LISTERIA MONOCYTOGENES IN READY-TO-EAT CHICKEN PRODUCTS, THEIR ANTIBIOTIC RESISTANCE AND VIRULENCE GENES

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ABSTRACT

Ready-to-eat (RTE) meat products are food items that are extremely popular worldwide. Albeit documented with the alarming prevalence of pathogenic multidrug-resistant *Listeria* species in these food items worldwide, studies from the perspective of Bangladesh are absent. This study was devised with the aim to detect pathogenic *Listeria monocytogenes* in some of the most popular RTE meat products in Dhaka, Bangladesh. Thirty-nine such samples were investigated using biochemical tests and the Polymerase Chain Reaction (PCR) analysis. Following confirmatory tests and detection of *Listeria* species, the isolates were subjected to the Kirby-Bauer disc diffusion test to investigate their antibiotic-susceptibility patterns against some of the most commonly used antibiotics to treat listeriosis – the infection caused by *Listeria.* Five *Listeria* species were detected using biochemical and PCR tests. This constituted an overall prevalence rate of 12.8% (n = 39). Four out of the five *Listeria* species were concluded to be *L. monocytogenes,* while the remaining one was an *L. innocua* isolate. PCR analyses revealed all four of the *L. monocytogenes* isolates to have the virulence genes *hlyA, plcB* and *actA,* although no polymorphism was observed for these genes. Despite the presence of these pathogenic genes, antibiotic susceptibility tests showed promising results as some of the most commonly prescribed drugs against listeriosis were highly effective against the isolates. Even though the isolates showed little to no antibiotic resistance against the antibiotics used in the study, the presence of three virulence genes in all four *L. monocytogenes* isolates is still a cause for concern. Even more so, the prevalence of these pathogenic strains in a food item that is increasing in popularity in a country like Bangladesh, where awareness and surveillance against listeriosis is limited, can have dire consequences should an outbreak occurs.

KEYWORDS: Ready-to-eat meat, *L. monocytogenes*, virulence, antibiotic resistance, Bangladesh

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Introduction

The world of science is currently aware of more than 200 diseases that may be transmitted through consuming contaminated food and food products. According to the World Health Organization (WHO), more than a million people are infected by foodborne pathogens every year (Mehlhorn, 2015). The production of food items has gone through many innovations, yet the hazards of foodborne infection have intensified over the last couple of decades. Estimates have showcased that about a quarter of the world population is at risk of foodborne diseases and hence the death rates due to these diseases are considered a significant public health concern (*Vital Signs: Listeria Illnesses, Deaths, and Outbreaks — United States, 2009–2011*, n.d.).

A foodborne disease may be defined as a disorder that has been incurred due to the consumption of foodstuffs contaminated with microorganisms (i.e., bacteria, viruses, parasites and fungi, *et cetera*). These diseases pose major threats to public health sectors worldwide and have had adverse impacts on consumer health as well as on the economies of those countries that import them (Scallan *et al*.,

2011). Several factors such as modifications to industrial production, globalization of food, and new trade agreements have been implicated in the rising number of foodborne disease cases worldwide. Moreover, the demand for fresher, healthier and ready-to-eat (RTE) food with minimal or moderate production and artisanal processing has also been added to the causes of increased foodborne illness cases (Bustamante *et al.*, 2020; Wallace *et al.*, 2018). Ready-to-eat food products made from meat pose considerable health risks because they have the potential to contain harmful microorganisms. The adoption of good manufacturing practices and proper hygiene control is of paramount importance throughout the entire process of production in order to preclude food contamination by microorganisms. Temperature control is essential during the distribution of food products, or it might culminate in the growth of pathogenic organisms to levels that can cause diseases (Gil *et al.*, 2009).

Listeria monocytogenes is one of the leading bacterium notorious for the recall of RTE food from supply chains all

over the world (Zhu *et al.*, 2017). Consumption of foods contaminated with *L. monocytogenes* gives rise to a condition called listeriosis (Kumar B. N, 2016). Compared to other foodborne diseases, listeriosis is relatively rare (Borucki *et al.*, 2005). Still, because of its high fatality rate of up to 30% (Bustamante et al., 2020), the US Food and Drug Administration (FDA) adopts a zero-tolerance policy against *L. monocytogenes* (Borucki *et al.*, 2005). Clinical manifestations of the condition include stillbirths, abortions, septicemia, meningitis, endocarditis, and febrile gastroenteritis. Newborn babies, elderly and immunocompromised individuals are at the highest risk of listeriosis (Borucki *et al.*, 2005; Mead *et al.*, 1999; Shourav *et al.*, 2020). The bacterium is Gram-positive, non-spore-forming, microaerophilic, and rod-shaped. It has a collection of complex physiological mechanisms that allow it a certain form of ubiquity in all sorts of environmental conditions (Larsen *et al.*, 2006). It has several crucial virulence factors such as hemolysin (*hlyA),* internalin (encoded by *inlA* and *inlB*), phosphatidylinositol-specific phospholipase C (PI-PLC, *plcA*), phosphatidylcholine-specific phospholipase C (PC-PLC, *plcB*) and actin polymerization protein (*actA*) (Jaradat *et al.*, 2002). Furthermore, the bacterium has the ability to survive and proliferate in the gastrointestinal tract enabling it to cause chronic infections (Shamloo *et al.*, 2019).

The main reason *Listeria monocytogenes* is a significant pathogen as far as RTE food is concerned is that studies have reported it to survive and proliferate at temperatures of 2/4 °C. This aspect makes it a suitable candidate to be present in foods with a long shelf-life, as RTE foods do (Todd & Notermans, 2011; Walker *et al.*, 1990). Ready-to-eat meat products are currently items of growing popularity in Bangladesh, but studies dedicated to the prevalence of *L. monocytogenes* in these foodstuffs are almost non-existent. This study was devised to investigate the scenario of *L. monocytogenes* in the RTE meat products in Bangladesh and promote more public awareness against a potentially fatal outbreak.

Materials and methods

Sampling and sample processing
A total of 39 samples comprising RTE meat products were collected randomly from various departmental stores in Dhaka City, Bangladesh. These samples constituted various RTE products of chicken origin such as chicken sausage, chicken salami, chicken loaf and chicken sandwich meat.

Processing of the samples was carried out according to the procedures followed by a previous study (Shourav *et al.*, 2020). Twenty-five grams of each sample was weighed, homogenized in a pre-sterilized homogenizer and mixed with 225 ml Listeria Enrichment Broth (Oxoid Ltd., Hampshire, UK). The mixture was shaken thoroughly to make it homogenous before incubation at 30℃ for 48 h. Prior to inoculation, the Listeria Enrichment Media was mixed with Listeria Selective Enrichment Supplement (Oxoid Ltd., Hampshire, UK) as per the manufacturer's instructions in an effort to prevent the growth of any other organism except *Listeria* spp. *(SR0141, Listeria Selective Enrichment Supplement / Oxoid - Product Detail, n.d.)*.

Isolation of Listeria spp.
Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM) Agar (Oxoid Ltd., Hampshire, UK) medium was used for the selective isolation of *Listeria* spp. Following enrichment, a loop-full of the culture was streaked on PALCAM agar plate and incubated at 37℃ for 24-48 h. On PALCAM agar plates, *Listeria* spp. colonies manifested as black or black-green color with a black halo and a sunken center having a growth diameter of 1-2 mm (Scotter *et al.*, 2001). Five suspected *Listeria* spp. colonies were carefully selected from a plate, and each was inoculated into Tryptose Soya Broth Yeast Extract (TSBYE, Oxoid Ltd., Hampshire, UK) and incubated at 37℃ for 1 hour. After that, the suspension was inoculated into Tryptone Soya Yeast Extract Agar (TSYEA, Himeadia, India) and incubated at 37℃ for 24 h. The colonies that grew on the TSYEA media were presumptively pure *Listeria* spp. The aforementioned several steps of isolation ensured a greater extent of precision to in the isolation of the *Listeria* spp. (Gebretsadik *et al.*, 2011).

Phenotypic identification of potential Listeria spp. isolates
The morphological characteristics of the isolates were examined using the Gram staining technique. Typical *Listeria* spp. isolates surfaced as Gram-positive short rods under a light microscope. These isolates were then screened further by means of a collection of confirmative biochemical tests that included catalase, oxidase, urease, acid formation, gas production and hydrogen sulfide production (Kligler's Iron Agar), indole formation, motility, methyl red and Voges-Proskauer (MR-VP) tests. *Listeria monocytogenes* ATCC 19117 (*Listeria Monocytogenes (Murray et Al.) Pirie ATCC ® 19117TM*, n.d.) was used as a reference strain (positive control) so as to compare with the results of the isolates under study.

PCR-based identification and virulence gene detection in the Listeria spp. isolates
The presumptive *Listeria* spp. isolates were subjected to the Polymerase Chain Reaction (PCR) based amplification of the *16S rRNA* gene followed by sequencing. An attempt was also made to detect the virulence-associated genes *hlyA, actA* and *plcB*. For every PCR reaction, the final reaction volume was 25 µl containing 12.5 µl Mastermix (DreamTaq Green PCR Mastermix, Thermo Fisher Scientific, USA), 0.5 µl forward primer, 0.5 µl reverse primer, 2.0 µl genomic DNA as a template and sterile nuclease-free water to make up the volume.

Amplification of 16S rRNA gene and sequencing
Whole genomic DNA was extracted by boiling method from each of the isolates deemed as *Listeria* by the colony morphology and biochemical tests (Bai *et al.*, 2010). Concentrations of the genomic DNA were measured using a NanoDrop™ 8000 spectrophotometer (Thermo Scientific, California, USA). The *16S rRNA* gene was amplified in a thermal cycler (Applied Biosystems® Veriti® 96-Well Thermal Cycler) using primer pairs shown in Table 1. The reaction conditions included initial denaturation at 94℃ for 5 minutes, 30 cycles of amplification at 94℃ for 1 minute and 30 seconds, annealing at 55℃ for 1 minute and final extension at 72℃ for 1 minute and 30 seconds. The PCR products were separated and visualized on agarose gel (1-2%) following ethidium bromide staining. With strict adherence to the

manufacturers' instructions, a PCR cleaning kit (Favorgen, Taiwan) was used to clean the amplified products and about 0.5-1.00 µg of the purified product was sent to Macrogen (Korea) for sequencing. The forward and reverse primers generated partial sequences that were cleaned manually and combined to obtain a full-length sequence (about 1000 bp) using the Seqman Genome Assembler (DNAstar, USA). Full-length sequences were compared with sequences in the GenBank database of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool (BLAST) to identify close phylogenetic relatives. The *16S rRNA* gene sequences and their respective reference sequences from NCBI were aligned using ClustalW program, available in MEGA-X (Kumar *et al.*, 2018), and a phylogenetic tree was constructed using the neighbor-joining analysis in the MEGA X software (Saitou & Nei, 1987). The full-length *16S rRNA* gene sequences were submitted to the NCBI GenBank nucleotide sequence database. The isolates were designated with accession numbers by NCBI GenBank after necessary verifications (Table 2)**.**

Molecular detection of virulence-associated gene

The PCR conditions for the amplification of the virulence-associated genes *hlyA, actA* and *plcB* are as follows: denaturation of DNA template at 94°C for 3 min, followed by 35 cycles of amplification (each cycle comprised of denaturation at 94°C for 1 min, annealing at 60 °C for 2 min and extension at 72 °C for 1 min) in a Thermal Cycler (Applied Biosystems® Veriti® 96-Well Thermal Cycler). Agarose gel electrophoresis (1-2%) and staining by ethidium bromide was used to visualize the PCR products (Jaradat *et al.*, 2002). Primer pairs used are indicated in Table 1. *Listeria monocytogenes* ATCC 19117 was used as a positive control while a lab owned random strain of *Escherichia coli* was used as a negative control.

Table 1. Primer pairs used for the PCR-based amplification of assorted genes in the isolated *Listeria* spp.

Targeted gene		Primer sequences (5`-3`)	Product size (bp)	Reference
16S rRNA	**27F**	AGA GTT TGG ATC M TGG CTC AG		
	907R	CCG TCA ATT CCT TTR AGT TT	1000	(Jiang *et al.*, 2006)
hlyA	**F**	CGG AGG TTC CGC AAA AGA TG		
	R	CCT CCA GAG TGA TCG ATG TT	234	(Jaradat *et al.*, 2002)
actA	**F**	GAC GAA AAT CCC GAA GTG AA		
	R	CTA GCG AAG GTG CTG TTT CC	268	(Jaradat *et al.*, 2002)
plcB	**F**	GGG AAA TTT GAC ACA GCG TT		
	R	ATT TTC GGG TAG TCC GCT TT	261	(Jaradat *et al.*, 2002)

Antimicrobial susceptibility pattern

The isolates were tested for antimicrobial susceptibility using the Kirby-Bauer Disc diffusion method (Hudzicki, 2012). Each *Listeria* isolate was grown in an LB broth until the log phase was reached and its turbidity was adjusted to 0.5 McFarland standard (OD_{625nm} = 0.08 – 0.13) spectrophotometrically. Freshly prepared standardized cultures of each isolate were spread on Mueller-Hinton (MH) agar plates using sterile cotton swabs and allowed to stand for 3-5 minutes inside the biosafety cabinet. No more than four antibiotic discs, approximately 40 mm apart from each other, were positioned on a single MH agar plate and incubated at 37°C for 16 h. The diameter of the clear zone around each antibiotic disc was measured. The data was used to work out the level of susceptibility of the isolates to a particular antibiotic (Hudzicki, 2012). The isolates were classified as sensitive (S), intermediate (I), and resistant (R) based on the diffusion zone breakpoints described by Clinical and Laboratory Standards Institute (CLSI) guidelines for *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (Issa *et al.*, 2011; Olaniran *et al.*, 2015; Shourav *et al.*, 2020). The Multiple Antibiotic Resistance (MAR) index was calculated as follows: MAR = a/b, where a = number of antibiotics to which the isolate was resistant; b = total number of antibiotics against which individual isolate was tested.

Results

Isolation of presumptive Listeria spp. isolates

Based on the growth pattern on PALCAM agar media, a total of 17 isolates (43.6, n = 39) were selected from plates belonging to the 39 RTE meat product samples. The colonies surfaced as black or black-green color with a black halo and a sunken center with a 1-2 mm (Scotter *et al.*, 2001). No discernible *Listeria*-like growth pattern was observed in the plates corresponding to the RTE chicken meat samples.

Identification of Listeria spp. isolates

The Gram reaction was used to confirm the morphology of the presumptive *Listeria* spp. isolates. Under a light microscope at 100X magnification, the isolates were visualized as Gram-positive short rods. Following Gram reaction and Biochemical tests, 10 isolates (25.6%, n = 39) showed results typical of *Listeria* spp. In the assorted biochemical tests (Table 2), the isolates presumed as *Listeria* spp. showed catalase positive, oxidase negative, urease negative, positive acid formation, negative gas formation, negative hydrogen sulfide formation, negative indole production, positive motility and positive MR-VP tests. *Listeria monocytogenes* ATCC 19117 was used as a reference strain to confirm the genus-level identification of isolates as *Listeria*.

Table 1. Biochemical tests result of the suspected *Listeria* spp. Isolates

Isolates	Biochemical Tests										
	Gram Reaction	Catalase	Oxidase	Kligler's Iron Agar (KIA)			Motility	Indole	Urease	Methyl red	Voges-Proskauer
				Acid	Gas	H_2S					
PSABD1	+	+	-	+	-	-	+	-	-	+	+
PSABD4	+	+	-	+	-	-	-	-	-	+	+
PSABD7	+	+	-	+	-	-	+	-	-	+	+
PSABD11	+	+	-	+	-	-	+	-	-	+	+
PSABD16	+	+	-	+	-	-	+	-	-	+	+
Positive control (*L. monocytogene* ATCC 19117)	+	+	-	+	-	-	+	-	-	+	+

***Molecular confirmation of potential Listeria spp. isolates by 16S rRNA gene sequencing***

Based on the homology search of the *16S rRNA* gene sequences using BLAST, 5 isolates showed 98 – 99% similarity with *Listeria* spp. sequences from NCBI GenBank. Table 3 depicts the accession number of the partial *16S rRNA* gene sequences designated by GenBank to the isolates of this study. Apart from the isolate PSABD4, the other isolates of the study showed high similarity (98 – 99%) with *Listeria monocytogenes* sequences available in GenBank. Only isolate PSABD4 was similar to a *Listeria innocua* sequence.

The partial *16S rRNA* gene sequences of the isolates in this study along with the reference sequences from GenBank were used to construct a phylogenetic tree (Figure 1). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.59974477 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. This analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 759 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). *Halobacterium volcanii* (Accession number: K0042.1) served as an outgroup for the tree.

Table 2. Identification of strains based on *16S rRNA* gene sequencing and their accession numbers published in DNA data base

Isolates (NCBI GeneBank Accession Number)	Sequence length (base pairs)	Source of isolation	16S rRNA similarity with *Listeria* species in GenBank (Accession number)	Percentage similarity
PSABD1 (MW940981)	898	RTE chicken	*Listeria monocytogenes* (CP006596.2)	98
PSABD4 (MW940982)	906	RTE chicken	*Listeria innocua* (HM007562.1)	99
PSABD7 (MW940983)	903	RTE chicken	*Listeria monocytogenes* (KU891822.1)	99
PSABD11 (MW940984)	897	RTE chicken	*Listeria monocytogenes* (CP032672.1)	99
PSABD16 (MW940985)	900	RTE chicken	*Listeria monocytogenes* (MT534107.1)	98
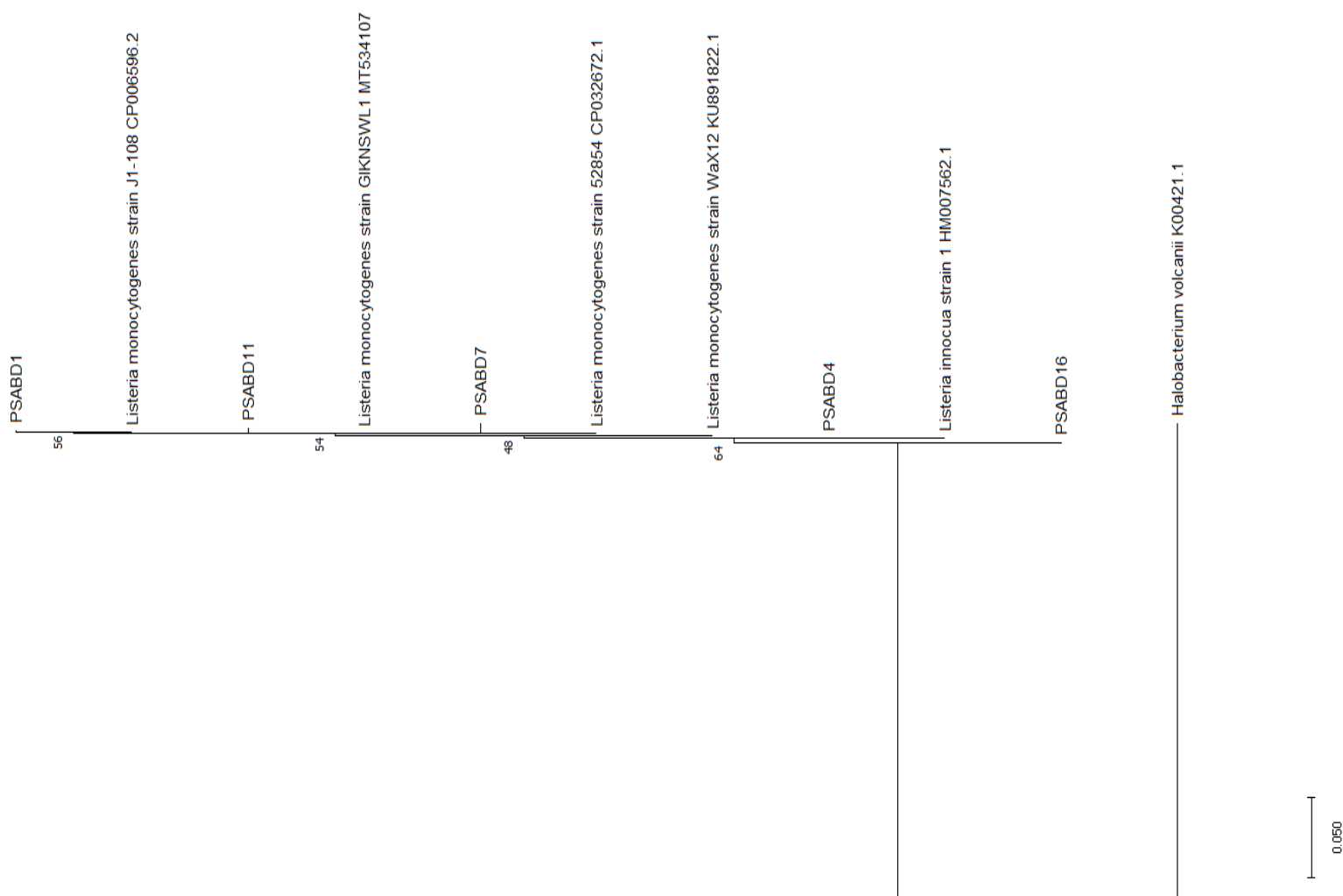
Figure 1. Phylogenetic analysis of partial 16S rRNA gene sequences and related species by neighbor-joining method

Detection of virulence-associated genes

The four suspected *L. monocytogenes* isolates (i.e., PSABD1, PSABD7, PSABD11, PSABD16) were subjected to PCR with a view to amplify three virulence-associated genes (*hlyA, actA* and *plcB*). Following analysis, all 4 isolates depicted the presence of *hlyA, actA* and *plcB* (Figure 2). However, none of the genes showed any polymorphism.
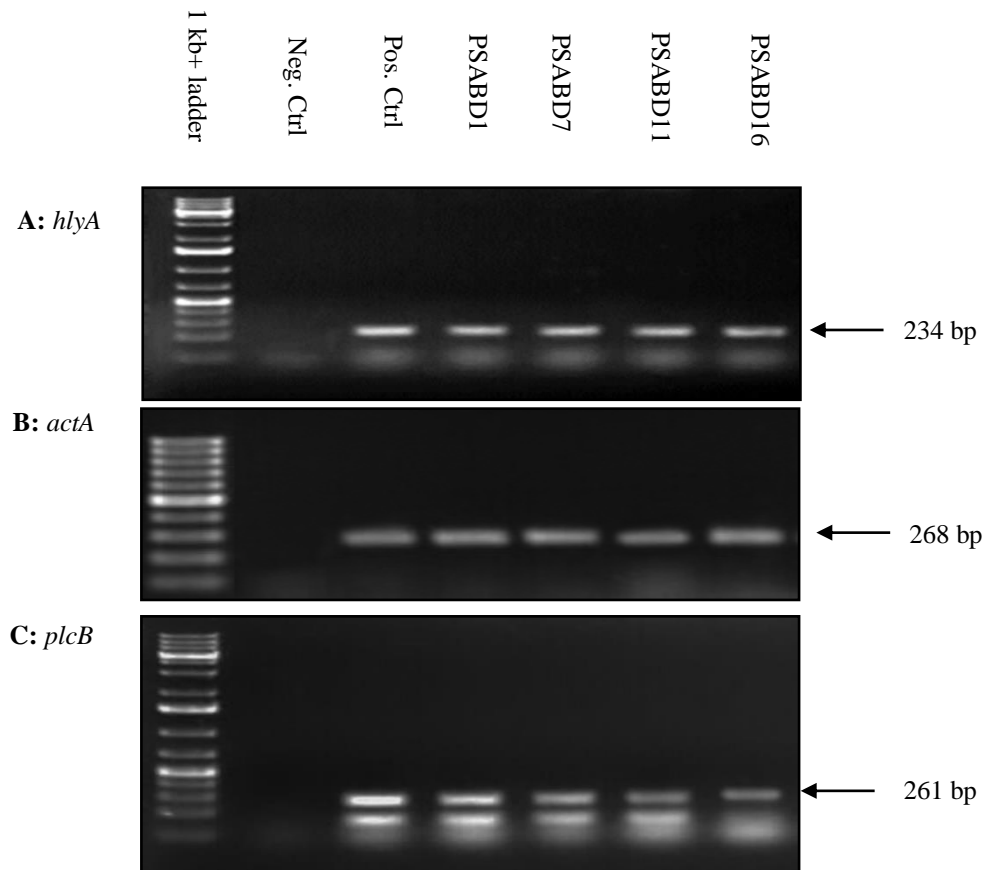

Figure 2. PCR amplification of the genes *hlyA* (picture A), *actA* (picture B) and *plcB* (Picture C). In all three gel pictures, lane 1 represents 1 kb+ DNA ladder, and lanes 2 through 7 represent the negative control, the positive control (*L. monocytogenes* ATCC 19117) and the isolates PSABD1, PSABD7, PSABD11 and PSABD16 respectively.

Antimicrobial susceptibility patterns of the isolated *Listeria* spp.

The results of antibiotic susceptibility test of the *Listeria* spp. isolates against a panel of eight antibiotics were tabulated in Table 4. The highest resistance, at 40%, was shown against both erythromycin and rifampicin. Complete susceptibility was depicted by all 5 *Listeria* spp. isolates of the study against streptomycin, sulfamethoxazole-trimethoprim, tetracycline and chloramphenicol. After these antibiotics, ampicillin showed the second-highest efficiency as 80% of the isolates were susceptible to it.

Table 3. Antibiotic susceptibility profile of the *Listeria* spp. Isolates

Classes of antibiotics	Antibiotics	Concentration (μg)	Susceptibility of the Listeria spp. isolates (n = 5)		
			Resistant (%)	Intermediate (%)	Susceptible (%)
β-Lactams	Penicillin (P)	10	20	40	40
	Streptomycin (S)	10	0	0	100
Macrolides	Erythromycin (E)	15	40	0	60
Penicillins	Ampicillin (AMP)	2	20	0	80
Sulfonamides	Sulfamethoxazole-trimethoprim (STX)	25	0	0	100
Tetracyclines	Tetracycline (TE)	30	0	0	100
Amphenicols	Chloramphenicol (C)	10	0	0	100
Ansamycin	Rifampicin (RD)	5	40	0	60

Data generated by the antibiotic susceptibility test was used to calculate the Multiple Antibiotic Resistance (MAR) indices for the isolates (Figure 3). Isolates PSABD 11 and PSABD 16 scored 0 MAR indices which means that these isolates were susceptible to all the antibiotics used on them. PSABD7 showed resistance against only 3 of the 8 antibiotics tested, giving it a MAR index of 0.375. Likewise, PSABD4 and PSABD1 were resistant against 2 and 1 antibiotics respectively.
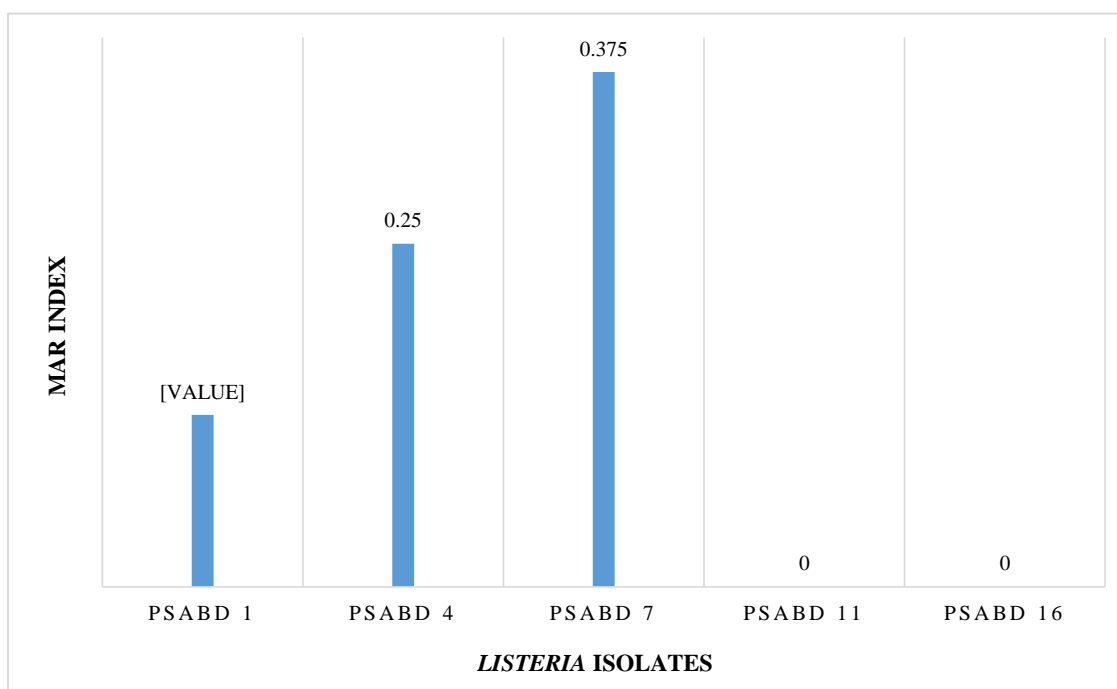
Figure 3. Multiple Antibiotic Resistance (MAR) index of the *Listeria* spp. Isolate.

Discussion

The remarkable ability of *Listeria monocytogenes* to persist in the harshest of conditions for great lengths of time has allowed it to easily breakthrough into food chains (Carpentier & Cerf, 2011). This has caused relevant authorities from several developed countries to implement strict guidelines against *L. monocytogenes* over the decades (Prevention, 2013). On the other hand, the epidemiological knowledge on listeriosis in developing countries is largely underappreciated as regulations against *L. monocytogenes* in these countries are almost non-existent. In Bangladesh, for instance, incidence rate of listeriosis is unknown and several factors can be attributed to it: lack of public awareness, overwhelmed medical sectors with other common diseases and underreporting of cases (Islam *et al.*, 2016). Previously, there have been a few studies in Bangladesh addressing the incidence of *Listeria* in dairy food products and the environment (Manjur *et al.*, 2018), cattle farm environment (Shourav *et al.*, 2020) and raw beef, chicken and chevon (Islam *et al.*, 2016). To the best of our knowledge, this is the first study investigating the prevalence of multi-drug resistant pathogenic *L. monocytogenes* in RTE meat products in Bangladesh.

The present study analyzed 39 samples of RTE chicken meat products for the presence of *L. monocytogenes*. By means of *Listeria*-specific biochemical and molecular detection methods, 5 *Listeria* spp. isolates were detected and characterized. Out of these, 4 isolates were confirmed to be *L. monocytogenes* (Figure 2). This made the overall prevalence rate of *Listeria* spp. 12.8% (n = 39) and the prevalence rate of *L. monocytogenes* is 10.3% (n = 39). The overall prevalence rate conforms with studies by Uyttendaele *et al.* (Uyttendaele *et al.*, 1999) and Islam *et al.* (Islam et al., 2016). The RTE food products investigated in this study are all sold as frozen food in Bangladesh, having a high shelf life. Although the other typical foodborne pathogens in the country may not find this environment as sustainable, *L. monocytogenes's* adaptability to survive in low temperatures, extremes of pH and water activity could be a reason for the documented prevalence in this study.

Previous investigations into the virulence factors of *L. monocytogenes* have observed that about 8% to 21% of the isolates can be either weakly virulent or completely avirulent (Bustamante *et al.*, 2020). The detection of *hlyA, actA* and *plcB* genes (Figure 2) in all four of the *L. monocytogenes* isolates in our research suggest that the isolates have the potential to be pathogenic. However, the genes detected did not show any polymorphism even though the manifestation of this phenomenon has been documented in these genes previously. Previous research has demonstrated *actA* gene to be polymorphic and that *L. monocytogenes* can be classified into two different categories based on polymorphism in *actA* (Wiedmann *et al.*, 1997). Polymorphism has also been demonstrated for *hlyA* gene in another study (Vines & Swaminathan, 1998). The absence of polymorphism in the genes detected in our study could be due to a few reasons: the target regions for the primer pairs used could have been too small for any variation to be detected or the target sequences might have been located outside the regions of polymorphism.

The accurate picture of polymorphism in these genes could be revealed by sequence analysis.

In the last few decades, there has been a rising number of cases of antibiotic resistance in *Listeria* spp. since it was first detected in 1990 (Poyart-Salmeron *et al.*, 1990). Despite that, treatment of listeriosis still involves the use of antibiotics and *Listeria* spp. are susceptible to those antibiotics that are generally aimed against Gram-positive bacteria (e.g. ampicillin, penicillin G, tetracyclines, amoxicillin, chloramphenicol *et cetera*) (Bustamante *et al.*, 2020). The AMR studies in our research revealed that the pattern of resistance in the isolates was not so high. Isolates PSABD11 and PSABD16 registered 0 scores of MAR, as shown in Figure 3. This means that these two isolates had no resistance whatsoever against the antibiotics tested on them. Isolate PSABD7 demonstrated the highest resistance with a MAR index of only 0.375, followed by PSABD4 and PSABD1 with MAR indices of 0.25 and 0.125. Moreover, all the isolates were completely susceptible (Table 4) to streptomycin, sulfamethoxazole-trimethoprim, tetracycline and chloramphenicol. The sensitivity pattern to sulfamethoxazole-trimethoprim is promising because it is used to treat listeriosis patients who are allergic to penicillin (Bajkó *et al.*, 2013) and penicillin, being the gold standard of listeriosis treatment (Martínez-Martínez *et al.*, 2001), was effective against only 40% of the isolates in the study. Ampicillin, one of the primary drugs of choice for the treatment of listeriosis (Shourav *et al.*, 2020), was effective against 80% of the isolates. This is particularly significant because two previous studies on *Listeria* conducted in Bangladesh (Islam *et al.*, 2016; Shourav *et al.*, 2020) found contrasting resistance patterns against ampicillin in their respective isolates.

Results demonstrated by the AMR pattern of the isolates in our study was not as alarming as other studies have reported in the past, but the detection of virulence-associated *hlyA, actA* and *plcB* genes in all 4 of the *L. monocytogenes* isolates could be a cause for concern, especially when the food items investigated are growing in popularity. Due to financial constraints, our study was limited to examining a relatively small number of samples, but the prevalence rates calculated are in close agreement with several published reports worldwide. Although the results of this study cannot be extrapolated to a larger number of samples, it still provides an insight into the risks associated with RTE chicken meat products in Bangladesh. Hence, food and health authorities in the country need to enforce more active control and surveillance of these food items.

Conclusion

Being a developing country, Bangladesh has a public health sector that is constantly overwhelmed with various foodborne diseases throughout the year. With hardly any prior scientific research conducted on *Listeria* spp. from the country's context, an outbreak of listeriosis could have a catastrophic effect on the population. Our study has demonstrated that pathogenic strains of *L. monocytogenes* are prevalent in RTE meat products commercially available in the country. As public popularity towards these food items increases, it is crucial that the government-run health and food authorities take a closer look at the quality control of these food products and enforce relevant regulations on their manufacturing process just as the developed countries of the world do.

References

1. Bai J, Shi X, Nagaraja T. G (2010) A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. Journal of Microbiological Methods 82:85–89. https://doi.org/10.1016/j.mimet.2010.05.003

2. Bajkó Z, Bălaşa R, Maier S, Moţăţăianu A, Treabă A, Macarie I, Gârbovan C, Chiriac C (2013) *Listeria monocytogenes* meningoencephalitis mimicking stroke in a patient with chronic lymphocytic leukemia. Neurology and Therapy 2:63–70. https://doi.org/10.1007/s40120-013-0009-y

3. Borucki MK, Gay CC, Reynolds J, McElwain KL, Kim SH, Call DR, Knowles DP (2005) Genetic diversity of *Listeria monocytogenes* strains from a high-prevalence dairy farm. Applied and Environmental Microbiology 71:5893–5899. https://doi.org/10.1128/AEM.71.10.5893-5899.2005

4. Bustamante F, Maury-Sintjago E, Cerda Leal F, Acuña S, Aguirre J, Troncoso M, Figueroa G, Parra-Flores J (2020) Presence of *listeria monocytogenes* in ready-to-eat artisanal chilean foods. Microorganisms 8:1–18. https://doi.org/10.3390/microorganisms8111669

5. Carpentier B, Cerf O (2011) Persistence of *Listeria monocytogenes* in food industry equipment and premises. International Journal of Food Microbiology 145:1–8. https://doi.org/ 10.1016/j.ijfoodmicro.2011.01.005

6. Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

7. Gebretsadik S, Kassa T, Alemayehu H, Huruy K, Kebede N (2011) Isolation and characterization of *Listeria monocytogenes* and other *Listeria* species in foods of animal origin in Addis Ababa, Ethiopia. Journal of Infection and Public Health 4:22–29. https://doi.org/10.1016/j.jiph.2010.10.002

8. Gil M I, Allende A, López Gálvez F, Selma MV (2009) Hay alternativas al cloro como higienizante para productos de IV Gama. Revista Horticultura. Mayo. http://www.horticom.com/revistasonline/extras/extra09/38_45.pdf. Accessed 3 May 2021

9. Hudzicki J (2012) Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. American Society For Microbiology, December 2009, 1–13. https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro. Accessed 1 May, 2021

10. Islam MS, Husna AA, Islam MA, Khatun M M (2016) Prevalence of *Listeria monocytogenes* in Beef, Chevon and Chicken in Bangladesh. American Journal of Food Science and Health 2:39–44. http://www.aiscience.org/journal/ajfshhttp://creativecommons.org/licenses/by/4.0/

11. Issa ZM, Yen LH, Radu S (2011) Antibiogram Profiles of *Listeria monocytogenes* isolated from foods. 2011 2nd International Conference on Biotechnology and Food Science 7:133–137. http://www.ipcbee.com/vol7/31-ICBFS2011S10043.pdf. Accessed 10 May, 2021

12. Jaradat ZW, Schutze GE, Bhunia AK (2002) Genetic homogeneity among *Listeria monocytogenes* strains from infected patients and meat products from two geographic locations determined by phenotyping, ribotyping and PCR analysis of virulence genes. International Journal of Food Microbiology 76:1–10. https://doi.org/10.1016/S0168-1605(02)00050-8

13. Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (2006) Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. Applied and Environmental Microbiology, 72:3832–3845. https://doi.org/10.1128/AEM.02869-05

14. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120. https://doi.org/10.1007/BF01731581

15. Kumar BNV (2016) Molecular characterization and antibiogram of *Listeria monocytogenes* isolated from chicken and mutton of retail markets. Journal of Microbiology and Infectious Diseases 6:65–68. https://doi.org/10.5799/ahinjs.02.2016.02.0218

16. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549. https://doi.org/10.1093/molbev/msy096

17. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1870–1874. https://doi.org/10.1093/molbev/msw054

18. Larsen, MH, Kallipolitis BH, Christiansen JK, Olsen JE, Ingmer H (2006) The response regulator ResD modulates virulence gene expression in response to carbohydrates in *Listeria monocytogenes*. Molecular Microbiology, 61:1622–1635. https://doi.org/10.1111/j.1365-2958.2006.05328.x

19. *Listeria monocytogenes (Murray et al.) Pirie ATCC ® 19117^{TM}*. (n.d.). https://www.atcc.org/products/all/19117.aspx#generalinformation. Accessed April 29, 2020

20. Manjur MSE, Siddique S, Ahmed S (2018) Multi-drug resistant pathogenic *Listeria monocytogenes* in surface water and soil samples of Dhaka city. Bangladesh Journal of Microbiology 33:39–42. https://doi.org/10.3329/bjm.v33i1.39602

21. Martínez-Martínez L, Joyanes P, Suárez AI, Perea EJ (2001) Activities of gemifloxacin and five other antimicrobial agents against *Listeria monocytogenes* and coryneform bacteria isolated from clinical samples. Antimicrobial Agents and Chemotherapy 45:2390-2392. https://doi.org/10.1128/AAC.45.8.2390-2392.2001

22. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerging Infectious Diseases 5:607–625. https://doi.org/10.3201/eid0505.990502

23. Mehlhorn, H (2015) Food-Borne Disease Burden Epidemiology Reference Group. Encyclopedia of Parasitology, 1–1. https://doi.org/10.1007/978-3-642-27769-6_3884-1

24. Olaniran AO, Nzimande SBT, Mkize NG (2015) Antimicrobial resistance and virulence signatures of *Listeria* and *Aeromonas* species recovered from treated wastewater effluent and receiving surface water in Durban, South Africa. BMC Microbiology 15:1–10. https://doi.org/10.1186/s12866-015-0570-x

25. Poyart-Salmeron C, Carlier C, Trieu-Cuot P, Courtieu AL, Courvalin P (1990) Transferable plasmid-mediated antibiotic resistance in *Listeria monocytogenes*. Lancet (London, England) 335:1422–1426. https://doi.org/10.1016/0140-6736(90)91447-i

26. Prevention, C. for D. C. and. (2013). *CDC. (2013) Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen Farms, Colorado*. https://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/index.html. Accessed 5 May 2021

27. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

28. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones, J L, Griffin PM (2011) Foodborne illness acquired in the United States-Major pathogens. Emerging Infectious Diseases 17:7–15. https://doi.org/10.3201/eid1701.P11101

29. Scotter SL, Langton S, Lombard B, Schulten S, Nagelkerke N, In't Veld PH, Rollier P, Lahellec C (2001) Validation of ISO method 11290 part 1--detection of *Listeria monocytogenes* in foods. International Journal of Food Microbiology 64:295–306. https://doi.org/10.1016/s0168-1605(00)00462-1

30. Shamloo E, Hosseini H, Moghadam AZ, Larsen HM, Haslberger A, Alebouyeh M (2019) Importance of *Listeria monocytogenes* in food safety: A review of its prevalence, detection, and antibiotic resistance. In Iranian Journal of Veterinary Research 20:241–254. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6983307/

31. Shourav AH, Hasan M, Ahmed S (2020) Antibiotic susceptibility pattern of *Listeria* spp. isolated from cattle farm environment in Bangladesh. Journal of Agriculture and Food Research 2:100082. https://doi.org/10.1016/j.jafr.2020.100082

32. *SR0141, Listeria Selective Enrichment Supplement | Oxoid - Product Detail*. (n.d.). http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp?pr=SR0141&cat=&c=UK&lang=EN. Accessed August 21, 2020

33. Todd ECD, Notermans S (2011) Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. Food Control 22:1484–1490. https://doi.org/10.1016/j.foodcont.2010.07.021

34. Uyttendaele M, De Troy P, Debevere J (1999) Incidence of *Listeria monocytogenes* in different types of meat products on the Belgian retail market. International Journal of Food Microbiology 53:75–80. https://doi.org/10.1016/S0168-1605(99)00155-5

35. Vines A, and Swaminathan B (1998) Identification and characterization of nucleotide sequence differences in three virulence-associated genes of *Listeria monocytogenes* strains representing clinically important serotypes. Current Microbiology 36:309–318. https://doi.org/ 10.1007/s002849900315

36. *Vital Signs: Listeria Illnesses, Deaths, and Outbreaks — United States, 2009–2011*. (n.d.). https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6222a4.htm. Accessed April 29, 2021

37. Walker SJ, Archer P, Banks JG (1990) Growth of *Listeria monocytogenes* at refrigeration temperatures. Journal of Applied Bacteriology 68:157–162. https://doi.org/ 10.1111/j.1365-2672.1990.tb02561.x

38. Wallace C., Sperber W., Mortimore S. Food Safety for the 21st Century, Managing HACCP and Food Safety Throughout the Global Supply Chain. 2nd ed. Wiley; Minnesota, MN, USA: 2018. p. 396.

39. Wiedmann M, Bruce JL, Keating C, Johnson AE, McDonough PL, Batt CA (1997) Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infection and Immunity 65:2707–2716. https://doi.org/10.1128/iai.65.7.2707-2716.1997

40. Zhu Q, Gooneratne R, Hussain MA (2017) *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. Foods 6:21. https://doi.org/10.3390/foods6030021