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Prevalence of *E. coli* in The Retailed Chicken Meat Products with Protective Trials Using *Carica papaya* and *Moringa oleifera Extracts*



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Abstract

Products made from chicken meat have gained popularity in recent years in both developed and developing countries, and they can help with the scarcity of the red meat. They provide abundant, easily digestible protein and offer essential vitamins and minerals like niacin, thiamine, and riboflavin, crucial for maintaining life, promoting development, and providing various health benefits. Despite having a high biological value, chicken meat products can nevertheless contain various food spoilage and poisoning organisms such as *E. coli*. This study was undertaken to investigate the prevalence of *E. coli* in the retailed chicken meat products in Egypt including chicken luncheon, nuggets, and pane. Besides, the anti-*E. coli* activities of natural products including papaya and moringa extracts were examined. The obtained results revealed coliform contamination of the examined chicken meat products. In addition, *E. coli* 02:H6, O26:H11, O78, O91:H21, O121:H7, O128:H2, and O146:H21 were the recovered serotypes with *E. coli* O128:H2 the most predominant serotype. The recovered serotypes harbored Shiga toxin coding genes. Both papaya and moringa extracts could significantly reduce *E. coli* load in an experimental trial.

Keywords: Chicken meat products, E. coli, Carica papaya, Moringa oleifera.

Introduction

Chicken meat products have been increasingly popular in recent decades, both in rich and developing countries. These products offer a potential solution to the issue of animal meat scarcity. They offer numerous health benefits because to their abundance of easily digestible, highquality protein. Additionally, they serve as an excellent source of essential minerals and vitamins. However, chicken meat can be contaminated with various types of pathogens. This is because there are plenty of essential nutrients available for the proliferation and expansion of these pathogens [1, 2].

Escherichia coli (E. coli) is a pathogen that has been associated with several outbreaks of human

sickness. E. coli is a common bacterium that is present in the intestinal tracts of people, poultry, and other animals. However, several strains of this bacterium have acquired genes linked to virulence, which enable them to cause disorders in the intestines or elsewhere in the body. These diseases affect avian, human, and other species. Diarrheic E. coli strains are responsible for causing enteric infections. The development of these infections is governed by several virulence features, which vary based on the specific pathotypes. Currently, E. coli isolates that lead to diarrhoea are classified into six main pathotypes based on their distinct virulence factors and pathogenic features. Shiga toxinproducing E. coli (STEC) group is among the most important pathotypes associated with human disease [3, 4].

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Globally, papaya (Carica papaya Linn.) is generally acknowledged for its gastronomic and nutritional attributes. The conventional medical system is also fully cognizant of the therapeutic advantages of papaya fruit and other components of plants. The papaya tree is cultivated for industrial purposes because of its comprehensive utility. Papaya has undergone considerable advancements in its biological activity and therapeutic applications in recent decades, establishing itself as a valuable fruit plant with nutraceutical characteristics. Papain derived from it can be utilized as a valuable resource for the development of various commercial and therapeutic goods aimed at treating diverse disorders [5]. Nevertheless, the utilization of papaya in the realm of food safety has received limited scrutiny.

Moringa oleifera, comprising several beneficial components, can serve as a food additive. The main function of this substance is to act as a preservative by preventing lipid oxidation and other chemical reactions that lead to the deterioration of products. Furthermore, it can improve the physical and chemical characteristics of the food, thereby prolonging its durability and overall quality. Furthermore, it improves nutrition by increasing the levels of vitamins, minerals, and proteins. However, the food industry encounters a difficulty due to the sensory characteristics of the plant, resulting in restricted customer approval of the fortified goods [6]. The utilization of moringa as an antibacterial ingredient in the chicken meat sector lacks sufficient knowledge.

The objective of the study was to study the prevalence of *E. coli* in three chicken meat products, namely nuggets, luncheon, and pane. In addition, detection of shiga toxin-coding and virulence-associated genes was screened using PCR. The anti-*E. coli* activities of *Carcia papaya* and *Moringa oliefera* extracts were additionally examined.

Material and Methods

This study was done according to the guidelines of Zagazig University, and no living animals were used in the present study.

Sample collection

Sixty samples of chicken meat products, such as nuggets, luncheon, and pane, were gathered randomly from different stores in Zagazig city, Sharkia Governorate, Egypt. These samples were immediately transported in a cooler to the Meat Hygiene Laboratory at the Faculty of Veterinary Medicine, Zagazig University, for bacterial analysis. Sample preparation

The samples, each weighing twenty-five grams, were thoroughly mixed using a sterile homogenizer. This was done by combining the samples with 225 ml of sterile buffered peptone water (BPW) 0.1% and homogenizing them at a speed of 2500 rpm for

duration of 3 minutes. The resulting mixture was a homogenate with an initial dilution of 1/10. Starting with a dilution of 1 in 10, 1 ml of the solution was transferred into a tube containing 9 ml of sterile BPW 0.1%. This diluted solution was then further diluted by a factor of ten through ten-fold dilution [7].

Most probable number (MPN) of Coliforms

A volume of one milliliter from the dilution prepared earlier was added separately to three MacConkey broth tubes, each with an inverted Durham's tube. These tubes were then placed in an incubator set at 37°C. After 24 and 48 hours, they were examined and assessed for the production of acid and gas in the inverted Durham's tubes as per ICMSF [8].

Most probable number (MPN) of E. coli

A volume of one milliliter from the tubes that initially tested positive for the Most Probable Number of Coliforms, demonstrating both acid and gas production, was introduced into pre-warmed tubes containing 7 milliliters of *E. coli* (EC) broth from Himedia, Mumbai. The tubes were then incubated at a temperature of 44.5°C for a period of 24 to 48 hours. The MPN of *E. coli* [9] was determined using positive tubes that exhibited acid and gas generation.

Isolation and identification of E. coli

A volume of one milliliter from the initial dilution was transferred to MacConkey broth tubes containing inverted Durham tubes. The inoculated tubes were incubated at 37°C for 24 hours. Next, one milliliter of the positive tube was pipetted into MacConkey broth enrichment tubes and place them in an incubator set at 44°C for a period of 24 hours. The loops containing bacteria from the positive enhanced broth tubes were spread onto eosin methylene blue agar (EMB) media and then placed in an incubator at a temperature of 37°C for duration of 24 hours. The alleged colonies exhibited a green metallic sheen. The colonies suspected were separated and grown on nutrient agar slopes in order to be further identified [10]. The E. coli isolates that were confirmed were identified serologically using rapid diagnostic E. coli antisera sets (DENKA SEIKEN Co., Japan), following the method published by Kok et al. [11].

Detection of shiga toxin-coding genes by PCR

A multiplex polymerase chain reaction (PCR) was performed to amplify the shiga toxin-coding genes including *stx1*, and *stx2*, intimin (*eaeA*), and hylA gene as virulence associated gene. Genomic DNA was extracted using the Gene JET Genomic DNA Purification Kit (Fermentas), following the protocol described by Sambrook et al. [12]. The purified DNA was promptly utilized in the subsequent application or stored at a temperature of -

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20°C. The identification of shiga toxins (stx1 and stx2), intimin (eaeA), and haemolysin (hylA) in E. coli was performed using Multiplex PCR. Specific primers from Pharmacia Biotech were used, and the cycling conditions for stx1 and stx2 were based on the method described by Dhanashree and Mallya [13]. The amplification conditions for eaeA were based on the method described by Mazaheri et al. [14], and for hylA, they were based on the method described by Fratamico et al. [15]. The amplified DNA fragments were examined using 2% agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer, stained with ethedium bromide, collected, and observed under a UV transilluminator. The size of the fragment was assessed using a 100-bp plus DNA ladder from Qiagen, Germany GmbH.

Anti-E. coli activity of papaya and Moringa ethanolic extracts:

The ethanolic extracts of papaya and Moringa were tested for their ability to inhibit the growth of E. coli. The ethanolic extracts of papaya and moringa were generously provided by the Faculty of Agriculture at Zagazig University. The extracts were made using the method described in a previously published study by Addai et al. [16]. The extracts were utilized at two different concentrations, specifically 1% and 2%, in distilled and deionized water (DDW). Twenty-five cubes of pane samples free from E. coli, each weighing 50 g, were partitioned into five groups, with each group including 5 cubes. The cubes were infected with E. coli O128:H2 at a concentration of 6 log cfu/g. Group 1 was submerged in deionized distilled water (DDW) for duration of 30 minutes and set as a control group. Group 2 was submerged in a solution of 1% papava extract in DDW for duration of 30 minutes, whereas group 3 was submerged in a solution of 2% papaya extract in DDW for the same duration. Group 4 was submerged in a 1% moringa extract solution in DDW for duration of 30 minutes; whereas group 5 was submerged in a 2% moringa extract solution in DDW for the same duration. Subsequently, the enumeration of E. coli was performed on Tryptic Soya Agar (TSA, Oxoid). The sensory attributes and rates of decline were assessed based on the methodology outlined by Bourdoux et al. [17].

Statistical analysis

The results were transformed into logarithmic values (log₁₀ CFU/g) and shown as the average values accompanied by the standard error (S.E). The numbers were subjected to statistical analysis using the SPSS-21 software, a statistical package for social sciences, based in Chicago, IL, USA. The Duncan Multiple Range test was used to analyze the differences among individual means. A significant level of 95% confidence was applied, with P<0.05 deemed as statistically significant. The experimental

reduction trial involved comparing *E. coli* counts among different treatment groups and determining statistical significance at a *P*-value of less than 0.05.

Results and Discussion

Products made from chicken meat may become contaminated at any stage of the processing, packing, and shipping process with various pathogens. The chicken products become hazardous to customers and inappropriate for human consumption due to these pathogens. The hygienic status of chicken products can be assessed using a variety of indicators; coliform is frequently used to assess the safety and cleanliness of chicken meat products [18].

The E. coli and coliform contamination in tested products of chicken meat were indicated by the results shown in Fig. 1. The average MPN of coliforms was 3.37 ± 0.11 in nuggets, 3.83 ± 0.27 in luncheon, and 3.64 ± 0.30 Log ₁₀ MPN/g, respectively (Fig. 1A). The prevalence rates of E. *coli* in the studied nuggets, luncheon, and pane were 65%, 35%, and 50%, respectively (Fig. 1B). The mean counts of the MPN of *E. coli* were 2.14 ± 0.17 in nuggets, 2.56 ± 0.30 in luncheon, and 2.64 ± 0.25 in pane Log 10 MPN/g (Fig. 1C). Ibrahim et al. [19] reported comparable MPN of coliform numbers. In the meantime, the luncheon and chicken nuggets had greater coliform counts (4.6 and 4.4 log10 CFU/g, respectively) [20]. El-Kewaiey [21] reported lower coliform levels (2.4 and 1.7 log_{10} CFU/g) that were in luncheon and nuggets of chicken, seen respectively. The elevated MPN of coliform in chicken products suggests that the product is of low sanitary quality. According to Yadav et al. [22], infected hands, shopping blocks, knives, and water can all lead to coliform contamination of chicken meat products. Chicken, farm animals, and humans naturally have E. coli in their gastrointestinal tracts; the existence of it in chicken meat products indicates that the meat may be contaminated with feces as well as possibly other intestinal pathogens. Numerous incidences of foodborne infections in humans have been linked to virulent strains of E. coli [23]. Previous reports of E. coli isolation from products of chicken meat include those by Ahmed [24], Awadallah et al. [25], and Sobieh [26]. It is likely that Ibrahim et al. [19] isolated E. coli at 13.3% and 16.6% from chicken meals and nuggets, while Edris [27] isolated E. coli at 25% from chicken nuggets. The E. coli numbers reported by Sharaf and Sabra [28] and Edris [27] were comparable to that recorded in the current study. In the meantime, Wardhana et al. [29] reported lower counts, while Ibrahim et al. [30] reported higher counts. According to Adevanju and Ishola [31], E. coli can enter the end chicken meat products by being handled improperly, such as cross-contamination during processing or human-tofood contamination by food handlers, or by using raw or undercooked chicken meat.

The results shown in Fig. 2 demonstrated the recovered E. coli serotypes from various chicken meat products. E. coli O2:H6, O26:H11, O78, O91:H21, O121:H7, O128:H2, and O146:H21 were recovered. Serotype E. coli O128:H2 was the most predominant serotype found in the samples that were analyzed. Fast food in Egypt was a probable source where E. coli serotypes O157, O158, O119, O128, O125, and O86 were isolated by Morshdy et al. [32]. The identification of the virulence-coding genes and the shiga toxin in the recovered E. coli isolates was displayed in Fig. 3 and Table 1. In E. coli O2:H6, O26:H11, O78, O91:H21, and O128:H2, stx1 was found. Nevertheless, O78, O91:H21, and O146:H21 all had stx2 detections. E. coli O26:H11 and O91:H21 included EaeA. Three pathotypes were found to contain the hylA coding gene: O26:H11, O91:H21, and O121:H7. Enterotoxigenic E. coli carrying the stx1 and stx2 genes was found in chicken meat products, such as burgers, luncheons, shawarma, and pane, in line with the recovered serotypes [33].

The obtained results of the current study revealed isolation of enterotoxigenic E. coli. Hospitalizations due to E. coli are commonplace worldwide. Fever, diarrhea, stomach pain, and dehydration are signs of an E. coli infection [34]. Both papaya and moringa extracts showed a pronounced and potent anti-E. coli effect in the protection testing, according to the current investigation. 1% and 2% papaya extracts, respectively, decreased E. coli O128 at 8.71% and 28.31%. Similarly, E. coli O128 was lowered by 1% and 2% of moringa extracts, respectively, at 5.01% and 21.68% (Fig. 4). Its high concentration of bioactive substances, such as flavonoids, carotenoids, and alkaloids-all known for their antibacterial and antioxidant qualities-may be the cause of such antimicrobial activities. The samples under examination retained their sensory qualities after using the extracts. Nirosha and Mangalanayaki [35] showed strong antibacterial properties for papaya leaf extracts against E. coli, Salmonella Typhi, Staphylococcus aureus, Pseudomonas aeruginosa,

and Bacillus cereus, which is consistent with the findings of the current work. Hidayati et al. [36] most likely showed that papaya seed extracts had antibacterial properties against Salmonella Typhi and E. coli. This is likely because of the high concentration of flavonoids, terpenoids, alkaloids, and saponins that they contain. In reference to the moringa extract, Viera et al. [37] reported the antibacterial properties of the ethanolic extract against Staphylococcus aureus and E. coli in vitro. Additionally, extracts from moringa leaves shown broad-spectrum antibacterial activity against Enterobacter aerogens, Pseudomonas aeruginosa, Staphylococcus aureus, and E. coli. The high concentration of tannin and saponins in the moringa extracts was blamed for these activities [38].

Conclusions

This study's results showed that handling chicken meat products was done without proper sanitation, which led to the contamination of such products with $E.\ coli$. As a result, while processing chicken meat products, strict cleanliness guidelines need to be followed. It is strongly advised to use 2% papaya and moringa extracts to reduce the $E.\ coli$ load that contaminates chicken meat products.

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Conflicts of interest

The authors declared no competing interests.

Funding statement

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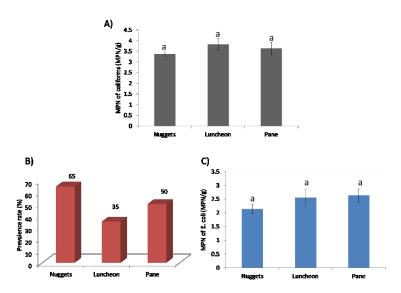


Fig. 1. A) Most probable number (MPN) of coliforms, B) Prevalence rate (%) of *E. coli*, C) Most probable number of *E. coli* in the examined chicken meat products. MPN values represent log 10 MPN/g of the mean \pm SE. Columns with similar letter are not significantly different at p < 0.05.

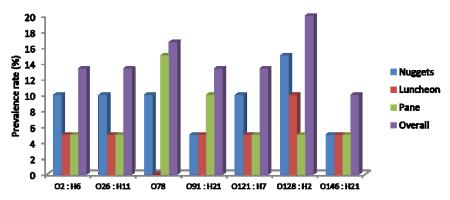


Fig. 2. Prevalence rates of different E. coli serotypes recovered from the examined chicken meat products.

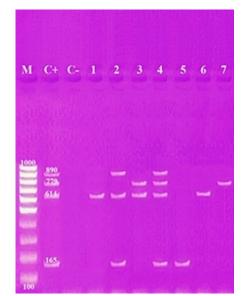


Fig. 3. Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp), eaeA (890 bp) and hlyA (165 bp) virulence genes of Enteropathogenic E. coli.

Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lane C-: Control negative.

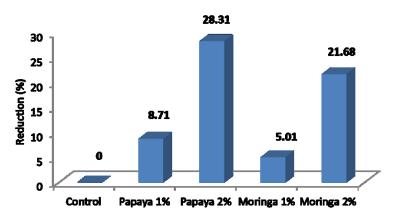


Fig. 4. Reduction (%) of papaya and moringa extracts on the *E. coli* load in experimentally-infected pane samples.

 TABLE 1. Incidence of virulence genes of Enteropathogenic E. coli isolated from the examined samples of chicken meat products

Virulence genes				
	stx1	stx2	eaeA	hylA
Serovars				
O2 : H6	+	-	-	-
O26 : H11	+	-	+	+
078	+	+	-	-
O91 : H21	+	+	+	+
O121 : H7	-	-	-	+
O128 : H2	+	-	-	-
O146 : H21	-	+	-	-

stx1: Shiga- toxin 1 gene; stx2: Shiga- toxin 2 gene; eaeA: intimin gene; hylA: hemolysin gene

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تواجد الايشريشيا القولونية في منتجات لحوم الدواجن المسوقة مع محاولة الوقاية باستخدام مستخلصات كاريكا بابايا والمورينجا اوليفيرا

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المستخلص

اكتسبت المنتجات المصنوعة من لحم الدجاج شعبية في السنوات الأخيرة في كل من البلدان المتقدمة والنامية، ويمكن أن تساعد في التغلب على قلة اللحوم الحمراء. أنها توفر بروتينًا وفيرًا وسهل الهضم وتوفر الفيتامينات والمعادن الأساسية مثل النياسين والثيامين والريبوفلافين، وهي ضرورية للحفاظ على الحياة وتعزيز التنمية وتوفير فوائد صحية متنوعة. على الرغم من قيمتها البيولوجية العالية، إلا أن منتجات لحوم الدجاج يمكن أن تحتوي على العديد من الكائنات الدقيقة المسببة للتسمم الغذائي مثل الإشريكية القولونية. أجريت هذه الدراسة لمعرفة مدى انتشار بكتيريا الإشريكية القولونية في منتجات لحوم الدجاج التي يتم بيعها بالتجزئة في مصر بما في ذلك لانشون الدجاج، والناجتس، والبانيه. بالإضافة إلى منتجات لحوم الدجاج التي يتم بيعها بالتجزئة في مصر بما في ذلك لانشون الدجاج، والناجتس، والبانيه. بالإضافة إلى ذلك، تم فحص الأنشطة المضادة للايشريشيا القولونية والخاصة ببعض المنتجات الطبيعية بما في ذلك مستخلصات دلك، تم فحص الأنشطة المضادة للايشريشيا القولونية والخاصة ببعض المنتجات الطبيعية بما في ذلك مستخلصات بالإضافة إلى ذلك، تم عزل بكتيريا الإشريكية القولونية مالانشون والناجتس والبانيه. بالإضافة إلى بالإضافة إلى ذلك، تم عزل بكتيريا الإشريكية القولونية من اللانشون والناجتس والبانيه بنوعة بالإضافة إلى ذلك، تم عزل بكتيريا الإشريكية القولونية من اللانشون والناجتس والبانيه بنسبة 35%، و50%. و1020: و101:118 مع مائولونية الاعثريكية القولونية من اللانشون والناجتس والبانيه بنسبة 35%، و50%. مالإضافة إلى ذلك، تم عزل بكتيريا الإشريكية القولونية من اللانشون والناجتس والبانيه بنسبة 35%، و50%. و1021:129، و112:129، و112:129، و111:200، و701، و121:200، و712:01، و121:20%. و121:200، والمليمي مالي منتقلل مسليمي المنيميين المسلي الكثري من ماميريا والمو

الكلمات الدالة: منتجات لحوم الدواجن ، الإشريكية القولونية ، البابايا ، المورينجا.