Original Article

Quantitative Polymerase Chain Reaction Identified a Multitude of *mcr*-1 to *mcr*-5 Genes in Fresh Cow Dung in Bangladesh: An Urgent Public Health Concern

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Abstract

Introduction: The emergence of antimicrobial resistance (AMR) in the food industry is a serious global public health threat. Colistin is frequently used as a growth promoter in livestock, which is a concern. The widespread use of colistin in the food industry is linked to the emergence of mobilised colistin resistance (*mcr*) genes. This must be avoided with colistin, an important Reserve antibiotic in humans. Consequently, there is an urgent need to investigate current variants of *mcr* gene in cattle faeces in Bangladesh. **Methods:** Cross-sectional study analysing *mcr-1* to *mcr-5* in fresh cow dung samples from 20 commercial farms and 6 individual houses. DNA was extracted from cow dung samples using commercial kits. Real-time quantitative polymerase chain reaction was used to assess the five *mcr* genes in the extracted DNA. **Results:** 40.8% (49/120) of the samples revealed the existence of at least one *mcr* gene, with *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* identified in 27.5% (33/120), 2.5% (3/120), 4.2% (5/120), 14.2% (17/120) and 8.3% (10/120) of samples, respectively. Co-occurrence of two or more genes was documented in 8.3% samples, with co-carriage of three genes in 1.7% of samples. No significantly higher numbers of *mcr* genes were identified between commercial farms and individual households. **Conclusion:** Excess use of antibiotics in cattle may result in increased prevalence of antibiotic-resistant genes. A comprehensive One Health approach is urgently needed in Bangladesh to reduce the spread of colistin resistance and meet the United Nation's targets for Access to antibiotics and AMR.

Keywords: Animal food production, Bangladesh, colistin, mobilised colistin resistance genes, quantitative polymerase chain reaction, reserve antibiotics

Introduction

Antimicrobial resistance (AMR), which includes antibiotic resistance (ABR), poses a serious and growing danger to global public health and food security in both human and veterinary medicine.[1-3] The extended use of antibiotics as growth promoters (AGPs) at subtherapeutic levels in cattle and other animal farms, including amongst low- and middle-income countries (LMICs), encourages the evolution of ABR in animal husbandry.[4,5] The emergence of AMR in livestock will impact on Sustainable Development Goal 3, which is to ensure healthy lives and promoting well-being for all across all age groups.[5-8] In addition, livestock-AMR challenges the goals of the recent United Nation's General Assembly (UN GA) on AMR to appreciably reduce the rate of AMR in the coming years.[5,7,9] This also includes encouraging greater use of antibiotics from the Access group, given current concerns with the growing use of Watch and Reserve antibiotics across sectors amongst LMICs in recent years, increasing AMR.[4,7,10,11]

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Overall in 2020, total global antimicrobial usage was estimated at 99,502 tons, with projections indicating an increase of 8.0%–11.5% by 2030 if current consumption trends continue, especially given continued growth in populations.[12-14] The most frequently used antimicrobials in animals reared for food include β-lactams, tetracyclines, aminoglycosides, lincosamides, quinolones, polypeptides, amphenicols, macrolides and sulphonamides, some of which are critically important antibiotics across sectors.[15-19] In view of this, the overuse of antibiotics outside of the health care sector is now considered one of the most important factors increasing AMR globally.[2,3,19-22] This needs to urgently be addressed if countries are looking to reduce their AMR rates in line with their public health goals, as well as meet the recent goals of the UN GA in September 2024.[8,9]

The use of antimicrobials in animals, including as AGPs, has surged in LMICs in recent years, including within South and Southeast Asia countries incorporating Bangladesh, which is an increasing public health concern.[17,18,23-25] In Bangladesh, it is estimated that over 94% of poultry farmers currently administer antimicrobials in their farms for disease prevention and as APGs, with other authors believing this practice currently occurs in up to 100% of broiler and layer chicken farms.[25-27] This is a major issue in Bangladesh with rising rates of AMR exacerbated by livestock-derived food consumption with resistant genes.[28-33]

Colistin (polymyxin E) is categorised by the World Health Organization (WHO) as one of the 'Highest Priority Critically Important Antimicrobials for Human Medicine' because it is a last-resort option for treating severe infections in humans caused by multidrug-resistant Gram-negative bacteria.[34-36] As a result, the WHO has designated colistin as a reserve antibiotic in their Access, Watch, Reserve (AWaRe) classification system, with increasing focus by the WHO and the UN GA to reduce the prescribing of colistin, together with other important antibiotics in the Watch and Reserve groups, to preserve their effectiveness in humans.[9,37] However, despite its critical role in human health, colistin continues to be widely used across countries, including Bangladesh, for livestock purposes, causing increasing concern.[36,38] This includes colistin being administered to animals to prevent and treat infections as well as for growth promotion.[38-42]

This extensive use of colistin, following appreciable imports into Bangladesh as well as other LMICs from countries such as China, has resulted in a global increase in the prevalence of colistin-resistant *Escherichia coli* strains in meat and food-producing animals, which needs to be urgently addressed going forward.[34,36,42,43] In Bangladesh, colistin resistance *E. coli* has been found in 78% of the broiler meat samples from carcasses collected from live bird markets in urban and rural areas.[44] In their study, Tanzin *et al.* found 74.92% of isolates from broiler meats were resistant to colistin, with a strong correlation between colistin resistance and the presence of the mobilised colistin resistance (*mcr-1*) gene.[45]

The first mobile colistin resistance gene, *mcr-1*, was reported in November 2015, found in an *E. coli* strain from a pig.[46] Since then, several other variants have been identified across countries, building on reports of *mcr-1* in animals in Bangladesh and beyond.[41,44,47-49] These include *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, *mcr-8*, *mcr-9* and *mcr-10*.[50-58] These *mcr* genes have been detected in various bacterial species from both human and animal sources, raising public health concerns in humans regarding the spread of mobile colistin resistance and the subsequent implications for managing infectious diseases.[36,38,59] *In vitro* studies have shown that *mcr* genes can transfer from animal bacteria to human pathogens;[59,60] with colistin-resistant bacteria linked to higher mortality rates in critically ill patients due to limited treatment options.[61-64]

As a result of growing concerns, identifying the main sources, factors and transmission routes of colistin resistance is a critical global priority. Traditional methods, including conventional PCR and Sanger sequencing, for detecting *mcr* genes are time-consuming and labour-intensive. Recently, more rapid and specific real-time quantitative PCR methods have been developed.[65] This advancement is particularly important in LMICs such as Bangladesh, where antibiotics from the WHO Reserve list, including polymyxins, are often available and used without a prescription, highlighting the need for improved monitoring and policy guidance to reduce growing rates of colistin resistance, as well as the resistance to other critical Watch and Reserve antibiotics, amongst patients.[66-72] Such advancements are part of a 'One Health' approach to reduce ABR for critical antibiotics such as colistin in priority countries, including Bangladesh.[36,73]

In view of this, there is a need to detect and quantify *mcr* genes (*mcr-1 to mcr-5*) in key animal species in Bangladesh using Real-time quantitative polymerase chain reaction (RT-qPCR) to provide future guidance to all key stakeholders going forward. This is because recent research has highlighted the spread of these genes from clinical settings to environmental areas.[38,74,75] Studying the colistin resistance gene distribution in the environment has potential implications for human health, with resistance to colistin already being reported amongst patients in Bangladesh.[70,71,76,77] However, this is not always the case.[78] This is important with Bangladesh previously banning the use of antibiotics in animal feeds, and seeking to reduce AMR, as part of its National Action Plan (NAP) against AMR.[79] However, there are concerns about whether actions and policies to improve the use of antimicrobials in animals, including monitoring current usage and resistance patterns, have actually been fully instigated and followed up in Bangladesh to reduce AMR in line with NAP goals.[79] Consequently, it is essential for the authorities in Bangladesh to research and monitor such activities to meet the AMR objectives outlined in its NAP as well as achieve the recent UN GA goals for AMR.[9,79,80]

To the best of our knowledge, we are not aware of any studies published in peer-reviewed journals to explore the prevalence of the *mcr* gene in cow dung samples in Bangladesh. We are

aware though of studies that have been published exploring carbapenemase genes in cow dung in Bangladesh.[81] This is critical for Bangladesh to meet its AMR goals, as well as UN GA goals, given ongoing concerns with AMR in the country.[9,32,33,80,82] Consequently, the objective of this study is to investigate the prevalence of mobile colistin resistance genes, *mcr-1 to mcr-5,* in fresh cow dung from cattle farms as well as from households in Bangladesh given the diverse situation. Subsequently, use the findings to offer future recommendations to all relevant stakeholder groups in Bangladesh.

Methods

Study aim, areas and sampling

A cross-sectional investigation was carried out between December 2021 and December 2022 to detect the presence and extent of different *mcr* genes in fresh cow dung samples in Bangladesh. This was built on the study of Al Asad *et al*., which analysed the presence of carbapenemase genes in these samples.[81] Briefly, 108 dung samples were gathered from 20 commercial cattle farms (90% of the sample) alongside 12 cow dung samples from six separate households. The commercial cattle farms rear a variety of dairy and beef cattle breeds, which include Holstein Friesian, Holstein Friesian-Jersey crosses, and Sahiwal. Some farms also bred well-known local cattle breeds including Pabna cattle, Red Chittagong cattle and Munshiganj cattle.[81] Each household raises locally sourced cattle of unspecified breeds.

Information regarding the animals' current health conditions, as well as any medication use, was collected through a previously used structured questionnaire.[81] Sampling took place in four districts of Bangladesh that were considered the dairy farming regions of the country, with details of sample collection and transportation previously described.[81]

DNA extraction from cow dung samples

The extraction method has previously been documented,[81] with the QIAamp DNA stool mini kit (Qiagen GmbH, Germany) used to manually extract DNA.

Primer design

Five pairs of *mcr* primer sequences (*mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5)*, their annealing temperatures and amplicon lengths, were obtained from previous literature.[81] The accuracy of the oligonucleotide sequences of primers was further checked in the NCBI BLAST (Basic Local Alignment Search Tool) database and then synthesised from an external manufacturer (Macrogen Inc., Teheran-ro, South Korea). A separate primer pair was synthesised to amplify the bacterial 16s rDNA gene as a positive control [Table 1].

Real-time polymerase chain reaction amplification programme

In line with our previously published study,[81] RT qPCR was used to determine relative (per 16S rRNA gene) abundances of ARGs in DNA extracted from cow dung samples. The qTOWER thermal cycler (Analytik Jena GmbH, Jena, Germany) was applied to conduct the qPCR amplification, with the amplification process completed with a melting step undertaken using the following cycling parameters: 60°C for 15s and 5°C temperature adjustments up to a final temperature of 95°C.[81] SYBR green's fluorescence energy was again used to measure the amount of amplified product.

A negative control (no template control) was used in each PCR run. The bacterial 16S rRNA gene was used for the positive control.[81]

Determination of specificity and sensitivity of the quantitative polymerase chain reaction

We calculated the sensitivity and specificity of each *mcr* gene detection by real-time qPCR, where deionised water was used as the negative control template, and the known bacterial 16S rRNA gene was used with the respective primer pair as a positive control.

The following equation was used to calculate specificity and sensitivity: specificity = (D/[C + D]) × 100 and sensitivity = (A/[A + B]) × 100, where A is a true positive, B is a false

Table 1: Primers utilised in quantitative polymerase chain reaction assays for identifying mobilised colistin resistance - 1 to mobilised colistin resistance - 5 genes and bacterial *16S* rDNA gene as positive control

Primers name	Nucleotides (5'–3')	Product length (bp)	References
mcr-1-qf[a]	AAAGACGCGGTACAAGCAAC	213	[65,81,83]
mcr-1-qr[b]	GCTGAACATACACGGCACAG		
mcr-2-qf	CGACCAAGCCGAGTCTAAGG	92	[65,81,83]
mcr-2-qr	CAACTGCGACCAACACACTT		
mcr-3-qf	ACCTCCAGCGTGAGATTGTTCCA	169	[65,81,83]
mcr-3-qr	GCGGTTTCACCAACGACCAGAA		
mcr-4-qf	AGAATGCCAGTCGTAACCCG	230	[65,81]
mcr-4-qr	GCGAGGATCATAGTCTGCCC		
mcr-5-qf	CTGTGGCCAGTCATGGATGT	98	[65,81]
mcr-5-qr	CGAATGCCCGAGATGACGTA		
16S-qf[c]	TCCTACGGGAGGCAGCAGT	467	[65]
16S-qr	GGACTACCAGGGTATCTAATCCT		

[a]Illustrates the forward primer for the mobilised colistin resistance gene, [b]Denotes the reverse primer for the mobilised colistin resistance gene, [c]Signifies the specific primer for *16S* rRNA, utilised as an internal control in qPCR. qPCR: Quantitative polymerase chain reaction, *mcr*: Mobilised colistin resistance

negative, C is a false positive, and D is a true negative.[81] The standard curve approach was used to obtain the correlation coefficients' R^2 values. A cut-off Ct value of 30 was selected as positive for ARG detection.

Statistical analysis

Descriptive and inferential statistics were used to assess the various *mcr* genes in different cow dung samples. Pearson's Chi-square test was used to test the association between ARG carriage in farm-based cow dung and household cow dung. A two-tailed $P = 0.05$ or lower was considered statistically significant. IBM SPSS version 20.0 was used to analyse all the data (IBM Corp., Armonk, NY, USA).

Ethics approval

This study was an extension of an earlier research project approved by the Ethics and Research Review Committee of the Jahangirnagar University Faculty of Biological Sciences (approval number: BBEC, JU/M 2017 12(4), approval date: 27 December 2017). The ethical committee subsequently waived the necessity for a new approval for this study.

Results

Study farms and samples

From the 120 cow dung samples, a small percentage (1.7%) of the cattle were infected with foot rot, and several (5.8%) had a fever during sample collection. Almost all of the cattle had been exposed to antibiotics within the last 6 months. The history of antibiotic use was similar between the cattle from both the commercial and household farms. Over 96% (116/120) of the cattle were vaccinated against anthrax, foot-and-mouth disease or blackleg.

Sensitivity and specificity of *mcr*-1 to *mcr*-5 real-time polymerase chain reaction assay

Overall, the newly optimised qPCR technique worked well in identifying all *mcr* genes in the test samples. We calculated the sensitivity and specificity of each *mcr* gene detection by real-time qPCR, where deionised water was used as the negative control template and the known bacterial 16S rRNA gene was used with the respective primer pair as a positive control.

No expected deviation was found in the amplification cycles for both the positive and negative controls. The correlation coefficients, R^2 values appeared 0.98, for detecting the sensitivity and specificity of the *mcr* gene variants. Consequently, the qPCR's specificity and sensitivity were 100% [Table 2].

Distribution of *mcr* genes in cow dung samples

In 71 cow samples no *mcr* gene was detected. In the remaining 49 samples, at least one *mcr* gene out of the five genes investigated was detected. Mobile colistin resistance genes, *mcr-1, mcr-2, mcr-3, mcr-4* and *mcr-5* were identified in 27.5% (33/120), 2.5% (3/120), 4.2% (5/120), 14.2% (17/120) and 8.3% (10/120), respectively [Table 3].

In more than 59% of the carriages, no *mcr* gene was detected. 40.8% of the tested samples carried at least one *mcr* genes; the remaining 8.3% of samples carried more than two *mcr* genes concurrently. Co-carriage of three *mcr* genes was found in only 1.7% of the samples. Co-occurrence of four and five *mcr* genes was not found in any of the samples [Figure 1].

Comparative distribution of *mcr* genes in farm-based and household cow dung

From the assessment of 108 cow dung samples from commercial farms, the prevalence of *mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5* was detected in 26.2%, 1.9%, 3.7%, 15.7% and 7.4%, respectively [Figure 2].

Four genes, namely *mcr-1, mcr-2, mcr-3* and *mcr-5,* were appreciably higher in household cow dung than commercial cow dung samples. Overall, the presence of mobile colistin resistance genes (*mcr-1, mcr-2, mcr-3,* and *mcr-5*) was found to be higher in household samples than commercial cow dung samples.

However, the differences in ARG prevalence were not statistically significant. One variant of the mobile colistin resistance gene, *mcr-4*, though was not identified in any household cow dung samples [Table 3].

Discussion

To the best of our knowledge, we believe this is the first study to explore the occurrence of the colistin resistance *mcr* gene in both commercial and household cow dung samples in Bangladesh using real-time quantitative PCR. The use of rapid real-time qPCR techniques to screen for ABR genes without the need for culture appears to work, which is beneficial going forward.

Table 2: Specificity and sensitivity of the quantitative polymerase chain reaction detection system for mobilised colistin resistance genes (%s)

ARG analysed	True (+)=A	False (−)=B	False (+)=C	True (−)=D	Percentage sensitivity; specificity*
mcr-1	33	0	0	87	100; 100
mcr-2	3	0	0	117	100; 100
mcr-3	5	0	0	115	100; 100
mcr-4	17	0	0	103	100; 100
mcr-5	10	0	0	110	100; 100

*Sensitivity and specificity of each *mcr* gene detection - determined based on negative control results where no template was added for RT qPCR amplification and negative control results, where known bacterial *16S* rRNA gene was used with respective primer pairs. A: True positive, B: False negative, C: False positive, D: True negative, RT qPCR: Real-time quantitative polymerase chain reaction, *mcr*: Mobilised colistin resistance

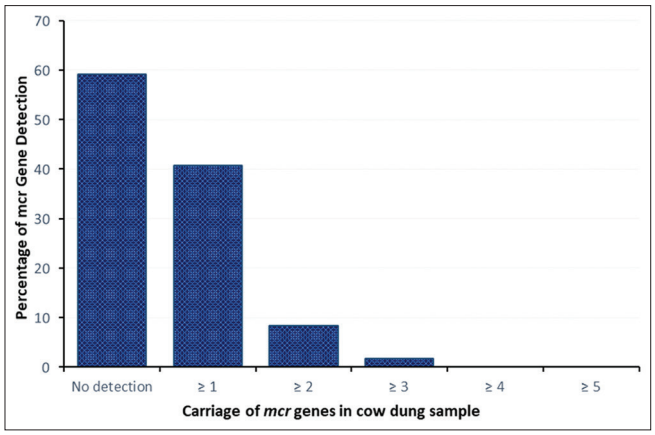

Figure 1: Cumulative distribution of mobilised colistin resistance genes in cow dung samples.

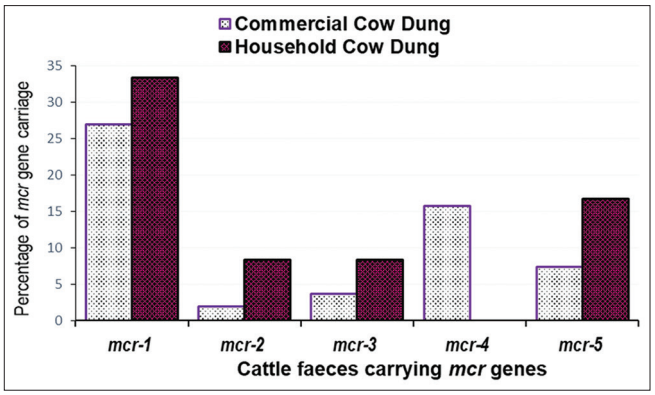

Figure 2: Comparative distribution of mobilised colistin resistance genes in farm-based and household cow dung samples. *mcr*: Mobilised colistin resistance.

Table 3: Comparative distribution of mobilised colistin resistance genes in farm-based and household cow dung samples

ARG analysed	Number of carriages (%)	
	Commercial cow dung sample (108)	Household cow dung sample (12)
mcr-1	29 (26.9)	4 (33.3)
mcr-2	2 (1.9)	1 (8.3)
mcr-3	4 (3.7)	1 (8.3)
mcr-4	17 (15.7)	0
mcr-5	8 (7.4)	2 (16.7)
Total	60 (55.6)	8 (66.6)

mcr: Mobilised colistin resistance

We explored the different ARGs in cattle faeces through optimised Sybr Green-based qPCR without the need for culturing. The qPCR system can identify the target ARGs rapidly with 100% sensitivity and 100% specificity, as confirmed by previous investigations.[65,81,84] This is important for developing countries such as Bangladesh with limited resources and infrastructure for continually monitoring AMR patterns.

In culture-based microbiology, some fastidious and injured bacteria are incapable of growing on culture media,[85] which is a concern. Consequently, culture-independent molecular microbiological methods, including RT-qPCR, can be useful for monitoring ABR profiles in environmental and clinical samples where conventional microbiology is challenging, and where increasing resistance to last resort antibiotics from the WHO Reserve list are a growing challenge. Consequently, this framework and approach appear to support the goal for sustainable development of AMR surveillance within the One Health approach in countries where resources are scarce, which benefits LMICs as emphasised by the WHO, FAO and OIE.[86,87]

More than 40% of the studied cow dung samples harboured at least one variant of *mcr* gene. The identification of colistin-resistant genes in food-producing animals again highlights the significance of the One Health approach to reduce AMR globally due to the risk of horizontal gene transmission.[36,38,87,88] However, there was appreciable variation, with low frequencies of *mcr-2 (*2.5%) and *mcr-3 (*4.2%) genes. Conversely, we found *mcr-1 (*27.5%) and *mcr-4 (*14.2%) to be highly prevalent, which was unexpected. More than 8% of the tested samples also had two or more colistin resistance genes together. Earlier studies had reported *mcr-1, mcr-2* and *mcr-3* genes in poultry, environment and clinical bacteria in Bangladesh;[71,89-91] however, the reporting of *mcr-4* and *mcr-5* had not yet been seen in Bangladesh. This is important going forward. The fact that the horizontal transfer of colistin resistance characteristics to nearby susceptible isolates may occur through natural reservoirs is a concern that needs to be urgently addressed in Bangladesh going forward.[92] Similarly, the prevalence of *mcr* genes in India, Pakistan and Nepal was reported to be between 9.2% and 27.6% by conventional PCR,[93-95] which also needs addressing by all key stakeholder groups in these countries.

Of interest is that we found no significant differences in the abundance and diversity of colistin-resistant genes between commercial cattle farms and household settings, which does not align with our initial hypothesis that the *mcr* genes would be more prevalent in commercial cattle farms than in households. In a number of countries, colistin has been used in livestock farming, including cattle, as a prophylactic and growth-promoting agent.[36,38,96] This practice raises concerns as the overuse of antibiotics in cattle farming can contribute to the development of ABR in humans.[38,97] Currently, both commercial and household cattle farms across countries, including Bangladesh, commonly use colistin as a prophylactic and growth-promoting agent, with its over-the-counter availability contributing to its widespread, and often, uncontrolled use.[36,38,97]

Consequently, future policy initiatives should prioritise addressing antibiotic overuse in household cattle and other farm operations in LMICs, including with colistin. This is because humans can be exposed to ARGs or ARG-carrying bacteria through direct interaction with contaminated environments, faecal waste, or

food and/or polluted water.[38,96] ARGs in cow dung faeces pose a significant risk of spreading resistance to other pathogens by vertical or horizontal gene transmission when dung is dumped in water or used as manure in agriculture or gardening, which should be avoided where possible going forward.[98]

We are also aware that antibiotics are readily available over-the-counter in Bangladesh, encouraging the overuse of colistin,[68,72,99] which also needs addressing going forward, alongside introducing measures to reduce inappropriate antimicrobial use generally amongst animals, including colistin. Consequently, implementing key measures in Bangladesh is crucial to lessen the burden of resistance to colistin currently seen in cow dung, with subsequent implications for the management of infectious diseases in humans. Potential measures include tightening of the current regulations banning the use of antibiotics in animal feeds in Bangladesh.[79] Multiple measures and initiatives have worked well in South Africa to reduce unnecessary use of colistin amongst farmers, and in China, measures such as fines have substantially reduced the extent of substandard medicines, including antibiotics, being produced and used across sectors to treat patients and animals with infectious diseases.[8,100,101]

Other activities include making all key stakeholders aware of the implications of the inappropriate use of antibiotics, especially those from the Watch and Reserve list, including colistin, given current concerns.[2,102-105] Such activities are important in Bangladesh, given the current high rates of AMR in the country, current extensive use of Watch and Reserve antibiotics across all sectors as well as variable knowledge of dispensers and the public regarding antibiotics and AMR.[33,68,70-72,106-109] Potential educational activities for all key stakeholders, including those involved in food production as well as the dispensing and purchasing of antibiotics, including drug dispensers and veterinarians, start in schools, colleges, and universities and followed up post qualification.[79,105,110,111] Potential activities also include training on antimicrobial stewardship (AMS) amongst all key stakeholder groups, which includes pharmacists and others dispensing antibiotics to farmers as well as amongst veterinarians.[69,105,110] Alongside this, continuing to monitor AMR patterns for key antibiotics as well as utilisation patterns. We acknowledge though, that AMS activities can be challenging in LMICs, including amongst farmers and associated dispensers of antibiotics.[8,69,112] However, this is now changing with multiple AMS programmes being introduced amongst LMICs to improve future antibiotic use.[8,69,105] Improving infection, prevention and control measures in farms is also necessary to help reduce infections and any associated overuse of antibiotics.[79]

Overall, AMS activities including monitoring the implementation of any educational and other activities introduced amongst key stakeholder groups to reduce the indiscriminate use of colistin in animals, and the associated impact, are needed building on previous bans in Bangladesh.[69,79,113] We will continue to monitor the situation, given the multiple concerns that we identified coupled with the goals of the NAP in Bangladesh. We will also continue to assess the novel technique of RT qPCR, and work towards its swift adoption in microbiological laboratory across Bangladesh. We believe this is important to align with the objectives of Bangladesh's NAP to combat AMR.[80]

We are aware that this study had several limitations. This included its cross-sectional design and lack of follow-up. We also did not investigate the acquisition of ARGs in bacterial communities through microbiological methods. Future research should focus on determining the presence of ARGs in bacteria and their phenotypic antimicrobial susceptibilities. While the study covered five variants of *mcr* (*mcr-1 to mcr-5*) genes, other gene variants, including *mcr-6 to mcr-9* were not explored. The sample size also limited the ability to perform robust statistical analyses. However, we believe internal validity was upheld through repeated independent experiments. Despite these concerns, we have confidence in this study providing insights into potential sources of colistin resistance genes at the animal–human interface and the implications for all key stakeholders in Bangladesh going forward.

Conclusions

The *mcr-1* variant was more common in Bangladeshi cow faeces compared to other variants including *mcr-2, mcr-3, mcr-4* and *mcr-5*. This current initiative has established a basis for monitoring ARG detection at the animal–human interface, which is important for LMICs such as Bangladesh.

Overall, our research has established the foundation for feasible, straightforward and cost-effective qPCR-based monitoring of AMR that can be adopted in other developing countries to help them achieve their NAP AMR goals. Enhanced AMR surveillance, alongside implementing other government strategies, is urgently needed in Bangladesh to specifically address rising colistin resistance in the country. As a result, seek to maintain the effectiveness of colistin as a last resort Reserve antibiotic in Bangladesh and beyond. This is a growing public health priority.

Data availability

Additional research data will be available upon reasonable requests to the corresponding authors.

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Conflicts of interest

There are no conflicts of interest.

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