Journal Pre-proof

Occurrence and genetic characteristics of *mcr*-1 positive colistin resistant *E. coli* from poultry environments in Bangladesh

Mohammed Badrul Amin Ajrin Sultana Sraboni Muhammed Iqbal Hossain Subarna Roy T.I.M. Amin Uddin Mozmader Leanne Unicomb Emily K. Rousham Mohammad Aminul Islam

PII: S2213-7165(20)30092-8

DOI: https://doi.org/doi:10.1016/j.jgar.2020.03.028

Reference: JGAR 1216

To appear in: *Journal of Global Antimicrobial Resistance*

Received Date: 8 January 2020

Revised Date: 30 March 2020

Accepted Date: 31 March 2020

Please cite this article as: Amin MB, Sraboni AS, Hossain MI, Roy S, Mozmader TIMAU, Unicomb L, Rousham EK, MAI, Occurrence and genetic characteristics of *mcr*-1 positive colistin resistant *E. coli* from poultry environments in Bangladesh, *Journal of Global Antimicrobial Resistance* (2020), doi: https://doi.org/10.1016/j.jgar.2020.03.028

Occurrence and genetic characteristics of *mcr*-1 positive colistin resistant *E. coli* from poultry environments in Bangladesh

Mohammed Badrul Amin[1#], Ajrin Sultana Sraboni[1], Muhammed Iqbal Hossain[1], Subarna Roy[1], TIM Amin Uddin Mozmader[1], Leanne Unicomb[2], Emily K. Rousham[3], Mohammad Aminul Islam[1,4#]

[1]Laboratory of Food Safety and One Health, Laboratory Sciences and Services Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka-1212, Bangladesh

[2]Environmental Intervention Unit, Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka-1212, Bangladesh

[3]Centre for Global Health and Human Development, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK

[4]Paul G. Allen School for Global Animal Health, Washington State University, Pullman, Washington, USA

Running title: *mcr*-1 *E. coli* from poultry environment in Bangladesh

[#]Corresponding author:
Mohammed Badrul Amin
Food Microbiology Laboratory, Laboratory Sciences and Services Division, icddr,b
GPO box 128, Dhaka 1000, Bangladesh

E-mail: badrul.amin@icddrb.org

Mohammad Aminul Islam

Paul G. Allen School for Global Animal Health, Washington State University

Pullman, Washington, USA

E-mail: amin.islam@wsu.edu

Abstract

Objectives

Colistin is one of the last-resort antibiotics for treatment of multi-drug resistant (MDR) Gram negative bacterial infections. We determined occurrence and characteristics of *mcr*-1-producing *E. coli* obtained from live bird markets (LBM), rural poultry farms (RPF) and rural household backyard poultry (HBP) in Bangladesh.

Methods

We tested 104 extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolated during 2017-2018 from poultry sources for colistin resistance. We analyzed the resistant isolates for *mcr* gene and characterized *mcr* positive isolates for antibiotic susceptibility, antibiotic resistance genes, transmissible plasmids and clonal diversity.

Results

Of 104 isolates, 98 (94%) had $MIC_{colistin}$ ≥4 µg/mL and 14 (13.5%) were positive for *mcr*-1 of which 10 were from LBM (n=10), 3 from RFP and 1 from HBP. All 14 *mcr*-1 *E. coli* were resistant to third generation cephalosporin and tetracycline, while 12 were resistant to fluoroquinolone and sulphamethoxazole, 10 were to aminoglycosides and 3 were to nitrofurantoin. Four isolates carried conjugative *mcr*-1 plasmid of 23 to 55 MDa in size. The 55 MDa plasmid found in 2 isolates carried additional resistant genes including $bla_{CTX\text{-}M\text{-}group\text{-}1}$ and $bla_{TEM\text{-}1}$ (ESBL), *qnrB* (fluoroquinolone) and *rmtB* (aminoglycoside). These plasmids belong to IncF family with additional replicons: HI1 and N. ERIC-PCR revealed a heterogeneous banding pattern of *mcr*-1 positive isolates.

Conclusion

We report a 13.5% prevalence of *mcr*-1 positive MDR *E. coli* in poultry fecal samples predominantly from LBMs in Bangladesh accentuating the need for safe disposal of poultry feces and hygiene practices among people exposed to poultry.

Key words: Colistin, *mcr*, ESBL, poultry, antibiotic resistance

1. Introduction

There is increasing concern regarding the potential for the emergence and transmission of antibiotic resistance to human populations through the food supply chain and the environment. Human infections with highly resistant organism are increasingly treated with drugs of last resort which often may not fall under the standard regimen of therapy. For instance, colistin, an antibiotic that was rarely used in patients even in the recent past due to its nephrotoxicity and

neurotoxicity, has now been widely used in countries with a high burden of AMR for the treatment of carbapenem resistant Gram negative bacterial infections [1-3]. Historically colistin has been used in the poultry industry and bacteria resistant to colistin have been detected from samples collected as early as the 1980s [4, 5].

Resistance to colistin was primarily attributed to mutations in chromosomal genes like *pmrAB*, *phoPQ*, and *mgrB* [6, 7] and the occurrence of colistin resistance among human pathogens has never been observed. The recent emergence of the plasmid mediated colistin resistance gene, *mcr*-1 has shifted the entire paradigm of antimicrobial resistance due to its rapid movement through horizontal transmission of plasmids that carry resistance genes to many other antibiotics critical for human health [8, 9]. Within 6 months of the first report of the emergence of *mcr*-1 from China, at least 30 other countries have reported the occurrence of the same gene from both animal and human sources [10-12]. Subsequently, other subtypes of *mcr* gene like *mcr*-2, -3, -4 and -5 were reported [13-15].

Poultry is considered by far the most common source of *mcr*-1-producing organisms which have been isolated from different stages of poultry production and the supply chain [16-20]. *mcr*-1-producing organisms have also been reported from other food-producing animals and food sources for human and animals [5, 20-22]. Emergence and transmission of *mcr*-1 are linked with excessive use of colistin and polymyxins in animal farms in many countries and Bangladesh is not an exception [4].

Recently, *E. coli* positive for *mcr*-1 gene was isolated from sludge samples in Dhaka, Bangladesh [23]. The source of these organisms in urban sludge was unknown, as it contained mixed waste but a large proportion comes from wet markets. It is essential to explore the possible sources of these organisms. This study therefore aimed to investigate the occurrence of colistin resistant *E. coli* among poultry in three different settings in Bangladesh including small/medium live bird markets in urban areas, rural poultry farms, and backyard poultry from rural households.

2. Methods and Materials

2.1. Study overview

Our current research was part of larger study which contemporaneously explored the dynamics of antimicrobial resistance (AMR) transmission from contaminated outdoor environments including animals, soil, drinking water, solid waste and waste water to humans in urban and rural Bangladesh [24]. In this study, we used the ESBL-producing *E. coli* isolates collected from poultry sources to investigate the occurrence of *E. coli* with *mcr* encoding colistin resistance as colistin has been used widely in the poultry farming industry.

2.2. E. coli isolates

We tested a total of 104 ESBL-producing *E. coli* that were isolated from poultry feces obtained from urban Live Bird Markets, LBM (n=66); Rural Poultry Farms, RPF (n=24); and Household Backyard Poultry HBP (n=14). Of these 104 fecal samples, 67 were from poultry pen feces and 37 were from caecum samples of individual chicken (*n*=37). The sample collection procedures

and isolation of ESBL-producing *E. coli* from these samples have been described previously [24].

2.3. Screening of ESBL-producing E. coli isolates for colistin resistance by analyzing minimum inhibitory concentration (MIC)

MIC of colistin for all *E. coli* was determined using broth micro dilution method recommended by joint European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines as described previously [25]. According to the guidelines, isolates having a MIC of ≤2 µg/mL is considered colistin susceptible while MIC of >2 µg/mL is considered colistin resistant.

2.4. Identification of antibiotic resistance genes by PCR

All *E. coli* isolates with a colistin MIC value of >2 µg/mL were tested for plasmid encoded *mcr* genes (*mcr*-1, *mcr*-2, *mcr*-3, *mcr*-4 and *mcr*-5) by a multiplex PCR program using primer sequences and PCR conditions as described previously [26]. The primers for clinically important major genes encoding ESBL: $bla_{\text{CTX-M-group-1}}$, $bla_{\text{CTX-M-group-2}}$, $bla_{\text{CTX-M-group-8}}$, $bla_{\text{CTX-M-group-9}}$, $bla_{\text{CTX-M-group-25}}$, bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-1,}}$ $bla_{\text{OXA-47}}$; quinolone resistance: *qnrA*, *qnrB* and *qnrS*; and aminoglycoside resistance: *rmtB* and *rmtC* were used following the PCR conditions described previously [27, 28]

2.5. Confirmation of mcr-1 amplified fragment by sequencing

The PCR amplified *mcr*-1 fragment from one of the *E. coli* strains (DL88FP1E1) was sequenced by using ABI PRISM BigDye Terminator Cycle Sequencing Reaction kit (Applied Biosystems;

CA, USA) using ABI PRISM 310 automated sequencer (Applied Biosystems; CA, USA). Briefly, the raw sequence was analyzed with BioEdit software and 241 bp deduced sequence was searched for homology determination by Basic Local Alignment Search Tool (BLAST). BLASTn homology comparison of 241 bp inferred sequence (Accession no. **MK738010**) from *mcr*-1 fragment confirmed their sequence similarity with the earlier reported *mcr*-1 in *E. coli* strain SHP45 [10].

2.6. Plasmid analysis

Plasmid DNA from the 14 *mcr*-1 positive *E. coli* isolates was prepared using the rapid alkaline lysis method and separated by horizontal electrophoresis in 0.7% agarose gels as described earlier [29]. The transferability of plasmids harboring *mcr*-1 was investigated by the broth mating experiment as described previously [28] in which colistin resistant *mcr*-1 positive *E. coli* isolates were used as donors and azide resistant *E. coli* J53 served as the recipient. The transconjugants were selected on Mueller–Hinton agar plate containing colistin (2 µg/mL) and sodium azide (150 µg/mL) after overnight mating of the donor (*mcr*-1 positive *E. coli*) and recipient (Na-Azide^{r} *E. coli*) at 37^{o}C. The transferable plasmid was assessed for incompatibility typing using 5 multiplex- and 3 singleplex-PCRs following the procedure described previously [30]**.** The PCR amplified products of plasmids were sequenced to confirm their replicon types using the BLAST tool available at NCBI web (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and plasmid multi locus sequence typing (pMLST database (http://www.pubmlst.org/plasmid).

2.7. Antibiotic susceptibility tests and antibiotic resistance gene analysis of transconjugant E. coli

All transconjugant *E. coli* isolates were tested for susceptibility against 14 clinically important antibiotics by disc diffusion method, MIC for colistin resistance and ESBL screening following the CLSI guidelines [25, 31]. Antibiotic resistant transconjugants were further analyzed for corresponding resistance genes by PCR as described previously [29].

2.8. Typing of mcr-1 *positive E. coli isolates by Enterobacterial Repetitive Intergenic Consensus* (*ERIC)-PCR*

Two primers: ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') were used to type *mcr*-1 positive *E. coli* isolates using ERIC-PCR according to the procedure described earlier [32]. PCR amplification was carried out in a C1000 Touch Thermal Cycler system (BioRad, CA, USA) and amplified products were separated in 1.5% agarose gel, stained with Midori Green, and visualized with a FastGene Blue/Green LED Gel Illuminator (Nippon Genetics, Tokyo, Japan). The image was analysed with diversity database finger-printing software, BioNumerics version 4.5 (Applied Maths; Kortrijk, Belgium) to determine the clonal relatedness among the strains. A dendrogram showing hierarchical representation of the level of linkage between the strains was used to estimate the degree of clonality. The degree of heterogeneity was determined by comparing of the Dice coefficients and clustering correlation coefficients calculated by unweighted-pair group method with arithmetic averages (UPGMA).

3. Results

3.1. Colistin resistance among ESBL-producing E. coli isolates from poultry sources

Of 104 ESBL-producing *E. coli* isolates 98 (94%) had an MIC for colistin ≥4 µg/mL suggesting that these isolates were phenotypically resistant to colistin according to CLSI-EUCAST breakpoint [25, 31]. A total of 52 (50%) isolates had an MIC of 4 µg/mL, 28 (27%) had an MIC of 8 µg/mL, 18 (17%) had an MIC of ≥16 µg/mL and only 6 isolates had an MIC ≤2 µg/mL. MIC_{50} and MIC_{90} among all the isolates tested were 4 µg/mL and 16 µg/mL, respectively (Table 1).

3.2. Detection of mcr genes

Multiplex PCR assay for *mcr* genes with *E. coli* isolates having MIC of ≥4 µg/mL revealed that 14 of 98 (14%) resistant isolates were *mcr*-1 positive (Supplementary Fig. 1). All *mcr*-1 positive isolates had an MIC of colistin ≥8 µg/mL (Table 1). None of the isolates was positive for other variants of *mcr* (*mcr*-2 to *mcr*-5). *mcr*-1 positive *E. coli* were predominantly isolated from LBM (n=10, 71.5%) followed by RFP (n=3, 21.5%) and HBP (n=1, 7%). Overall, 12% (8 of 67) of isolates from poultry pen feces and 16% (6 of 37) of isolates from caecum samples were positive for *mcr*-1, highlighting that gastrointestinal tracts of chicken are important sources of colistin resistant *E. coli*.

3.3. Antibiotics susceptibility and antibiotic resistance genes

All ESBL-producing *E. coli* carrying *mcr*-1 (*n*=14) were resistant to multiple classes of antibiotics (Fig. 1). All isolates were resistant to ampicillin and tetracyclines followed by 85% (*n*=12) to fluoroquinolone and sulphamethoxazole-trimethoprim, 71% (*n*=10) to aminoglycosides, and 21% (*n*=3) to nitrofurantoin. None of the isolates was resistant to carbapenem. PCR results showed that 85% (*n*=12) of the *mcr*-1 isolates were positive for bla_{CTX}-

206 $_{\text{M-group-1}}$ followed by 71% (*n*=10) for *bla*$_{\text{TEM}}$, 21% (*n*=3) for *bla*$_{\text{OXA-1,}}$ 14% (n=2) for *bla*$_{\text{OXA-47}}$ and
207 7% (n=1) for *bla*$_{\text{CTX-M-group-9}}$. Plasmid mediated quinolone resistance gene *qnr* was detected in 6
208 isolates (*qnr*S in 28%, n=4; and *qnr*B in 14%, n=2 isolates) whereas, aminoglycoside resistance
209 gene *rmt*B was found in 28% (n=4) of the isolates. None of the *mcr*-1 *E. coli* isolates were
210 positive for *bla*$_{\text{CTX-M-group-2}}$, *bla*$_{\text{CTX-M-group-8}}$, *bla*$_{\text{CTX-M-group-25,}}$ *bla*$_{\text{SHV}}$, *qnrA* and *rmtC*.
211

212 ***3.4. Plasmid analysis of mcr-1 carrying E. coli isolates***

213 All *mcr*-1-producing *E. coli* isolates (n=14) carried multiple plasmids ranging from 1.2 to 140
214 MDa and isolates were heterogenous in their plasmid patterns (Supplementary Table 1**)**. The
215 conjugation experiment showed that 4 out of 14 *mcr*-1 positive isolates had *mcr*-1 gene in
216 transmissible plasmids of different sizes ranging from 23 MDa to 55 MDa (Fig. 2). All
217 transconjugants (*n*=4) had a colistin MIC of 16 µg/mL which was similar to their corresponding
218 donor strains. Antibiotic susceptibility of these 4 transconjugants showed diverse antibiotic
219 resistance patterns along with different combinations of antibiotic resistance genes (Table 2).
220 Plasmid incompatibility typing of these 4 transconjugants revealed that 2 were double-replicon
221 type: IncHI1, IncFIB, and IncN, IncFIA; and the remaining 2 were untypeable (Table 2)
222 (Supplementary Fig. 2).
223

224 ***3.5. ERIC-PCR typing***

225 ERIC-PCR of *mcr*-1 positive isolates showed diverse banding patterns except for two isolates
226 (DL166FP1 and DL166FP2). Of 14 isolates, 8 were differentiated into four close pairs of clusters
227 designated as E1 to E4 with 100% homology in one pair of isolates followed by 74 to 90% of
228 homogeneity between other pairs (Fig. 3).

4. Discussion

In this study, we found that 94% (98/104) of ESBL-producing *E. coli* isolates were phenotypically resistant to colistin and 13.5% were positive for *mcr*-1 in live bird markets, rural poultry farms and household backyard poultry environment. Despite side effects, colistin is used increasingly to treat patients with infections caused by multi-drug resistant organisms against which colistin is still active [33]. This crucial drug of last resort is becoming ineffective as plasmid mediated colistin resistance *mcr*-1 has emerged in bacteria that are already resistant to many antibiotics. To the best of our knowledge, this is the first report of the occurrence of *mcr*-1-producing *E. coli* isolates from poultry sources in Bangladesh. Recently, other Asian countries including Nepal, China, South Korea and Vietnam reported *mcr*-1 presence in *E. coli* from poultry [13, 16, 17, 34]. The prevalence of *mcr*-1 among colistin resistant isolates in our study was very low compared to other studies. For example, more than 94% of colistin resistant (MIC: >2 µg/mL) isolates obtained from turkey and broiler feces in Germany were positive for *mcr*-1 [35]. The underlying reason for the low prevalence in isolates from Bangladesh is not clear but other resistance mechanisms, including chromosomal mutations through amino acid substitution in *pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB* might be involved [7, 36, 37]. Besides, other variants of *mcr* such as -6, -7 and -8 could also be present in these isolates but these were not tested in this study [22]. Further investigations for other resistance markers or chromosomal mutations in *mcr* negative but colistin resistant isolates would provide new insights into the mechanisms of resistance. Notably, in this study, *mcr*-1 was only positive among isolates showing an elevated level of colistin MIC (≥8 µg/mL) and the majority of isolates (9 out of 14) had an MIC of 16 µg/mL (Table 1). Similar results were reported by a study in China [38] though discordant

252 results were also reported by a previous study in Bangladesh which showed that the only *mcr*-1
253 positive *E. coli* strain had an MIC of colistin 4 µg/mL [23].
254
255 In addition to colistin and 3rd and 4th generation cephalosporins, these isolates were resistant to
256 most other clinically important antibiotics including fluoroquinolone, aminoglycoside and
257 sulphamethoxazole. More interestingly, we found that genes conferring resistance to all these
258 antibiotics are carried by the *mcr*-1 plasmid in two isolates, which is not unusual for this plasmid
259 according to previous work [8, 9]. Although we did not explore the potential drivers for this
260 resistance among poultry isolates, excessive use of antibiotics in commercial poultry farming
261 might be a possible reason. However, we also found one colistin resistant *E. coli* strain in a free
262 ranging chicken (Table 1) that are rarely given antibiotics. Free ranging chicken might be
263 exposed to antibiotic residues and antibiotic resistant bacteria from the environment where they
264 forage for foods. A previous study in Bangladesh (Dhaka and Gazipur) reported that a majority
265 of poultry farmers (215 of 260) use antibiotics for growth promotion and disease prevention in
266 their poultry farm without a veterinarian prescription. Antibiotics that are commonly
267 administered to poultry include tetracycline, doxycycline, ampicillin, colistin sulphate, nalidixic
268 acid, neomycin, ciprofloxacin, and sulfonamides with trimethoprim [39].
269
270 We confirmed that at least 4 of the 14 *mcr*-1 positive isolates carried transmissible *mcr*-1
271 plasmids of which two transconjugants co-harbor resistance determinants to multiple antibiotics
272 including 3rd and 4th generation cephalosporins, fluoroquinolones, aminoglycosides and
273 trimethoprim-sulphamethoxazole (Table 2). Plasmid replicon typing revealed that the *mcr*-1
274 plasmids belonged to IncF type which is one of the most prevalent plasmid families found in *E.*

coli and they commonly carry a wide range of genes conferring resistance to all major classes of antibiotics including β-lactams, aminoglycosides, tetracyclines, chloramphenicol, and quinolones [40, 41]. We found that 2 of the 4 transconjugants had IncF type plasmid (FIA, FIB) along with additional replicon types; N and HI1 respectively in a single conjugative plasmid indicating fusion of plasmids, similar to a previous report [42]. The presence of these highly promiscuous plasmids carrying multiple antibiotic resistance genes in *E. coli* isolates from poultry feces in live poultry markets in Dhaka city indicates the possible sharing of plasmids with other environmental organisms and their widespread dissemination in the environment. Human exposure to contaminated environments increases the risk of colonization or infection with these pathogens given the poor waste management and sanitary conditions in Dhaka slums. Widespread sharing of *mcr*-1 plasmids via horizontal gene transmission (HGT) among other organisms in the environment is further attested by the diverse ERIC-PCR types among the isolates, which suggest that clonal expansion of specific lineages is unlikely the driver of the high prevalence of *E. coli* strains with acquired antibiotic resistance.

In this study, we only screened ESBL-producing *E. coli* for colistin resistance rather than testing all *E. coli* isolates, therefore the estimation of prevalence of plasmid mediated colistin resistant *mcr*-1 may be an underestimation of the true prevalence. However, 94% prevalence of colistin resistance among all ESBL-producing *E. coli* from poultry sources with 13.5% of isolates carrying *mcr*-1 highlights a serious public health concern emphasizing the need for regular surveillance of this organism and interventions to reduce their transmission.

5. Conclusions

Our study showed that more than 94% of ESBL-producing *E. coli* from poultry feces collected from urban live bird market, rural poultry farm and backyard poultry were resistant to colistin and around 14% of these isolates carried plasmid mediated colistin resistance gene *mcr*-1. *mcr*-1-producing isolates were resistant to many antibiotics that are critical for human health and possessed corresponding resistance genes in self-transmissible *mcr*-1 plasmids. People are frequently exposed to poultry and poultry environments in urban live bird markets, in farming practices and in rural households in Bangladesh and thus colonization/infection with these multidrug-resistant organisms/pathogens are likely. High rate of colonization with ESBL-producing organism has already been reported among healthy infants in rural Bangladesh [31]. It is essential to control the prophylactic use of antibiotics especially those that are critical for human health to reduce the abundance of these organisms in poultry and its further dissemination to the environment. At the same time, systematic surveillance of AMR considering a One Health approach should be implemented to guide the intervention strategies directed to control AMR.

Author contributions

MBA and MAI contributed in concept development and initial draft writing; ASS, MIH, SR, MR and TAU performed sampling and laboratory experiment; MBA and MAI were involved in data analysis and designing of figure; MAI, EKR and LU contributed to the initial study concept, design, development and funding acquisition; MBA, MAI, EKR and LU were involved in final editing and review of the manuscript.

DECLARATIONS

Funding: This research was funded by the Antimicrobial Resistance Cross Council Initiative supported by the seven research councils in partnership with the Department of Health and Department for Environment Food & Rural Affairs (Grant number: NE/N019555/1). icddr,b acknowledges with gratitude the commitment of Natural Environment Research Council (NERC), UK to its research efforts. icddr,b is also grateful to the Governments of Bangladesh, Canada, Sweden and the UK for providing core/unrestricted support.

Conflict of interest: None

Ethical approval: This research protocol was submitted to the Institutional Review Board of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), where it received clearance from the Research Review Committee and Ethical Review Committee (protocol number PR-16071). Approval from the Human Participants Ethical Subcommittee was also obtained from Loughborough University (R17-P037).

References

1. Falagas, M.E., D.E. Karageorgopoulos, and P. Nordmann, *Therapeutic options for infections with Enterobacteriaceae producing carbapenem-hydrolyzing enzymes.* Future Microbiol, 2011. **6**(6): p. 653-66.
2. MacNair, C.R., et al., *Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics.* Nat Commun, 2018. **9**(1): p. 458.
3. Temkin, E., et al., *Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management.* Ann N Y Acad Sci, 2014. **1323**: p. 22-42.
4. Kempf, I., E. Jouy, and C. Chauvin, *Colistin use and colistin resistance in bacteria from animals.* International journal of antimicrobial agents, 2016. **48**(6): p. 598-606.
5. Shen, Z., et al., *Early emergence of mcr-1 in Escherichia coli from food-producing animals.* Lancet Infect Dis, 2016. **16**(3): p. 293.
6. Olaitan, A.O., S. Morand, and J.-M. Rolain, *Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria.* Frontiers in microbiology, 2014. **5**: p. 643.
7. Luo, Q., et al., *Molecular Epidemiology and Colistin Resistant Mechanism of mcr-Positive and mcr-Negative Clinical Isolated Escherichia coli.* Front Microbiol, 2017. **8**: p. 2262.
8. Malhotra-Kumar, S., et al., *Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid.* The Lancet infectious diseases, 2016. **16**(3): p. 283-284.
9. Shafiq, M., et al., *High incidence of multidrug-resistant Escherichia coli coharboring mcr-1 and bla CTX-M-15 recovered from pigs.* Infect Drug Resist, 2019. **12**: p. 2135-2149.

10. Liu, Y.Y., 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): p. 161-8.
11. Lu, X., et al., *Epidemiologic and genomic insights on mcr-1-harbouring Salmonella from diarrhoeal outpatients in Shanghai, China, 2006-2016.* EBioMedicine, 2019.
12. Joshi, P.R., et al., *Molecular Characterization of Colistin-Resistant Escherichia coli Isolated from Chickens: First Report from Nepal.* Microb Drug Resist, 2019.
13. Zhang, J., et al., *Molecular detection of colistin resistance genes (mcr-1, mcr-2 and mcr-3) in nasal/oropharyngeal and anal/cloacal swabs from pigs and poultry.* 2018. **8**(1): p. 3705.
14. Carattoli, A., et al., *Novel plasmid-mediated colistin resistance mcr-4 gene in Salmonella and Escherichia coli, Italy 2013, Spain and Belgium, 2015 to 2016.* Eurosurveillance, 2017. **22**(31).
15. Borowiak, M., et al., *Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting Salmonella enterica subsp. enterica serovar Paratyphi B.* Journal of Antimicrobial Chemotherapy, 2017. **72**(12): p. 3317-3324.
16. Trung, N.V., et al., *Zoonotic transmission of mcr-1 colistin resistance gene from small-scale poultry farms, Vietnam.* Emerging infectious diseases, 2017. **23**(3): p. 529.
17. Joshi, P.R., et al., *Molecular Characterization of Colistin-Resistant Escherichia coli Isolated from Chickens: First Report from Nepal.* Microbial Drug Resistance, 2019.

18. Dominguez, J.E., et al., *Simultaneous carriage of mcr-1 and other antimicrobial resistance determinants in Escherichia coli from poultry.* Frontiers in microbiology, 2018. **9**: p. 1679.

19. Grami, R., et al., *Impact of food animal trade on the spread of mcr-1-mediated colistin resistance, Tunisia, July 2015.* Eurosurveillance, 2016. **21**(8): p. 30144.

20. Chen, K., et al., *Widespread distribution of mcr-1-bearing bacteria in the ecosystem, 2015 to 2016.* Eurosurveillance, 2017. **22**(39).

21. Liu, Y.-Y., et al., *Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study.* The Lancet infectious diseases, 2016. **16**(2): p. 161-168.

22. Yang, F., et al., *Plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli and Klebsiella pneumoniae isolated from market retail fruits in Guangzhou, China.* Infect Drug Resist, 2019. **12**: p. 385-389.

23. Islam, A., et al., *Colistin resistant Escherichia coli carrying mcr-1 in urban sludge samples: Dhaka, Bangladesh.* Gut Pathog, 2017. **9**: p. 77.

24. Rousham, E., et al., *Spatial and temporal variation in the community prevalence of antibiotic resistance in Bangladesh: an integrated surveillance study protocol.* BMJ Open, 2018. **8**(4): p. e023158.

25. Chew, K.L., et al., *Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive Enterobacteriaceae: comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution.* Journal of clinical microbiology, 2017. **55**(9): p. 2609-2616.

26. Rebelo, A.R., et al., *Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes.* Eurosurveillance, 2018. **23**(6).

27. Woodford, N., E.J. Fagan, and M.J. Ellington, *Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases.* Journal of Antimicrobial Chemotherapy, 2005. **57**(1): p. 154-155.

28. Islam, M.A., et al., *Occurrence and characterization of multidrug-resistant New Delhi metallo-β-lactamase-1-producing bacteria isolated between 2003 and 2010 in Bangladesh.* Journal of medical microbiology, 2013. **62**(1): p. 62-68.

29. Islam, M.A., et al., *Emergence of multidrug-resistant NDM-1-producing Gram-negative bacteria in Bangladesh.* Eur J Clin Microbiol Infect Dis, 2012. **31**(10): p. 2593-600.

30. Carattoli, A., et al., *Identification of plasmids by PCR-based replicon typing.* Journal of microbiological methods, 2005. **63**(3): p. 219-228.

31. Islam, M.A., et al., *Fecal colonization with multidrug-resistant E. coli among healthy infants in rural Bangladesh.* Frontiers in microbiology, 2019. **10**: p. 640.

32. Morales-Erasto, V., et al., *ERIC-PCR genotyping of emergent serovar C-1 isolates of Avibacterium paragallinarum from Mexico.* Avian diseases, 2011. **55**(4): p. 686-688.

33. Aghapour, Z., et al., *Molecular mechanisms related to colistin resistance in Enterobacteriaceae.* Infection and drug resistance, 2019. **12**: p. 965.

34. Lim, S.-K., et al., *First detection of the mcr-1 gene in Escherichia coli isolated from livestock between 2013 and 2015 in South Korea.* Antimicrobial agents and chemotherapy, 2016: p. AAC. 01472-16.

35. Irrgang, A., et al., *Prevalence of mcr-1 in E. coli from Livestock and Food in Germany, 2010-2015.* PLoS One, 2016. **11**(7): p. e0159863.

36. Kempf, I., et al., *What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe?* Int J Antimicrob Agents, 2013. **42**(5): p. 379-83.

37. Olaitan, A.O., S. Morand, and J.M. Rolain, *Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria.* Front Microbiol, 2014. **5**: p. 643.

38. Lim, S.K., et al., *First Detection of the mcr-1 Gene in Escherichia coli Isolated from Livestock between 2013 and 2015 in South Korea.* Antimicrob Agents Chemother, 2016. **60**(11): p. 6991-6993.

39. Hasan, B., et al., *High prevalence of antibiotic resistance in pathogenic Escherichia coli from large-and small-scale poultry farms in Bangladesh.* Avian diseases, 2011. **55**(4): p. 689-692.

40. Liao, X.-P., et al., *Comparison of plasmids coharboring 16S rRNA methylase and extended-spectrum β-lactamase genes among Escherichia coli isolates from pets and poultry.* Journal of food protection, 2013. **76**(12): p. 2018-2023.

41. Liu, B.-T., et al., *Dissemination and characterization of plasmids carrying oqxAB-blaCTX-M genes in Escherichia coli isolates from food-producing animals.* PloS one, 2013. **8**(9): p. e73947.

42. Carattoli, A., et al., *Identification of plasmids by PCR-based replicon typing.* J Microbiol Methods, 2005. **63**(3): p. 219-28.

Legend information:

446 **Fig. 1.** Heat map showing antibiotic resistance patterns of colistin resistant *mcr*-1 positive *E. coli*
447 isolates.

448

449 **Fig.2**. Agarose gel electrophoresis showing *mcr*-1 plasmids extracted from *E. coli* donor and
450 transconjugant isolates. Lanes: 1= *E. coli* PDK9 strain having 140, 105, 2.7 and 2.1 MDa
451 plasmid (M); 2= DL86FP2 (D); 3= DL86FP2 (T); 4= TF136FP (D); 5= TF136FP (T); 6= *E. coli*
452 V-517 strain having 35.8, 4.8, 3.7, 2.0, 1.8 and 1.4 MDa plasmid (M); 7= DL190CL (D); 8=
453 DL190CL (T); 9= TF122FP (D); 10= TF122FP (T). M, marker; D, donor; T, transconjugant.

454

455 **Fig. 3**. ERIC-PCR based dendrogram showing clonal diversity of *mcr*-1 positive *E. coli* isolates
456 obtained from poultry feces in Bangladesh. The dendrogram was constructed by Bionumerics
457 (version 4.5) software using the Unweighted Pair Group Method with Arithmetic means
458 (UPGMA), where the tolerance for banding position was 1%. Five ERIC types, E1 to E5, were
459 observed in 10 isolates ranging from 60 to 100% sequence homology.

460

461 **Table 1.** Minimum Inhibitory Concentration (MIC) of colistin for *mcr*-1 positive *E. coli* isolates
462 obtained from poultry sources in Bangladesh

463 *LBM, Live Bird Market; RPF, Rural Poultry Farm; HBP, Household Backyard Poultry; MIC_{col}, MIC of
464 colistin

465

466 **Table 2.**

Isolates	Sources	Sample types	MIC_{col} (µg/mL)
DL86FP2	LBM	Poultry pen feces	16
DL86CL1	LBM	Cecum feces	16
DL87CL2	LBM	Cecum feces	16
DL87CL1	LBM	Cecum feces	16
DL88FP1	LBM	Poultry pen feces	16
TR107CL	HBP	Cecum feces	8
TF122FP	RPF	Poultry pen feces	16
TF122CL	RPF	Cecum feces	16
TF136FP	RPF	Poultry pen feces	8
DL166FP1	LBM	Poultry pen feces	16
DL166FP2	LBM	Poultry pen feces	8
DL170FP1	LBM	Poultry pen feces	8
DL190CL	LBM	Cecum feces	16
DL190FP1	LBM	Poultry pen feces	16

467 Characteristics of colistin resistant *mcr*-1 positive transconjugant *E. coli* strains

rain ID	Parent strain			Transconjugants			
	AMR phenotypes	AMR genotypes	Plasmid Profile (MDa)	AMR phenotypes	AMR genotypes	Plasmid profile (MDa)	Plasmid Inc/rep typ
L86FP2	AMP-CRO-CTX-CFM-FEP-CIP-NA-SXT-GEN	*bla*$_{CTX-M-group-1}$, *bla*$_{TEM-1}$, *qnr*B, *qnr*S, *mcr*-1	140, 55	AMP-CRO-CTX-CFM-FEP-CIP-NA-SXT-GEN	*bla*$_{CTX-M-group-1}$, *bla*$_{TEM-1}$, *qnr*B, *mcr*-1	55	HI1, FIB
L190CL	AMP-CRO-CTX-CFM-FEP- CIP-NA-SXT-GEN	*bla*$_{CTX-M-group-1}$, *bla*$_{TEM-1}$, *rmt*B	140, 60, 55, 2.5	AMP-CRO-CTX-CFM-GEN	*bla*$_{CTX-M-group-1}$, *bla*$_{TEM-1}$, *rmt*B, *mcr*-1	55	N, FIA
F136FP	AMP-CRO-CTX-CFM-FEP-SXT	*bla*$_{CTX-M-group-1}$, *qnr*S, *mcr*-1	23, 50, 1.6	All sensitive except colistin	*mcr*-1	23	Unidentifi
F122FP	AMP-CRO-CTX-CFM-FEP- CIP-NA-SXT-GEN	*bla*$_{CTX-M-group-1}$, *bla*$_{TEM-1}$, *mcr*-1	105, 50, 2.4	All sensitive except colistin	*mcr*-1	50	Unidentifi

AMP, ampicillin; CRO, ceftriaxone; CTX, cefotaxime; CFM, cefixime; FEP, cefepime; CIP, ciprofloxacin; NA, nalidixic acid; SXT, sulphamethoxazole-trimethoprim; GEN, gentamicin; $MIC_{col,}$ minimum inhibitory concentration of colistin

Highlights:

- About 94% extended spectrum beta lactamase (ESBL) producing *E. coli* from poultry sources are colistin resistant and 13.5% colistin resistant *E. coli* are positive for *mcr*-1 gene.
- *mcr*-1 positive isolates are resistant to many antibiotics that are critical for human health.
- Resistance to other antibiotics including 3rd generation cephalosporin, aminoglycoside and fluroquinolone are carried by the *mcr*-1 plasmid
- This is the first report on occurrence of plasmid mediated *mcr*-1-producing *E. coli* from poultry sources in Bangladesh