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Surveillance of Food Poisoning Escherichia coli (STEC) in Ready-to-Eat Meat Products in Aswan, Egypt

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READY to eat meat was defined as case of meat being ready for immediate consumption. Food diseases of microbial origin are associated with serious health problems due to consumption of ready to eat meat and meat products in many places in the world. Conventional processing methods used in preparation, improper storage / conservation are the main factor contributing to food contamination. One of the common pathogenic bacteria that causes the food-borne diseases is *E. coli* .Some strains produce toxins that can lead to a major health problem such as Shiga toxin-producing *E. coli* (STEC). *E. coli* O157:H7 is the most common strain of STEC, but there are many other strains of STEC as well. The current study was designed for screening of the ready to eat meat samples for *E.coli*, 120 Samples of (sausage ,hamburger ,minced beef and fried chicken 30 of each of them) through culture and biochemical tests and confirmation isolates by PCR. Depending upon culture, staining and biochemical tests *E.coli* isolates were detected in 12/30 (40%) samples of sausage, 4/30 (13%) of hamburger, 8/30 (27%) of minced beef and 1/30 (3%) of fried chicken with prevalence of all isolates were confirmed in 25/120 (20.83%) .According to PCR on positive isolates 10/12 (83.3%) in sausage , 4/4 (100%) in hamburger, 7/8 (87.5%) in minced beef and 1/1 (100%) in fried chicken. After serological identification isolates positive for both stx2 and eaeA were O26:H11 strain and isolates positive for eaeA only were O119:H6 strain.

Aim of the study: screening commercial samples of the ready to eat meat for contamination of (STEC) *E. coli* which are causing food borne illness.

Keywords: *E. coli*, (STEC), Ready to eat meat, PCR.

Introduction

Aswan government one of biggest lower Egypt area, living over it many millions people, and they depend upon ready to eat meat as rapidly prepared meal and has delicious taste. Ready to eat meat which are available in markets in Aswan area are sausage, hamburger, minced beef and fried chicken which may contain many microorganisms liable to be transmitted to humans and causing many catastrophes.

E.coli normally live in the intestines of healthy cattle, and contamination by STEC may occur during the slaughtering process. Infection occurs by eating raw or undercooked meat and meat product. Infected people can spread *E. coli* to other people if they do not wash their hands after using the toilet. Conventional processing methods used in the preparation, improper withhold/ preservation are the major contributing factor for the contamination of food [1]. Because foodborne disease outbreaks can be under-reported by up

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to a factor of 30, the number of food-associated gastroenteritis cases is estimated to be between 68 million and 275 million annually [2]. Although the changing in food production practices , well-known foodborne pathogens as Escherichia coli appear to evolve and exploit new opportunities and develop antimicrobial resistance to currently used agents [3]. Processing of meat may cause contamination due to a lack of knowledge on how to improve conditions in the meat industry. About 45.5% of retailers and 64% of slaughterhouse workers knew that contamination of meat lead to serious food poisoning in their hosts [4].

The possibility of *Escherichia coli* to induce health risks occurring mainly during preparation and storage of contaminated ready to eat meat [5].

E. coli is one of the basic bacterial pathogens of food origin. Most *E. coli* are not pathogenic, but some are highly pathogenic and causing food poisoning and food intoxication with serious symptoms as diarrhea (watery or bloody) , *E. coli* strains (STEC) that cause serious human illnesses as hemorrhagic colitis, hemolytic uraemic syndrome, and thrombotic thrombocytopenic purpura [6]. *E. coli* (STEC), the most common pathogen associated with minced beef, causes approximately 96,000 diseases, 3,200 hospitalizations, and 31 deaths annually in the United States, representing an annual health care cost of $ 405 million. The CDC (Centers for Disease Control and Prevention) reported 391 cases of E. coli over a ten-year period from 2003 to 2012. Between these epidemics, the Agency confirmed 4 930 cases, of which 1, 274 (26%) were hospitalized, 300 (6%) cases of hemolytic-uremic syndrome (HUS) and 34 died. The food is the most common source of E. coli (STEC) infection, which is the cause of 65% of cases **[7]**.A 40 years of research reviewed about enteric antimicrobial resistance in East Africa indicates that *E. coli* (STEC) has a great potency for the transmission of zoonosis to humans and has developed a high degree of resistance to available treatment manner [8].

All enterohemorrhagic *Escherichia coli* (EHEC) strains possess at least one Shiga-like toxin (stx1 or stx2) gene causing serious disease in human [9].

Shiga toxin–producing *E. coli* isolated from one sample from raw minced beef [10]. Outbreak of *E. coli* (STEC) was associated with salami and minced beef. Seasonal trends were observed for the incidence of bowel disease, with peaks consistently observed during the peak summer months of E. coli infection [2]. The consumption of meat around the world is becoming increasingly important and a challenge for meat hygiene and safety. These concerns are biological in their essence, and Contains bacterial pathogens such as *E.coli* (STEC) strains [11].

First isolate of Shiga toxin-producing *Escherichia coli* O157:H7 in 1982 ,turn into the important food and water-borne pathogen. Entero-hemorrhagic *Escherichia coli* O157:H7 induces diseases as a result to Shiga toxins production, which cause hemolytic uremic syndrome (HUS), which can cause a variety of gastrointestinal diseases, from watery diarrhea to hemorrhagic colitis, and cause systemic diseases in humans [12].

Serodicity of *E. coli* 0157:H7 and other Shiga-like toxin-producing Escherichia coli (SLT-EC) is pathogenic to humans isolated from meat and meat products, raw milk, and cow feces with or without diarrhea. This evidence is that cows are reservoirs of SLT-EC, and raw milk, meat and meat products are the main mediums of human infection by such pathogens [13]. About 10% of children with *E.coli* O157: H7 infection, especially infants under 5 years of age, develop hemolytic urethrosis syndrome (HUS). It has a 5% annual mortality rate, Who survive remain under risk of chronic kidney illness [14]. Among the strains of *E. coli* there is a remarkable serotype O157: H7. This serotype, which includes highly virulent strains, has been the focus of much importance more than 10 years ago because of its association with a number of highly publicized food-derived outbreaks and its ability to survive acidic conditions that were previously known to be fatal to *E. coli.* [15].

Strains of *E. coli* O157:H7 have been found to be relatively acid tolerant, and the infectious dose can be less than 50 cells. Important virulence factors include the production of Shiga toxins 1 and 2 (Stx1/Stx2) and genetic variants of these toxins and the *eae* (encoding for the intimin outer membrane protein) and other genes involved in the production of attaching and effacing lesions and cytoskeletal damage of intestinal cells. Other STEC/EHEC virulence genes are carried on mobile genetic elements, such as pathogenicity islands and plasmids [16].

However, conventional microbiological

methods for detecting bacterial-contaminated foods usually involve multiple subcultures and steps to identify a biotype or serotype, and are therefore laborious and time consuming. Rapid detection of these pathogens in many samples at the same time is required; this is very easily facilitated by PCR [17].

Study in ready to eat meat and meat products ,Out of 33 samples meat curry, 4(12.12%) and from 25 samples of non-veg momo, 1(4.0%) were found to be positive for Escherichia coli, All *E. coli* isolates belonged to four different serotypes (O8, O89, O60 and O Rough), this reveals that the contamination of ready to eat foods of animal origin with *E. coli* could be an important factor of gastrointestinal illness in the consumers [18].

Continuous attentiveness, maintained by monitoring and surveillance, is indispensable to tolerate food safety standard [3]. Detection of food-borne pathogen enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) through its shiga toxins and eae genes is the most important step towards ensuring food safety ,it is our aim of this study.

Materials and Methods

Sample collections: 120 samples from 4 groups of ready to eat meat including 30 samples from every group including are sausage , hamburger, minced beef and fried chicken were obtained from different markets in Aswan city, presence of *E. coli* was documented. *E. coli* detected by culture on selective media, biochemical test and confirm isolates by PCR using specific primers for target genes of pathogens. These samples were collected from period of February to August 2019. Samples are collected in sterile sampling jars. All the samples were carried to Central Veterinary Research Laboratory at Aswan University in insulated ice – box and immediately managed for identification and isolation of isolates , PCR occurred in (Animal health research institute & Agriculture research center, Giza , Egypt) and serotyping identify in Benha University Faculty of Veterinary Medicine Food Analysis Center.

Sample Preparation

Twenty five (25g) from solid samples were weighed and triturated in sterile pestle and mortar aseptically and added to 225 ml of sterile Nutrient broths for samples Likewise, liquid products, 10 ml of each sample was diluted with 90 ml of sterile Nutrient broth. All this process was carried near the flame under vertical laminar flow bench which was disinfected by ultra-violet radiation, observing all possible aseptic precautions.

Isolation of E. coli and its identification

After homogenization of samples transferred each in 5ml nutrient broth tube, thin culture on nutrient agar and others specific and selective media (MacConkey agar, EMB agar and tryptic soy agar). In every step, samples were incubated at 37°C for 24 hours. The positive samples were taken and sub cultured for several times to obtain a pure culture. Lactose fermenting pink colonies were identified as *E. coli*. using Gram negative staining identified as negative-bacilli or coccobacilli by optical microscopy. Making biochemical tests to confirm *E. coli* mainly indole positive and citrate negative [19]. All media and chemical used for cultural and chemical characterization of *Escherichia coli* were procured from Titan Biotech LTD, India.

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer’s recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer’s recommendations. Nucleic acid was eluted with 100 µl of elution buffer. Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in Table 1.

PCR amplification

Uniplex PCR. Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Stx1,2 duplex PCR. Primers were utilized in a 50- µl reaction containing 25 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 15 µl of water, and 6 µl of DNA template. The reaction was performed in

TABLE1. Primers sequences, target genes, amplicon sizes and cycling conditions .

Target bacteria	Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
E. coli	*Stx1*	ATGTTCCCAAAAATAATGAA TCATGCCGCCACTTCGGTGC	614	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[20]
	Stx2	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	779						
	eaeA	TTTCGATTGTCTGGCTGTATG CTTCAGATTCAGCGTCGTC	248	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	[21]

an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the Uuniplex PCR products and 40 µl of the duplex PCR products were loaded in each gel slot. A generuler 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Serological identification of E. coli

The isolates were serologically identified according to Kok et al. [22] by using rapid diagnostic *E.coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

Technique

-Two separate drops of saline were put on a glass slide and a portion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.

-To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of undiluted antiserum was added and titled back and forward for one minute.

-Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated onto a nutrient agar slant and incubated at 37°C for 24 hours to grow as a culture for testing with mono-valent sera.

-A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O-antigen.

N.B.

-Colonies from nutrient agar were tested rather than those from the MacConkey agar because the latter may give misleading reactions.

-Non-specific agglutination may be appeared rather than specific one with the slide technique, particularly when it is carried out on bacteria taken from selective media. This agglutination was appeared slowly and broken up on stirring.

-When the saline control suspension was granular in the slide agglutination test, the suspension was not suitable for typing by that method.

The diagnostic *E.coli* antisera sets used for identification include the following sets:

Set 1 : O- antisera:

Polyvalent antisera 1: O1, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O2, O6, O27, O78, O148, O159 and O168.
Polyvalent antisera 5: O20, O25, O63, O153 and O167.
Polyvalent antisera 6: O8, O15, O115 and O169.
Polyvalent antisera 7: O28ac, O112ac, O124, O136 and O144.
Polyvalent antisera 8: O29, O143, O152 and O164.

Set 2 : H- sera.

H2, H4, H6, H7, H11, H18 and H21.

Results

Identification of microorganisms

Depending upon staining ,culture and biochemical tests E.coli isolates were detected in 12(40%) samples in sausage ,4(13%) In huamberger,8 (26.7%)in minced beef and 1(3.3%) in fried chicken . Prevalence of all isolates of E.coli was 20.83%.

Detection of microorganisms by PCR

In our study out of 12 *E.coli* isolates in sausage detected by culture and biochemical tests, 10 isolates were confirmed by PCR, all 4 isolates of *E.coli* in hamburger were confirmed by PCR and in minced beef 7 out of 8 isoltes were confirmed by PCR, and in fried chicken the only one isolate detected by culture and biochemical tests was confirmed by PCR.

TABLE 2. Rates of microorganisms in ready to eat meat by culture and biochemical test.

Type of meat	Sausage	Hamburger	Minced beef	Fried chicken	Total
No of samples	30	30	30	30	120
Positive for *E.coli.*	12 (40%)	4 (13%)	8 (26.7%)	1 (3.3%)	25 (20.83%)

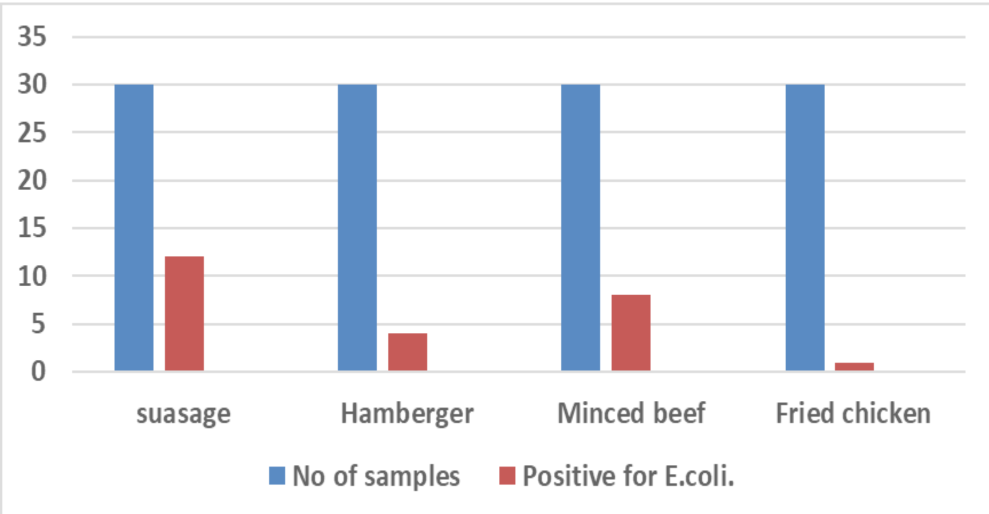

Fig. 1. Rates of microorganisms in ready to eat meat by culture and biochemical test.

TABLE 3. Rates of microorganisms in PCR .

Microorganisms	Sausage Total 30	Hamburger Total 30	Minced beef Total 30	Fried chicken Total 30	Total
No of samples	**12**	**4**	**8**	**1**	**No. 25**
Positive for *E.coli.*	10 83.3%	4 100%	7 87.5%	1 100%	No. 22 88%

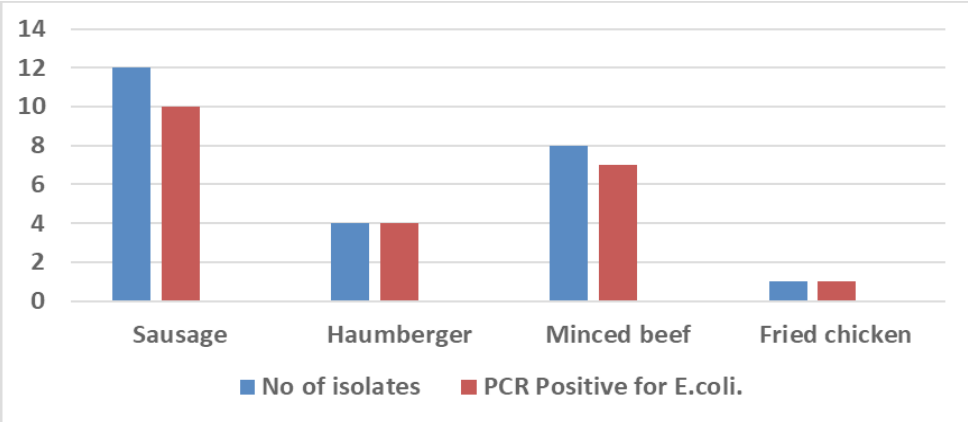

Fig. 2. Rates of microorganisms in PCR.

TABLE 4. Rates of pathogenic genes in isolates .

Sample No. 25	Stx1	Stx2	eaeA
6	-	+	+
16	-	-	+
3	-	-	-

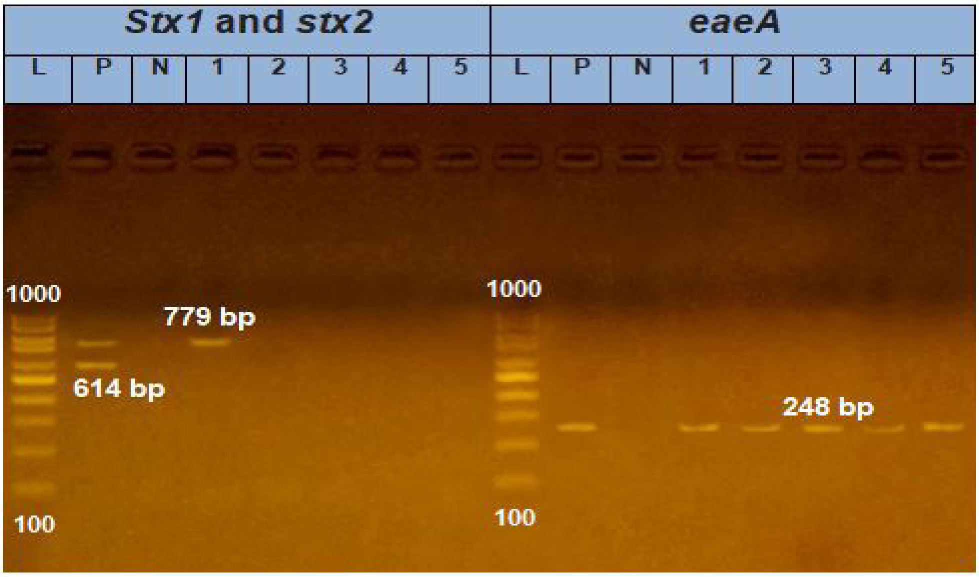

Photo 1. Detection of genes (Stx1,Stx2 and eaeA) by PCR.

TABLE 5. Serological identification of isolated *E. coli* .

Key No.	Identified bacterium	Sero diagnosis	Strain characterization
1	***E. coli***	**O26 : H11**	**EHEC**
2	***E. coli***	**O119 : H6**	**EPEC**

Discussion

In India presented that out of 33 meat curry samples and 25 of non-veg momo samples, 4(12.12%) and 1(4.0%) were positive *Escherichia coli.* respectively [23].

The isolated *E.coli* from food samples were Gram negative staining, biochemical identification test positive and conventional Polymerase Chain Reaction (PCR) positive. In all food samples, the prevalence of *E.coli* presented was 37.86%. As follow, 32 (29.63%) milk, 25 (49.02%) chicken and 7 (70%) beef samples were positive through conventional method [24].

One sample of raw ground beef only positive for STEC. PCR result give both genes *stx*1 and *stx*2, and determined the serotype of *E.coli* O22:H8 [10]. From 24 samples of *E. coli* O157:H7 were tested for Shiga toxin genes, give 17 sample positive for both stx1 and stx2, 4 samples positive for stx2, one positive for stx1 and one negative for both stx1 and stx2 genes [9]. The federally inspected establishments in Canada making a survey on ground beef revealed a presence of *E. coli* O157 in low prevalence (from 0.25 to 2.1%) [25]. Another report on ground beef from retail stores in France ,the prevalence of STEC 11% [26] where in United States was 16.8% [27]. The distribution of *E. coli* in meat homogenates samples from different sources give high incidence (65%) in open butcher shops, (40%) groceries and (20%) hypermarkets, this results confirmed by PCR [28].

In Egypt, *Escherichia coli* O157 was detected in water samples using PCR targeting virulence genes (stx1 , stx2 and eae genes) indicated that 57 out of 175 examined water samples (32%) contained E. coli O157 [29] also, The virulence genes stx1 , stx2 , eae and the enterohemorrhagic E. coli hemolysin (hlyA) genes in five E. coli O157:H7 strains were isolated from Egyptian food [30]. Other isolates positive for both stx1 and stx2 were one O111:H− strain and one O113:H21 strain. Isolates of one O157:H7 strain, two O103:H2 strains, two O26:H11 strains, two O111:H8 strains, and one O45:H2 strain were positive only for stx1 and negative for stx2; whereas four O157:H7 strains, two O91:H21 strains, and one O157:NM strain were positive for the stx2 gene only. One O157:H7 strain, one O2:H6 strain, one O2:H8 strain, and one *E. coli* strain of unknown serotype were negative for both stx1 and stx2 [9]. From bovine mastitic milk samples. Out of 73 positive sample of STEC , 15 (20.54%) were O26 and 11 (15.06%) were O157 while O111 not detected in any sample and out of 73 STEC strains, 11 (15.06%) were EHEC and 36 (49.31%) were AEEC,. All of the EHEC strains had stx1, eaeA, and ehly, virulence genes, while in AEEC strains had stx1 and eaeA [31]. Out of 197 meat samples, 23.4% and 9.1% were contaminated with *Escherichia coli* in general and *Escherichia coli* O157 : H7, respectively [32]. Isolated five *E. coli* (STEC) strains belonging to serotypes O26:H11, O103:H2, O111: H8 , O145: H28 and O157: H7 are known classically EHEC types which present in different countries over the world (www.sciencenet.com.au/vtectable.htm [33].

Some of the previously mentioned studies agreed with our result which revealed that prevalence of all isolates of *E.coli* was (20.83%). Our *E.coli* isolates were detected in 12 (40%) samples of sausage, 4 (13%) of hamburger, 8 (27%) of minced beef and, 1 (3%) samples of fried chicken. The 10 (83.3%) isolates were confirmed by PCR, 4 (100%) isolates of *E.coli* in hamburger were confirmed by PCR and in minced beef 7 (87.5%) isolates were confirmed by PCR, and in fried chicken the only one isolate (100%) detected by culture and biochemical tests was confirmed by PCR . according to serological identification 6 isolates O26:H11 strains and 16 isolates O119:H6 strains.

Another study differ due to its practice in water, milk and raw meat not in ready to eat meat. Therefore workers who are processing ready to meat should be educated about food hygiene. This finding indicates that poor hygienic and sanitary measures were practiced while processing, handling and serving the meat and meat products to the consumers. It also indicates that microbiological quality of ready-to-eat meat and meat products is associated with meat type and hygienic practices.

Conclusion and recommendations

The higher prevalence of *E. coli* in ready to eat meat indicates unhygienic production and processing of these foods.

In restaurants, send back undercooked ground beef for more cooking.

Be aware that bacteria from undercooked ground beef could have contaminated other foods on the plate and even the plate itself.

Reducing Risks from Ground Beef at Home Keep raw meat separate from ready-to-eat foods.

Wash hands, counters, and utensils with hot soapy water after they touch raw ground beef.

Wash meat thermometers between rounds of testing the temperature of ground beef being cooked .

Further studies on pathogenicity and detection of antibacterial resistant genes as well as genetic evolution can be performed.

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Conflict of interest

No conflict of interest

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مراقبة التسمم الغذائي بالإشريكية القولونية (STEC) في منتجات اللحوم الجاهزة للأكل في أسوان ، مصر

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يتم تعريف اللحوم الجاهزة للأكل بأنها حالة اللحوم الجاهزة للاستهلاك الفوري. وهذه اللحوم يمكن أن يؤدي استهلاكها الى الأمراض التي تنقلها الأغذية الناتجة عن التلوث بالإيكولاي مما يسبب مشكلة صحية كبيره .
فحص عينات اللحوم الجاهزة للأكل بحثًا عن مسببات الأمراض التى تنتقل عن طريق الغذاء مثل بكتريا الايكولاى.
مجموعات العينات ١٢٠(السجق ، اللحم البقري ، اللحم المفروم وشرائح الدجاج مسبق التجهيز) جميعها من منافذ بيع متفرقه فى مدينة اسوان ومن خلال العزل والاستنبات على المزارع البكتيريه وصبغة الجرام والكيمياء الحيوية والاختبارات التأكيدية للعينات الإيجابية بواسطة اختبار البلمره .
تم الكشف عن عزلات الإشريجية القولونية في ٣٠/١٢ (٤٠٪) عينات في السجق ،
في الهمبورجر ٣٠/٤ (١٣٪) وفى اللحم المفروم ٣٠/٨ (٢٦,٧ ٪) و في الدجاج المقلي ٣/١ (٣,٣٪) و نسبة انتشار جميع العزلات (١٢٠/٢٥) ٢٠,٨٣٪.
وفقًا لاختبار البلمره على العينات الإيجابية (٢٥) تم الكشف عن ١٢/١٠ (٨٣,٣٪) في السجق و٤/٤ (١٠٠٪) وفى الهبورجر ٨/٧ (٨٧,٥٪) و فى الدجاج مسبق التحهيز ١/١ (١٠٠٪) .
تم تصنيف العزلات الايجابيه من اختبار البلمره (٢٥) عينه تبين وجود السلاله انتيروباثوجنك المفرزه لسموم الشيجا٢ والانتيمين بنسبة ٢٥/٦ (٢٤٪) والسلاله انتيروهيمورجك المفرزه لسموم الانتمين بنسبة ٢٥/١٦ (٦٤٪) وسلالات لم يتم تصنيفها بنسبة ٢٥/٣ (١٢٪) .
فرضية الدراسة: فحص عينات اللحوم الجاهزة للأكل بحثًا عن مسببات الأمراض التي تنقلها الأغذية وعنيت الدراسه ببكتيريا الاشريجيه القولونيه السلالات المنتجه لسموم الشيجا والانتيمين .