, CTX, CXMR, FEP, SXT |
| 14      | 55       | HA           | No            | Yes                    | 6              | 2              | 2              | AMR, CF, CIP, CXMR, MIR, SXT                       |

*OP* outpatients, HA clients coming for health assessment, IP inpatients

ESBL extended-spectrum β-lactamase

PB MIC, Polymyxin B minimum inhibitory concentration by Etest

PE MIC, Polymyxin E (colistin) minimum inhibitory concentration by broth microdilution (SensiTest Colistin)

PE MIC, Polymyxin E (colistin) minimum inhibitory concentration by Vitek® 2 AST N-338

The subjects had received antibiotic treatment other than colistin prior to fecal specimen collection

Non-susceptible antibiotics (based on CLSI guidelines)

| Subject | Age/Year | Subject type | ESBL producer | Carbapenem susceptible | PB MIC (μg/mL) | PE MIC (μg/mL) | PE MIC (μg/mL) | Non-susceptible antibiotics (based on CLSI guidelines) |
|---------|----------|--------------|---------------|------------------------|----------------|----------------|----------------|--------------------------------------------------|
| 1       | 45       | OP           | No            | Yes                    | 8              | 4              | 8              | AMR, CF, SXTR                                      |
| 2       | 54       | HA           | No            | Yes                    | 8              | 2              | 8              | SXTR                                             |
| 3       | 42       | IP           | No            | Yes                    | 8              | 4              | 8              | CFR, MTR, SXTR                                    |
| 4       | 54       | HA           | No            | Yes                    | 12             | 4              | 8              | AMR, AMC, AN, MIR, SXTR                           |
| 5       | 4        | IP           | No            | Yes                    | 4              | 4              | 4              | AMR, AMC, AN, CF, CIP, CN, CXMR, LEV, SXT, TIM, TOBR, TZIP |
| 6       | 45       | HA           | No            | Yes                    | 8              | 4              | 8              | AMR, AMC, CIP, CN, CXMR, LEV, SXT, TIM, TOBR      |
| 7       | 43       | HA           | No            | Yes                    | 8              | 4              | 4              | AMR, AMC, CIP, CN, CXMR, LEV, MIR, SXTR, TIM, TZIP |
| 8       | 31       | HA           | No            | Yes                    | 12             | 4              | ≥16            | All susceptible                                   |
| 9       | 52       | HA           | No            | Yes                    | 4              | 2              | 4              | CFR, CXMR                                        |
| 10f     | 1        | IP           | No            | Yes                    | 6              | 4              | 8              | AMR, AN, CF, CXMR, MIR, TOBR                      |
| 11      | 58       | HA           | No            | Yes                    | 8              | 4              | 8              | AMR, SXT                                           |
| 12      | 7        | OP           | Yes           | Yes                    | 12             | 4              | 4              | AMR, AMC, CAZ, CF, CIP, CTX, CXMR, FEP, SXT       |
| 13      | 49       | HA           | Yes           | Yes                    | 8              | 4              | 4              | AMR, AMC, AN, CAZ, CF, CIP, CN, CTX, CXMR, FEP, SXT |
| 14      | 55       | HA           | No            | Yes                    | 6              | 2              | 2              | AMR, CF, CIP, CXMR, MIR, SXT                       |
the suggestion of using a lower susceptible breakpoint of ≤ 1 μg/mL by Chew and coworkers [32] for improving detection of mcr-1-possessing Enterobacteriaceae. On the other hand, it was noticeable that more than half of the isolates (57.1%) displayed a ‘baseline’ insusceptibility to 5 or more antibiotics, and 2 of which were ESBL producers. The choice of usable antibiotics may be further limited for these isolates upon acquisition of antibiotic resistance genetic elements, for instance, carbapenemase genes.

**Molecular characteristics of mcr-1-positive isolates**
The results are summarized in Table 2.

**mcr-1 gene sequences**
The mcr-1 gene sequences of 12 isolates were 100% identical to that of E. coli strain SHP45 (NG_050417), with exception of the isolate from Subject 4 harboring C>T substitution at nucleotide position 27 and the isolate from Subject 2 with mixed nucleotides (R) at position 1263, indicating possible coexistence of ‘wild type’ and 1263G>A variant in the same bacterium. Both of these nucleotide substitutions were silent mutations.

**E. coli sequence types**
Thirteen isolates were assigned to 12 E. coli STs by Achtman or Pasteur scheme, including ST442 (Achtman), ST201 (Pasteur), ST88 (Achtman)/ST66 (Pasteur), ST1716 (Pasteur), ST155 (Achtman)/ST21 (Pasteur), ST10 (Achtman)/ST2 (Pasteur), ST34 (Achtman)/ST638 (Pasteur), ST226 (Achtman)/ST486 (Pasteur), ST5995 (Achtman), ST95 (Achtman)/ST1 (Pasteur), ST746 (Achtman) and ST48 (Achtman, 2 ESBL-producing strains). The strain from Subject 4 could not be assigned to currently known ST by both Achtman and Pasteur schemes. Generally, the STs of these strains were diversified. Albeit both the strains from Subject 12 and 13 were assigned to ST48, their antibiotic susceptibility patterns, genetic context and Pasteur ST profiles were different, which appeared that they were divergent over years rather than a short period of time. Among these STs, ST10, ST34 and ST48 have been reported to possess mcr-1 gene [33], with ST10

| Subject | Sequence Type | Plasmid Replicons | Virulence Genes | Acquired Resistance Genes |
|---------|---------------|-------------------|----------------|--------------------------|
| 1       | 442 (Achtman) | Unknown (Pasteur) | IncI1          | cma, iroN, iss, lpfA     | aadA1, aadA2, blaox-1b, blaox-1d, mcr-1, mcr-1, QnrS1, sul3, tet(A) |
| 2       | Unknown      | 201 (Pasteur)     | IncX4, IncP1   | astA, iss, lpfA, mchF, tsh | aadA1, aadA2, blaox-1b, mcr-1 (R’ at nt. 1263), sul3, tet(A) |
| 3       | 88 (Achtman) | 66 (Pasteur)      | Not detected   | cma, iroN, iss, lpfA     | aadA5, mcr-1, mph(A), strA, strB, sul1 |
| 4       | Unknown      | Unknown (Pasteur) | IncX4          | iss                      | aadA1, aadA2, blaox-1b, mcr-1 (27C>T), sul3, tet(B) |
| 5       | Unknown      | 1716 (Pasteur)    | IncI1          | astA                     | aaco(6)bc-cr, aadA5, ARR-3, blaox-1b, mcr-1, qnrA, qnrB, QnrS2 |
| 6       | 155 (Achtman)| 21 (Pasteur)      | IncN           | lpfA                     | aadA1, aadA2, aph(3)3-lq, blaox-1b, mcr-1, mph(A), qnrB, sul2, sul3 |
| 7       | 10 (Achtman) | 2 (Pasteur)       | IncN           | Not detected             | aadA1, aadA2, aph(3)3-lq, blaox-1b, mcr-1, mph(A), sul2, sul3 |
| 8       | 34 (Achtman) | 638 (Pasteur)     | Not detected   | Not detected             | mcr-1 |
| 9       | 226 (Achtman)| 486 (Pasteur)     | IncR, IncX4    | Not detected             | mcr-1, strA, strB, tet(B) |
| 10      | 5995 (Achtman)| Unknown (Pasteur)| IncQ1          | Not detected             | aph(3)3-lq, blaox-1b, mcr-1, strA, strB |
| 11      | 95 (Achtman) | 1 (Pasteur)       | IncI1, IncI2, IncQ1 | iss, stfA      | aph(3)3-lq, blaox-1b, mcr-1, mph(A), qnrB, sul2, tet(B) |
| 12      | 48 (Achtman) | Unknown (Pasteur) | Not detected   | Not detected             | aadA2, blaox-1b, mcr-1, mcr-1, sul3 |
| 13      | 48 (Achtman) | Unknown (Pasteur) | IncN, IncH1    | Not detected             | aadA1, blaox-1b, mcr-1, mcr-1, sul3 |
| 14      | 746 (Achtman) | Unknown (Pasteur) | Not detected   | astA                     | aadA1, aadA2, aph(3)3-lq, mcr-1, mcr-1, sul3 |
being the most common among mcr-1-possessing E. coli [7]. On the other hand, ST95, ST155, ST226, ST442 and ST746 have been associated with human infection [34–38].

**Virulence genes**

Virulence genes were detected in 8 isolates, including astA (21.4%), which encodes EAST-1 heat-stable toxin; cma (14.3%), which encodes colicin-M; iroN (21.4%), which encodes enterobactin siderophore receptor protein; iss (35.7%), which correlates with increased serum survival of E. coli; lpfA (28.6%), which encodes long polar fimbriae; mchF (7.1%), which encodes ABC transporter protein; sfaS (7.1%), which encodes S-fimbrial adhesion protein and tsh (7.1%), which encodes temperature-sensitive hemagglutinin. These genes are associated with mild virulence of E. coli.

**Plasmid incompatibility groups**

Eight plasmid replicon types were identified in 10 isolates, with IncI1 (21.4%), IncX4 (21.4%) and IncN (21.4%) being the predominant incompatibility groups, followed by IncQ1 (14.3%), IncP1 (7.1%), IncR (7.1%), IncI2 (7.1%) and IncHI2 (7.1%). Previous study has shown that IncX4, IncI2 and IncHI2 incompatibility groups comprised 90.4% of identified mcr-1 plasmids [7]. Significant geographical clustering was observed with regional spread of IncI2 and IncHI2 types of mcr-1 plasmids in Asia and Europe, respectively, while there was no significant difference between distribution of these major replicon types from different sources of isolation [7]. In this study, albeit further work is needed to confirm the genomic locations of mcr-1 genes, plasmid replicon types were diversified and not confined to IncI2, which was detected in a single isolate from Subject 11. Further study with larger cohort of isolates from different sources is needed to determine local characteristics of mcr-1 plasmid incompatibility groups.

**Acquired antibiotic resistance genes**

With exception of mcr-1, acquired antibiotic resistance genes were identified in 13 out of 14 isolates. Aminoglycoside resistance-related genes were the most commonly observed, including aadA1 (50%), aadA2 (50%), aadA5 (14.3%), aph(3’)-Ia (35.7%), strA (28.6%) and strB (28.6%). This was followed by genes related to sulphonamide resistance (sul1, 14.3%; sul2, 21.4%; sul3, 57.1%), quinolone resistance (aac(6’)-Ib-cr, 7.1%; oqxA, 28.6%; oqxB, 28.6%; QnrS1, 21.4%; QnrS2, 7.1%), β-lactam resistance (blaOXA-1, 7.1%; blaTEM-1B, 42.9%; blaCTX-M-9-like, 14.3%), trimethoprim resistance (dfrA12, 50%; dfrA17, 14.3%), tetracycline resistance (tet(A), 28.6%).
14.3%; \textit{tet}(B), 21.4%), macrolide resistance (\textit{mph}(A), 21.4%), rifampicin resistance (\textit{AAR}-3, 7.1%) as well as fosfomycin resistance (\textit{fosA3}, 7.1%). Carbapenemase genes were not detected in these \textit{mcr}-1 possessing \textit{E. coli} isolates.

Further study on the isolate harboring 2 \textit{mcr}-1 genes

Genetic structures of the \textit{mcr}-1 plasmids

The \textit{E. coli} strain from Subject 2 possessed both `wild type’ and 1263G>A variant of \textit{mcr}-1 gene. As a matter of interest, we have further studied the genomic locations of \textit{mcr}-1 genes in this isolate. WGS data revealed 2 sets of contigs linking to both ends of \textit{mcr}-1 sequence. The first set comprised a 20,329-bp contig upstream and a 25,592-bp contig downstream of \textit{mcr}-1; the second set included a 3,508-bp contig upstream and a 3,608-bp contig downstream of \textit{mcr}-1. These 2 contig combinations best matched to plasmid sequences with GenBank accession numbers KY352406 and KX570748, respectively. Therefore, mapping of contigs was based on these 2 reference sequences.

The complete \textit{mcr}-1 plasmids were 47,818 bp (average depth of coverage = 65.0, GC content = 47.2%) and 33,309 bp (average depth of coverage = 54.8, GC content = 41.8%) in length, possessing single \textit{IncP}-1 and \textit{IncX4} replicon, respectively. No acquired virulence and antibiotic resistance genes were found on both plasmids except \textit{mcr}-1. The \textit{IncP}-1 plasmid, pHKSHmcr1\textsubscript{-P2}_p1 (MF136778), possessed 61 coding sequences (CDS), including the 1263G>A \textit{mcr}-1 variant, \textit{trfA} (plasmid replication initiation), genes for various cellular functions and 23 hypothetical proteins. No insertion sequence was found on the \textit{IncP}-1 plasmid. The genetic map is shown in Fig. 1. The \textit{IncX4} plasmid, pHKSHmcr1\textsubscript{-P2}_p2 (MF136779), contained 41 CDS, including the `wildtype’ \textit{mcr}-1 gene, \textit{pir} (initiation of plasmid DNA replication), genes for various cellular functions and 20 hypothetical proteins. IS26 insertion sequence was present at 3,444 bp upstream of \textit{mcr}-1. The genetic map is shown in Fig. 2.

Comparative sequence analysis

We have extracted all nucleotide sequences of \textit{IncP}-1 and \textit{IncX4} types of \textit{mcr}-1 plasmids from GenBank (complete sequences only, dated on April 23, 2017) for comparative sequence analysis. Among 104 \textit{mcr}-1 plasmid entries from 10 regions, 2 sequences (1.9%) possessed \textit{IncP}-1 replicons, whereas 30 sequences (28.8%) belonged to \textit{IncX4} incompatibility group. Details of these sequences are summarized in Table 3. The two \textit{IncP}-1 GenBank sequences originated from mainland China. The 57,278-bp plasmid pMCR\textsubscript{-1511} (KX377410) was isolated from \textit{Klebsiella pneumoniae}.
and the 47,824-bp plasmid pMCR16_P053 (KY352406) was identified in Salmonella enterica subsp. enterica. pMCR_1511 was used as reference for comparative sequence analysis (Fig. 3). The IncP-1 plasmid of this study was very similar to pMCR16_P053 (99% sequence homology), while both of them were different from reference by the absence of 9 genes and insertion sequences, including \( \text{bla}_{\text{TEM}} \) (non-ESBL), \( \text{ble} \) (bleomycin resistance), \( \text{dgkA} \) (diacylglycerol kinase), \( \text{eptA} \) (phosphoethanolamine transferase), \( \text{higA} \) (antitoxin), \( \text{higB-1} \) (probable mRNA interferase), \( \text{ybaQ} \) (transcriptional regulator), \( \text{IS26} \) and \( \text{ISApl1} \).

### Table 3 Details of IncP-1 and IncX4 types of \( mcr-1 \) plasmids from GenBank and this study (complete sequences only, retrieved on April 23, 2017)

| Accession No. | Plasmid size/bp | Species | Location | Carbapenemase/ESBL genes | Insertion sequences (complete/partial) |
|--------------|----------------|---------|----------|--------------------------|---------------------------------------|
| **IncP-1 plasmids** | | | | | |
| MF136778 (this study) | 47,818 | *E. coli* | Hong Kong | No | No |
| KX377410 | 57,278 | *K. pneumoniae* | China | No | IS26, ISApl1 |
| KY352406 | 47,824 | *S. enterica* subsp. *enterica* | China | No | No |
| **IncX4 plasmids** | | | | | |
| MF136779 (this study) | 33,309 | *E. coli* | Hong Kong | No | IS26 |
| KX084392 | 33,298 | *E. coli* | China | No | IS26 |
| KU647721 | 48,350 | *E. coli* | China | No | IS26 |
| KX727777 | 33,309 | *E. coli* | China | No | IS26 |
| KY120363 | 42,941 | *S. enterica* subsp. *enterica* | Taiwan | No | IS8so1, ISApl1 |
| KY120364 | 33,308 | *S. enterica* subsp. *enterica* | Taiwan | No | IS26 |
| KY471146 | 47,038 | *E. coli* | Korea | No | IS26, IS679, ISApl1 |
| LC227558 | 33,304 | *E. coli* | Japan | No | IS26 |
| CP015977 | 33,304 | *E. coli* | Brazil | No | IS26 |
| CP017246 | 34,992 | *E. coli* | Brazil | No | IS26, IS1294 |
| KU761327 | 33,287 | *K. pneumoniae* | Brazil | No | IS26 |
| KY770023 | 33,051 | *E. coli* | Brazil | No | IS26 |
| KY770024 | 33,304 | *E. coli* | Brazil | No | IS26 |
| KY770025 | 34,975 | *E. coli* | Brazil | No | IS26, IS1294 |
| CP018773 | 33,305 | *E. coli* | USA | No | IS26 |
| KX447768 | 33,395 | *E. coli* | USA | No | IS26 |
| LTB38201 | 33,304 | *E. coli* | France | No | IS26 |
| KX129783 | 34,640 | *E. coli* | Switzerland | No | IS2, IS126 |
| KU473383 | 33,311 | *E. coli* | Estonia | No | IS26 |
| KX236309 | 33,303 | *K. pneumoniae* | Italy | No | IS26 |
Fig. 3 Comparative sequence analysis of IncP-1 type of mcr-1 plasmids. The circular image was generated with BRIG using pMCR_1511 (KX377410) as reference. The 2 inner rings reveal GC content and GC skew and the outer ring represents the coding sequences (CDS) of reference. Unlabeled CDS represents genes encoding hypothetical proteins.

Fig. 4 Comparative sequence analysis of IncX4 type of mcr-1 plasmids. The circular image was generated with BRIG using pFS170G (KX711707) as reference. The 2 inner rings reveal GC content and GC skew and the outer ring represents the CDS of reference. Unlabeled CDS represents genes encoding hypothetical proteins.
The 30 IncX4 GenBank sequences were diversified in geographical origins (from 10 regions, with 18 entries from Asia, 8 from America and 4 from Europe). There were 2 ranges of plasmid sizes, with 27 sequences between 31,229 and 34,997 bp (> 40,000 bp) and 3 sequences from 42,941 to 48,350 bp (> 40,000 bp). The hosts of these plasmids included E. coli (86.7%), K. pneumoniae (6.7%) and S. enterica subsp. enterica (6.7%). Plasmid pFS170G (KX771707) was used as reference for comparative sequence analysis (Fig. 4). Majority of these sequences were very similar, and the IncX4 plasmid of this study shared highest homology (99%) with pICBE-C3AM (KY770024). IS26 was the predominant type of insertion sequence (78.6%). Carbapenemase/ ESBL genes were absent in most of these plasmids (96.8%), while pCQ02-121 (KU647721) was the only plasmid harboring IS26 was the predominant type of insertion sequence (78.6%). Carbapenemase/ ESBL genes were absent in most of these plasmids (96.8%), while pCQ02-121 (KU647721) was the only plasmid harboring carbapenemase gene (blaNDM-5). In fact, it was an IncX3-IncX4 hybrid plasmid identified in an E. coli isolate [39], which might account for its difference from majority.

Implications from coexistence of IncP-1 and IncX4 plasmids
Coexistence of IncP-1 and IncX4 types of mcr-1 plasmids in the same E. coli was indeed a combination of stability and high transmissibility - the broad-host-range IncP-1 plasmids can transfer to, replicate in and stably maintained in virtually all species of Gram-negative bacteria [40, 41], while IncX4 plasmids are self-transferable at high frequency (~ 10^-1 to ~ 10^-3) which have contributed to intercontinental spread of mcr-1 genes [42]. These 2 incompatibility groups of mcr-1 plasmids could be harbored by at least E. coli, K. pneumoniae and S. enterica subsp. enterica, and the list is expected to be expanding. Another aspect of concern is the potential of IncP-1 and IncX4 plasmids to acquire a wide spectrum of antibiotic resistance genes, for instance, ESBL and carbapenemase genes [39, 43–45], which may contribute to pan-drug resistance under selective pressure [46, 47]. Nevertheless, our study was not the sole report on Enterobacteriaceae harboring more than 1 copy of mcr-1 gene [42, 48]. Interestingly, colistin MICs of these isolates appeared to be unaffected by gain of extra copies of mcr-1 gene, which was coherent with the finding in our study. While the rationale behind this phenomenon remains obscure, it may reflect the active nature of transposons carrying mcr-1 gene, for instance, ISAPl1 transposons [49]. The mechanism behind mobilization of mcr-1 genes and its integration into a variety of plasmids warrant further investigation.

Limitations of this study
Our study had several limitations. First, we presented the surveillance data of a regional hospital. Further study with larger cohort of experimental subjects from representative geographical locations is warranted to have a more complete picture on prevalence and characteristics of mcr-possessing Enterobacteriaceae in Hong Kong. Second, we could not assess any information regarding antibiotic usage by experimental subjects outside our hospital. In addition, albeit not within the scope of this study, further curation and analysis of WGS data can provide insights into actual genomic locations of mcr-1 in remainder E. coli isolates.

Conclusions
In this study, we have further revealed asymptomatic carriage of mcr-1-harboring Enterobacteriaceae by both patients and healthy individuals, with fecal carriage of 2.08% which was higher than expected in our region. This asymptomatic carriage is alarming because it potentially facilitates acquisition of mcr-1 by multidrug-resistant bacteria in future infection, which severely limits the choice of usable antibiotics for treatment. Prevalence of mcr-2-harboring Enterobacteriaceae, on the contrary, is believed to be very low at present. This surveillance data on emerging antibiotic resistance is important for infection control, especially for patients under intensive care. In addition, we have unraveled the phenotypic and molecular traits of 14 mcr-1-possessing E. coli strains. Further studies on mobilization and resistance mechanisms of this recently-discovered resistance gene family may shed light on strategies limiting emergence and spread of pan-drug-resistant bacteria.

Abbreviations
AM: Ampicillin; AMC: Amoxicillin-clavulanate; AN: Amikacin; BMD: Broth microdilution; BRIG: BLAST Ring Image Generator; CAZ: Cefazidime; CDS: Coding sequence; CF: Cephalothin; CIP: Ciprofloxacin; CLSI: Clinical & Laboratory Standards Institute; CN: Gentamicin; CTX: Cefotaxime; CXM: Cefuroxime; ESBL: Extended-spectrum β-lactamase; EUCAST: The European Committee on Antimicrobial Susceptibility Testing; FEP: Cefepime; HA: Clients coming for health assessment; hp: Hypothetical protein; I: Intermediate; IP: Inpatients; LEV: Levofloxacin; MALDI-TOF MS: Matrix-assisted laser desorption/ionization-time of flight mass spectrometry; mcr-1 and mcr-2: Mobilized colistin resistance genes; MI: Minocycline; MIC: Minimum inhibitory concentration; OP: Outpatients; PB: Polymyxin B; PCR: Polymerase chain reaction; PE: Colistin; R: Resistant; ST: Sequence type; SXT: Trimethoprim/sulfamethoxazole; TIM: Ticarcillin-clavulanate; TOB: Tobramycin; TZIP: Piperacillin-tazobactam; VRE: vancomycin-resistant enterococci; WGS: Whole genome sequencing

Acknowledgements
Not applicable

Funding
No funding was obtained for this study.

Availability of data and materials
Full plasmid sequences of pHKSHmcr1_P2_p1 and pHKSHmcr1_P2_p2 have been deposited under GenBank accession numbers MF136778 and MF136779, respectively.

Authors’ contributions
BST and WC conceived and designed the study. BST, ESM, TC, WC, CA and DNH were involved in data collection and analysis. WC wrote the first draft. All authors critically reviewed the manuscript and approved the final manuscript.
Ethics approval and consent to participate
This study was approved and consent to participate was waived by Research Ethics Committee (REC) of Hong Kong Sanatorium & Hospital. The REC reference number is REC-2018-02. The data of this study was generated as part of routine surveillance of emerging antibiotic resistance. No patient-identifying data was collected.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 28 June 2017 Accepted: 31 January 2018
Published online: 13 February 2018

References
1. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16(2):161–8.
2. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016. Euro Surveill. 2016;21(27).
3. Castanheira M, Griffin MA, Deshpande LM, Mendes RE, Jones RN, Flamm RK. Detection of mcr-1 among Escherichia coli clinical isolates collected worldwide as part of the SENTRY antimicrobial surveillance program in 2014 and 2015. Antimicrob Agents Chemother. 2016;60(9):5623–4.
4. Wang Q, Sun J, Li J, Ding Y, Li XP, Lin J, et al. Expanding landscapes of the diversified mcr-1-bearing plasmid reservoirs. Microbiome. 2017;5(1):70.
5. Brauer A, Telling K, Laht M, Kalms P, Lutsch J, Remm M, et al. Plasmid with Colistin Resistance Gene mcr-1 in Extended-Spectrum-β-Lactamase-Producing Escherichia coli Strains isolated from Pig Stuny in Estonia. Antimicrob Agents Chemother. 2016;60(11):5963–6.
6. Jelovcan S, Leekhtseroohnpren P, Weissensteiner G, Hendriksen RS, Lassnig H, Allerberger F, et al. Detection of plasmid-mediated colistin resistance (mcr-1) in E. coli isolated from pig caecum in Austria. Int J Infect Dis. 2016;49:855–9.
7. Matamoros S, van hattem JM, Arcilla MS, Willems N, Melles DC, Penders J, et al. Global phylogenetic analysis of Escherichia coli and plasmids carrying the mcr-1 gene indicates bacterial diversity but plasmid restriction. Sci Rep. 2017;7(1):15364.
8. Wong SC, Tse H, Chen JH, Cheng YC, Ho PL, Yuen KY. Colistin-resistant Enterobacteriaceae carrying the mcr-1 gene among patients in Hong Kong. Emerg Infect Dis. 2016;22(9):1667–9.
9. Blackburn J, Tsimiklis C, Laverenge V, Pilote J, Grenier S, Gilbert A, et al. Carabapenem disks on MacConkey agar in screening methods for detection of carabapenem-resistant Gram-negative rods in stools. J Clin Microbiol. 2013;51(1):331–3.
10. Ye H, Li Y, Li Z, Gao R, Zhang H, Wen R, et al. Diversified mcr-1-harboung plasmid reservoir confer resistance to colistin in human gut microbiota. MBio. 2016;7(2):e00177.
11. SPAdes Genome Assembler, Algorithmic Biology Lab, St. Petersburg Academic University of the Russian Academy of Sciences. 2016. Available from http://cab.spbu.ru/software/spades/. Accessed on 08-03-2017.
12. Escherichia coli MLST Database, The University of Warwick. 2017. Available from: http://mlst.warwick.ac.uk/mlst/dbs/Ecoli. Accessed on 25-03-2017.
13. Sequence query – MLST locus/ sequence definitions (Escherichia coli (Pasteur)), PubMLST. 2017. Available from: https://pubmlst.org/bigdb/1?db=pubmlst_mlst_seqdef. Accessed on 25-03-2017.
14. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67(11):2640–4.
15. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol. 2014;52(5):1501–10.
16. Maue version 2.40, the Darling lab computational (metagenomics). 2014. Available from: http://darlinglab.org/maue/download.html. Accessed on 08-03-2017.
17. Prokka: rapid prokaryotic genome annotation. 2017. Available from: https://github.com/defmann/prokka. Accessed on 08-03-2017.
18. Casartelli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58(7):3895–903.
19. IS Finder, Laboratoire de Microbiologie et Génétique Moleculaires. 2017. Available from: https://isagabiotools.if. Accessed on 25-03-2017.
20. Allikhan NF, Petty NK, Ben Zakour NL, Beaton SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011;12:402.
21. Zhong LL, Phan HT, Shen C, Doris-Vihta K, Sheppard AE, Huang X, et al. High rates of human fecal carriage of mcr-1-positive multi-drug resistant Enterobacteriaceae isolates emerge in China in association with successful plasmid families. Clin Infect Dis. 2017;54:798–82.
22. Bi Z, Berglund B, Sun Q, Nilsson M, Chen B, Tärnberg M, et al. Prevalence of the mcr-1 colistin resistance gene in extended-spectrum β-lactamase-producing Escherichia coli from human faecal samples collected in 2012 in rural villages in Shandong Province, China. Int J Antimicrob Agents. 2017;49(4):493–7.
23. Zurfluh K, Stephan R, Widmer A, Pöirel L, Nordmann P, Nüesch HL, et al. Screening for fecal carriage of MCR-producing Enterobacteriaceae in healthy humans and primary care patients. Antimicrob Resist Infect Control. 2017;6:28.
24. Saly M, Jayol A, Pöirel L, Megraud F, Nordmann P, Dubois V. Prevalence of fecal carriage of colistin-resistant Gram-negative rods in a university hospital in western France, 2016. J Med Microbiol. 2017;66(6):842–3.
25. Teneer EM, Nijhuis RHT, Crebach MTJ, Knetoch CW, Veldkamp KE, Goossens J, et al. Prevalence of colistin resistance gene (mcr-1) containing Enterobacteriaceae in feces of patients attending a tertiary care hospital and detection of a mcr-1 containing, colistin susceptible E. coli. PLoS One. 2017;12(6):e0178598.
26. Huang X, Yu L, Chen X, Zhi C, Yao X, Liu Y, et al. High prevalence of colistin resistance and mcr-1 gene in Escherichia coli isolated from food animals in China. Front Microbiol. 2017;8:562.
27. Kawanishi M, Abo H, Ozawa M, Uchimaya M, Shirakawa T, Suzuki S, et al. Prevalence of colistin-resistance gene mcr-1 and absence of mcr-2 in Escherichia coli isolated from healthy food producing animals in Japan. Antimicrob Agents Chemother. 2016;61(1). pii: e02057-e02016.
28. Zurfluh K, Nüesch-Inderbinen M, Klumpp J, Poirel L, Nordmann P, Stephan R. Key features of mcr-1-bearing plasmids from Escherichia coli isolated from humans and food. Antimicrob Resist Infect Control. 2017;6:591.
29. Corbella M, Mariani B, Ferrari C, Comandatore F, Scalfitti E, Marone P, et al. Three cases of mcr-1-positive colistin-resistant Escherichia coli bloodstream infections in Italy, August 2016 to January 2017. Euro Surveill. 2017;22(16).
30. Cinniglataelli A, Tani A, Antinelli A, Principe L, Luzzaro F, Rossolini GM. First Description of MCR-1 in Escherichia coli from Hospital Sewage with the Identification of Two Variants. Front Microbiol. 2017;8:2094.
31. Homberg C, Flaxman SD, Glass R, et al. Comparison of Sensititre, MicroScan, Vitek 2, and Etest with Broth Microdilution (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011;12:402.
32. Zapp L, Schmalz V, Bauduin C, et al. Prevalence of mcr-1-containing, colistin susceptible E. coli and mcr-1 gene in Escherichia coli isolated from food animals in China. Front Microbiol. 2017;8:562.
33. Zapp L, Schmalz V, Bauduin C, et al. Prevalence of mcr-1-containing, colistin susceptible E. coli and mcr-1 gene in Escherichia coli isolated from food animals in China. Front Microbiol. 2017;8:562.
34. Mellmann A, Fruth A, Friedrich AW, Wieler LH, Harmsen D, Werber D, et al. Phylogenetic and disease association of Shiga toxin-producing Escherichia coli from Hospital Sewage with the Identification of Two Variants. Front Microbiol. 2017;8:2094.
35. Zapp L, Schmalz V, Bauduin C, et al. Prevalence of mcr-1-containing, colistin susceptible E. coli and mcr-1 gene in Escherichia coli isolated from food animals in China. Front Microbiol. 2017;8:562.
36. Chen Y, Chen X, Zheng S, Yu F, Kong H, Yang Q, et al. Serotypes, genotypes and antimicrobial resistance patterns of human Enterobacteriaceae
diarrhoeagenic *Escherichia coli* isolates circulating in southeastern China. Clin Microbiol Infect. 2014;20(1):52–8.

37. Johnson TJ, Wannemuehler Y, Johnson SJ, Stell AL, Doetkott C, Johnson JR, et al. Comparison of extraintestinal pathogenic *Escherichia coli* strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. Appl Environ Microbiol. 2008;74(22):7043–50.

38. Gedeberg A, Hasman H, Sørensen CM, Wang M. An OXA-48-producing *Escherichia coli* isolated from a Danish patient with no hospitalization abroad. Infect Dis (Lond). 2015;47(8):593–5.

39. Sun J, Yang RS, Zhang Q, Feng Y, Fang LX, Xia J, et al. Co-transfer of *bla* 

*NDM-5* and *mcr-1* by an IncX3-X4 hybrid plasmid in *Escherichia coli*. Nat Microbiol. 2016;1:16176.

40. Adamczyk M, Jagura-Burdzy G. Spread and survival of promiscuous IncP-1 plasmids. Acta Biochim Pol. 2003;50(2):425–53.

41. Norberg P, Bergström M, Jethava V, Dubhashi D, Hermansson M. The IncP-1 plasmid backbone adapts to different host bacterial species and evolves through homologous recombination. Nat Commun. 2011;2:268.

42. Sun J, Fang LX, Wu Z, Deng H, Yang RS, Li XP, et al. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the mcr-1 acquisition. Sci Rep. 2017;7(1):424.

43. Li G, Zhang Y, Bi D, Shen P, Ai F, Liu H, et al. First report of a clinical, multidrug-resistant Enterobacteriaceae isolate coharboring fosfomycin resistance gene *f0sA3* and carbapenemase gene *bla* 

*KPC-2* on the same transposon, Tn1721. Antimicrob Agents Chemother. 2015;59(1):338–43.

44. Dang B, Mao D, Luo Y. Complete nucleotide sequence of IncP-1B plasmid pDTC28 reveals a non-functional variant of the *bla* 

*KPC-2*-type Gene. PLoS One. 2016;11(5):e0154975.

45. Stokes MO, Abuoun M, Umur S, Wu G, Partridge SR, Mevius DJ, et al. Complete sequence of pSAM7, an IncX4 plasmid carrying a novel *bla* 

*CTX-M-14b* transposition unit isolated from *Escherichia coli* and Enterobacter cloacae from cattle. Antimicrob Agents Chemother. 2013;57(9):4590–4.

46. Beyrouthy R, Robin F, Lessene A, Lacombat I, Dortet L, Naas T, et al. MCR-1 and OXA-48 in vivo acquisition in KPC-producing *Escherichia coli* after colistin treatment. Antimicrob Agents Chemother. 2017;61(8):e02540–16.

47. Paveenkittiporn W, Kerdsin A, Chokngam S, Bunthi C, Sangkitporn S, Gregory CJ. Emergence of plasmid-mediated colistin resistance and New Delhi metallo-

β-lactamase genes in extensively drug-resistant *Escherichia coli* isolated from a patient in Thailand. Diagn Microbiol Infect Dis. 2017;87(2):157–9.

48. Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X, et al. Genetic characterization of mcr-1-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. J Antimicrob Chemother. 2017;72(2):393–401.

49. Snesrud E, Ong AG, Corey B, Kwak YI, Clifford R, Gleeson T, et al. Analysis of serial isolates of mcr-1-positive *Escherichia coli* reveals a highly active ISApl1 transposon. Antimicrob Agents Chemother. 2017;61(5).