

SHORT COMMUNICATION

Emergence of colistin-resistant *Escherichia coli* in poultry, house flies, and pond water in Mymensingh, Bangladesh

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ABSTRACT

Objective: Emergence of colistin-resistant *Escherichia coli* (CREC) has generated a sense of public alarm. The objective of this study was to detect the CREC and identification of the gene responsible for such resistance.

Materials and Methods: A total of 150 samples comprising poultry cloacal swab, house flies (*Musca domestica*), and pond water were collected randomly from Mymensingh, Bangladesh and analyzed. Isolation and identification of *E. coli* were done based on culture and *E. coli* 16S rRNA gene-specific polymerase chain reaction (PCR). Phenotypic detection of CREC was done by disk diffusion method. Finally, colistin resistance genes were detected by PCR by using colistin resistant gene *mcr3* specific primers.

Results: Among the 150 samples, phenotypically 18.00% ($n = 27/150$) isolates were found as colistin resistant. By PCR, 8.00% of the *E. coli* isolates were found positive for the presence of *mcr3* gene.

Conclusions: Colistin resistant *E. coli* carrying *mcr3* are detected in poultry, house flies and water that are of great public health concern.

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Introduction

Colistin is a reserve group of antibiotic and considered as one of the last-resort antimicrobials used for the treatment of multidrug-resistant Gram-negative bacteria. However, reports are now available on the development of resistance against colistin [1,2]. Five colistin resistance genes (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*) have been described so far [3], many of which are located in plasmid and therefore are mobile in nature [1]. Mobilized resistance to colistin is rapidly evolving globally and has emerged as a threat to human health. Transferable colistin resistance gene *mcr-3* was first identified in Shandong, China, whose product interferes the binding of colistin to target the bacterial cell membrane [2].

Livestock and poultry have been recognized as the major reservoir for colistin resistance and transmission reference [4]. The colistin resistant *Escherichia coli* (CREC) having the *mcr-3* gene has been isolated from

nasal/oropharyngeal and anal/cloacal swabs of pigs and poultry in China [1]. The higher prevalence of animal samples harboring *mcr* genes in animal isolates compared to other origins has raised alarm bell about the impact of colistin use in food animals and the spread of colistin resistance. We speculate that fly available in the poultry houses may be responsible for the spread of antibiotic-resistant bacteria to other animal and poultry [5]. In addition, there is a possibility for the spread of these resistant bacteria to nearby water source through sewage disposal system. As far as we know, no data are available in Bangladesh on the molecular detection of *E. coli* from poultry resistant to colistin harboring *mcr-3* gene. In this study, we describe the CREC carrying *mcr3* gene in poultry cloacal swabs, house flies, and pond water in Bangladesh.

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Materials and Methods

No ethical approval was required although all applicable international, national, and institutional guidelines for the care and use of animals were followed during sample collection.

A total of 150 samples comprising poultry cloacal swab, house flies (*Musca domestica*), and pond water were collected randomly from Mymensingh division, Bangladesh for bacteriological analysis during August and September 2018. Isolation and identification of *E. coli* were done based on morphology, staining, cultural, and biochemical characteristics as per previously published procedures [6]. Confirmation of isolation of *E. coli* was done by polymerase chain reaction (PCR) targeting *E. coli* 16S rRNA genes as described by others [7]. Phenotypic detection and interpretation of CREC were done by disk diffusion method following the guidelines of Clinical and Laboratory Standards Institute on Muller Hinton media [8]. Along with colistin, the sensitivity of the isolated *E. coli* was also detected against the carbapenem group of antibiotics, namely imipenem, meropenem, and ertapenem. Finally, PCR was carried out to confirm the isolates as colistin resistant by detecting *mcr-3* gene as described by others [2].

Results and Discussion

Escherichia coli is a widely distributed organism in nature. It is also a member of the intestinal microflora of animal and birds. Among the 150 samples, 96 (64.0%) were found positive for *E. coli* by PCR. Out of these 96 *E. coli*, antibiogram study phenotypically revealed the presence of 18.00% CREC (Fig. 1). Based on PCR, 8.00% of the *E. coli* isolates were found positive for the *mcr-3* gene (Fig. 2). Out of 60 cloacal swabs, 7 (11.67%) were found positive for *mcr-3* gene. Recently, a report from China shows the prevalence of *mcr-3* gene in chickens as 5.2% [1], which may be due to different amount or concentration of colistin used in these two different countries and the quality of the colistin itself.

Antimicrobial resistance is a serious global health threat. Few treatment options are left to overcome the complication associated with multidrug resistance, including the use of colistin. Colistin has been used extensively in veterinary medicine and agriculture for decades, both as a curative treatment and as a prophylactic drug [9]. Despite being a reserve group of antibiotics, colistin is a widely used antibiotic for poultry in Bangladesh. Previously from Vietnam, it has been shown that observed increasing colistin resistance in commensal *E. coli* is associated with the extensive use of colistin in the livestock and poultry industry [10].

Indiscriminate use of colistin may be associated with the occurrence of these CREC in poultry. House flies collected from food corners and dustbin located in the vicinity of the poultry farm were also found positive (4/60) for CREC. One of the interesting findings of this study is the detection of one CREC from 30 water samples collected from ponds draining from the poultry farm. Recently *mcr-3* gene has been detected from the aquatic environment in China [11]. Previous report is available on the detection of *mcr-1* gene carrying *E. coli* from sludge disposed into the water bodies in Dhaka, Bangladesh [12]. Further investigation is required to reveal the source for the presence of these CREC in files and pond water.

In this study, the CREC were also found resistant to carbapenem group of antibiotics (Table 1). The emergence and dissemination of colistin and carbapenem-resistant *E. coli* strains is a major risk for human health [13]. From poultry

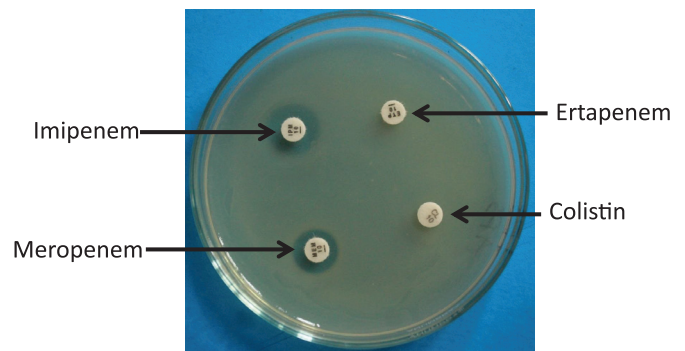


Figure 1. Phenotypic detection of colistin-resistant *E. coli* isolated from poultry.

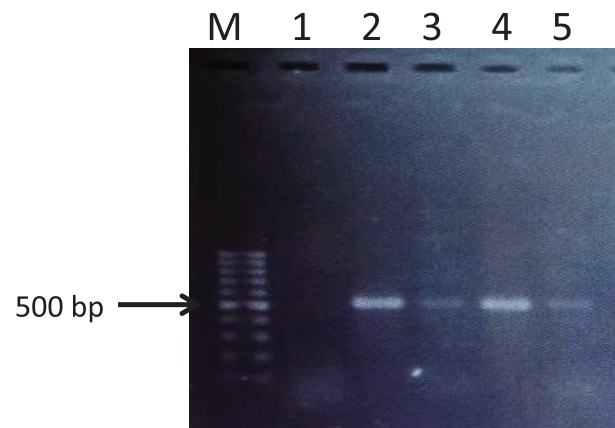


Figure 2. Amplification of *mcr-3* gene from colistin resistant *E. coli*. Lane M: 100 bp DNA marker, 1 = negative control, 2 = positive control, lane 3 = fly isolates, lane 4 = poultry isolate, lane 5 = water isolate.

Table 1. Occurrence of *mcr3* in colistin resistant *E. coli* isolated from various samples.

Sample Source (n)	Samples found positive for <i>E. coli</i> by PCR	Antibiogram (Resistance profile)				Samples found positive for <i>mcr-3</i> gene
		ETP (10 µg)	MEM (10 µg)	IPM (10 µg)	CS (10 µg)	
Cloacal swab (60)	44	29	19	6	13	7
Fly (60)	40	23	13	12	12	4
Pond water (30)	12	3	2	3	2	1
Total: 150	96 (64.00%)	55 (36.36%)	34 (22.66%)	21 (14.00%)	27 (18.00%)	12 (8.00%)

Here, ETP = ertapenem, MEM = meropenem, IPM = imipenem, and CS = colistin sulfate.

and water and through flies these CREC could enter the food chain to infect humans. The real scenario is more dangerous because of the mobile nature of *mcr-3* gene that can be transmitted to other Gram-negative bacteria horizontally [10]. To the best of our knowledge, this is the first report in Bangladesh on the detection of carbapenem-resistant *E. coli* carrying colistin resistance *mcr-3* gene from poultry and flies.

Colistin is widely used in the production of food animals to enhance growth, particularly poultry. Colistin sulphate can also be easily purchased from the pharmaceutical shop and veterinary drug dealers without any prescription. In addition, veterinary practitioners frequently prescribe colistin for treating diseases in poultry. It is therefore now time for the Government to adopt effective surveillance and monitoring for antibiotic-resistant bacteria and restrict the use of colistin in poultry to prevent or reduce colistin resistance.

Conclusions

The present study confirms the occurrence of CREC in a variety of samples, including poultry in Bangladesh with molecular-based evidence. It is now time to be careful in the use of colistin in the poultry industry in Bangladesh.

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Conflict of Interest

The authors have no conflict of interest.

Authors' contribution

MAS, SBZ and MTR designed and did the actual works. SI, ZFH, AN drafted the manuscript. MTR critically checked and improved the manuscript. All the authors read and approved finally for publication.

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