Research in
Agriculture, Livestock and Fisheries
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

Article Code: 444/2024/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 11, No. 2, August 2024: 115-124.

EMERGENCE OF MULTIDRUG RESISTANT MOBILE COLISTIN RESISTANCE (*mcr*) Carrying *Aeromonas* spp. ISOLATED FROM RETAIL FOODS IN MYMENSINGH DISTRICT, BANGLADESH

Mst. Tarifa Khatun[1], Mahmudul Hasan Rakib[1], Md. Mehedi Hasan[1], Md. Mahmudul Hasan[1], AKM Azharul Islam[2], Rajib Hossain[1], Md. Mahbub Alam[1], and Azimun Nahar[1*]

[1]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [2]Department of Livestock Services, Government of the People's Republic of Bangladesh, Farmgate, Dhaka, Bangladesh.

*Corresponding author: Azimun Nahar; E-mail: azimunripa.vm@bau.edu.bd

ARTICLE INFO

Received
18 May, 2024

Revised
26 May, 2024

Accepted
08 June, 2024

Online
September, 2024

Key words:
Antimicrobial resistance pattern
Chicken meat
PCR
Prevalence
mcr gene

ABSTRACT

The emergence of plasmid-mediated mobile colistin resistance (*mar*) in bacteria under Enterobacteriaceae, and aeromonads isolated from foods has been reported in many parts of the world. So far, we know there is no report of *mcr* carrying *Aeromonas* spp. Isolated from retail foods in Bangladesh. Therefore, this study was carried out to determine the prevalence of *mcr* carrying *Aeromonas* spp. isolated from retail foods (meat, milk, and fish) in Mymensingh district and their characterization for antimicrobial resistance patterns. The prevalence of *mcr-1* gene carrying *Aeromonas* spp. was 30 % (15/50) in chickens, 23 % (8/35) in fish, and 10 % (2/20) in milk. No other tested *mcr* gene variants (*mcr*-2 to 4) were detected. The minimum inhibitory concentration (MIC) for colistin was determined to be 80% (12/15) and 75% (6/8) of the *mcr-1* gene carrying *Aeromonas* spp. isolated from chicken and fish, respectively, had an MIC of 8-16 µg/ml whereas 100% (2/2) of the isolate from milk showed a MIC of 4–8 µg/ml. Most of the *mcr-1* gene-carrying isolates from chicken meats, fish, and milk were resistant to oxytetracycline, and ampicillin (80%–100%). Apart from multidrug resistant (MDR), extensive MDR was detected in *mcr-1* gene carrying *Aeromonas* spp., irrespective of the sample's origin. Overall, these data suggested that *the mcr-1* gene carrying *Aeromonas* spp. in retail foods could be a potential reservoir for antimicrobial resistance determinants. To the best of our knowledge, this is the first report of *the mcr* gene carrying *Aeromonas* spp. isolated from retail foods such as chicken meat, fish, and milk in Bangladesh.

To cite this article: Khatun M. T., M. H. Rakib, M. M. Hasan, M. M. Hasan, A. K. M. A. Islam, R. Hossain, M. M. Alam and A. Nahar, 2024. Emergence of multidrug resistant mobile colistin resistance (*mcr*) carrying *Aeromonas* spp. isolated from retail foods in Mymensingh district, Bangladesh. Res. Agric. Livest. Fish. 11(2): 115-124.

DOI: https://doi.org/10.3329/ralf.v11i2.75243

INTRODUCTION

Colistin (CST; Polymixin E), is the drug of last resort to treat infections caused by multidrug-resistant (MDR) pathogens like carbapenem-resistant Enterobacteriaceae. CST has been widely used for the prevention and treatment of bacterial infections in animals, birds, and in some critical cases in humans. Moreover, it has been widely used as a growth promoter in the animal and poultry industry (Catry *et al.*, 2015). However, recent studies have revealed that the MDR bacteria can also develop resistance to CST (Rossolini, 2015). Conventionally, CST resistance is conferred by chromosomally encoded enzymes, with minimum possibility of horizontal transfer. However, CST resistance mediated by a plasmid-harboring gene called *mcr* (mobile CST resistance), coding for phosphoethanolamine (PEtN) transferases has been recently reported from China (Liu *et al.*, 2016). Soon after publishing this report, the prevalence of *mcr-mediated* CST resistance was reported in various bacteria including *Escherichia coli*, *Klebsiella pneumoniae* (Zhang *et al*., 2019), *Salmonella enterica*, *Raoultella sp* and *Aeromonas* spp. from animals, food products, human and the environment. Over time, several variants from *mcr-1* to *-9* have been reported in various species of Enterobacteriaceae (Zając *et al*., 2019).

Aeromonads are ubiquitous organisms found in aquatic environments; food items, including meat, milk, fish, and vegetables; and the intestines of apparently healthy humans and humans with diarrhea (Albert *et al*., 2000). Aeromonads can infect humans via the consumption of contaminated food or water. The bacteria are associated with several infections, including bacteremia, meningitis, wound infections, and lung infections in humans (Albert *et al*., 2000). Particularly, Aeromonas induced gastroenteritis is more prevalent in Asian countries like India, Bangladesh, Thailand, and Japan where motile aeromonads have been isolated from about 7–11% of child diarrhea and 31% of traveler's diarrhea cases (Nishikawa and Kishi 1988; Albert *et al*., 2000). Gastroenteritis is characterized by acute profuse watery diarrhea in all age groups, but more frequently in young children (Albert *et al*. 2000). This pathogen carries several virulence factors like phospholipases, proteases, hemolysins, enterotoxins, adherence and invasion to host cells, cytotoxicity, enolase, quorum-sensing molecules, DNA adenine methyltransferase, etc., have been described (Manna *et al*., 2013). Besides, multidrug resistance in *Aeromonas* spp. has also been reported in many countries including Bangladesh. In Bangladesh, MDR has been observed in *Aeromonas* spp. isolated from freshwater prawns in Bangladesh (Lijon *et al.*, 2015) and in *A. hydrophila* from broiler chicken (Sarker *et al.* 2020). Thus, the prevalence of MDR pathogens like *Aeromonas* spp. in the food chain should be considered a serious threat to public health.

In Bangladesh, broiler meat is an important and low-cost source of animal protein that encourages the consumption of broiler meat by a large number of consumers. Besides, milk and fish are also important sources of protein for all levels of people including children. Thus, chicken meat, milk and fish contaminated with MDR, especially *mcr* carrying *Aeromonas* spp. should be considered a serious threat to public health. Until now, most investigations on antimicrobial resistance (AMR) of bacteria focused on mainly on *E. coli*, *Salmonella* spp., *Klebsiella* spp., and *Campylobacter* spp. in Bangladesh. In contrast, little information was obtained on the AMR of *Aeromonas* spp. isolated only from pawn and broiler chicken but no information about the contamination of retail milk and fish with this pathogen. Most importantly, there is no information on the prevalence of *mcr* gene carrying *Aeromonas* spp. isolated from food samples and their AMR profiles in Bangladesh. Thus, the present study was designed to determine the prevalence of *mcr* carrying *Aeromonas* spp. isolated from retail foods (meat, milk, and fish) and characterize *mcr* carrying *Aeromonas* spp. for their antimicrobial resistance pattern.

MATERIALS AND METHODS

Sample collection

Samples including 100 chicken meats, 100 milk, and 50 fish samples were purchased from supermarkets and local markets of randomly selected 4 upazillas (Mymensingh Sadar, Muktagaccha, Tarakanda and Fulpur) of the Mymensingh district. All the samples (40ml/gm of each sample) were collected in sterilized 50 ml falcon tube and transported to the laboratory with necessary precautions.

Bacterial Isolation and identification

Bacterial isolation was done according to the method (Shen *et al*., 2018). About 25 g of each food sample was enriched in 225 ml 1% alkaline peptone water (Himedia, India) for 24 h at 37 ˚C without shaking. Thereafter, the enrichment was streaked onto *Salmonella-Shigella* agar (Himedia, India), followed by incubation at 30 ˚C for 18 h. The resulting colonies will be picked up and subcultured onto nutrient agar at 37 ˚C for 24 h. Gram's staining method, motility test, sugar fermentation, and biochemical tests (oxidase test, catalase test, citrate test, indole test, and methyl red-Voges–Proskauer test) was performed to identify bacteria as *Aeromonas* spp.

PCR confirmation of *Aeromonas* spp.

A genus-specific PCR assay was performed to identify *Aeromonas* spp. by amplifying a 461-bp fragment of the 16S rRNA gene (Persson *et al*., 2015). Briefly, DNA templates were obtained from overnight agar cultures by thermal cell lysis. Each PCR reaction consisted of 12.5 µL One Taq PCR Master Mix (TaKaRa, Japan), 5.5 µL of nuclease-free water, 0.5 µL of each of the 10 primers solutions (10 µM), and 2 µL DNA lysate. The thermocycler conditions were as follows: 2-min hot start at 95°C, 30 cycles of denaturation at 94°C for 40 s, annealing at 65°C for 50 s, and elongation at 72°C for 40 s , and a final cycle of elongation at 72 °C for 5 min. The amplification was visualized by electrophoresis using 1.5% agarose gel at 130V followed by staining in ethidium-bromide. The DNA bands were photographed using a UV transilluminator (Cell biosciences, Australia)

Detection of *mcr* gene in *Aeromonas* spp. by PCR

Isolates were screened for *mcr-1* to *mcr-5* gene by PCR with the primers and conditions as described by Rebelo *et al*., 2017. Briefly, DNA templates were obtained from overnight agar cultures by thermal cell lysis. Each PCR reaction consisted of 12.5 µL One Taq PCR Master Mix (TaKaRa, Japan), 5.5 µL of nuclease-free water, 0.5 µL of each of the 10 primers solutions (10 µM), and 2 µL DNA lysate. Running conditions were: 1 cycle of denaturation at 94 °C for 15 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s and elongation at 72 °C for 60 s, and a final cycle of elongation at 72 °C for 10 min. The amplification was visualised by electrophoresis using 1.5% agarose gel at 130V followed by staining in ethidium-bromide.

Determination of minimum inhibitory concentration (MIC) for colistin of *mcr* gene carrying *Aeromonas* spp.

The *mcr* gene carrying *Aeromonas* spp. isolates were subjected to determination of MIC for colistin (CST) by broth microdilution method according to CLSI guidelines (CLSI, 2018) and results were interpreted following recommendations published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016). Briefly, the *mcr* gene carrying *Aeromonas* spp. isolates kept as glycerol stock at 80°C were sub-cultured on Luria-Bertani (LB) agar (Becton, Dickinson and Company, USA) at 37°C for 18 hours, and 5 to 6 colonies were collected and suspended in 1 ml of sterilized saline. After adjustment of OD_{600} = 0.3, 100 µl of suspension was added to 900 µl of sterilized saline to adjust a bacterial suspension to approx. 10^7 CFU/ml. Then 5 µl of the suspension was inoculated in 100 µl of cation-adjusted Mueller-Hinton (MH) broth (Becton Dickinson and

Company) supplemented with CST at different concentrations ranging from 0 to 32 µg/ml. After 14-18 hours of incubation at 37°C, the MIC was determined at the lowest concentration of CST that completely inhibited the growth of *mcr* gene carrying *Aeromonas* spp. in microdilution well as detected by unaided eye as well as microplate photometer (Thermo Scientific, Singapore). *E. coli* ATCC 25922 was used as a control strain in the susceptibility test.

Determination of antimicrobial susceptibility

Antimicrobial susceptibility for antibiotics (commonly used for treatment and as growth promoters in livestock and agricultural production in Bangladesh) of *mcr* gene-positive *Aeromonas* spp. isolated from retail foods were analyzed by the double disk diffusion method (CLSI, 2018). Antimicrobial susceptibility of *mcr* gene-positive *E. coli* were tested by double disk diffusion method (CLSI 2018) against 14 antimicrobial agents using commercially available discs (Nihon Becton Dickinson, Japan) such as ampicillin (AMP, 10 µg), cefoxitin (FOX, 30 µg), cefotaxime (CTX, 30 µg,), ceftazidime (CAZ 30 µg,), imipenem (IPM, 10 µg), gentamicin (GEN, 10 µg), kanamycin (KAN, 30 µg,), streptomycin (STR, 300 µg,), ciprofloxacin (CIP, 30 µg), nalidixic acid (NAL 30 µg,), tetracycline (TET, 30 µg), chloramphenicol (CHL, 30 µg), fosfomycin (FOS, 30 µg) and sulfamethoxazole-trimethoprim (SXT/ TMP, 25 µg). In brief, *mcr-1* gene carrying *Aeromonas* spp. kept as glycerol stock at -80°C were sub-cultured on LB agar at 37°C for 18 hr and 3 to 5 *mcr-1* gene carrying *Aeromonas* spp. Colonies were collected and suspended in 5 ml of sterilized saline. After adjustment of the optical density at 600 nm (OD_{600}) =0.3, an evenly distributed bacterial lawn was prepared on MH agar plates. Antimicrobial discs were placed on each bacterial lawn. The inhibition zone of each antimicrobial agent was analyzed after 16-18 hr of incubation at 37°C. Results were interpreted according to the CLSI guidelines (CLSI, 2021). *E. coli* ATCC 25922 was used as a control strain in the susceptibility test. MDR was defined as resistance to at least one antimicrobial agent in three or more classes of antimicrobial agents (Cantón *et al.*, 2011).

RESULTS

Isolation and identification of *Aeromonas* spp.

Aeromonas spp. was isolated from 50 out of 100 chicken meats, 35 out of 50 fish, and 20 out of 100 milk samples as shown in Table 1. Figure 1 showed the representative PCR results of identification *Aeromonas* spp. isolated from retail foods (chicken meats, fish, and milk) by PCR.

Table 1. Isolation of *Aeromonas* spp. from retail foods

Sample type	Number of samples	Number of *Aeromonas* spp. isolated (%)
Chicken meat	100	50 (50)
Fish	50	35 (70)
Milk	100	20 (20)
Total	**250**	**105 (42)**

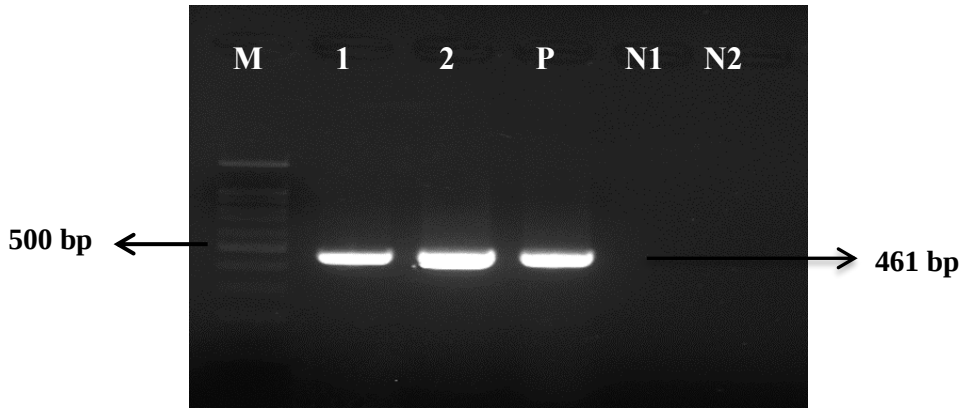

Figure 1. Molecular identification of *Aeromonas* spp. by PCR. M: 100 bp ladder, N1: Negative control (Dw), N2: *E. coli* (CI-32-1, Laboratory strain), P: Positve control (Aeromonas spp. (Laboratory strain); Lane 1 to 2: isolates from samples, Specific for *Aeromonas* spp. (Size-461 bp)

Detection of *mcr* gene in *Aeromonas* spp.

Prevalence of *mcr-1* gene carrying *Aeromonas* spp. was 30 % (15/50) in chicken, 23 % (8/35) in fish, and 10 % (2/20) in milk. However, *mcr-2*, *-3*, *-4* were not detected in either of the samples as shown in Table 2. Figure 2 showed representative PCR results of *mcr-1* gene detection in *Aeromonas* spp. isolated from retail foods (chicken meats, fish, and milk).

Table 2. Prevalence of *mcr-1* carrying *Aeromonas* spp. from retail foods

Sample type	Number of *Aeromonas* spp. isolated samples	Prevalence of *mcr-1* carrying *Aeromonas* spp. isolated (%)
Chicken meat	50	15 (30)
Fish	35	08 (23)
Milk	20	02 (10)
Total	**105**	**24 (23)**

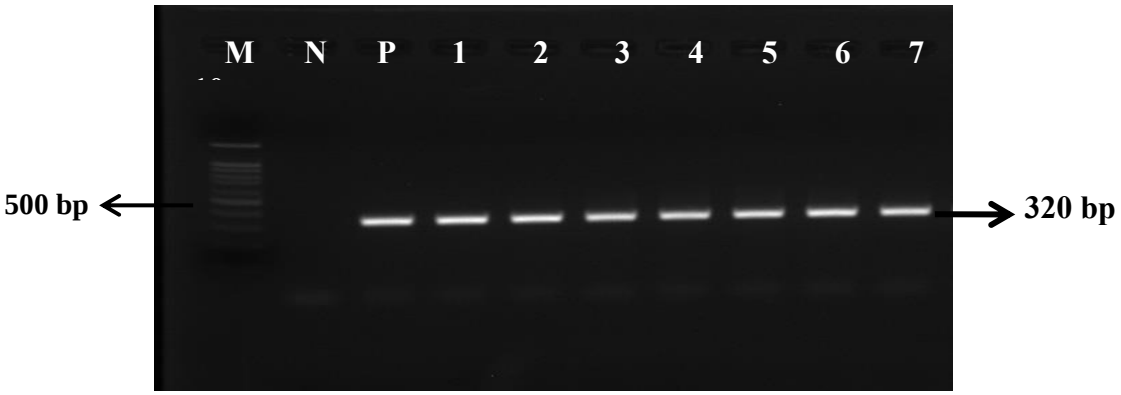

Figure 2. Representative PCR result showing mcr-1 gene detection in Aeromonas spp. isolated from retail foods (chicken meats, fish, and milk). M: 100 bp ladder (100 ng/lane, Takara Bio. Inc.) P: positive control (Laboratory strains: E. coli strain CI-32-1, mcr-1 gene-positive, N: negative control (E. coli strain C600). Lanes: 1, Ae-Cm1-1 (mcr-1 gene-positive); 2, Ae-Cm5-1 (mcr-1 gene-positive); 3, Ae-Cm12-1 (mcr-1 gene-positive); 4, Ae-Cm30-1 (mcr-1 gene-positive); 5, Ae-Cm55-1 (mcr-1 gene-positive); 6, Ae-F4-1 (mcr-1 gene-positive); 7, Ae-M12-1 (mcr-1 gene-positive)

Colistin resistance pattern of *mcr-1* gene carrying *Aeromonas* spp.

The minimum inhibitory concentration of CST for *mcr-1* gene-carrying *Aeromonas* spp. was determined. The results showed that 80% (12/15) and 75% (6/8) of the *mcr-1* gene carrying *Aeromonas* spp. isolated from chicken and fish, respectively had a MIC of 8-16 µg/ml whereas 100% (2/2) of the isolate from milk showed a MIC of 4-8 µg/ml as shown in Figure 3.

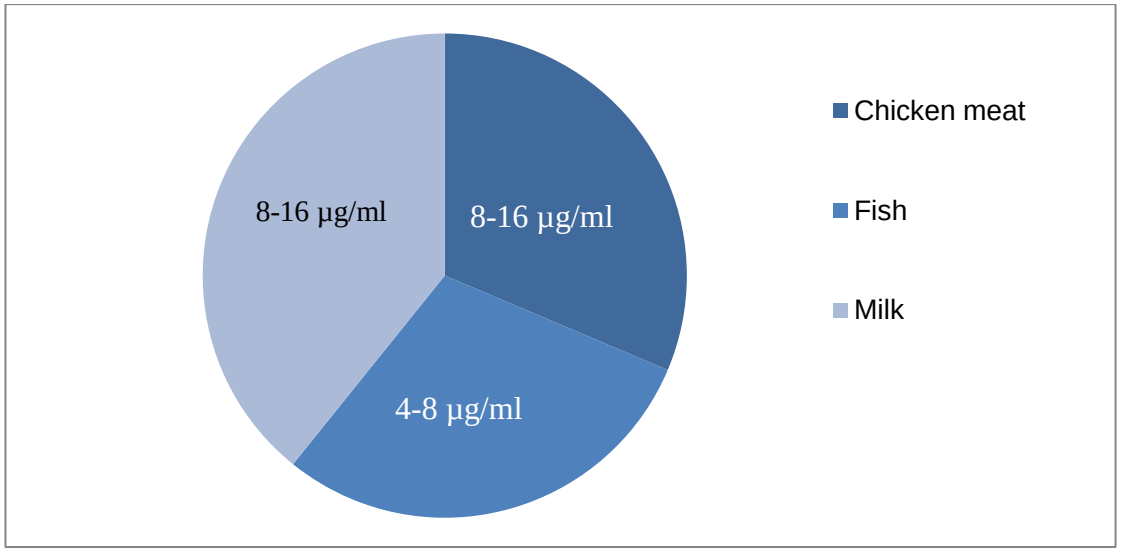

Figure 3. MIC of CST for *mcr-1* gene carrying *Aeromonas* spp. isolated from retail foods

Antimicrobial resistance pattern of *mcr* carrying Aeromonas spp.

Antimicrobial susceptibility test for the representative isolates with other antimicrobials showed that most of the *mcr-1* gene-carrying isolates from chicken meats were resistant to TET, AMP (12/15; 80%) followed by, KAN, STR (10/15; 67%), SXT/TMP (8/15; 54%), NAL (7/15; 47%) and CHL (2/15; 14%). Similarly, most of *mcr-1* carrying isolates from fish were resistant to TET, AMP (6/8; 75%) followed by KAN, STR, CHL (5/8; 63%), and NAL, SXT/TMP (4/8; 50%). Interestingly, 100 % of *mcr-1* gene-carrying isolates from milk were resistant to AMP (6/8; 75%) followed by CTX, CAZ, KAN, STR, NAL, CIP, SXT/TMP (1/2; 50%) as shown in Table 3. All the mcr carrying Aeromonas spp. isolated from retail foods showed MDR including 52% of the isolates were extensive MDR (XDR) as shown in Table 4.

Table 3. Antimicrobial resistance of colistin-resistant *mcr-1*gene carrying *Aeromonas* spp. isolated from retail foods

Antimicrobial agents	No. of resistant isolates (%)		
	Chicken (n=15)	Fish (n=8)	Milk (n=2)
AMP	12 (80)	6 (75)	2 (100)
FOX	0 (0)	0 (0)	0 (0)
CTX	0 (0)	0 (0)	1 (50)
CAZ	0 (0)	0 (0)	1 (50)
IPM	0 (0)	0 (0)	0 (0)
STR	10 (67)	5 (63)	1 (50)
KAN	10 (67)	5 (63)	1 (50)
GEN	0 (0)	0 (0)	0 (0)
CIP	0 (0)	3 (38)	1 (50)
NAL	7 (47)	4 (50)	1 (50)
TET	12 (80)	6 (75)	1 (50)
CHL	2 (14)	5 (63)	1 (50)
FOS	0 (0)	0 (0)	0 (0)
SXT/TMP	8 (54)	4 (50)	0 (0)

Table 4. Multidrug resistance (MDR) pattern in *mcr-1*gene carrying *Aeromonas* spp. isolated from retail foods

Isolate (%) (n=25)	[a)]MDR pattern	[b)]NARC
5 (20)	AMP, CIP, STR, TET, CHL, NAL,TET, SXT/TMP	8 (XDR)
8 (32)	AMP, KAN,TET, CHL, NAL, IPM, SXT/TMP	7 (XDR)
12 (48)	AMP, STR, TET, CHL, IPM	5 (MDR)

a) AMP: Ampicillin, STR: streptomycine, CIP: ciprofloxacin, CHL: chloramphenicol, IPM: imipenem, NAL: nalidixic acid, SXT/TMP: sulfamethoxazole-trimethoprim, KAN: kanamycin, TET: oxytetracycline,

b) b) No. of antimicrobial resistance classes according to CLSI (CLSI, 2018).

DISCUSSION

This study showed that the prevalence of *Aeromonas* spp. was 50 % (50/100) in chicken, 70 % (35/50) in fish, and 20 % (20/100) in milk collected from retail supermarkets in Mymensingh district. The prevalence of *Aeromonas* spp. in chicken meat was correlated with the findings of Sarker *et al*., 2020 who reported 45% *Aeromonas* spp. in chicken meats in Mymensingh Sadar, Bangladesh. On the other hand, Lijon et al. (2015) reported 17 % *Aeromonas* spp. in freshwater prawns in Khulna, Bangladesh, which is lower than the prevalence in fish in the present study. In this study, the isolation media was supplemented with CST to specifically target CST-resistant *Aeromonas* spp. Another possibility for this finding might be prevalence of *Aeromonas* spp. in retail foods, especially in fish in Bangladesh is emerging. This study showed that the prevalence of the *mcr-1* gene carrying *Aeromonas* spp. was 30 % (15/50) in chicken, 23 % (8/35) in fish, and 10 % (2/20) in milk collected from retail supermarkets in, the Mymensingh district. Nagar et al. (2011) showed highest percentages of Aeromonas spp. were from chicken (28.6%) followed by fish (20%) and sprout (2.5%) samples in India. Ling *et al*. (2017) reported 17 % mcr-3 carrying *Aeromonas* spp. in chicken meats in China, which is lower than the prevalence of *mcr-1* gene-carrying *Aeromonas* spp. in chicken in the present study. Monte *et al.* (2017) conducted a study on poultry meat between August-October 2016 in Brazil and reported that the prevalence of *mcr-1* gene-positive *E. coli* in poultry meat was 19.5% (8/41 samples). This is probably the first report of the prevalence of *mcr-1* gene-carrying *Aeromonas* spp. in retail chicken meats, fish, and milk in Bangladesh. In this study, we could obtain only a limited number of fish samples. To know the true prevalence of *mcr-1* gene carrying *Aeromonas* spp. in retail fish, it is necessary to investigate more samples. However, we could not detect mcr-2 to *mcr-5* in any of the samples.

Furthermore, MIC of CST for *mcr-1* gene carrying *Aeromonas* spp. in this study was determined to be 4-16 µg/ml indicating that all isolates were phenotypically colistin-resistant. In this study, the *mcr-1* gene-carrying isolates from chicken meats were highly resistant to TET and AMP (12/15; 80%). In a previous study from Bangladesh, Lijon et al. (2015) also showed that *Aeromonas hydrophila* isolated from freshwater prawns was the highest resistant to AMP. Besides, Sarker et al. (2020) reported *Aeromonas hydrophila* isolated from chicken meats in Bangladesh was the highest resistant to TET. In a previous study from Japan, Tada *et al.* (2017) reported that *mcr-1* gene-positive *E. coli* isolated from patients showed resistance to CHL, CST, penicillins, and TET. These findings indicated that may be not only TET but also AMP might be widely used to optimize poultry, fish, and dairy animal production and thus result in resistance development against these antibiotics. Among *mcr-1* carrying *Aeromonas* spp., the majority of the isolates were MDR and some isolates showed extensive MDR phenotype (Table 4).These findings highlights the potential for these bacteria to serve as a reservoir of resistance genes and pose a threat to public health.

CONCLUSION

Retail foods (chicken meat, fish, and milk) in Mymensingh are the potential reservoir of *mcr-1* gene carrying *Aeromonas* spp. which is potentially MDR. Therefore, retail foods (chicken meat, fish, and milk) in Mymensingh district in terms of contamination with *mcr-1* gene carrying *Aeromonas* spp. might be harmful to public health.

ACKNOWLEDGMENT

We express our gratitude to the food retailers who participated in this study by providing samples and for their cooperation. We also acknowledge the support provided by the Ministry of science and technology, Bangladesh through funding for this research (Project ID No. SRG-221101).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

AUTHORS CONTRIBUTION

Azimun Nahar, Md. Mahbub Alam, and AKM Azharul Islam: Conceptualization. Azimun Nahar, Mst. Tarifa Khatun, Md. Mahmudul Hasan, Rajib Hossain, Md. Mehedi Hasan, and Mahmudul Hasan Rakib: Methodology. Azimun Nahar, AKM Azharul Islam, and Md. Mehedi Hasan: Original draft preparation. Mahmudul Hasan Rakib and Azimun Nahar: Editing and supervision. All authors have read, reviewed, and approved the final manuscript.

REFERENCES

1. Albert MJ, Ansaruzzaman M Ansaruzzaman, KA Talukder, AK Chopra, I Kuhn, M Rahman, AS Faruque, MS Islam, RB Sack and R Mollby, 2000. Prevalence of Enterotoxin Genes in Aeromonas spp. Isolated From Children with Diarrhea, Healthy Controls, and the Environment. Journal of Clinical Microbiology, 38(10): 3785–3790.
2. Cantón R and P Ruiz-Garbajosa, 2011. Co-resistance: an opportunity for the bacteria and resistance genes. Current Opinion in Pharmacology, 11(5): 477–485.
3. Catry B, M Cavaleri, K Baptiste, K Grave, K Grein, A Holm, H Jukes, E Liebana, A Lopez Navas, D Mackay, AP Magiorakos, MA Moreno Romo, G Moulin, C Muñoz Madero, MC Matias Ferreira Pomba, M Powell, S Pyörälä, M Rantala, M Ružauskas, P Sanders, C Teale, I EJ Threlfal, K Törneke, E van Duijkeren andJ Torren Edo, 2015. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. International Journal of Antimicrobial Agents, 46(3):297–306.
4. CLSI, 2018. Performance Standards for Antimicrobial Susceptibility Testing. CLSI, Wayne, NJ, USA, p1–260
5. Lijon MB, MM Khatun, A Islam, MM Khatun and MA Islam, 2015. Detection of multidrug resistance Aeromonas hydrophila in farm raised fresh water prawns. Journal of Advanced Veterinary and Animal Research, 2(4): 469–474.
6. Ling Z, W Yin, H Li, Q Zhang, X Wang, Z Wang, Y Ke, Y Wang and J Shen, 2017. Chromosome-Mediated mcr-3 Variants in Aeromonas veronii from Chicken Meat. Antimicrobial Agents and Chemotherapy, 61(11).
7. Liu YY, Y Wang, TR Walsh, LX Yi, R Zhang, J Spencer, Y Doi, G Tian, B Dong, X Huang, LF Yu, D Gu, H Ren, X Chen, L Lv, D He, H Zhou, Z Liang, JH Liu and J Shen. 2016. 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, 16(2):161-168.
8. Manna SK, P Maurye, C Dutta and G Samanta, 2013. Occurrence and Virulence Characteristics of Aeromonas Species in Meat, Milk and Fish in India. Journal of Food Safety, 33(4): 461-469.
9. Monte DF, A Mem, MR Fernandes, L Cerdeira, F Esposito JA, Galvão, BDGM Franco, N Lincopan and M Landgraf, 2017. Chicken Meat as a Reservoir of Colistin-Resistant Escherichia coli Strains Carrying mcr-1 Genes in South America. Antimicrobial Agents and Chemotherapy, 61(5): e02718-16.

10. Nagar V, R Shashidhar and JR Bandekar, 2011. Prevalence, Characterization, and Antimicrobial Resistance of Aeromonas Strains from Various Retail Food Products in Mumbai, India. Journal of Food Science, 76(7): 501-507
11. Nishikawa Y and Kishi T, 1988. Isolation and characterization of motile Aeromonas from human, food and environmental specimens. Epidemiology and Infection, 101(2): 213-223.
12. Persson S, Al-Shuweli S, Yapici S, Jensen JN and Olsen KE, 2015. Identification of clinical aeromonas species by rpoB and gyrB sequencing and development of a multiplex PCR method for detection of Aeromonas hydrophila, A. caviae, A. veronii, and A. media. Journal of Clinical Microbiology, 53(2):653-6.
13. Rebelo AR, V Bortolaia, JS Kjeldgaard, SK Pedersen, P Leekitcharoenphon, IM Hansen, B Guerra, B Malorny, M Borowiak, JA Hammerl, A Battisti, A Franco, P Alba, A Perrin-Guyomard, SA Granier, C De Frutos Escobar, S Malhotra-Kumar, L Villa, A Carattoli, and RS Hendriksen, 2018. 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, 23(7): 180215-1.
14. Rossolini GM, 2015. Extensively drug-resistant carbapenemase-producing Enterobacteriaceae: an emerging challenge for clinicians and healthcare systems. Journal of Internal Medicine, 277(5): 528-531.
15. Sarker B, M Arif, N Eashmen, MR Akter, and SL Kabir, 2020. Isolation, identification and antibiogram profile of Aeromonas hydrophila from broiler chickens in Mymensingh Sadar, Bangladesh. Asian-Australasian Journal of Food Safety and Security, 4(1): 22-30.
16. Shen Y, C Xu, Q Sun, S Schwarz, Y Ou, L Yang, Z Huang , I Eichhorn, TR Walsh, Y Wang, R Zhang and J Shen, 2018. Prevalence and Genetic Analysis of mcr-3-Positive Aeromonas Species from Humans, Retail Meat, and Environmental Water Samples. Antimicrobial Agents and Chemotherapy, 62(9): e00404-18.
17. Tada T, K Uechi, I Nakasone, K Shimada, M Nakamatsu, T Kirikae and J Fujita, 2017. 'Emergence of a colistin-resistant Escherichia coli clinical isolate harboring *mcr-1* in Japan', International Journal of Infectious Diseases, 63: 21-22.
18. Zając M, P Sztromwasser, V Bortolaia, P Leekitcharoenphon, LM Cavaco, A Ziętek-Barszcz, RS Hendriksen and D Wasyl, 2019. Occurrence and Characterization of mcr-1-Positive Escherichia coli Isolated From Food-Producing Animals in Poland, 2011–2016. Frontiers in Microbiology, 10: 1753.
19. Zhang R, YY Hu, HW Zhou, SL Wang, LB Shu and GX Chen, 2019. Emergence of *mcr-1* and the tet(A) variant in a Klebsiella pneumoniae isolate from the faeces of a healthy person. Journal of Medical Microbiology, 68(9): 1267-1268.