

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Detection of Bacteria Secreting Toxins in Shawarma and Their Sensitivity to Antibiotics Using VITEK



Eman F. Mohamed* and Dina I. Al-Zahabi

Food Hygiene Department, Animal Health Research Institute (AHRI), Agriculture Research Center of Egypt (ARC), Shebin El-Koom Branch, Egypt.

Abstract

TOTAL OF 200 of chicken and beef shawarma sandwiches (100 for each group) were collected from several restaurants in El Menofiya **Governorate** to analyze the prevalence of *E. coli*, *S. aureus*, and Salmonellae spp. It was 15%, 21% and 14% for chicken shawarma sandwiches, respectively while, it was 21%, 30% and 9% for beef shawarma sandwiches, respectively. The chicken shawarma samples showed a higher acceptability rate based on the presence of *E. coli* and *S. aureus*. The study also detected a number of serotypes of *E. coli* EHEC (O₂₆: H₁₁, O₁₀₃: H₂ andO₁₁₇: H₄), ETEC (O127: H6) and EPEC (O146: H21) has been detected and Salmonellae serotypes were S. *typhimurium*, *S. infantis*, *S. montevideo* and *S. enteritidis*. In the examined samples the isolates produced toxins was genetically tested by PCR to detect genes that encode for enterotoxin production. Antibiotic Susceptibility Testing (AST) of isolated strains by VITEK revealed that the pathogenic strains of *E. coli* and *S. aureus* showed resistance to several antibiotics, while non-pathogenic strains showed sensitivity to most antibiotics. Salmonella spp. was sensitive to most tested antibiotics, except for Cefalotin and Trimethoprim/Sulfamethoxazole. The study provides information on the incidence of harmful microbes in shawarma sandwiches and their antibiotic sensitivity, which can help in improving food safety measures.

Keywords: E. coli, Salmonella, S. aureus, Shawarma, PCR, VITEK2.

Introduction

Ready-to-eat meat (RTE) was characterized as meat that is ready for immediate intake, with traditional processing procedures employed in preparation and incorrect storage/conservation being the primary contributors to food contamination. The biological value, affordable cost, palatable taste, and ease of preparation, ready to eat meat (RTE) is in high demand [1]. Meat products are considered a good source of high-quality protein, minerals, and vitamins. These meals don't need a drawn-out pretreatment process, which makes them shelf-stable, tasty, inexpensive, and immediately available to customers [2]. On the other hand, foods of animal origin can serve as a means of transportation and a medium for the spread of a wide variety of germs that can result in sickness, disease, and even death [1]. These bacteria cause 3000 mortality and 48 million illnesses annually in the United States. On a spinning vertical skewer, slices of fat and chunks of seasoned meat are alternately layered to produce a wrap of chopped meat (beef, lamb, or marinated chicken). The majority of the meat's interior is left raw while the exterior is roasted [3]. It does not require any additional preparation, with the exception of warming, and these RTE foods are frequently consumed without any additional heat treatment [4, 5].

Meat processing may get contaminated as a result of a lack of information about how to improve conditions in the industry. Between 68 million and 275 million instances of food-associated gastroenteritis are thought to occur each year [6]. It is known that handling, preparing, and selling these products might lead to outbreaks [5]. Escherichia coli is a major food-borne bacterial disease. The majority of E. coli strains are not pathogenic, but some are extremely pathogenic and can cause food poisoning and intoxication, as well as significant symptoms such as diarrhoea (bloody or clear). These *E. coli* strains (STEC) can cause significant human disorders. The processing and storage of tainted RTE meat is when *E. coli* can potentially cause health hazards [7].

Concern over *Staphylococcus aureus* spreading through the food supply chain has grown because of its discovery in food products with an animal origin. The presence of multidrug-resistant *S. aureus* in food, notably ready-to-eat foods, could endanger consumers [8]. Furthermore, the Salmonella genus continues to be a major global public health concern and is the primary contributor to foodborne outbreaks. According to Cheng et al. [9], Salmonella organisms can enter the small intestine through the gut lumen and cause acute gastrointestinal illnesses like gastroenteritis, organ focal infections, and systemic febrile infections. Meat and poultry are the main sources of Salmonella infections in people.[10],

Using VITEK Commercial automated systems are utilized in the United States for the identification and susceptibility testing of bacteria owing to its low cost and ease of use. Additionally, PCR techniques have been routinely employed to identify foodborne infections [11].

RTE foods are frequently utilized in restaurants and at home. Therefore, the goal of our study was to look into any potential microbial contamination in RTE meat products (chicken and meat shawarma sandwiches) using the VITEK2 system for detection of pathogens, antibacterial susceptibility testing, and PCR for virulence gene detection.

Material and Methods

Collection of samples

Two hundred samples from two groups of chicken and meat shawarma sandwiches (one hundred for each group), were collected from several restaurants in the El Menofiya governorate, Egypt. These samples gathered between June and August of 2023. All samples were brought in an insulated icebox to the Animal Health Research Institute Laboratory at Shebin El Koom, right away for isolation, identification, and antibiotic sensitivity testing using the Vitek 2 system for isolates, and the Animal Health Research Institute & Agriculture Research Centre, Giza, Egypt for serological tests and PCR.

Preparation of samples

In a stomacher (Seward Medical, London, UK), 25 g of each sample were accurately homogenized aseptically for 1.5 minutes with 225 ml of 0.1% sterile peptone for the isolation of *E. coli and S. aureus* and 1% sterile buffer peptone for Salmonellae (ISO 4833-1, 2013)[12].

Screening for E. coli

Identification of Enteropathogenic E. coli

According to the manufacturer's instructions, a VITEK® 2 systems (GN product information. bioMérieux, Inc., Durham, NC. USA, 18868) was used for the biochemical confirmation for *E. coli* (ISO 16649-2: 2001) [13].

Serological identification of E. coli

The serological identification was performed by using quick diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) [14].

Detection of virulence genes of STEC by PCR

The reaction was performed in an Applied Biosystem 2720 Thermal cycler. Then, analysis of the PCR products was done on 1.5% agarose gel (Applichem, Germany, GmbH). Control positive for E. coli was kindly supplemented by media unit of Food Hygiene Lab., Animal Health Research Institute, Cairo, Egypt. DNA extraction using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), the Oligonucleotide primers were supplied from PCR amplification was Metabion (Germany). performed according to Dipineto et al. [15]. The reaction was carried out in an Applicable Biosystem 2720 Thermal cycler. The PCR results then analyzed on a 1.5% agarose gel (Applichem, Germany GmbH). Primers sequences, target genes, amplicon sizes and cycling conditions were according to Dipineto et al. [15].

Isolation and identification of S. aureus

The isolation was performed according to ISO 6888 [16]. The biochemical confirmation test for *S. aureus* was performed using a VITEK® 2 system bioMérieux, Inc., Durham, NC. USA, 18868 (GP card).

Detection of toxin producing genes in isolated S. aureus strains using PCR

DNA Extraction using *QIA* amp kit [17]. Amplification of enterotoxin genes of *S. aureus* was done according to Mehrotra et al. [18]. Primers sequences, target genes, amplicon sizes and cycling conditions were done according to Mehrotra et al. [18]. Control positive for *S. aureus* was kindly supplemented by media unit of Food Hygiene Lab., Animal Health Research Institute, Cairo, Egypt. Isolation and identification of *Salmonellae spp.* carried out reference to ISO (6579-1) [19]. The biochemical identification of salmonellae was performed using a VITEK® 2 system, bioMérieux, Inc., Durham, NC. USA, 18868 (GN card, 2411756).

Serological identification of Salmonella spp.,

It was done according to Kauffman – White scheme [20] for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

Detection of virulence genes of Salmonellae by (PCR)

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations.

Oligonucleotide Primers

Primers used were supplied from Metabion (Germany). Primers sequences, target genes, amplicon sizes and cycling conditions were done according to Yang et al. [21] and Olivera et al. [22].

PCR amplification

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 concentrations, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler. Control positive for Salmonellae was kindly supplemented by media unit of Food Hygiene Lab., Animal Health Research Institute, Cairo, Egypt.

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

Antibiotic Sensitivity Testing

Sensitivity testing was performed using VITEK® 2 bioMérieux, Inc., Durham, NC. USA, 18868 (AST-GN96) against *E. coli* and Salmonellae while VITEK ®2 (AST-GP79) for *S. aureus*.

Statistical analysis

The incidence of pathogens are calculated using graph pad prism 8.0.2 and the significance difference was calculated using t- test is at confidence level 99% (p<0.01).

Results

The incidence of the recovered *E. coli*, *S. aureus* and Salmonellae from the examined chicken shawarma sandwiches samples was 15%, 21% and 14%, respectively, while it was 21%, 30% and 9% for meat shawarma, respectively. The results proved the significance difference between the isolated microbes from chicken and meat shawarma with confidence level 99% (p<0.01). Also, chicken shawarma samples were more acceptable than meat shawarma samples as the acceptability of samples according to the isolated E. coli and S. aureus were 85% and 79% for chicken shawarma, respectively and 79 % and 70% for meat shawarma, respectively. While the acceptability of samples based on the presence of salmonellae were 86% for chicken and 91% meat shawarma (Table 1).

Based on the somatic lipopolysaccharide (O) and flagellar (H) antigens of E. coli a number of serotypes contained EHEC (O_{26} : H_{11} (10.34%), O_{103} : H_2 (3.44%) and O_{117} : H_4 (10.34%)), ETEC (O127: H6 (6.89%)) and EPEC (O146: H21 (10.34%)) for chicken shawarma has been detected. However for meat shawarma the serotypes were EHEC (O_{26} : H_{11} (20.68%), O_{103} : H_2 (13.79%) and O_{117} : H_4 (3.44%)), ETEC (O127: H6 (10.34%)) and EPEC (O146: H21 (10.34%)) (Figure 1).

The serotypes of Salmonellae isolated in the examined samples of chicken shawarma was *S. Typhimurium*, *S. Infantis, S. Montevideo* and *S. Enteritidis* with incidence 6%, 2%, 2% and 4%, respectively and from meat Shawarma with incidence 2%, 3%, 2% and 2%, respectively (Table 2)

The studied isolates produced shiga toxins (stx1) shiga-toxin 1 and (stx2) shiga-toxin 2). Only two strains were positive for stx1 (614bp) and one isolate was positive for stx2 (779bp) (Fig. 2).

Also, genes that responsible for production of enterotoxin in *S. aureus* microorganism are presented in (Fig. 3) (A, B, C, D) as six samples were positive for seb (164bp), one sample was positive for sec (451bp) and another one samples was positive for sed (278bp). All the examined samples were negative for sea (102 bp). The prevalence of *S. aureus* enterotoxins (enterotoxins B, and C) that were detected in chicken shawarma samples were 25, 12.5, respectively. While enterotoxins B and D were detected in meat shawarma samples with prevalence of 50 and 12.5, respectively. But enterotoxin A failed to be detected (Fig.4).

The studied isolates for *hilA* and *invA* genes for characterization of *Salmonellae* revealed that all examined strains were positive for *hila* (150 bp) and *invA* (284 bp) genes (Fig.5).

The antibiotic sensitivity test against the isolated strains of E. coli reavealed that, as the pathogenic strains were resistant to Ampicillin, Amoxicillin/Clavulanic acid, Cefalotin, Cefoperazone. Ceftiofur and Trimethoprim/Sulfamethoxazole also, they showed intermediate resistance against Flumequine and Enrofloxacin while, they showed sensitivity against Cefalexin, Gentamicin, Neomycin, Marbofloxacin, Tetracycline and Imipenem (Table 3).

The non-pathogenic strains showed resistance only to Ceftiofur and showed intermediate resistance against Ampicillin and Cefalotin while, they showed Cefalexin. Cefoperazone. sensitivity against Flumequine, Enrofloxacin, Gentamicin, Neomycin, Marbofloxacin, Tetracycline, **Imipenem** Trimethoprim/Sulfamethoxazole. The toxigenic strains were resistant to Benzylpenicillin, Oxacillin, Gentamicin, Erythromycin, Clindamycin, Tilmicosin,

Tylosin, Tetracycline while, they showed sensitivity against Cefquinome, Amikacin, amikamycin, Florfenicol, Kanamycin, Trimethoprim/ Sulfamethoxazole (Table 4).

The non-pathogenic strains showed resistance only to TilmicosinTylosin and showed intermediate resistance against Ceftifur, Erythromycin and Clindamycin while, they showed sensitivity against Benzylpenicillin, Oxacillin, Gentamicin. Cefquinome, Amikacin, amikamycin, Tetracycline, Trimethoprim/ Florfenicol. Kanamycin, Sulfamethoxazole. The antibiotic sensitivity testing against Salmonella showed that it was sensitive for all tested antibiotics that were listed at (Table 5) Cefalotin and Trimethoprim/ Sulfamethoxazole showed intermediate sensitivity.

Discussion

In terms of the microbiological quality and safety of food, problems with foodborne infections continue to be a major concern worldwide [23]. In Egypt, ready-to-eat food is frequently purchased from restaurants, but a significant portion is also purchased from street sellers, where the food is not adequately shielded from flies and dust. Furthermore, it is challenging to keep food at a safe temperature for storage. The results are worrying because the majority of samples tested were positive for harmful microorganisms such E. coli, S. aureus, and Salmonella, which are important for public health [24, 25]. Furthermore, the acceptability of the tested samples exceeded the CFS [26] and WHO [27] upper limits of acceptable acceptability. Similar findings were made by Ajaja et al. [28], who isolated S. aureus from 0.7% of the analyzed shawarma samples while isolating E. coli from the examined samples with an incidence of 19.9%. Additionally, E. coli and S. aureus were recovered from ready-to-eat meat products by Nethathe et al. [23], but Salmonella was not isolated from these items. Additionally, E. coli was recovered from RTE meals by Abebe et al. [29] and from ready-to-eat sandwiches by Ema et al. [1]. Shawarma in Sri Lanka had 32.0% S. aureus, according to Wimalasekara and Gunasena [30]. Food-borne illness outbreaks have been linked to sandwich fillings and ingredients like bread, chicken, salad, and sauce [31].

Salmonella from a ready-to-eat beef product was also recovered by Tîrziu et al. [32] and Castrica et al. [33]. Contamination of fast food can happen at any stage of preparation. During production, distribution, retail, handling, and presentation, pathogens may be introduced. People working in the food industry should get education about how their personal hygiene influences food safety and how they can contribute to a reduction in the spread of foodborne illnesses. The cooking, preparation, seasoning, and storage of chicken shawarma sandwiches all present potential areas of infection [28]. Food contamination

that results in foodborne illnesses may be caused by improper handling and poor hygiene practices [23]. The majority of E. coli strains are not harmful, but a small number are dangerous and can cause bloody and watery diarrhea [34]. E. coli, a member of the Enterobacteriaceae family, is primarily found in the digestive tracts of both humans and animals. As a fecal and enteric pathogen indicator species, E. coli has been discovered. Enterohaemorrhagic (EHEC), Enteropathogenic (EPEC), and Enterotoxigenic (ETEC) serotypes of *E. coli* were isolated from the samples that were under examination; these serotypes are known to produce toxins that might result in diarrhea [35]. The most prevalent pollutants the examination samples under enterohaemorrhagic (EHEC), followed enteropathogenic (EPEC), and then enterotoxigenic (ETEC). In tested chicken shawarma, Morshdy et al. [36] isolated E. coli O26 and O127 with incidences of 3(6%) and 3(6%), respectively. While O91:H21 and O121:H7 are found in the tested chicken shawarma by Afaf et al. [3]. Although the majority of E. coli strains are not harmful to humans, some of them have been linked to serious gastrointestinal illnesses such hemorrhagic colitis and hemorrhagic uremic syndrome. According to Bruyand et al. [37], virulence factors called shiga toxins (stx1 and stx2) are crucial in the development of these illnesses. E. coli and other pathogens in RTE food has been the subject of several investigations [38]. Similar findings were made by Afaf et al. [3], who identified the virulence genes stx1 and stx2 in chicken shawarma.

Furthermore, PCR is used to identify enterotoxins. Enterotoxin B was the enterotoxin that was most isolated, but enterotoxin A was not found. Enterotoxins A and D have been found in samples of shawarma by Fathalla et al. [8]. Additionally, samples of ready-to-eat food include several forms of enterotoxins, according to Nethathe et al. [23]. Enterotoxins, which *S. aureus* is capable of producing, can result in vomiting and diarrhoea when consumed [39].

Salmonella is the most common cause of foodborne outbreaks and remains a serious public health problem around the world. Salmonella organisms penetrate the gut lumen and enter the small intestine epithelium, causing acute gastrointestinal illnesses such [40]. Currently, more than 2,000 Salmonella serotypes are being recognized. [41]. Moreover, hilA and invA are two examples of Salmonella-specific target genes that have been discovered Salmonella **Typhimurium** [42]. internalization in mammalian epithelial cells has been linked to the Salmonella invasion gene invA, according to research. The DNA sequence of this gene is highly conserved throughout Salmonella spp., and it is specific to Salmonella [40].

Using Vitek® 2, examine the relationship between the virulence genes of isolated pathogens and the sensitivity of various antibiotic groups against those genes. The findings showed that the presence of virulence genes caused the sensitivity to change as the virulence-carrying strains evolved antibiotic resistance and changed the MIC (minimum inhibitory concentration). Because local RTE foods have the potential to act as the vectors for pathological E. coli with the multi-drug resistant characteristic, allowing these resistant bacteria or genes to spread to humans via food and establish themselves in the intestinal flora. The presence of multidrug-resistant (MDR) ETEC in local RTE food is a public health concern [43]. Multiple antibiotics are used to combat infections in hens and enhance their growth, allowing 100% of chicken isolates in Oman to display multidrug resistance [44]. As a result, chickens are potential sources of MDR (multidrug resistance) bacteria. Multiple drug resistance is challenging to overcome, especially with routinely used antibiotics. This improves the bacteria's capacity to colonize their hosts and causes

the creation of more virulent substances, which boost their survival [45].

Conclusion

In this study, shawarma samples that analyzed contain different pathogenic microorganism as *E. coli, S. aureus* and *Salmonella spp.* Therefore, it is essential for the health authorities to raise awareness, train, and educate food handlers and groundskeepers. These requirements will guarantee that food safety and hygiene standards are met by retailers, protecting those who rely on RTE for convenience.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that they have no conflict of interest.

TABLE 1. The incidence and acceptability of the examined shawarma samples according to the microbiological guide lines (CFS, 2014) for *E. coli*, *S. aureus* and Salmonellae spp. Counts cfu/g (n=100 of each)

Microorganism	Chicken shawarma				Beef shawarma						
	Incidenc acceptable e		table	Un acceptable		Incidence	acceptabl e		Un acceptable		Range
	No. (%)	No.	%	No.	%	No. (%)	No.	%	No.	%	_
E. coli	15 (15) ^a	85	85	15	15	21 (21) ^a	79	79	21	21	Free
S. aureus	21 (21) ^b	79	79	21	21	30 (30) ^b	70	70	30	30	>10 ²
Salmonellae	14 (14) ^c	86	86	14	14	9 (9) ^c	91	91	9	9	Free

^{*}Acceptability according to Centre for Food Safety, 2014. The results are significantly different when the superscripted letters are different (p<0.01)

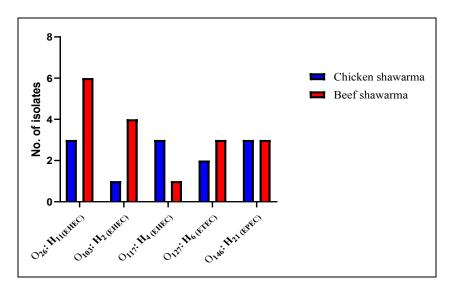


Fig. 1. Serological characterization of E. coli isolates from chicken and beef shawarma samples

TABLE 2. Incidence of serotypes of Salmonellae isolated from chicken and beef shawarma (n=100).

Products	Chicken	shawarma	Beef shawarma		
	No	%	No	%	
S. Typhimurium	6	6	2	2	
S. Infantis	2	2	3	3	
S. Montevideo	2	2	2	2	
S. Enteritidis	4	4	2	2	
Total	14	14	9	9	

[%] calculated in relation to total samples of each group (n=100)

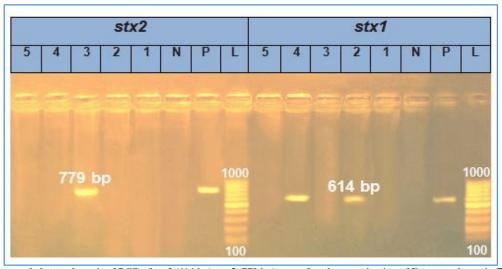


Fig. 2. Agarose gel electrophoresis of PCR of stx1 (614 bp), stx2 (779 bp) genes for characterization of Enteropathogenic E. coli, 100 bp ladder as molecular size DNA marker (Lane L), control positive E. coli for stx1, stx2 (Lane P), control negative (Lane N) and the examined samples (lanes 1-5).

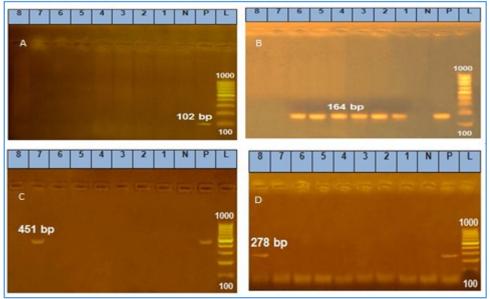


Fig. 3. (A, B, C & D) Agarose gel electrophoresis of PCR of sea (102 bp), (164 bp), (451 bp) and (278 bp) enterotoxins genes for characterization of *S. aureus*, 100 bp ladder as molecular DNA marker (Lane L), control positive for sea, seb, sec and sed genes (Lane P). Control negative (Lane N) and the examined samples (Lanes 1-8).

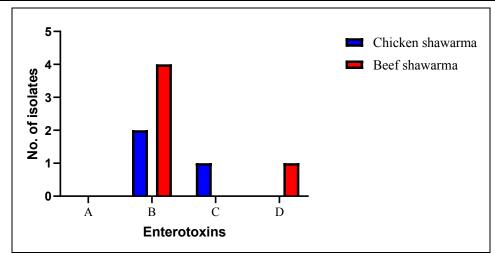


Fig. 4. Occurrence of enterotoxins secreted by S. aureus isolated from chicken and beef shawarma (n=8).

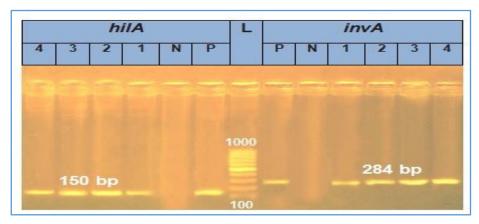


Fig. 5. Agarose gel electrophoresis of PCR of hilA(150 bp), invA (284 bp) genes for characterization of Salmonellae, 100 bp ladder as molecular size DNA marker (Lane L), control positive salmonella for hilA and invA genes (Lane P), control negative (Lane N) and the examined samples (Lanes 1-4).

TABLE 3. Performance of AST-GN 96 card for pathogenic and non-pathogenic E. coli

Antimicrobial		MIC	Interpretation		
	pathogenic strains	Non-pathogenic strains	pathogenic strains	Non-pathogenic strains	
ESBL	NEG	NEG	-	-	
Ampicillin	>=32	16	R	I	
Amoxicillin/ Clavulanic acid	8*	R*	R	R	
Cefalexin	<=8	<=4	S	S	
Cefalotin	>=64	16	R	I	
Cefoperazone	>=64	16	R	S	
Flumequine	2	<=1	I	S	
Ceftiofur	>=8	>=8	R	R	
Gentamicin	<=3	<=1	S	S	
Neomycin	<=2	<=1	S	S	
Enrofloxacin	0.25	<=0.12	I	S	
Marbofloxacin	0.5	1	S	S	
Tetracycline	<=1	<=1	S	S	
Imipenem	0.5	<=0.25	S	S	
Trimethoprim/ Sulfamethoxazole	>=320	<=0.5	R	S	

ESBL: Extended Spectrum Beta-Lactamase. MIC Interpretation Guideline: global Clinical & Laboratory Standards Institute (CLSI- based), Therapeutic Interpretation Guideline: natural resistance

TABLE 4. Performance of AST-GP79 card for toxigenic and non-toxigenic S. aureus

Antimicrobial		MIC	Interpretation		
	Toxigenic strains	Non -Toxigenic strains	Toxigenic strains	Non -Toxigenic strains	
Cefoxitin	POS	NEG	+	-	
Benzylpenicillin	>=0.5	0.06	R	S	
Oxacillin	>=4	0.5	R	S	
Gentamicin	>=16	<=0.5	R	S	
Ceftifur	>=8	4	R	I	
Cefquinome	<=1	<=1	S	S	
Amikacin	<=2	<=2	S	S	
Clindamycin Resistance	NEG	NEG	-	-	
Erythromycin	>=8	<=2	R	I	
Clindamycin	>=4	<=2	R	I	
Florfenicol	8	8	S	S	
Tilmicosin	>=4	>=2	R	R	
Tylosin	>=32	>=16	R	R	
Tetracycline	>=16	<=1	R	S	
Kanamycin	<=4	<=4	S	S	
Trimethoprim/ Sulfamethoxazole	<=10	<=10	S	S	

TABLE 5. Performance of AST-GN 96 card for pathogenic Salmonellae spp.

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Gentamicin	<=2	S
Ampicillin	<=4	S	Neomycin	<=1	S
Amoxicillin/	6	S	Enrofloxacin	<=0.5	S
Clavulanic acid					
Cefalexin	<=4	S	Marbofloxacin	<=0.5	S
Cefalotin	12	I	Pradofloxacin	<=0.12	S
Cefpodoxime	<=8	S	Chloramphenicol	<=0.5	S
Cefovecin	<=4	S	Tetracycline	<=1	S
Ceftiofur	<=8	S	doxycycline	<=16	S
Imipenem	<=0.25	S	Nitrofurantoin	<=2	S
Amikacin	<=2	S	Trimethoprim/Sul famethoxazole	12	I

ESBL: Extended Spectrum Beta-Lactamase. MIC Interpretation Guideline: global Clinical & Laboratory Standards Institute (CLSI- based), Therapeutic Interpretation Guideline: natural resistance

References

- Ema, F.A., Shanta, R.N., Rahman, M.Z., Islam, M.A. and Khatun, M.M. Isolation, identification, and antibiogram studies of *Escherichia coli* from ready-toeat foods in Mymensingh, Bangladesh. *Veterinary World*, 15, 1497 (2022).
- Jaroni, D., Ravishankar, S. and Juneya, V. Microbiology of ready-to-eat foods. In: Hwang, A. and Huang, L., editors. Ready-to-eat Foods Microbial Concerns and Control Measures. Ch. 1. CRC Press, Boca Raton. 1–6 (2010).
- 3. Afaf, A.M.A., Samah, E., Shimaa, E. and Soad, A.N. Detection of Shiga-Like Toxin Producing Escherichia coli in Food of Animal Origin by Street Vendors at

- Luxor City. Animal Health Research Journal, 7, 216-226 (2019).
- 4. Bagumire, A. and Karumuna, R. Bacterial contamination of ready-to-eat meats vended in highway markets in Uganda. *African Journal of Food Science*, **11**, 160–170 (2017).
- Oje, O.J., Ajibade, V.A., Fajilade, O.T. and Ajenifuja, A. Microbiological analysis of RTE foods vended in mobile outlet catering units from Nigeria. Advanced *Journal of Food Science and Technology*, 5, 15–19 (2018).

- Islam, S., Thangadurai, D., Sangeetha, J. and Cruz-Martins, N. Global Food Safety: Microbial Interventions and Molecular Advancements. CRC Press (2023).
- 7. Mokhtar, A. and Karmi, M. Surveillance of food poisoning *Escherichia coli* (STEC) in ready-to-eat meat products in Aswan, Egypt. *Egyptian Journal of Veterinary Sciences*, **52**, 41–50 (2021).
- Fathalla, S. M., Mahyudin, N.A., Mohamad, G. F., Rukayadi, Y. and Jaafar, S.N. Detection of enterotoxin gene (sea) and biofilm formation ability among multidrug resistant Staphylococcus aureus isolated from shawarma sandwich sold at selected kiosks in Klang Valley, Malaysia. *Food Research*, 4, 1234 – 1244 (2020).
- Cheng, C.M., Lin, W., Van, K.T., Phan, L., Tran, N.N. and Farmer, D. Rapid detection of Salmonella in foods using real-time PCR. *Journal of Food Protection*, 71, 2436-2441 (2008).
- Myint, M. S., Johnson, Y. J., Tablante, N. L. and Heckert, R. A. The effect of pre-enrichment protocol on the sensitivity and specificity of PCR for detection of naturally contaminated Salmonella in raw poultry compared to conventional culture. *Food Microbiology*, 23, 599–604 (2006).
- Riyaz-Ul-Hassan, S., Verma, V. and Qazi, G. N. Rapid detection of Salmonella by polymerase chain reaction. *Molecular Cell Probes*, 18, 333–339 (2004).
- 12. ISO "International Standards Organization" (4833-1). Microbiology of food chain- Horizontal method for the enumeration of microorganisms. Part I; Colony count at 30°C by the pour plate technique. International Standards Organization, Geneva, Switzerland (2013).
- 13. ISO "International Standards Organization" (16649-2). Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. Geneva, International Organization for Standardization. 8 p (2001).
- Kok, T., Worswich, D. and Gowans, E. Some serological techniques for microbial and viral infections. In Practical Medical Microbiology, Collee, J., Fraser, A., Marmion, B. and Simmons, A. (Ed.), 14th ed., Edinburgh, Churchill Livingstone, UK (1996).
- Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A. and Menna, L.F. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. *Letters in Applied Microbiology*, 43, 293– 295 (2006).
- 16. ISO "International Standards Organization" (6888). Microbiology of the food chain — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) (2018).
- Mehrotra, M., Wang, G. and Johnson, W.M. Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome

- toxin 1, and methicillin resistance. *Journal of Clinical Microbiology*, **38**, 1032-1035 (2000).
- Shah, D., Shringi, S., Besser, T. and Call, D. Molecular detection of foodborne pathogens, Boca Raton: CRCPress, In Liu, D. (Ed). Taylor & Francis group, Florida, USA. 369-389 (2009).
- ISO "International Standards Organization" (6579-1).
 Microbiology of the food chain Horizontal method for the detection, enumeration and serotyping of Salmonella Part 1: Detection of Salmonella spp. (2017).
- Kauffman, G. Kauffmann white scheme. *Journal Acta Pathological and Microbiological Sciences*, 61, 385 (1974).
- 21. Yang, X., Brisbin2, J., Yu, H., Wang, Q., Yin, F., Zhang, Y., Sabour, P., Sharif, S. and Gong, J. Selected Lactic Acid-Producing Bacterial Isolates with the Capacity to Reduce Salmonella Translocation and Virulence Gene Expression in Chickens. *PLOS ONE*, 9, e93022 (2014).
- Olivera, S.D., Rodenbusch, C.R., Ce, M.C., Rocha, S.L.S. and Canal, C.W. Evaluation of selective and non-selective enrichment PCR procedures for Salmonella detection. *Letters in Applied Microbiology*, 36, 217-221 (2003).
- Nethathe, B., Matsheketsheke, P.A., Mashau, M.E. and Ramashia, S.E. Microbial safety of ready-to-eat food sold by retailers in Thohoyandou, Limpopo province, South Africa. *Cogent Food & Agriculture*, 9, 2185965 (2023).
- 24. Geletu, U.S., Usmael, M.A. and Ibrahim, A.M. Isolation, identification, and susceptibility profile of E. coli, Salmonella, and S. aureus in dairy farm and their public health implication in Central Ethiopia. Veterinary Medicine International, 1887977 (2022).
- Aworh, M.K., Kwaga, J.K., Hendriksen, R.S., Okolocha, E.C., Harrell, E. and Thakur, S. Quinoloneresistant Escherichia coli at the interface between humans, poultry and their shared environment-a potential public health risk. *One Health Outlook*, 5, 1-16 (2023).
- 26. CFS (Centre for Food Safety). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). In Food and Environmental Hygiene Department, Hong Kong (2014).
- WHO (World Health Organization). Interim practical manual: Supporting national implementation of the WHO guidelines on core components of infection prevention and control programmes (No. WHO/HIS/SDS/2017.8) (2017).
- Ajaja, S., Bozakoukb, I., Abdallac, I., Bumadianb, M., Bleiblob, A. and Keand, I. Bacterial contamination of fast food shawarma sandwiches in local restaurants in Ben-ghazi city, Libya. *Libyan Journal of Science and Technology*, 11, 2 (2020).
- Abebe, E., Gugsa, G. and Ahmed, M. Review on major food-borne zoonotic bacterial pathogens. *Journal of Tropical Medicine*, 2020, 4674235 (2020).
- Wimalasekara, S.G.M.R.L. and Gunasena, G.D.D.K. Microbiological quality of ready-to-eat meat-based

- food available in temporary food outlets in Gall Face Green, Colombo, Sri Lanka. *International Journal of Agriculture and Forest Plant*, **4**, 38-44 (2016).
- 31. Jang, H. G., Kim, N. H, Choi, Y. M. and Rhee, M. S. Microbiological quality and risk factors related to sandwiches served in bakeries, cafés, and sandwich bars in South Korea. *Journal of Food Protection*, 76, 231-238 (2013).
- 32. Tîrziu, E., Bărbălan, G., Morar, A., Herman, V., Cristina, R.T. and Imre, K. Occurrence and antimicrobial susceptibility profile of Salmonella spp. in raw and ready-to-eat foods and Campylobacter spp. in retail raw chicken meat in Transylvania, Romania. *Foodborne Pathogenic Diseases*, 17, 479-484 (2020).
- 33. Castrica, M., Andoni, E., Intraina, I., Curone, G., Copelotti, E., Massacci, F.R., Terio, V., Colombo, S. and Balzaretti, C.M. Prevalence of *Listeria monocytogenes* and *Salmonella spp.* in different ready to eat foods from large retailers and canteens over a 2-year period in Northern Italy. *International Journal of Environmental Research and Public Health*, 18, 10568 (2021).
- 34. Rahman, M.A., Rahman, A.K.M., Islam, M.A. and Alam, M.M. Antimicrobial resistance of Escherichia coli isolated from milk, beef and chicken meat in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 15, 141-146 (2017).
- Nataro, J.P. and Kaper, J.B. Diarrheagenic Escherichia coli. Clinical Microbiological Reviews, 11, 142-201 (1998).
- Morshdy, A.E., Hussein, M.A., Tharwat, A.E., Moustafa, N.A. and Hussein, O.K. Prevalence of shiga toxigenic and multi drug resistant *Escherichia coli* in ready to eat chicken products' sandwiches. *Slovenian Veterinary Research*, 55 (2018).
- 37. Bruyand, M., Mariani-Kurkdjian, P., Gouali, M., de Valk, H., King, L.A., Hello, S.L., Bonacorsi, S. and Loirat, C. Hemolytic uremic syndrome due to Shiga

- toxin-producing Escherichia coli infection. Médecine et Maladies Infectieuses, 48, 167–174 (2018).
- Secim, Y. and Ucar, G. Evaluation of the desserts, which are hosmerim, cheese halva, Kunafah produced in Turkish cuisine-in aspect of tourism. *International Journal of the Society of Economic Research*, 3, 1478– 1484 (2017).
- Liu, C., Shen, Y., Yang, M., Chi, K. and Guo, N. Hazard of Staphylococcal enterotoxins in food and promising strategies for natural products against virulence. *Journal of Agriculture and Food Chemistry*, 70, 2450-2465 (2022).
- Swamy, S. C., Barnhart, H. M., Lee, M. D. and Dreesen, D. W. Virulence determinants invAand spvCin salmonellae isolated from poultry products, wastewater, and human sources. *Applied Environmental Microbiology*, 62, 3768–3771 (1996).
- 41. Popoff, M. Y. Antigenic formulas of the Salmonella serovars, 8th ed. Institute Pasteur, Paris (2001).
- 42. Sanchez-Jimenez, M. M. and Cardona-Castro, N. Validation of a PCR for diagnosis of typhoid fever and salmonellosis by amplification of the hila gene in clinical samples from Colombian patients. *Journal of Medicinal Microbiology*, 53, 875–878 (2004).
- 43. Taha, Z.M., Mustafa, S.I., Ahmed, C.J., Mikaeel, F.B., Ali, M.M., Khudr, A.I., Ali, S.Z. and Haji, M.L. Multidrug-resistant and clonal dispersion of enterotoxigenic Escherichia coli from ready-to-eat meat products in Duhok province, Iraq. *Iraqi Journal of Veterinary Science*, 37, 275-282 (2023).
- Al-Bahry, S.N., Mahmoud, I.Y., Paulson, J.R. and Al-Musharafi, S.K. Antibiotic resistant bacteria in terrestrial and aquatic environments: A review. *International Arabic Journal Antimicrobial Agents*, 4, 754 (2015).
- Frieri, M., Kumar, K. and Boutin, A. Antibiotic resistance. *Journal of Infection and Public Health*, 10, 369-3978 (2017).

الكشف عن البكتيريا المفرزة للسموم في الشاورما وحساسيتها للمضادات الحيوية باستخدام الفايتك 2

إيمان فتحى محمد * ودينا إبراهيم الذهبي

قسم صحة الأغذية - معهد بحوث صحة الحيوان - فرع شبين الكوم - مركز البحوث الزراعية المصري - مصر.

الملخص

أجريت الدراسة الحالية لتقصى وجود بكتيريا الايشريكية القولونية والمكور العنقودي الذهبي والسالمونيلا في شطائر شاورما الدجاج واللحم الجاهزة للأكل (100 لكل مجموعة) من عدة الدجاج واللحم بمحافظة المنوفية. وكانت 15% و 21% و 14% لسندويشات شاورما الدجاج على التوالي بينما كانت 21% و 30% مطاعم بمحافظة المنوفية. وكانت 15% و 12% و 14% لسندويشات شاورما الدجاج على التوالي بينما كانت 21% و 30% و 9% لسندويشات شاورما اللحم على التوالي وأظهرت النتائج أن عينات شاورما الدجاج كانت أكثر قبولا من عينات شاورما اللحم، كما كشفت الدراسة عن عزل عدد من الأنماط المصلية للإشريكية القولونية والسالمونيلا. أنتجت العزلات المسببة المعوية، والجينات المسؤولة عن إنتاج تلك السموم. أظهر اختبار الحساسية للمضادات الحيوية أن السلالات المسببة للأمراض من بكتيريا الايشريكية القولونية والمكور العنقودي الذهبي كانت مقاومة للعديد من المضادات الحيوية، في حين أظهرت السلالات غير المسببة للأمراض حساسية لمعظم المضادات الحيوية. كانت السالمونيلا حساسة لمعظم المضادات الحيوية التي تم اختبارها، باستثناء سيفالوتين وتريميثوبريم/سلفاميثوكسازول. توفر الدراسة معلومات قيمة عن وجود الميكروبات الضارة في شطائر الشاورما وحساسيتها للمضادات الحيوية، والتي يمكن أن تساعد في تحسين تدابير سلامة المغذة ت

الكلمات الدالة: الشاور ما، الإشريكية القولونية، المكورات العنقودية الذهبية، السالمونيلا.