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Multidrug-resistant *E. coli* and *Salmonella* Isolated from Raw and Ready-to-Eat Meat Products, Raising the potential of Future Foodborne Illness and Treatment Challenges

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Abstract

THE STUDY aimed to determine *Escherichia coli* and *Salmonella* species frequency, and their phenotypic and molecular multidrug-resistance (MDR), in raw and ready-to-eat (RTE) meat products. Using golden standard culture and serotyping techniques, one hundred raw (hamburgers and sausages) and RTE meat (hawawshi and kofta) products were screened for targeted pathogens. The genetic elements that correlate with MDR characteristics were identified by PCR. *Escherichia coli* and *Salmonella* were identified in 26% and 14% of the samples, respectively, and both were prevalent in the raw products, at 57.7% and 57.14%. MDR *Escherichia coli* (73.1%) and *Salmonella* (35.71%) were more frequently found in raw foods. The *blaCTX* and *blaSHV* genes were present in all five tested *Escherichia coli*, and *mcr1* was expressed in three of them—two raw and one RTEM. Two raw-derived *Escherichia coli* co-expressed *blaTEM*, *blaCTX*, and *blaSHV*, and one of them also shared *mcr1*. The *norA* gene predominated in four of five MDR *Salmonella* isolates, raw (3) and RTE meat (1), whereas blaCTX or mcr1 occurred in three isolates, raw (2) and RTE meat (1). Two MDR *Salmonella* co-expressed the *blaTEM* and *blaCTX* or *blaCTX* and *blaSHV* genes, while also exhibiting *mcr1* and/or *norA*. These antibiotic-resistant genes of vital importance imply veterinary mistreatment.

Keywords: Raw and ready-to-eat meat, *Escherichia coli, Salmonella,* multidrug-resistance, *blaTEM*/*blaCTX*/*blaSHV*, *mcr1*, *norA*.

Introduction

The origins of ready meat meals and snacks, as well as the expansion of urban fast food cultures, were directly related to the emergence of novel socio-cultural expectations and feelings of time scarcity, as well as other preferences like nutritional quality optimization and the immediate inclusion of more unmeasurable aspects like variation, hedonic virtues, extended shelf life, and authenticity [1]. However, the evolution of these products and their technologies for quick meat preparation and consumption was associated with safety and health implications. Meat products present one of the most pressing problems with regard to microbial safety because they make an ideal environment for the growth of microorganisms, particularly pathogenic bacteria[2].

The foodborne disease burden epidemiology reference group (FERG) of the World Health Organization recently estimated that 31 foodborne diseases (FBDs) caused over 600 million illnesses and 420,000 deaths globally in 2010, resulting in the loss of 33 million healthy life years (DALYs) [3,4].

Salmonella spp. were estimated to be the primary cause of diarrheal and invasive foodborne illness globally, accounting for 93.8 million gastroenteritis cases, 111,000 deaths, and around 8.6 million Disability Adjusted Life Years (DALYs) [5]. Between 2006 and 2013, typhoid fever had the second highest incidence (12.7 cases /100.000) among 15 notifiable communicable diseases, according to epidemiological data from the Egyptian Ministry of Health and Population's surveillance

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system [6]. While *Salmonella enterica* subspecies *enterica* was discovered in 35 (6.99%) of the 501 samples in our most recent cross-sectional investigation in Al-Qalyubia, Egypt, between November 2019 and May 2021. Surprisingly, ready-to-eat food/drink [REF/D] had a greater prevalence (8.93) than raw food (7.67) [7]. Foodborne *Salmonella* spp. was the leading bacterial cause of illness, hospitalizations, and deaths in the United States in 2011[8], and the incidence remained fairly consistent in 2015 [9]. The high annual incidence of foodborne *Salmonella* spp. and associated direct costs in the United States resulted in a total annual loss of 1.66 million quality-adjusted life days (QALDs), with a monetary loss of $5.49 million considered to be the highest among all known illnesses [10]. In 2017, *Salmonella Virchow* was responsible for a nationwide outbreak of 210 cases reported in five EU/EEA countries, the United Kingdom, and the United States. The aforementioned incidents were all linked to restaurants that provided kebab meat with infected chicken flesh, and the clone has been circulating in the EU poultry meat production chain [11]. In low-income subregions, particularly in Africa, EPEC and enterotoxigenic *E. coli* (ETEC) were the second most common causes of diarrhea and the burden of foodborne illness after NTS [3]. Additionally, it was found that the main source of infections with Shiga toxin-producing *E. coli* (STEC) was beef. Foodborne STEC, according to the World Health Organization (WHO), resulted in more than 1 million illnesses, more than 100 fatalities, and about 13 000 years of life with a disability-adjusted between 2007 and 2015 (DALYs) [3,5].

Food poisoning is not the only health hazard produced by foodborne priority pathogens; antibiotic resistance transmission is also a major worry. Food animals, inevitably, contribute significantly to the continued expansion of this challenge because they are the principal source of animal protein in the food supply. Bacterial antimicrobial resistance (AMR) develops when bacteria go through genetic changes that reduce the effectiveness of antibiotics. Human factors such as the incorrect use of antimicrobials in human and veterinary medicine, as well as inadequate hygienic conditions and practices in healthcare settings or in the food chain, all contribute to the spread of resistant microbes [12]. According to previous predictive statistical study, AMR globally caused 4.95 million fatalities in 2019, of which 1.27 million were attributable to bacterial AMR. With 27.3 fatalities per 100 000, the developing world, particularly western Sub-Saharan Africa, has the highest rate of all-age deaths caused by resistance [13]. That number is anticipated to increase to 10 million by 2050, with a total cost of $100 trillion, if the challenge would not be addressed [14]. According to the World Bank, an additional 28 million people will be pushed into extreme poverty by 2050 if AMR is not controlled.

Governmental and official surveys are less common in Egypt than they are in developed countries, so research is constantly required to provide information on the microbiological safety of meat products. Furthermore, higher outbreaks and contamination rates of priority pathogens, specifically *Escherichia coli* (*E. coli*), and *Salmonella*, have recently been linked to ready-to-eat foods [7], including kebab meat [11] and ground beef [15]. Therefore, the objective of this study was to determine the prevalence of the foodborne pathogens *E. coli* and *Salmonella* in raw sausage and kofta products and ready-to-eat Hawawshi and Kofta meat products. Antibiotic resistance risks associated with isolated pathogens were also estimated.

Experimental

Ethical approval

All methods used in this study were approved by the Benha University Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee Research Ethics number (BUFVTM50-06-23).

Sample collection

In summary, a total of one-hundred random samples of four meat products (25 of each) were purchased from various butchers and restaurants in Benha city, Egypt, between July to December 2021. Burger and sausage were among the raw meat categories, while Hawawshi and kofta were included in the RTE category. Within one hour, samples were collected and delivered in sterile plastic bags in iceboxes to the lab for microbiological investigation [16].

Isolation and identification

Isolation and identification of Escherichia coli

MacConkey broth Enrichment at 37°C and 44°C and plating, respectively, at 37°C and 44°C on Eosin Methylene Blue agar (EMB) and Tryptone Bile Xglucoronide agar *(TBX agar)* were used for the screening of enteropathogenic *E. coli* in accordance with [17]. The presumptive *E. coli* was identified using the GNI card (Gram-negative identification) of the automated VITEK2 system (compact model, bioMérieux).

Isolation and identification of Salmonella

A standard cultivation method recommended by (ISO, 2017) was used for the isolation and identification of Salmonella, with some modifications applied in our previous study [7]. The samples were incubated overnight at 37°C the same day they arrived in the laboratory. The pre-enrichment broth was then transferred to 10 ml of Rappaport Vassiliadis (RV) broth (Lab M, UK) and incubated for 18-24 hours at 41°C. Each suspected turbid tube from the selectively enriched medium

was streaked onto selective xylose lysine deoxycholate (XLD) agar plates (BioLife, USA) and incubated at 37°C for 18-24 hours to isolate *Salmonella* species. To confirm atypical colonies, Rappaport Vassiliadis medium (MSRV) semi-solid agar, Hektoen enteric (HE) agar, and Bismuth Sulfite Agar (Wilson Blair) were used. The presumptive *Salmonella* isolates were identified using the GNI card (Gram-negative identification) of the automated VITEK2 system (compact model, bioMérieux).

Serological Identification

Presumptive positive samples of *E. coli* and *Salmonella* were subcultured onto nutrient agar slopes and sent to the Animal Health Research Institute Laboratory (Dokki Giza, Egypt) for serotyping. Serotyping of *E. coli* followed (Ewing, 1986) for *Enterobacteriaceae* identification. *Salmonella* serology was done according to Kauffmann–White serotyping scheme [18] using slide agglutination tests with commercial polyvalent and monovalent somatic and flagellar antisera (DENKA SEIKEN Co., Japan).

Safety assessment of studied meat products

All raw and ready-to-eat (RTE) meat products were tested for safety in accordance with Egyptian Organization of Standardization (EOS) safety standards, ES:1973/2005 frozen balls (Kofta) specifications [19], ES:1972/2005 frozen sausage specifications [20], ES:1688/2005 specified for frozen burger [21], ES: 1694/ 2005 minced meat specification [22] ES: 4334:2004 fresh meat specification[23] and ES:2911/2005 frozen poultry sausage specifications [24], to determine whether they were fit for human consumption.

Disc diffusion antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was used to test antimicrobial susceptibility. All findings were interpreted in accordance with the Clinical and Laboratory Standards Institute [25]. The current experiment attempted to follow the recommended CLSI antibiotics criteria for characterizing the phenotypic resistance of the targeted pathogens; however, the use of these guidelines was affected by the disc's availability during the study time. Here, all *E. coli* (n=26) and *Salmonella* (n=14) isolates were tested for phenotypic resistance to nine commonly used antibiotics, which are both important and critical in the Egyptian veterinary and medical sectors. The five antibiotic classes included beta-lactams such as ampicillin (AMP, 30 µg) and penicillin (PCN,10 IU); aminoglycoside such as gentamicin (GEN,10 µg), kanamycin (KAN, 5 µg) and neomycin (NEO, 30 µg); fluoroquinolones such as ciprofloxacin (CIP, 5 µg) and enrofloxacin (ENR, 5 µg); macrolide such as erythromycin (ERY, 15 µg); and third-generation cephalosporin that involved ceftriaxone (CTR, 30 µg). Bacterial isolates that showed resistance to at least three different classes of antimicrobial drugs were deemed multidrug-resistant (MDR).

Molecular Characterization of targeted pathogens for antimicrobial resistance

The QIAamp DNA Mini Kit (Cat. No. 51304, Qiagen, Hilden, Germany) was used to perform totally practical nucleic acid purification from several types of bacterial colonies according to the manufacturer's procedure in 20 minutes. Table S1 lists all primers and conditions for polymerase chain reaction (PCR) amplification of various targeted genes from *E. coli* isolates and *Salmonella* isolates, including *blaTEM*, *blaCTX*, *blaSHV*, *norA*, and *mcr1*. For PCR, a 25 µL reaction mixture containing 12.5 µL of Emerald Amp GT PCR Master Mix (Cat. No. RR310A, Takara Bio, Shiga, Japan), 1 µL (20 pmol / µL) of each primer (Midland Certified Reagent Company_ oilgos, USA), 5 µL target DNA, and the remaining volume adjusted with deionized PCR grade water was prepared. The reaction was carried out using a thermal cycler T3 Biometra Trio. Analytik Jena, Jena, Germany (Biometra). Following the completion of the amplification, the PCR products (6 µL) were processed through 1.5% agarose gel electrophoresis, stained with ethidium bromide, and viewed under UV light in a gel documentation system. Alpha Innotech is based in Kasendorf, Germany.

Statistics analysis

Statistical analysis was performed using SPSS Statistics 20 (SPSS Inc., USA). The collected results from various sources were computed using descriptive statistics such as frequency, percentage, and/or proportion

Results

The prevalence of various pathogens in samples of raw and ready-to-eat (RTE) meat products is compared in Table 1. The overall prevalence of the pathogens investigated *E. coli*, and *Salmonella*, was 26% and 14%, respectively. Raw meat products had the highest *E. coli* prevalence (57.7%, 15/26) as compared to RTE meat products. Similarly, raw meat products had a higher *Salmonella* isolation rate (57.14%, 8/14) than RTE meat products. Within the raw meat products category, no significant pathogen relationship was seen, with Burger samples containing higher rates of *Salmonella* contamination and sausage samples generated higher rates of *E. coli* contamination. In compared to Hawawshi, Kofta was found to have higher levels of *Salmonella* contamination, and lower levels of *E. coli*, upon a closer assessment of RTE meat items. Table 1 and Table S2 also depict the serotypes and categories of the targeted pathogen isolated from raw and RTE meat products. All 26 *E. coli* isolates were of five different serotypes (O26, O55, O111:H4, O124, O126) and three pathotypes, enterohemorrhagic (EPEC) 34.6%, enteropathogenic (EPEC) 46.15%, and enteroinvasive (EIEC) 19.23%. Serotype O26

had the highest prevalence (34.6%) and distribution in the four products evaluated, notably RTE. *Salmonella enterica* serovar Enteritidis had the highest prevalence and distribution (57.14%) in the four products studied, followed by *Salmonella enterica* serovar Typhimurium, which was evenly distributed between raw and RTE categories.

All ready-to-eat (RTE) meat products, including Hawawshi and kofta, that are contaminated with one of the four pathogens, *E. coli* (22%, 11/50), *Salmonella* (12%, 6/50), are not permitted for human consumption under Egyptian Organization of Standardization (EOS) safety standards, ES:1973/2005 frozen balls (Kofta) specifications, ES:1972/2005 frozen sausage specifications, ES:1688/2005 specified for frozen burger and ES:2911/2005 frozen poultry sausage specifications. All *Salmonella*-contaminated Hawawshi and Kofta samples were positive for *E. coli* contamination. As a result, the overall number of inappropriate RTE meat products is 22% (11/50) (Table S2). *E. coli* (30%, 15/50) and *Salmonella* (16%, 8/50) shall be free in raw meat products according to EOS criteria (Table S2). Except for two burger samples, all *Salmonella*-contaminated raw samples tested positive for *E. coli*. The total number of incompatible raw meat items was 30% (15/50). A total of 28 raw and ready-to-eat (RTE) beef items were deemed unfit for human consumption.

All *E. coli* and *Salmonella* isolates were subjected to phenotypic and genetic characterization in accordance with the current study's goal and earlier investigation findings [7] to clarify patterns of priority pathogens resistance, isolated from raw compared to RTE meat products, to important antibiotics utilized in the veterinary and medical sectors. Following the disc diffusion test, five patterns of antibiotic resistance were identified: 19 (73.1 %, *n*=26) *E. coli* isolates were multidrug resistant, two were resistant to all five antibiotic classes utilized in the current investigation, and seven and ten isolates were resistant to four and three classes, respectively (Fig. 1 and Table S2). The remaining seven isolates were resistant to two types of antibiotics. Ten MDR *E. coli* isolates were identified from raw meat (four burgers and six sausage samples), whereas nine were obtained from RTE products (six hawawshi and three kofta). The multiple antibiotic resistance index (MAR) average for raw meat products was 0.48, which was lower than the MAR average for RTE items of 0.55. Additionally, one of the RTE Hawawshi *E. coli* isolates received the highest MAR values of 0.89, while the Burger isolate recorded 0.78 (Table S2). Burger and sausage exhibited MAR index ranges of 0.30 to 0.78 and 0.30 to 0.67, but Hawawshi and kofta produced ranges of 0.30 to 0.89 and 0.30 to 0.78, respectively. Most *E. coli* isolates (20-26), particularly those isolated from raw products, were resistant to beta-lactam and macrolide classes, while approximately half of the isolates (11-13) were resistant to gentamicin, neomycin, and enrofloxacin, and only a few isolates (3-5) were resistant to cephalosporins, ciprofloxacin, and kanamycin (Fig. 1 and Table S2).

A 35.71% (5/14) of the *Salmonella* isolates were MDR; two of these were from RTE meat products (one hawawshi and one kofta), while the other two were obtained from raw meat (two burgers and one sausage). *Salmonella* only displayed two MDR patterns; four isolates showed resistance to four classes, whereas one strain showed resistance to three classes. The remaining nine were made up of five that were resistant to two classes and the other four to one. The isolates of raw sausage and burger items had the highest MAR index values, 0.67, as well as higher mean values (0.38 vs. 0.31 for RTE products) (Table S2). *Salmonella* isolates showed the highest level of resistance to the beta-lactam class and the lowest level of resistance to gentamicin, cephalosporins, kanamycin, and fluoroquinolones (Figure S1 and Table S2).

Five MDR *E. coli* isolates were selected for genetic characterization of genes that confer resistance to important antibiotics, including *bla*TEM, *bla*CTX, *bla*SHV, *norA*, and *mcr1* (Table 2 and Figure S1). The selection criteria considered all pathogen serotypes recovered from the four products under investigation, as well as the highest MAR score and resistance patterns. Fortunately, there were only five MDR *Salmonella* isolates, roughly distributed among the four products under examination. The findings revealed that all of the *E. coli* isolates under investigation carried the *bla*CTX and *bla*SHV genes, and that *mcr1* was expressed in two of the raw-derived isolates and one of the RTE-derived isolates. Only two raw recovered *E. coli* isolates expressed *bla*TEM, and *norA* was identified solely in the RTE Hawawshi *E. coli* isolate. Two isolates derived of raw burger and sausage samples co-expressed extended spectrum beta-lactams resistance (ESBL) conferring genes, *bla*TEM, *bla*CTX, *bla*SHV, where the sausage derived isolate shared also *mcr1*.

The genetic analysis of MDR *Salmonella* isolates revealed that *norA* was expressed in four isolates, three raw-derived isolates and one RTE, whereas *bla*CTX or *mcr1* was expressed in three samples, raw (2) and RTE (1) isolates (Table 2 and Fig. S1). None of the MDR *Salmonella* isolates co-expressed the three β-lactamase genes; they either had *bla*TEM and *bla*CTX or *bla*CTX and *bla*SHV genes, but these two isolates additionally displayed *mcr1* and/or *norA*. All of the targeted genes were expressed by various raw-derived isolates, however *bla*TEM was not demonstrated by any of the RTE-derived *Salmonella* isolates.

Discussion

The results of the current study showed that the incidence rates of *E. coli* were higher in raw meat products (30%) than in RTE meat products (22%), but since RTE meat products will not undergo further processing, the risk associated with receiving such contaminated RTE meat products would be significantly higher. Among the identified *E. coli* isolates, serotypes such as O26 (34.62%, 9/26) and O111 (19.23%, 5/26) have the potential of Shiga-toxin (Stx) production. Furthermore, serogroups O26 and O111, coupled with serotypes O45, O103, O121, and O145, are among "the big six" EHEC and have been clinically linked to human disease [26]. In humans, Stxs induces severe EHEC disease by cleaving ribosomal RNA, limiting protein synthesis, and killing poisoned epithelium or endothelial cells [27,28]. These strains, known collectively as non-O157 strains, together with serotypes O26:H11 or H-, O103:H2, O111:H-, O117:H7, O121:H19, and O146:H21, have been linked to significant illness in humans [29]and have been found to be more widespread in animals and as food pollutants [26,30]. Interestingly, *E. coli* O111:H-, like O146:H21and O26, has been designated as atypical enteropathogenic *E. coli* (aEPEC), classically cause diarrhea in children, and both were previously classified as enterohaemorrhagic *E. coli* that evolved the ability to generate shiga toxin and incriminated in bloody diarrhea and hemolytic uremic syndrome (HUS) in various areas of the world [31–34]. Despite the fact that the majority (59.2%) of STEC-infected patients, such as O26 and O111, had nonbloody diarrhea, 14.3%, 3.5%, and 8.7% of patients experienced bloody diarrhea (BD), HUS, and stomach discomfort without diarrhea. Asymptomatic excreters could also be generated (11.0%) from recovered patients [32]. In developing countries, EPEC, including O55 and O126 strains, is the most frequent bacterial cause of infants' prolonged diarrhea, sporadic and outbreak cases [35]. Here, the *E. coli* O55 (23.1%, 6/26) were only isolated in raw products, in contrast to the one isolate of *E. coli* O126 that was identified in RTE meat products. Despite belonging to the same O serogroup, the strains O55: H6 and O55: H7 were found to contain typical and atypical lineages. Previous sequencing studies showed that the O55:H7, which acquired the Stx2 gene and has expanded globally and is a growing public health threat in Europe, was the progenitor of both motile O157:H7 (beta-glucuronidase and Sorbital negative features) and nonmotile O157:H clones [36,37]. Similarly, previous research suggested that O126 serogroup could contain ETEC and Enteroaggregative *E. coli* (EAggEC) virulence factors in addition to the traditional tEPEC O126:H2 subtype [35]. Atypical EPEC is more closely related to Shiga-toxin producing *E. coli* (STEC), and both strains considered to be emerging pathogens [31,34]. However, if they were to acquire the EPEC attaching and effacing (A/E) factor plasmid encoding bundle-forming pilus (BFP), which is only seen in tEPEC [38], the public health relevance of such emerging pathogens would be amplified 32. In addition, unlike typical EPEC, which is limited to human reservoir, atypical EPEC could adopt humans as well as animals [38]. The fact that aEPEC, like tEPEC, both have a chromosomal pathogenicity island called the locus of enterocyte effacement (LEE), which encodes important virulence proteins such intimin proteins that are necessary for EPEC adhesion to epithelial host cells, makes it even more problematic [39]. ECDC considered that any RTE product contaminated with an isolate of one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) and molecularly contain *vtx* either with *eae* (intimin production)- or [*aaiC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes presenting a potentially high risk for diarrhea and HUS [40].

In the current study, five isolates of Enteroinvasive *E. coli* O124 were identified, accounting for 19.23% of the overall *E. coli* incidence rate. Enteroinvasive *E. coli* is mostly spread through oral-fecal route from the primary reservoir, which is a human carrier [41]. This pathotype lacks animal reservoirs, and its spread is primarily attributed to poor personal hygiene, particularly in developing nations. *E. coli* O124 was the most frequent isolated biliary pathogenic bacterium, and it was the major pathogen implicated in the development and/or progression of acute cholecystitis. Previous research demonstrated that *E. coli* O124, K72 strain, damages intestinal membrane major integral proteins, CLDN2 and Occludin, at tight junctions for invasion to host organs, and that it may possibly play a role in colon carcinogenesis [42,43]. The existence of this pathotype indicates carrier faecal contamination of raw and ready-to-eat meat products, which could have serious health consequences for consumers.

Salmonella is mostly found in animals, and animal-based foods are the principal route of infection to humans [3]. Understanding the global epidemiology of *Salmonella* serovars is so critical for controlling and tracking this pathogen [44]. The most frequently isolated serovars from foodborne outbreaks worldwide linked to the consumption of contaminated poultry, pig, and beef products were *Salmonella* Enteritidis and Typhimurium [45,46]. According to the most recent surveillance in Egypt's Al Qalyubia province, RTE food products had a higher *Salmonella* incidence rate (8.93 %) than raw foodstuffs (7.67 %) [7]. Current findings based on animal-derived foods indicate that ready-to-eat meat products have a somewhat lower rate (12%) than raw meat products (16%), but this rate in RTE meat products poses a potentially significant risk of Salmonellosis. The current *Salmonella* recovery

rates, either raw or RTE, are higher than in prior studies in the same [7], other Egyptian regions (4.3%) [47] and across 27 African countries (5.3%) [48]indicating a rising pattern. The overall prevalence of *Salmonella* in raw and ready to eat (RTE) turkey from retail outlets in the United States was 2.2% (21/959), with contamination being substantially connected to raw samples (4.1%,14/345) rather than RTE (1.1% [7/614]) and sampling month ($p < 0.05$) [49]. *Salmonella* excretion from carrier livestock is one of the principal sources of *Salmonella* in farms and slaughterhouses, leading in contamination of surroundings and, of course, associated raw materials [50]. Foodborne diarrhoeal disease agents, primarily diarrhoeal and invasive non-typhoidal *Salmonella enterica* (NTS), were accountable for 230,000 deaths [3].

The strategies established by *Enterobacteriaceae*, particularly *E. coli*, to combat antibiotics are the most powerful and diverse [51]. *E. coli* is *recognized* as a key reservoir of antibiotic resistance because it is capable of rapidly acquiring and distributing genetic materials and, when stressed, readily transmits those genetic materials to enteric pathogens share the same living environment such as *Salmonella*, *Yersinia*, *Vibrio*, and *Shigella* species [52–54]. Thus, the prevalence of antibiotic resistance in *E. coli* is an excellent predictor of antibiotic resistance in each community [51,55]. More than 90% of *E. coli* isolates from major food animals (including healthy broiler chickens, cattle, and pigs) in Korea between 2010 and 2020 exhibited high resistance to quinolones and cephalosporins [56], and comparable commensal isolates were also detected from multiple hosts and environmental compartments [57]. These earlier findings may help to explain the current high proportion of MDR phenotype in the studied *E. coli* isolates (73.1%, 19/26) as well as the distribution and co-expression of genetic determinants of resistances to multiple critical antibiotic classes. The most similar results were in KSA, where 120 *E. coli* isolates from food had a prevalence of 22.22% and included O26: K60, O128: K67, O111: K58, O126: K58, O55: K59, O86: K61, and O157: H7, all serotypes had 100% resistance to erythromycin, amoxicillin-clavulanic acid, and penicillin, and genetically *blaTEM* and *blaSHV* were most prevalent genes [58]. In India, 27 isolates were verified as *E. coli*, 5 of which were ESBL positive; the most abundant genes included *blaTEM* (40.68%), *blaCTX* (32.20%), *blaSHV* (10.17%), and *blaNDM* (10.17%) [59]. A genomic analysis of *E. coli* isolated from infected poultry in the Czech Republic [60]and outpatients in Egypt [61]with community-acquired UTIs showed that the majority of the sequenced strains had the MDR phenotype 69.5% and 62.5%, respectively, with beta-lactam and quinolone resistance being the most prevalent [60]. They found that chromosomal *gyrA* mutations and TEM-type beta-lactamase genes were among the most prevalent resistance gene combinations [60], in contrast to recent findings indicating *blaCTX* and *blaSHV* were abundant and associated with *mcr1* in most studied isolates. ESBLs hydrolyze a variety of β-lactam antibiotics, including some that are resistant to newer β-lactams, carbapenems, which totally render β-lactams therapy options ineffective. Plasmids, but also other mobile genetic elements such as transposons and gene cassettes, have been widely recognized to play a significant role in the spread of resistance genes, ESBL/*AmpC*, produced by commensal and pathogenic *E. coli* [62]widely distributed in multiple food sources [63–67]. Colistin resistance in *E. coli* appears to be linked to the global usage of colistin in veterinary medicine [68]. At first, chromosomal gene mutations led to colistin-resistant mechanisms, but plasmid-mediated and transmissible colistin resistance (*mcr*) led to more significant problems [68]. In an earlier Egyptian study, out of 210 *E. coli* strains (150 from raw beef and 60 from RTE beef products), eight (six strains from five raw beef and two from two RTE sausage sandwiches) were colistin-resistant and carried the *mcr*-1 gene, while five were cefotaxime-resistant and carried the *blaCTX*-M-28 gene, and three carried both *mcr*-1 and ESBL [69]. Quinolones are commonly used antimicrobials for the treatment of bacterial infections. There are three mechanisms that contribute to quinolone resistance: chromosomal mutations and/or plasmid gene uptake that change the topoisomerase sites, modify the quinolone, and/or diminish drug accumulation by either decreased uptake or greater efflux. The current study focused on *norA*-mediated efflux-pump resistance mechanisms, while previous research found that mutations in the *gyrA* and *parC* genes, coupled with the transmission of plasmid-mediated quinolone resistance genes, are the most common mechanisms implicated in high-level quinolone resistance [70,71]. ESBL-producing genes mostly co-circulate with genes encoding resistance to other kinds of antibiotics, such as fluoroquinolones, trimethoprim-sulfamethoxazole, and aminoglycosides, reducing antibiotic options even further [72]. Earlier investigations demonstrated that many cattle-derived EHEC isolates, such as *E. coli* O26 and O111 strains in Korea and O157 in the United States [73], were resistant to many different antibiotics, with the majority of them being Shiga toxin-producing *E. coli* (STEC) [74]. Antibiotic resistance was also widespread in *E. coli* O26, O103, O111, O128, and O145 strains isolated from humans, food animals, and food from diverse countries [73,75]. The current study's notable finding is that RTE-derived *E. coli* co-expresses genetic resistance determinants such as ESBL, *mcr1*, and *norA*, as well as a high percentage of MDR phenotype, which could indicate an increasing trend in antibiotic resistant *E. coli* inhabiting raw and RTE meat products in the area under investigation. The high prevalence and

resistance found here raises the risk of transmission between animals and people, complicating treatment choices. However, due to gene availability limitations, the current study still requires sequencing of MDR *E. coli* isolates to completely define other virulence and resistance features that were not addressed here.

On the other hand, compared to *E. coli*, the current study's *Salmonella* isolates had a lower MDR to administered antibiotics (35.71%). The current MDR rate is lower than the rate found in earlier studies conducted in the Egyptian governorate of Mansoura (68.1%) [47], and it was 100% for *Salmonella* recovered from broiler carcasses and humans [76], and retail fish [77]. Additionally, none of the MDR *Salmonella* isolates co-expressed the three β-lactamase genes; rather, they either had the genes for *blaTEM* and *blaCTX* or *blaCTX* and *blaSHV*. Unfortunately, these two isolates additionally carried *mcr1* and/or *norA*. Three MDR isolates also originated from raw foods. In a recent Egyptian study, all cases of colistin resistance in *Salmonella enterica* were found in raw meat (cattle and rabbit) [7], but the situation is much worse here because *mcr1* was found in three different isolates from three different products, including RTE meat kofta. This indicates that colistin has been used continuously in food animals and that colistin resistance is on the rise. In the USA, *Salmonellae* from raw turkey showed stronger antimicrobial resistance (53%) compared to those from RTE products (33%), but 62% of *Salmonellae* (86% from RTE, 50% from raw meats) showed multidrug resistance [49]. Meat samples are known to be one of the main sources of *Salmonella* infections, so the frequent survey is crucial for preventing and controlling *Salmonella* contamination and serious illnesses [78].

E. coli was first among the top six pathogens causing resistance-related death. In 2019, the top six AMR pathogens caused 929000 deaths; overall, AMR caused 3.57 million deaths. six additional pathogen-drug combinations, including but not limited to third-generation cephalosporin-resistant *E. coli* and fluoroquinolone-resistant *E. coli*, caused 50.000-100.000 deaths. All of these AMR-related dangers have elevated AMR to the forefront of public health concerns in the twenty-first century [13,14]. *Salmonellae* have been estimated to have high risk levels in all food categories (raw and processed), with the exception of preserved meat products such dry fermented sausages. Ingredients that are susceptible to early-stage *Salmonella* spp. and EHEC contamination and poor fermentation were predicted to have intermediate risk ratings [79]. Globally, ESBL-producing Gram-negative organisms, particularly *E. coli*, will continue to be an important root cause of antibiotic resistance [72].

Conclusion

Escherichia coli and *Salmonella* were identified in 26% and 14% of the samples, respectively, and both were more prevalent in the raw products, at 57.7% and 57.14%. All twenty-six *E. coli* isolates belonged to one of five serotypes (O26, O55, O111:H4, O124, O126) and one of three pathotypes: enterohemorrhagic (EHEC) (34.6%), enteropathogenic (EPEC) (46.15%), and enteroinvasive (EIEC) (19.23%). A total of twenty-eight were ruled unfit for human consumption, including 17 raw and 11 RTE meat. MDR *E. coli* (73.1%) (35.71%) and *Salmonella* were more frequently found in raw foods. The multiple antibiotic resistance index (MAR) was 0.48 on average for raw goods and 0.55 for RTE meat. The *blaCTX* and *blaSHV* genes were present in all *E. coli* isolates, and *mcr1* was expressed in three of them—two raw and one RTE meat. Only the RTE Hawawshi *E. coli* isolate had *norA*, and only two raw recovered *E. coli* isolates expressed *blaTEM*. Two raw-derived isolates co-expressed *blaTEM*, *blaCTX*, and *blaSHV*, which conferred extended spectrum beta-lactams resistance (ESBL), and one of them also shared *mcr1*. The *norA* gene predominated in four MDR *Salmonella* isolates, raw (3) and RTEM (1), whereas *blaCTX* or *mcr1* occurred in three isolates, raw (2) and RTEM (1). The MDR *Salmonella* isolates co-expressed the *blaTEM* and *blaCTX* or *blaCTX* and *blaSHV* genes, while these two isolates also harbored *mcr1* and/or *norA*. All of the targeted genes were expressed by different raw-derived isolates, but none of the RTEM *Salmonella* isolates exhibited *blaTEM*. The current findings of high resistance levels in the studied pathogen confirm that antibiotics are still used in food-producing animals, and if transmitted directly from animal to human or indirectly through the food chain, can cause serious diseases in humans and complicate future therapeutic options under development.

List of abbreviations

Ready-to-eat (RTE); Shiga toxin-producing *Escherichia coli* (STEC); enterohaemorrhagic *Escherichia coli* (EHEC); enteropathogenic (EPEC); enteroinvasive *Escherichia* coli (EIEC); Shiga toxin (Stx); typical EPEC (tEPEC); atypical EPEC (aEPEC); heat-labile (LT); heat-stable (ST); ready-to-eat food/drink [REF/D].

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Conflicts of interest

Competing Interests: the authors have no relevant financial or non-financial interests to disclose.

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TABLE 1. Pathogen occurrences, serotypes, and categories detected in raw and ready-to-eat (RTE) meat products.

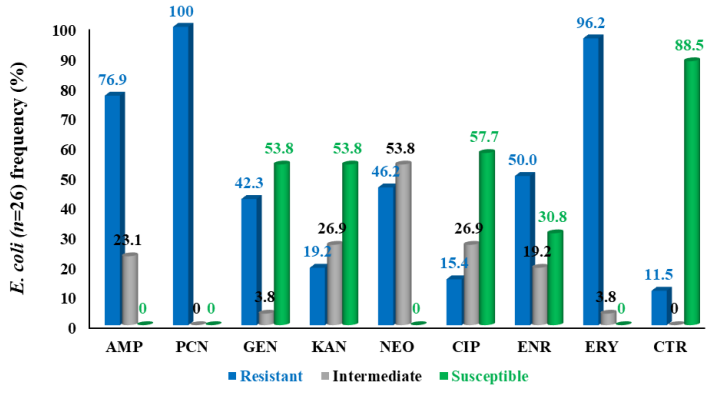
Pathogen		Raw (n=50)					RTE (n=50)				
		Burger[1] (n=25)		Sausage[1] (n=25)		Subtotal[1] (n=50)	Hawawshi[1] (n=25)		Kofta[1] (n=25)		Subtotal[1] (n=50)
Serotypes	**Groups**	**No.**	**%[1]**	**No.**	**%**	**%**	**No.**	**%**	**No.**	**%**	**%**
E. coli											
O26	EHEC[2]	2	8	2	8	8	2	8	3	12	10
O55	EPEC[2]	4	16	2	8	12	ND	ND	ND	ND	ND
O111:H4	EPEC	ND[4]		3	12	6	2	8	ND	ND	4
O124	EIEC[2]	1	4	1	4	4	2	8	1	4	6
O126	EPEC	ND	ND	ND	ND	ND	ND	ND	1	4	2
Total		7	28	8	32	30	6	24	5	20	22
Salmonella species	Serogroup										
S. Enteritidis [3]	D1	3	12	2	8	10	1	4	2	8	6
S. Typhimurium [3]	C1	2	8	1	4	6	1	4	2	8	6
Total		5	20	3	12		2	8	4	16	

[1] The incidence was determined per product by dividing positive samples by 25, and the category subtotal was obtained by dividing positive samples of either raw or RTE products by 50.
[2] EPEC = Enteropathogenic *E. coli*; EIEC = Enteroinvasive *E. coli*.
[3] *Salmonella enterica* Serovar Enteritidis O antigens were 1,9,12; while H antigens were g, m:-; the *Salmonella enterica* Serovar Typhimurium O antigens were 6,7,14; while H antigens were r:1,5.
[4] ND, not detected

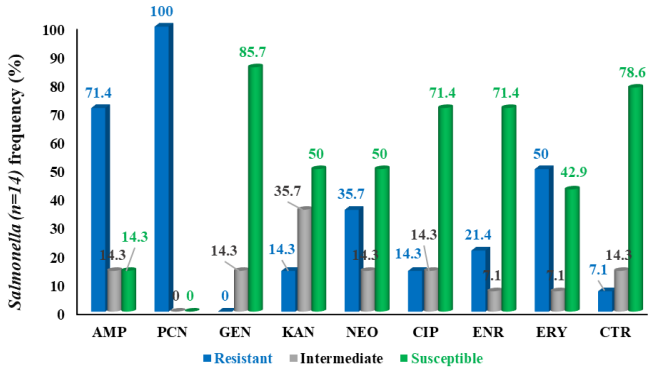
TABLE 2. The phenotypic and genotypic antibiotic resistance profiles of *E. coli* (n = 5) and *Salmonella* (n = 5) isolated from raw and ready-to-eat (RTE) meat products.

Pathogen/ Serotypes	Origin	Resistance	
		Phenotypes	**Genes**
Escherichia coli			
E. coli O26	Burger	AMP, PCN, GEN, NEO, ERY, CTR	*bla*TEM, *bla*CTX, *bla*SHV
E. coli O55	Burger	AMP, PCN, GEN, KAN, NEO, ERY	*bla*CTX, *bla*SHV, *mcr1*
E. coli O111:H4	Sausage	AMP, PCN, GEN, NEO, ERY, CTR	*bla*TEM, *bla*CTX, *bla*SHV, *mcr1*
E. coli O124	Hawawshi	AMP, PCN, NEO, ENR, ERY	*bla*CTX, blaSHV, *norA*, *mcr1*
E. coli O126	Kofta	AMP, PCN, GEN, NEO, ERY, CTR	*bla*CTX, *bla*SHV
Salmonella			
S. Enteritidis [2] (n=3)	Burger	AMP, PCN, NEO, ENR, ERY	*bla*TEM, *norA*, *mcr1*
	Sausage	AMP, PCN, KAN, NEO, CIP, ERY	*bla*TEM, *bla*CTX, *norA*, *mcr1*
	Hawawshi	NEO, ENR, ERY	*norA*
S. Typhimurium (n=2)	Burger	AMP, PCN, NEO, CIP, ENR, ERY	*bla*CTX, *norA*
	Kofta	AMP, PCN, NEO, ERY, CTR	*bla*CTX, *bla*SHV, *mcr1*

E. coli, *Escherichia coli*; S. Typhimurium, *Salmonella* Typhimurium
AMP, ampicillin; PCN, penicillin; GEN, gentamicin; KAN, kanamycin; NEO, neomycin; CIP, ciprofloxacin; ENR, enrofloxacin; ERY, erythromycin; CTR, ceftriaxone.



A



B

Fig. 1. The antimicrobial susceptibility profile of *E. coli* (A) and *Salmonella* (B) isolated from raw and ready-to-eat (RTE) meat products using disc diffusion test. Antibiotics tested: ampicillin (AMP, 30 µg), penicillin (10 IU), gentamicin (GEN,10 µg), kanamycin (KAN, 5 µg), neomycin (NEO, 30µg), ciprofloxacin (CIP, 5 µg), enrofloxacin (ENR, 5 µg), erythromycin (ERY, 15 µg), and ceftriaxone (CTR, 30 µg).

Figures legends

Fig. 1. The antimicrobial susceptibility profile of *E. coli* (*n* = 26) and *Salmonella* (*n* = 14) isolated from raw and ready-to-eat (RTE) meat products using disc diffusion test. Antibiotics tested: ampicillin (AMP, 30 µg), penicillin (10 IU), gentamicin (GEN,10 µg), kanamycin (KAN, 5 µg), neomycin (NEO, 30µg), ciprofloxacin (CIP, 5 µg), enrofloxacin (ENR, 5 µg), erythromycin (ERY, 15 µg), and ceftriaxone (CTR, 30 µg)

Fig. S1. PCR characterization of five antibiotic resistant genes in ten *Escherichia coli* and *Salmonella* isolates from raw and ready-to-eat meat products with expected amplicon size. The amplified genes were a: *blaCTX* gene at 307 bp; b: *blaTEM* gene at 516 bp; c: *blaSHV* gene at 1233bp; d: *mcr1*gene at 305 bp; e: *norA* gene at 704 bp. Lane M: 100 bp DNA ladder; C+: Positive control; C-: Negative control; Isolates of lanes from 1-10 in each gel were recorded for each targeted gene. The codes for *E. coli* isolates are 1, BE4, 2, BE19, 3, SE13, 4, HE11, and 5, KE6, while *Salmonella* isolates are 6, BS3, 7, BS12, 8, SS15, 9, HS1, and 10, KS6.

Supplementary Tables and Figures

TABLE S1. PCR primers and conditions for *Escherichia coli*, and *Salmonella* species gene amplification

Target gene	Primer	Sequences (5' to 3')	Amplicon size (bp)	Annealing Temperature	Reference
blaTEM	Forward	ATCAGCAATAAACCAGC0	516	55˚C	[1]
	Reverse	CCCCGAAGAACGTTTTC			
blaCTX	Forward	CGC TTT GCC ATG TGC AGC ACC	307	54˚C	[2]
	Reverse	GCT CAG TAC GAT CGA GCC			
blaSHV	Forward	GGTTATTCTTATTTGTCGCTTCTT	1233	54˚C	[3]
	Reverse	TACGTTACGCCACCTGGCTA			
norA	Forward	TTCACCAAGC CATCAAAAAG	704	60˚C	[4]
	Reverse	GCACATCAAA TAACGCACCT			
mcr1	Forward	CGGTCAGTCCGTTTGTTC	305	60˚C	[5]
	Reverse	CTTGGTCGGTCTGTAGGG			
	Reverse	TGCTTGACCACTTTTATCAGC			

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TABLE S2. Antibiogram and multidrug resistance (MDR) profiles of *Escherichia coli* and *Salmonella enterica* subspecies *enterica* isolated from raw and ready-to-eat meat products.

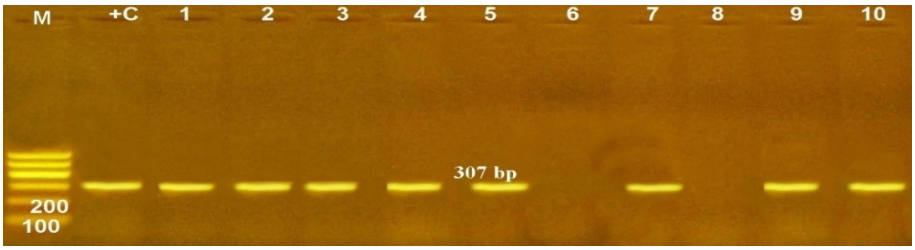
		E. coli Antibiogram										
Sample ID	Serotypes	AMP[1]	PCN	GEN	KAN	NEO	CIP	ENR	ERY	CTR	MAR[1]	Resistance genes
B1	O26	I	R	R	I	R	R	R	R	S	0.67	
B3	O55	R	R	S	S	I	I	R	I	S	0.33	
B4	O26	R	R	R	I	R	I	R	R	R	0.78	*bla*TEM, *bla*CTX, *bla*SHV
B11	O55	R	R	S	S	I	S	S	R	S	0.33	
B12	O55	I	R	S	S	I	R	R	R	S	0.44	
B17	O124	R	R	S	S	I	S	S	R	S	0.33	
B19	O55	R	R	R	R	R	S	I	R	S	0.67	*bla*CTX, *bla*SHV, *mcr1*
S5	O111:H4	I	R	R	I	R	I	R	R	S	0.56	
S13	O111:H4	R	R	R	I	R	I	I	R	R	0.67	*bla*TEM, *bla*CTX, *bla*SHV, *mcr1*
S15	O55	R	R	S	S	I	S	S	R	S	0.33	
S16	O111:H4	I	R	S	I	R	S	I	R	S	0.33	
S20	O26	R	R	S	S	I	R	R	R	S	0.56	
S22	O55	R	R	S	S	I	S	S	R	S	0.33	
S23	O26	R	R	R	S	I	S	I	R	S	0.44	
S25	O124	R	R	R	S	I	S	S	R	S	0.44	
H1	O26	R	R	R	S	I	S	S	R	S	0.44	
H3	O26	R	R	I	R	R	I	R	R	S	0.67	
H8	O111:H4	R	R	R	R	R	S	S	R	S	0.67	
H11	O124	R	R	R	R	R	R	R	R	S	0.89	*bla*CTX, *bla*SHV, *norA*, *mcr1*
H12	O124	I	R	S	S	I	S	R	R	S	0.33	
H16	O111:H4	R	R	S	S	I	S	R	R	S	0.44	
K2	O26	R	R	S	S	I	S	I	R	S	0.33	
K3	O124	I	R	S	R	R	I	R	R	S	0.56	
K6	O126	R	R	R	I	R	I	R	R	R	0.78	*bla*CTX, *bla*SHV
K13	O26	R	R	S	S	I	S	S	R	S	0.33	
K18	O26	R	R	S	I	R	S	R	R	S	0.56	
Resistant	Raw	11	15	7	1	6	3	6	14	2		
	RTE	9	11	4	4	6	1	7	11	1		
	Total	20	26	11	5	12	4	13	25	3		
Intermediate		6	0	1	7	14	7	5	1	0		
Susceptible		0	0	14	14	0	15	8	0	23		

Multidrug resistance (MDR)	
No. of classes	positive isolates
5	2
4	7
3	10
2	7
1	0

Salmonella Antibiogram												
Sample ID	Serotypes	AMP	PCN	GEN	KAN	NEO	CIP	ENR	ERY	CTR	MAR	Resistance genes
B3	*S*. Enteritidis	R	R	S	I	R	I	R	R	I	0.56	*bla*TEM, *norA*, *mcr1*
B8	*S*. Enteritidis	R	R	S	S	I	S	S	S	S	0.22	
B12	*S*. Typhimurium	R	R	S	I	R	R	R	R	S	0.67	*bla*CTX, *norA*
B17	*S*. Typhimurium	S	R	S	S	S	S	S	R	S	0.22	
B24	*S*. Typhimurium	R	R	S	S	S	S	S	S	S	0.22	
S15	*S*. Enteritidis	R	R	I	R	R	R	I	R	I	0.67	*bla*TEM, *bla*CTX, *norA*, *mcr1*
S22	*S*. Enteritidis	R	R	S	S	S	S	S	S	S	0.22	
S25	*S*. Typhimurium	I	R	I	R	S	S	S	S	S	0.22	
H1	*S*. Enteritidis	S	R	S	I	R	I	R	R	S	0.44	*norA*
H3	*S*. Typhimurium	R	R	S	S	S	S	S	R	S	0.33	
K2	*S*. Typhimurium	R	R	S	S	S	S	S	S	S	0.22	
K3	*S*. Enteritidis	I	R	S	S	S	S	S	S	S	0.11	
K6	*S*. Typhimurium	R	R	S	I	R	S	S	R	R	0.56	*bla*CTX, *bla*SHV, *mcr1*
K13	*S*. Enteritidis	R	R	S	I	I	S	S	I	S	0.22	
Resistant	Raw	6	8	0	2	3	2	2	4	0		
	RTE	4	6	0	0	2	0	1	3	1		
	Total	10	14	0	2	5	2	3	7	1		
Intermediate		2	0	2	5	2	2	1	1	2		
Susceptible		2	0	12	7	7	10	10	6	11		

Multidrug resistance (MDR)	
No. of classes	positive isolates
5	0
4	4
3	1
2	5
1	4

MAR, Multiple antibiotic resistance index = number of ineffective antibiotics/total number of antibiotics tested.
AMP, ampicillin; PCN, penicillin; GEN, gentamicin; KAN, kanamycin; NEO, neomycin; CIP, ciprofloxacin; ENR, enrofloxacin; ERY, erythromycin; CTR, ceftriaxone.



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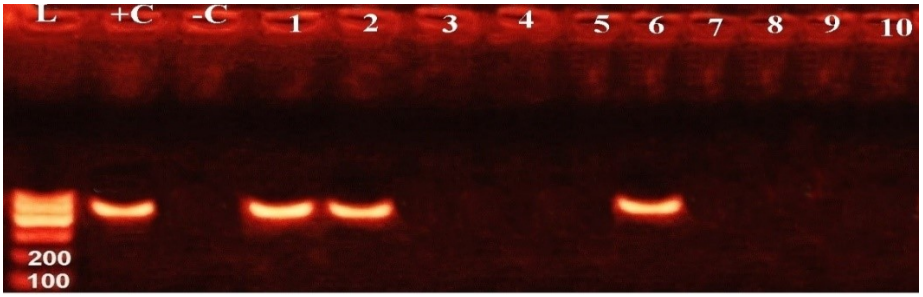

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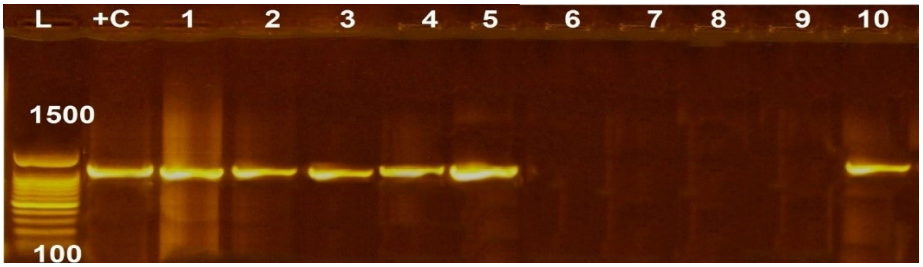

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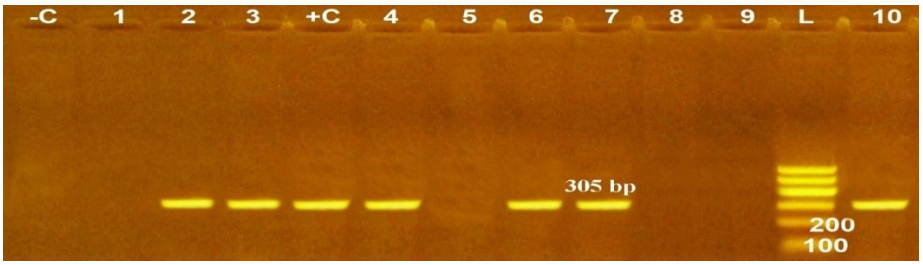

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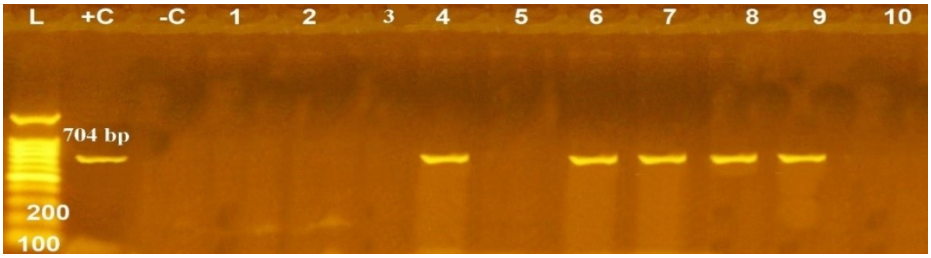

e.

Figure S1. PCR characterization of five antibiotic-resistant genes in ten *Escherichia coli* and *Salmonella* isolates from raw and ready-to-eat meat products with expected amplicon size. The amplified genes were a: *blaCTX* gene at 307 bp; b: *blaTEM* gene at 516 bp; c: *blaSHV* gene at 1233bp; d: *mcr1*gene at 305 bp; e: *norA* gene at 704 bp. Lane M: 100 bp DNA ladder; C+: Positive control; C-: Negative control; Isolates of lanes from 1-10 in each gel were recorded for each targeted gene. The codes for *E. coli* isolates are 1, BE4, 2, BE19, 3, SE13, 4, HE11, and 5, KE6, while *Salmonella* isolates are 6, BS3, 7, BS12, 8, SS15, 9, HS1, and 10, KS6.

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تواجد الإشريكية القولونية والسالمونيلا متعددة المقاومة للمضادات الحيوية من منتجات اللحوم النيئة والجاهزة للأكل، يزيد احتمالات الأمراض المنقولة بالغذاء في المستقبل ومن صعوبة العلاج

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الملخص

تهدف الدراسة إلى تحديد تواتر أنواع الإشريكية القولونية والسالمونيلا، ومقاومتها المظهرية والجزيئية المتعددة للمضادات الحيوية، في منتجات اللحوم النيئة والجاهزة للأكل. وباستخدام طرق العزل القياسية وتقنيات التنميط المصلي، تم فحص مئة منتج خام (الهامبرغر والنقانق) والجاهزة للأكل (الحواوشي والكفتة) بحثًا عن مسببات الأمراض المستهدفة. تم تحديد العناصر الجينية المرتبطة بخصائص المقاومة للمضادات الحيوية بواسطة PCR. تم التعرف على الإشريكية القولونية والسالمونيلا في 26% و14% من العينات على التوالي، وكان كلاهما منتشراً في المنتجات الخام بنسبة 57.7% و57.14%. وكانت الإشريكية القولونية متعددة المقاومة للمضادات الحيوية (73.1%) والسالمونيلا (35.71%) موجودة بشكل متكرر في الأطعمة النيئة. كانت جينات *blaCTX* و*blaSHV* موجودة في جميع عزلات الإشريكية القولونية الخمسة التي تم اختبارها، وتم التعبير عن mcr1 في ثلاثة منها - اثنان خام وواحد في الجاهزة للأكل. شارك اثنان من الإشريكية القولونية المشتقة من المنتجات الخام في التعبير عن *blaTEM* و*blaCTX* و*blaSHV*، كما شارك أحدهما أيضًا في *mcr1*. ساد جين *norA* في أربع من خمس عزلات من السالمونيلا متعددة المقاومة للمضادات الحيوية، خام (3) والجاهزة للأكل (1)، بينما تواجد blaCTX أو mcr1 في ثلاث عزلات، خام (2) والجاهزة للأكل (1). شارك اثنان من السالمونيلا متعددة المقاومة للمضادات الحيوية في التعبير عن جينات *blaTEM* و*blaCTX* أو *blaCTX* و*blaSHV*، بينما يحتويان أيضًا على *mcr1* و/أو *norA*. هذه الجينات المقاومة للمضادات الحيوية ذات الأهمية الحيوية تعني سوء الاستخدام في الحقل البيطري، يزيد احتمالات الأمراض المنقولة بالغذاء في المستقبل ومن صعوبة العلاج.