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| RESEARCH ARTICLE

Bacteriological Assessment of *Escherichia coli* and *Salmonella spp.* from Local Meat Shops in Dhaka, Bangladesh

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| ABSTRACT

Retail meat and contact surfaces of meat have been identified as reservoirs of foodborne pathogens and can be used to transmit antimicrobial-resistant bacteria to consumers. In this research, the prevalence, phenotypic identification, molecular confirmation, antimicrobial resistance, and biofilm-forming capability of Escherichia coli and Salmonella spp. were determined in local meat shop in Dhaka, Bangladesh. Twelve samples (raw meat portions and environmental swabs) were sampled, 8 of which were subjected to microbiological analysis. Presumptive isolates were subcultured on MacConkey (MAC) and Xylose Lysine Deoxycholate (XLD) agar and identified by Triple Sugar Iron (TSI), citrate utilization and Motility-Indole-Urease (MIU) assays. The biochemical characteristics of most XLD-derived isolates were similar to Salmonella spp. and the majority of MAC-derived isolates were similar to E. coli. Molecular verification was added with the help of Polymerase chain reaction (PCR) on the invA gene of Salmonella spp. and the uidA gene of E. coli. The Kirby-Bauer disk diffusion method of antimicrobial susceptibility testing showed widespread resistance, with about 70% of the tested isolates having a multidrug-resistant phenotype and no isolates being completely susceptible to all antibiotics tested. Biofilm formation, as measured using the microtiter plate assay, revealed that all the isolates tested were biofilm formers with optical densities of 0.105 to 0.35. It was also found that stronger biofilm formation was mostly seen in XLD-derived isolates compared to MAC-derived isolates. The results indicate an important food safety issue and the necessity of heightened hygiene, regular monitoring, and sensible antimicrobial usage.

| KEYWORDS

Escherichia coli, *Salmonella* spp., PCR, antimicrobial resistance, biofilm, retail meat

| ARTICLE INFORMATION

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1. Introduction

Meat is a key part of human diet; it includes high-quality protein, essential amino acids, vitamins and micronutrients like iron and zinc. Nevertheless, it can easily be microbially contaminated with a high content of moisture, which is why its nutrient content makes it highly vulnerable to microbial food spoilage. Foodborne diseases remain a significant worldwide public health burden, and contaminated food significantly contributes to the prevalence of illness and death annually (F. Shaltout, 2026; F. A. E. Shaltout, 2025). The risk is further compounded in developing nations like Bangladesh due to poor hygiene when slaughtering, handling, and a lack of temperature control, as well as a lack of strong regulatory actions in informal meat markets. These environments provide good conditions that facilitate the growth and contamination of microbes and heighten the chances of transmitting the pathogen to consumers (Sultana et al., 2020; WHO, 2024).

Newer investigations in Bangladesh and other developing countries have shown that food borne pathogens in retail food systems are becoming commonly prevalent and have huge repercussions on the health of the population. As an example, Rafiq et al. (2024) found multidrug-resistant Escherichia coli and Salmonella in poultry meat, eggs, and feces, which implies a high level of distribution of resistant bacteria throughout the food production chain. Likewise, Amin et al. (2024) reported antimicrobial-resistant foodborne pathogens in fish sold at the Dhaka markets, which showed that microbial contamination and resistance are not limited to a particular type of food but are common among various food commodities. The findings of these indicate that there is a system wide problem with food safety management where contamination remains throughout the food production process and as far as retail outlets.

Contamination of meat in retail is a multifactorial issue, which may happen at various critical stages such as slaughtering, transportation, processing, storage, and retail handling. Poor infrastructure and absence of cold chain management and poor hygiene practices are some of the factors that contribute to microbial contamination in most local meat markets especially in developing countries. The use of contaminated equipment and materials, including knives, cutting boards, weighing scales, and the hands of handlers, is a key element in the increase of the spread of pathogens (Rabby et al., 2021; Siddiky et al., 2022). Moreover, wholesale and wet markets, such as contaminated water sources, unsanitary working conditions, and environmental contamination, contribute to the perpetuation and spread of pathogens to various food products and surfaces (Parvin et al., 2022). All these interdependent reasons outline the complexity of the contamination pathways and the necessity of overall monitoring of not only the food products but also their immediate environment.

Among the microbial contaminants of meat, Escherichia coli and Salmonella spp. are the most important ones, as they are closely related to gastrointestinal tract infections, foodborne outbreaks, and evidence of poor hygiene. The occurrence of E. coli in meat can be commonly discussed as a sign of fecal contamination and poor sanitation in the slaughtering and processing stages, and Salmonella spp. are one of the most common causes of zoonotic foodborne disease across the globe(Siddiky et al., 2021; Zhao et al., 2001). Various studies have continued to document the presence of antibiotic-resistant E. coli and Salmonella in poultry meat, retail setting, and processing facilities in Bangladesh (Hossain et al., 2022; Parvin et al., 2020). Such data suggest that not only is contamination common, but there is also an upsurge in contamination being linked to antimicrobial resistance, further complicating treatment and control.

Although the evidence supporting this is increasing, most of the literature has concentrated on pathogen prevalence or antimicrobial resistance per se without further consideration of other factors like biofilm formation, environmental persistence and molecular confirmation in a cohesive framework. This absence of combination analysis inhibits a full comprehension of the risk presented by the total of foodborne pathogens in the retail setting. Hence, the purpose of the current paper was to examine the presence of Escherichia coli and Salmonella spp. on retail meat and meat-contact surfaces in a local meat shop in Dhaka, Bangladesh, by biochemical identification, antimicrobial susceptibility testing, biofilm analysis, and PCR-based confirmation of the presence of the bacterial type using uidA and invA genes. Through the combination of phenotypic and molecular methods, this research offers a holistic assessment of foodborne pathogens and adds to the body of knowledge of the overall effect of antimicrobial resistance and biofilm formation in retail meat settings.

2. Foodborne Pathogens in Retail Meat

Retail meat is commonly known as a significant source of foodborne pathogens because it is contaminated at various points in the production and distribution process. Between slaughtering and end retail store display, meat can be subjected to a variety of microbial contamination sources, such as animal intestinal contents, contaminated equipment, water, and direct human contact. These dangers are further enhanced in developing nations like Bangladesh by poor hygiene, infrastructures, and little regulatory control in the old markets. This has led to the high incidences of microbial contamination in meat products sold in such facilities (Rani et al., 2023). As an example, MDR Escherichia coli and Salmonella spp. have been found in poultry meat, eggs, and feces, which is an indication of widespread distribution of resistant pathogens within the food production system (Rafiq et al., 2024). Likewise,

antimicrobial-resistant foodborne bacteria were detected in fish sold in Dhaka, which proves that the issue of contamination and resistance is not specific to meat but spans various food industries (Amin et al., 2024).

Even retail settings are crucial nodes in the transmission of pathogens, with contamination being not only introduced but also sustained and enhanced. An inadequate sanitation, inefficient waste management and the lack of standardized hygiene practices in a lot of the local markets are some of the factors which have led to the survival and multiplication of the pathogenic bacteria (Hasan et al., 2025). Rabby et al. (2021) observed that wet markets and supermarkets in Dhaka contain Salmonella in poultry meat, with the level of contamination related to the handling practices and the environment. Moreover, it has been found that one of the significant reservoirs of Salmonella spp. is the poultry processing environment in wet markets, where meat products can be constantly contaminated (Siddiky et al., 2022). Pathogen transmission in retail stores is also enhanced by cross-contamination of tools and surface contacts such as knives, cutting boards, and weighing scales with contaminated surfaces (Hassan Ali et al., 2010).

Escherichia coli and Salmonella spp. are important microbial indicators in the retail setting as they are common and can endure in a variety of conditions. Their occurrence indicates contamination incidents as well as the hygienic condition of meat handling. These pathogens have been consistently documented in poultry meat and retail outlets in studies in Bangladesh and are frequently related to antimicrobial resistance phenotypes, thereby enhancing their significance to human health (Parvin et al., 2022; Siddiky et al., 2021). The further occurrence of resistant strains emphasizes the increasing concerns about the spread of antimicrobial-resistant pathogens in the food chain and the necessity of more powerful surveillance and control plans.

The environmental contamination also has a significant role in the maintenance and transmission of foodborne pathogens in retail meat systems. Meat-contact surfaces like knives, cutting boards, display tables are some of the surfaces on which bacteria can survive and create persistent reservoirs that perpetuate contamination of meat products. These pathogens may last long under favorable conditions such as high moisture content and availability of nutrient. Thames and Theradiyil Sukumaran (2020) pointed out that the issue of Salmonella contamination of poultry systems is hard to manage, and previous research showed that meat and environmental samples often contain E. coli and Salmonella spp. (Zhao et al., 2001). Such results highlight that food safety control measures should not be limited to the product level test but should also be expanded to the environmental level and enhanced sanitation measures as well as effective hygiene control in retail outlets.

3. Biofilm of Antimicrobial Resistance and Molecular Analysis

The problem of antimicrobial resistance (AMR) has become one of the key worldwide health issues, especially in food-borne bacteria related to the animal food. Bacteria have become resistant to multiple drugs, which can be transmitted to humans via food production due to the widespread and uncontrolled use of antibiotics in livestock production (Rahman & Ahmed, 2022; WHO, 2023). In Bangladesh, Escherichia coli and Salmonella isolates of meat, poultry, and retail foods have shown high levels of antimicrobial resistance (Parvin et al., 2020). The same results have been noted in other parts of the world with antimicrobial-resistant E. coli and Salmonella being detected in poultry feed, fish, and dairy settings that implies the prevalence of resistance among the various food industries (Lautan et al., 2025; Tilahun & Efa, 2026). These discoveries underscore the increasing challenge of resistant foodborne pathogens on the health of the population.

The formation of biofilms also increases the persistence and survival of the bacteria in food processing and retail environment. Biofilms are organized microbial communities situated in an extracellular matrix which offers protection against environmental stress, disinfectants and antimicrobial agents (O'Toole, 2011; Shahid et al., 2025). In butchers, biofilms on the surfaces like knives, cutting boards and other equipment may cause chronic contamination and repeated expose to the consumer. Biofilm development and stability can be affected by environmental factors (moisture, availability of nutrients, surface properties) and this is a crucial consideration in food safety. Antimicrobial resistance and biofilm-forming capacity together contributes greatly to the resilience and transmissibility of foodborne pathogens (Cappitelli et al., 2014; Tran et al., 2020).

Besides the phenotypic features, molecular confirmation is important in the proper identification of pathogens. Traditional biochemical techniques can yield inconclusive or ambiguous results especially with unusual strains. Polymerase chain reaction (PCR) offers a specific and dependable method through the focus on universal genetic markers. invA gene is a common molecular marker of Salmonella, and uidA gene is a common molecular marker of E. coli (Hadi et al., 2023) (Bej et al., 1991; Rahn et al., 1992). Through the combination of antimicrobial susceptibility testing, biofilm testing, and PCR-based molecular confirmation, research can provide a more detailed and precise estimation of microbiological risks of retail meat environments.

4. Methods

4.1. Study design and sample collection

A cross-sectional laboratory experiment was done based on samples taken in a local meat store in Dhaka, Bangladesh. A total of 12 samples were collected to examine the presence of Salmonella spp. and Escherichia coli. The samples included raw meat samples and environmental swabs collected on meat-contact surfaces, such as knives, cutting boards, and meat-handling surfaces.

Sterile swab sticks and sampling bags were used to collect all the samples in a sterile condition. After collection, samples were immediately transferred to microbiology laboratory in an insulated ice box stored at around 4-8 C within 2-3 h so as to maintain the integrity of the microbes. Sample preparation and initial suspension steps were conducted in accordance with the common food microbiology procedures, such as using a 25 g test portion and 1:10 dilution ratio with 225 mL pre-enrichment medium. Owing to the limitations of laboratory processing, 8 out of 12 samples collected were picked and further analyzed using microbiological techniques. Meat samples (25 g) were aseptically transferred to a 225 mL sterile stomacher bag with 25g of buffered peptone water (BPW) and swab samples were immersed in 10 mL BPW. Pre-enrichment was done to all the samples at 37 o C, 18-24 hours to promote recovery of stressed or low-level bacterial populations before selective culturing.

4.2. Isolation and phenotypic identification.

After pre-enrichment, aliquots of each of the samples were streaked on selective and differential media, such as MacConkey agar (MAC) and Xylose Lysine Deoxycholate agar (XLD). The plates inoculated were kept at 37° C 18 to 24 hrs. Selective isolation of distinct colonies was done on the basis of morphological features and subculture to obtain pure isolates.

The standard biochemical tests of E. coli and Salmonella spp. such as Triple Sugar Iron (TSI) agar, Simmons citrate agar, and Motility-Indole- Urease (MIU) medium was done as per the instructions of the manufacturer to identify them presumptively. TSI agar was stab-and-streaked and incubated at 37° C 18 to 24 h and the results were interpreted by changes in the slant and butt color, gas production, and the production of hydrogen sulfide (H_2S). The use of citrate was evaluated on Simmons citrate agar following incubation at 37° C between 24 and 48 h and the transformation of green color to blue was taken as a positive test. A single stab was used to inoculate MIU medium, which was incubated at 37° C in 24 h to assess motility, indole production (at the end of incubation with the addition of the reagent of Kovac) and urease activity. Phenotypic identification was done using characteristic biochemical reaction profiles which were typical of enteric Gram-negative bacteria with E. coli generally having acid reactions and no H_2S production and Salmonella spp. having alkaline slant/acid butt reactions with H_2S production.

4.3. PCR molecular confirmation

PCR was done on the presumptive isolates to verify the identity of the isolates. Fresh overnight cultures were then subjected to the boiling extraction technique or a commercially packaged DNA extraction kit to extract genomic DNA as per the standard protocols. In the case of Salmonella spp., invA gene was amplified with the help of the primer pair presented by Rahn et al. (1992), which resulted in the 284 bp amplicon. In the case of E. coli, primers to target the uidA gene were designed by Bej et al. (1991), and resulted in a 147 bp fragment. These are the gene targets that are commonly known to be specifically molecularly confirmed as Salmonella spp. and E. coli, respectively. Table 1 shows the primer sequences that were employed in this study.

Table 1: Sequences of primers to amplify PCR

Target organism	Gene	Primer sequence (5'–3')	Expected amplicon
Salmonella spp.	invA	F: GTGAAATTATCGCCACGTTCGGGCAA	284 bp
		R: TCATCGCACCGTCAAAGGAACC	
E. coli	uidA	F: AAAACGGCAAGAAAAAGCAG	147 bp
		R: ACGCGTGGTTAACAGTCTTGCG	

PCR amplification was carried out in the end volume of 25 µL using 12.5 µL PCR master mix, 1 µL forward and reverse primer, 2 µL template DNA, and nuclease-free water. Thermal cycling conditions used to amplify invA were the following: initial denaturation at 95° C, 35 cycles of denaturation at 95° C, 30 s, annealing at 60° C, 30 s, and extension at 72° C, 30 s, and finally, extension at 72° C, 5 min. In the case of uidA amplification, the same cycling conditions were employed with the exception that the annealing temperature was altered to 55° C. PCR products were separated by electrophoresis in 1.5-2.0% agarose stained with ethidium bromide or a different less toxic DNA stain and observed under UV transillumination.

4.4. Antimicrobial susceptibility testing

Kirby-Bauer disk diffusion was used to test the antimicrobial susceptibility on Mueller-Hinton agar (MHA). New cultures of bacteria were put into sterile saline and readjusted to 0.5 McFarland turbidity standard. Sterile cotton swabs were used to a uniformly spread the standardized inoculum across MHA plates.

Agar was aseptically inoculated with antibiotic discs, such as ampicillin, cefixime, meropenem, azithromycin, erythromycin, tetracycline, chloramphenicol, kanamycin, sulfonamide and colistin. Incubation of plates was done at 37° C over a period of 18-24 h. The size of the inhibition zone was measured in millimeters and interpreted as sensitive, intermediate, or resistant as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

4.5. Biofilm formation assay

The crystal violet microtiter plate assay was used to determine biofilm formation. Overnight cultures were diluted (1:100) on fresh broth and transferred into sterile 96-well flat-bottom microtiter plates. The plates were left to incubate at 37° C in a period of 24 h to enable the formation of biofilms.

After incubation, wells were softly rinsed 3 times with phosphate-buffered saline (PBS) to eliminate non-adherent cells and dried using air. The adherent biofilms had been stained with 0. 1% crystal violet 15 min, and the stain was removed by washing the biofilm with distilled water. The remaining stain was dissolved in ethanol or acetic acid and the optical density (OD) was determined at 570 nm to measure biofilm biomass.

4.6. Data interpretation and data handling.

The data were summarized descriptively and tabulated in Microsoft Excel. Since the research was based on a small number of processed samples and isolate-level observations, the outcomes were only considered with regard to phenotypic profiles, frequency of resistance categories and biofilm trends instead of a conventional inferential statistical test. Unclear biochemical reactions were left in the story but were explained with reservations.

5. Results Analysis and Discussion

5.1. Recovery and phenotypic characterization of isolates

Triple Sugar Iron (TSI), citrate utilization and Motility Indole Urease (MIU) tests were used to determine the biochemical nature of the isolates obtained on Xylose Lysine Deoxycholate (XLD) agar and MacConkey (MAC) agar. These are routine microbiological tests that are employed in the identification of enteric Gram-negative bacteria. TSI test is used to identify bacteria according to the ability to ferment carbohydrates (glucose, lactose, sucrose), the ability to produce gas, and the ability to produce hydrogen sulfide (H_2S). The citrate utilization test identifies the organism as having a particular metabolic capacity or not, based on its ability to use citrate as its only source of carbon. The MIU test is used to measure three valuable characteristics at the same time, they are; bacterial motility, production of indole using tryptophan and activity of urease enzyme. In general, TSI test was useful in the process of differentiating presumptive Salmonella spp. against E. coli thus affirming its usefulness as a screening test. Table 1 shows the specific TSI reaction profiles of the isolates of XLD and MAC media.

The TSI test revealed a clear biochemical differentiation between the isolates grown in XLD and MAC media. The majority of the isolates derived with XLD showed a red slant and yellow butt (K/A) phenotype, which implies the presence of glucose fermentation with the subsequent alkaline reversion on the slant on the basis of the peptone utilization. H_2S production was also positive in these isolates as demonstrated by the presence of black precipitate which is a characteristic of Salmonella spp. Conversely, the isolates of MAC-derived were always positive (yellow slant and yellow butt) (A/A) which indicates the capacity to ferment glucose and lactose and/or sucrose, thus leading to the production of acid during the entire medium. The active fermentation processes were observed in most of the isolates and this was due to the production of gas. Nevertheless, some of the samples did not provide clear or consistent results, which is probably associated with the technical variability or mixed cultures or observed limitations. Altogether, the TSI test was a reliable screening tool as it was able to differentiate presumptive Salmonella spp. and E. coli.

Table 2: TSI reaction profiles of XLD and MAC media isolates (Source: Author)

Sample	Medium	Slant	Butt	Gas	H_2S	Presumptive identity
1	XLD	Red	Yellow	+	+	*Salmonella* spp.
	MAC	Yellow	Yellow	+	–	*E. coli*
2	XLD	Red	Yellow	–	+	*Salmonella* spp.
	MAC	Not clear	Not clear	+	–	Ambiguous
3	XLD	Not clear	Not clear	+	+	Ambiguous
	MAC	Yellow	Yellow	+	–	*E. coli*
4	XLD	Red	Yellow	+	+	*Salmonella* spp.
	MAC	Yellow	Yellow	+	–	*E. coli*
5	XLD	Red	Yellow	+	+	*Salmonella* spp.
	MAC	Yellow	Yellow	+	–	*E. coli*
6	XLD	Red	Yellow	+	–	*Salmonella* spp.
	MAC	Yellow	Yellow	+	–	*E. coli*
7	XLD	NC	NC	+	+	Ambiguous
	MAC	Yellow	Yellow	+	–	*E. coli*
8	XLD	Red	Yellow	+	+	*Salmonella* spp.
	MAC	Yellow	Yellow	+	–	*E. coli*

Table 2 shows the findings of citrate utilization and MIU tests. These tests were additional evidence of the biochemical identity of the isolates and they reinforced the differentiation in the TSI test. The usage of citrate showed a distinct trend with isolates obtained on XLD agar (presumptive Salmonella spp.) displaying a strong trend towards being citrate positive as shown by a change in color of the media to blue with the production of alkaline by products. Isolates on MAC agar (presumptive Escherichia coli) was typically citrate negative (remaining green) and this finding is consistent with the observation that most E. coli strains do not use citrate as a sole carbon source. The

difference underlines the metabolic dissimilarities between the two groups of bacteria and the preliminary identification of these groups.

The results of the MIU test also helped in differentiation and validation of the isolates. All the isolates were found to be motile as evidenced by diffuse growth around the stab line implying that motility is not a distinguishing feature of these enteric bacteria and therefore not dependable as a differentiator. Nonetheless, the indole production was highly discriminatory: MAC-derived isolates were always indole positive and formed a red ring when Kovacs reagent was added, as a result of tryptophan metabolism, and XLD-derived isolates were indole negative, as was expected of Salmonella spp. The urease activity was mostly negative in all isolates, with some weak or intermittent reactions which could be due to variation in the experiment and not necessarily due to the presence of the enzyme. Combined, these complementary patterns of citrate positivity and indole negativity in XLD isolates, and citrate negativity and indole positivity in MAC isolates, indicate high internal consistency of the tests when done collectively, and show the strength and reliability of the biochemical identification with multiple tests.

Table 3: Citrate and MIU test profiles (Source: Author)

Sample	Medium	Citrate	Motility	Indole	Urease
1	XLD	+	+	–	–
	MAC	–	+	+	–
2	XLD	+	+	–	–
	MAC	–	+	–	–
3	XLD	+	+	–	±
	MAC	+	+	+	–
4	XLD	+	+	–	–
	MAC	–	+	+	±
5	XLD	+	+	–	–
	MAC	–	+	+	–
6	XLD	+	+	–	±
	MAC	+	+	+	–
7	XLD	+	+	–	–
	MAC	–	+	+	–
8	XLD	+	+	–	–
	MAC	–	+	+	–

5.2. PCR confirmation

Molecular confirmation using species-specific genetic markers by PCR was conducted to confirm the identity of putative Salmonella spp. and Escherichia coli isolates. Salmonella spp. invA and E. coli uidA genes were chosen as being highly specific and previously used in molecular diagnostics. The amplification outcome was shown in Figure 2 that gives the agarose gel electrophoresis pattern of both targeted genes. The presence of Salmonella spp. in XLD-derived isolates and the presence of E. coli in MAC-derived isolates are confirmed by distinct bands at the expected 284 bp and clear bands at 147 bp positions, respectively. The positive controls were strongly amplified at their respective positions and no bands were seen in the negative control indicating specificity and reliability of the PCR assay. These species-specific amplicons are consistently detected, which gives solid molecular evidence in support of the biochemical identification and confirms the presence of Salmonella spp. and E. coli in the meat and meat-contact surfaces samples.

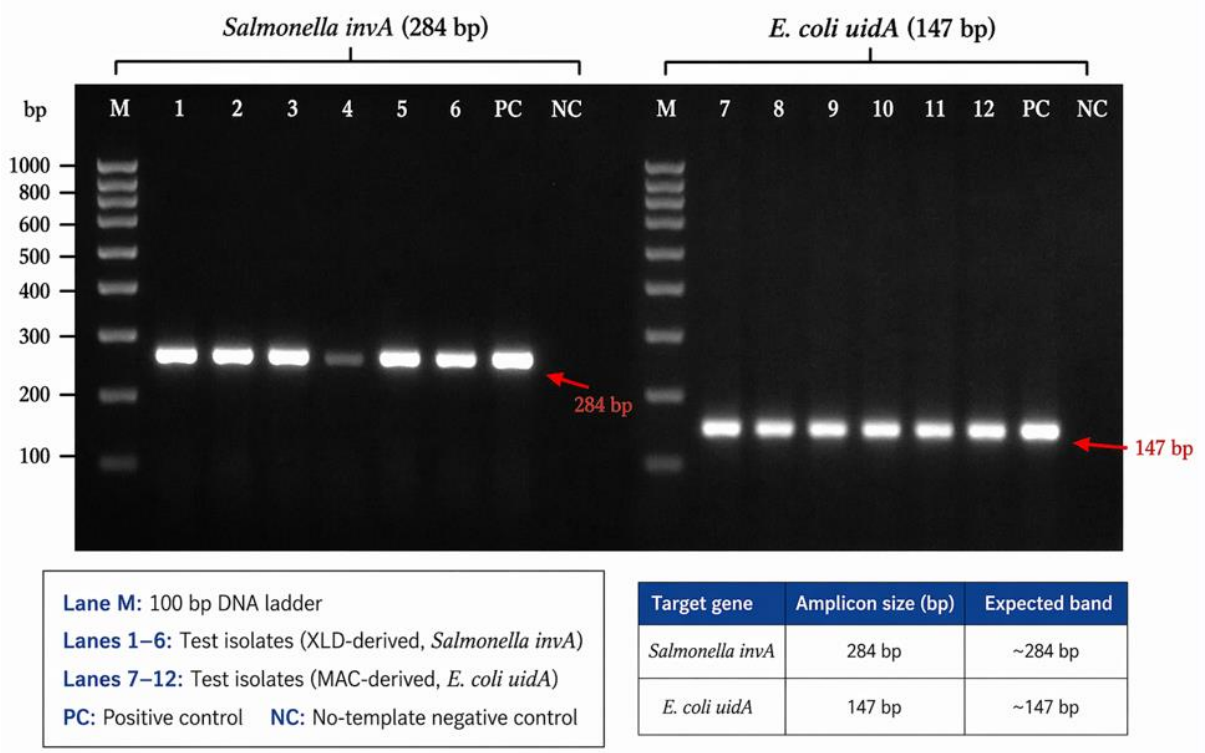

Figure 1: Agarose gel electrophoresis of PCR products that displayed amplification of Salmonella invA (284 bp) and E. coli uidA (147 bp) genes (Source: Present study)

5.3. Antimicrobial susceptibility profile

The antimicrobial susceptibility test showed that the isolates obtained at the meat shop samples had a high level of resistance. About 70 per cent of the isolates were multidrug-resistant (MDR) phenotypes and the rest were intermediate-resistant to one or more antibiotics. Notably, all the isolates were not completely susceptible to all the antibiotics tested implying a high prevalence of antimicrobial non-susceptibility. All the antibiotics in the panel, such as ampicillin, cefixime, meropenem, azithromycin, erythromycin, tetracycline, chloramphenicol, kanamycin, sulfonamide, and colistin, showed resistance. These patterns of resistance were identical in both XLD-derived (presumptive Salmonella) and MAC-derived (presumptive E. coli) isolates indicating that antimicrobial resistance is widely spread across bacterial populations. The detailed antibiotic resistance profiles of the representative isolates are shown in Table 3.

Table 4: Profiles of representative isolates

Sample	Medium	AMP	CEFI	MERO	AZITH	ERYTH	TETRA	CHLOR	KAN	SULF	COL
1	XLD	R	IR	R	R	R	R	R	R	R	R
	MAC	R	R	IR	R	R	R	R	R	R	IR
3	XLD	R	R	R	R	R	R	R	R	R	R
	MAC	R	IR	R	R	R	R	R	R	R	R
4	XLD	R	R	R	R	R	R	R	R	R	R
	MAC	R	R	IR	R	R	R	R	R	R	IR
6	XLD	R	R	R	R	R	R	R	R	R	IR
	MAC	R	IR	R	R	R	R	R	R	R	R
8	XLD	R	IR	IR	R	R	R	R	R	R	R
	MAC	R	R	R	R	R	R	R	R		

The results of the antibiogram show that the prevalence of resistance in various classes of antibiotics is observed, which proves the existence of the MDR bacterial population in the meatshop environment. There is high resistance to commonly used antibiotics (ampicillin, tetracycline, erythromycin and chloramphenicol) implying potential selective pressure when antimicrobial use is prolonged or not used properly in food-animal production. It was also found to be resistant to critical antibiotics, such as cefixime, meropenem and colistin, which is of concern due to the development of reduced susceptibility to last-resort therapy. The patterns of consistent resistance in both E. coli and Salmonella spp. suggest exposure to the environment and possible spread of the resistance factors. In general, the presence of the multidrug resistance among these foodborne pathogens is a major issue of public health concern and reflects the necessity of ongoing monitoring and judicious use of antimicrobials.

5.4. Biofilm-forming ability

The microtiter plate assay was used to quantify biofilm formation and the optical density (OD 90) of isolates growing in the MacConkey (MAC) and Xylitol Lysine Deoxycholate (XLD) media were measured and are indicated in Figure 1. Biofilm forming capacity was found in all test isolates, and OD 5 values were between 0.105 and 0.35 which showed that there exist different adherence and biomass levels. The production of biofilm was continually elevated in the XLD-derived isolates relative to the MAC-derived isolates in all the samples with the greatest biomass being in sample 8 (XLD; OD ≈ 0.35), sample 4 (OD ≈ 0.31), and sample 7 (OD ≈ 0.30) and the lowest biomass in sample 2 (MAC; Overall, MAC-derived isolates showed fewer and more consistent OD values, whereas XLD-derived isolates were less uniform with a higher level of biofilm. This tendency indicates that culture conditions and medium composition can affect biofilm expression, possibly via stress-induced physiological changes or increased production of extracellular polymeric substance (EPS). On the whole, the results demonstrate that biofilm formation is a widespread characteristic of the isolates and is under the control of growth conditions which could be the reason of bacterial persistence and contamination in meat-processing facilities.

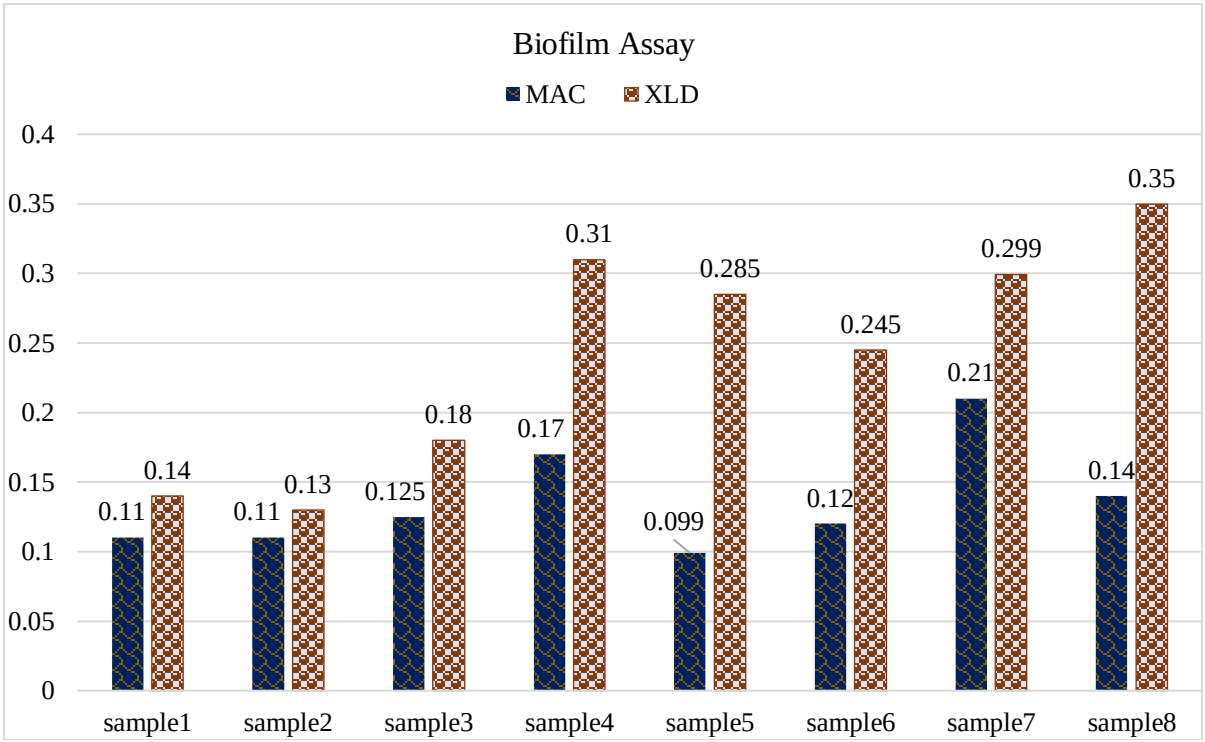

Figure 2: XLD and MAC derivatives isolates that form biofilm as determined by OD in the microtiter plate assay

5.5 Discussion

This research shows that the local meat shop in Dhaka has retail meat and meat-contact surfaces that harbor bacteria with phenotypic and molecular features that are aligned to Escherichia coli and Salmonella spp. The fact that these organisms can both be recovered after being in contact with meat and their environmental contact

points underlines that contamination is not a singular product but is perpetuated and reinforced by the handling environment around it. This observation is consistent with the past research which suggests that retail meat environments support persistence and inter-contact transfer of enteric pathogens between food and contact surfaces (Hassan Ali et al., 2010; Zhao et al., 2001).

The biochemical identification of TSI, citrate and MIU tests yielded reaction profiles that were in line with the classical metabolic characteristics of enteric Gram-negative bacteria. The majority of XLD-derived isolates had alkaline slant/acid butt (K/A) reactions (indicating presumptive Salmonella spp.) and citrate positive reactions (indicating E. coli), but MAC-derived isolates had acid/acid (A/A) reactions (indicating E. coli). These trends have been attributed to variations in metabolic pathways, with Salmonella spp. fermenting glucose to oxidative amino acid metabolism resulting in alkaline reversion, whereas E. coli sustains acid production by fermenting a variety of carbohydrates. But the fact that there are occasional ambiguous reactions demonstrates the weaknesses of phenotypic methods, which could be influenced by mixed cultures, fluctuation of incubation, or unusual strains.

In order to counteract these constraints PCR-based molecular confirmation to Salmonella spp. invA gene and E. coli uidA gene was included. These species-specific gene targets are highly conserved and the most accurate at the genetic level (Hadi et al., 2023; Rahn et al., 1992). The combination of biochemical and molecular techniques will improve the reliability of the diagnosis and minimize the chances of misidentification, especially in complicated environmental samples. This integrated method enhances the overall validity of the pathogen detection in this study.

One of the key issues that have been found in this research is the high occurrence of antimicrobial resistance. About 70 percent of isolates had multidrug-resistant (MDR) phenotypes and none of them were completely susceptible to all antibiotics examined. The result aligns with recent observations in Bangladesh where MDR E. coli and Salmonella have been commonly identified in the retail food systems (Hasan et al., 2025; Rafiq et al., 2024). Mechanistically, resistance can occur by decreasing outer membrane permeability, active efflux systems, enzyme breakdown of antibiotics (e.g., β-lactamases) and horizontal gene transfer in plasmids. There are no complete susceptible isolates implying that there is a continued selection pressure, but probably relevant to the widespread and largely uncontrolled use of antimicrobials in food-animal production, a matter of global concern highlighted by the World Health Organization.

Every isolate in this paper was able to form biofilms with the optical density values reflecting different amounts of biomass. The formation of biofilms offers a strong ecological benefit as it allows bacteria to stick to surfaces and generate extracellular polymeric substances (EPS), a protective framework. This matrix restricts the penetration of antibiotics, stimulates metabolic heterogeneity and allows persisted cells to form, thus increasing resistance to antimicrobial agents. Biofilms also facilitate quorum sensing and horizontal gene transfer, which helps to spread resistance to microbial communities (O'Toole, 2011). The relatively increased biofilm formation in isolates of the XLD indicates that the environmental or nutritional stress factors could trigger adaptive changes that lead to increased adhesion and EPS generation.

Antimicrobial resistance and the presence of biofilm-forming capacity are two critical issues of food safety. Biofilms allow bacteria to survive on meat-contact surfaces even after regular cleaning and disinfection, and results in repeated cycles of contamination. This continuity adds to the danger of unremitting exposure of the consumers to the resistant pathogens. The same observations were made in other studies performed in the past where infected equipment and surfaces served as reservoirs of transmission of pathogens (Hassan Ali et al., 2010). These results indicate the necessity of better hygienic procedures, successful decontamination plans on surfaces, and frequent microbiological checks in retail meat settings.

The confirmation of the presence of pathogens in meat and environmental samples also contributes to the importance of cross-contamination as a major process in the spread of pathogens. Poor hygiene of knives, cutting boards and surfaces of handling aids in the transmission of bacteria between products, increasing contamination in

the retail environment. Thus, intervention measures must not be limited to safety of products but also on environmental hygiene and on handling practices.

Although these are significant findings, there are a few limitations of this study. The processing of a relatively small sample (eight) and the relativity of the sample size can be a limitation to the generalizability of the results. Also, PCR confirmation was used, but molecular characterization of resistance genes and strain typing were not carried out in detail. To give an all-encompassing perspective of the dynamics of pathogen transmission and resistance within retail meat systems, future research must include larger sample sizes, genomic research, and quantitative risk analysis.

6. Conclusion

The current paper suggests that presumptive Escherichia coli and Salmonella spp. with alarming antimicrobial resistance and biofilm-forming capabilities are likely to be present on retail meat and meat-contact surfaces in a meat shop located in Dhaka, Bangladesh. Phenotypic identification confirmed the existence of both organisms, representative isolates were generally non-susceptible to various antibiotics, and all isolates produced biofilms, with better biofilm production relative to XLD-derived isolates. Adding PCR confirmation would provide a stronger description of the presence of pathogens, molecular identities, AMR burden, and persistence-related biofilm behavior within a small retail meat environment to the manuscript. These results justify the necessity to enhance the sanitary handling, surface decontamination, periodic microbiological testing, and rational antimicrobial stewardship in the meat supply chain. This conclusion is directly based on the summary of data uploaded and discussed in your draft.

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