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Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon

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Abstract

The aim of this study was to characterize at the molecular level the different stains of *Salmonella* spp. and *Escherichia coli* that were isolated from meat-based fast food in Lebanon. In addition, this study evaluated the resistance of those strains to different antimicrobials that are commonly used. The foods included were Lahm-bi-Ajeen (LBA, meat pies) and Shawarma (Lebanese meat sandwiches similar to Gyros and Donairs, containing meat, vegetables, and sesame seeds-oil-based sauce). Polymerase chain reaction (PCR) was used to characterize and identify the strains of both bacteria. *Salmonella* species characterization was performed using *rfb* genes cluster genetic marker, while that of *E. coli* strains were carried out based on stx1, stx2, eaeA, fliC, and ehlyA virulence markers. The characterized strains were then tested for their response to various antimicrobials. The results showed that the tested foods were contaminated with *Salmonella paratyphi* (serogroup A) and Shiga Toxin (Stx)-producing *E. coli* (*STX-EC*). The PCR showed that 75% of *E. coli* tested strains was positive in PCR performed with stx1 primers, one of which was eaeA positive. Two of the tested strains were positive using PCR with fliC primers.

The resistances of the various strains were evaluated using the following antimicrobials: Oxacillin, Teicoplanin, Trimethoprim/sulfamethoxazole, Gentamicin, Clindamycin, Cefotaxime, Cefuroxime, Erythromycin, and Vancomycin. Bacteria were highly resistant to one or more of the tested antimicrobials. Approximately 69% of *E. coli* and 77.8% of *Salmonella* spp. exhibited resistance. *Salmonella* spp. were shown to be 100% resistant to four antimicrobials: Oxacillin, Teicoplanin, Clindamycin, Vancomycin, and Erythromycin, while *E. coli* was 100% resistant to Teicoplanin and Trimethoprim/sulfamethoxazole. The most interesting findings were the high susceptibility of the *E. coli* to Gentamicin (100%). Highest

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resistance in the case of *Salmonella* spp. was seen against Cefotaxime (74%). Those two antimicrobials are commonly used for the treatment of enteric infections caused by gram-negative bacteria. The results showed that meat-based fast foods in Lebanon could be a public health hazard, especially Shawarma, as they may act as a potential vehicle for many antimicrobial-resistant pathogenic organisms. Improper hygienic standards and indiscriminate use of antimicrobials are two of the main causes for the prevalence of these pathogenic resistance strains in Lebanon. These results will emphasize the need to implement protective measures and more emphasis will be placed on the application of hygienic practices to reduce the levels of food contamination.

Keywords: Lebanese fast foods; Antimicrobial resistance; *Escherichia coli* (*STX-EC*); *Salmonella paratyphi*

1. Introduction

The importance of food as a vehicle for the transmission of many diseases has been documented for a long time especially in the developing countries where hygienic standards are not strictly followed and enforced. The presence of these microorganisms can lead to many food-borne outbreaks. Furthermore, the wide application of antimicrobials has led to large-scale dissemination of bacteria resistant to antimicrobials in the environment. Infection caused by those resistant strains usually lead to a high fatality rate than especially among immuno-comprised individuals (Holmberg et al., 1984). The identification of pathogenic organisms is highly crucial for surveillance, prevention, and control of food-borne diseases. In addition, studying antimicrobial resistance in humans and animals is important in order to (a) detect changes patterns in resistance, (b) implement control measures on the use of antimicrobial agents, and (c) prevent the spread of multidrug-resistant strains of bacteria (Duijkeren et al., 2003).

Considering the marked importance of *Salmonella* spp. and *Escherichia coli* (*E. coli*) organisms as food-borne pathogens, we aimed in this study at characterizing the different strains of *Salmonella* spp. and *E. coli* that are present in meat-based fast food in Lebanon and to evaluate their antimicrobial resistance patterns.

Salmonellosis has become one of main causative agents of enteric infections in humans and animals (Tauxe, 1996). Several Salmonellosis outbreaks have been documented worldwide due to the consumption of contaminated meat in the last decade (Liewellyn et al., 1998).

In *Salmonella* spp., the *rfb* gene cluster encodes for the O antigens of *Salmonella* spp. polysaccharides (Lee et al., 1992). Variations among different O antigen structures are manifested in the types of sugar present or the arrangement of sugars. This variability provides the basis for serotyping *Salmonella* spp. into serogroups (Lee et al., 1992). In our study, this highly polymorphic *rfb* gene cluster has been targeted as a molecular marker to detect *Salmonella* spp. serovars in contaminated meat-based fast food in Lebanon. Polymerase chain reaction (PCR) procedure was performed using four sets of primers that amplify the *rfb*(B), *rfb*(C1), *rfb*(C2) and *rfb*(D) genes of the *rfb* cluster, respectively (Luk et al. 1993; Lee et al., 1992).

Escherichia coli on the other hand is common, usually harmless, bacteria of the human intestinal flora. However, five groups of *E. coli*-causing diarrhea in humans and other warm-blooded animals have been identified (Brook et al., 1994; Wasteson, 2001). These include enterotoxinogenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC). The later includes Shiga Toxin (Stx)-Producing *E. coli* (*STX-EC*).

Shiga toxin (Stx)-producing *E. coli* (*STX-EC*), also known as Verotoxin-producing *E. coli*, is associated with infant diarrhea, haemorrhagic colitis, thrombiotic thrombocytic purpura, and hemolytic uremic syndrome in humans (Griffin and Tauxe, 1991). *E. coli* O157:H7, which belongs to *STX-EC* group, is the most common serotype isolated from individuals with haemorrhagic colitis (*E. coli* O157:H7). Outbreaks of infection with those bacteria have emerged due to the consumption of contaminated animal-derived food products (Belongia et al., 1991; Borezyk et al., 1987). Research reveals that *E. coli* O157:17 infections are endemic in cattle, goat, sheep, and other farmed

animals, with the cattle being the primary source of this pathogen (Langreid et al., 1999).

Different molecular markers throughout *STX-EC* genomic DNA are now being used for the detection of *STX-EC*, using mainly Polymerase chain reaction (PCR) for this purpose (Jackson, 1991; Karch and Meyer, 1989). In this study, five genes were targeted for amplification (*stx1*, *stx2*, *eaeA*, *ehlyA*, and *fliC*), using eight different sets of oligonucleotide primers. Sometimes, two different sets of primers were used to amplify the same gene, as described in the literature.

To achieve our aim, different bacterial isolates were tested for their susceptibility to nine different antimicrobials. Moreover, the role of plasmids in conferring antimicrobial resistance was studied by transforming plasmid preparations from different isolates into *E. coli* wild type bacteria, MG1655.

2. Materials and methods

2.1. Sample collection

Lahm-bi-Ajeen (LBA, meat pies) and Shawarma (Lebanese meat sandwiches similar to Gyros and Donairs, containing meat, vegetables, sesame seeds oil, and other ingredients) samples were randomly collected from five areas in Lebanon. These areas included Beirut, Mount Lebanon, north (Tripoli mainly), south (Sidon mainly), and east (the Bekaa Valley). The distributions of samples were as follows: 40% of the samples obtained from Beirut, 20% from Mount Lebanon, and 13% from each of the north, the south, and the east of Lebanon. Samples were collected over an 11-month period from 37 food establishments. These food establishments ranged from restaurants to fast food outlets to small kiosks and street vendors. Each establishment was visited on at least two occasions and during the summer and winter seasons. A total of 95 samples were aseptically collected in the same way delivered to the consumer. Samples were then packaged in sterile Whirl-Pak bags and brought to the laboratory on ice in a cooler (<8 °C) within 2 h from the time of purchase. Duplicate samples were obtained whenever possible. In the Shawarma sampling, a sharp knife was used to cut meat from the surface. All samples were collected during lunchtime; no samples were obtained after 3:00 p.m. All samples were analyzed within 2 h after their arrival to the laboratory.

2.2. Bacterial isolation and bacteriological analysis

Samples of Lahm-bi-Ajeen (LBA) and Shawarma were subjected to various biological tests. To isolate bacteria, a 25-g portion was weighed aseptically in a sterile stomacher bags (Seward Medical Stomacher® Bags), diluted with 225 ml of sterilized 0.1% w/v peptone water (Oxoid Media) and macerated in a stomacher (Mix 1 AES Laboratories, ref: AESAP1040) for 3 min. For isolation and counting, serial dilutions ranging from 10^{-1} to 10^{-6} were performed by adding 1 ml of homogenate to 9 ml sterilized peptone water (1:10 dilution factor). Bacteriological analyses were performed by plating 0.1 ml of each dilution on agar plates. Plates were then incubated at 37 °C for 48 h, and colonies were counted. The following bacteria were isolated: indicator bacteria (Aerobic plate count and *E. coli*) and pathogenic organisms (*Salmonella* spp. and *STX-EC*). Bacteriological analyses were done according to American Public Health Association (APHA, 2001). All bacteriological media used were of Oxoid. Plate Count Agar (PCA) was used for aerobic plate count (APC), MacConkey Agar (MCA) for *E. coli* detection, Sorbitol MacCankoky Agar (SMAC) for *STX-EC*, and Bismuth Sulfate Agar (BSA) for *Salmonella* spp. detection. Purple colonies on MCA were identified as *E. coli*, whereas white colonies on SMAC were presumptive *STX-EC* and were therefore stereotyped by PCR for confirmation. On the other hand, brown to black colonies on BSA were identified as presumptive *Salmonella* spp. and were then subjected to PCR tests for further confirmation.

2.3. DNA extraction

Total bacterial DNA was extracted as described by Ausubel et al. (1987). Briefly, bacteria were grown on Brain Heart Infusion (BHI; Oxoid media) broth over night at 37 °C, and then bacterial cells were harvested by centrifugation and lysed with sodium lauryl sulfate. DNA was extracted from the lysate with phenol-chloroform and precipitated with isopropanol. A 100–200-ng DNA template was used per 25 μl Polymerase chain reaction (PCR) reaction.

Table 1
Primers used in identifying *Salmonella* serogroups

Target gene	Primers	Sequence	Resource	Predicted size of the amplified product (bp)
rfb(B)	*rfb*(B)	F: GAGAATATGTAATTGTCAG R: GTAACCGTTTCAGTAGTTC	Luk et al. (1993, 1997)	851
rfb(C1)	*rfb*(C1)	F: AAGTGTGTTTGATTGTTGG R: GTAACCGTTTCAGTAGTTC	Lee et al. (1992)	781 and 410
rfb(C2)	*rfb*(C2)	F: ATGCTTGATGTGAATAAG R: CTAATCGAGTCAAGAAAG	Luk et al. (1993, 1997)	795
rfb(D)	*rfb*(D)	F: AGTCACGACTTACATCCTAC R: ACCTGCTATATCAGCACAAC	Luk et al. (1993, 1997)	703

2.4. PCR assays

For *Salmonella* spp. detection, primers for PCR were selected based on the *rfb* gene clusters specific for *Salmonella* spp. serogroups B, C2, and D, as reported by Luk et al. (1993, 1997). The primers for *Salmonella* spp. serovar C1 were designed based on the sequence of *Salmonella enterica* group C1 *rfb* gene cluster, as described by Lee et al. (1992). Primer sequences used in the PCR are listed in Table 1. DNA amplification was performed following the protocol of Luk et al. (1993, 1997) with some modifications: detection of PCR products by gel electrophoresis and photography of agarose gels were carried out as indicated by Gillespie et al. (1997). Briefly concerning the detection for *STX-EC*, primers were selected based on five genes specific for *STX-EC* strains. Primers to amplify Shiga toxins 1 and 2 (*stx1* and *stx2*) genes, also named as *slt1* and *slt2*, were selected as shown by Osek and Gallein (2002) and Victor et al. (1992) (Table 2). Due to mutations that may occur spontaneously in nature, many gene sequences might be affected. For this reason, two sets of primers were used to amplify each of *stx1* and *stx2* genes (Table 2). This is in addition to the fact that different sets of primers were reportedly used to amplify the above-mentioned genes. *STEC* primers were used to amplify both *stx1* and *stx2* in combination, as described by Reischl et al. (2002). The three other genes that were targeted for amplification were *eacA* gene, encodes for intimin gamma, *ehlyA*, encodes for enterohemolysin, and *fliC*, encodes for H7, as described by Reischl et al. (2002) and Osek and Gallein (2002), respectively (Table 2). Amplification of bacterial

Table 2
Primers used in identifying *STX-EC* strains

Target gene	Primers	Sequence	Resource	Predicted size of the amplified product (bp)
stx1	stx1	F: CAGTTAATGTCGTGGCGAAGG R: CACCAGACAATGTAACCGCTG	Osek and Gallein (2002)	348
stx2	stx2	F: ATCCTATTCCCGGGAGTTTACG R: GCGTCATCGTATACACAGGAGC	Osek and Gallein (2002)	584
slt1	slt1	F: ACACTGGATGATCTCAGTGG R: CTGAATCCCCCTCCATTATG	Gannon et al. (1992)	614
slt2	slt2	F: CCATGACAACGGACAGCAGTT R: CCTCTCAACTGAGCACTTTG	Gannon et al. (1992)	779
stx1+stx2	STEC	F: gA(Ag) C(Ag)A AAT AAT TTA TAT gTg R: TgA TgA Tg(Ag) CAA TTC AgT AT	Reischl et al. (2002)	520
eaeA	eaeA	F: gAC CCg gCA CAA gCA TAA gC R: CCA CCT gCA gCA ACA AgA gg	Reischl et al. (2002)	383
fliC	flicH7	F: GCTGCAACGGTAAGTGAT R: GGCAGCAAGCGGGTTGGT	Reischl et al. (2002)	984
ehlyA	ehlyA	F: gCA TCA TCA AgC gTA CgT TCC R: AAT gAg CCA AgC Tgg TTA AgC T	Reischl et al. (2002)	532

DNA was performed in 50-μl volumes containing 150–200-ng DNA templates; 2 mM MgC12 (AB-gene products); 1X reaction buffer (AB-gene products); 0.2 mM each of dATP, dGTP, dCTP, and dTTP (AB-gene products); 1.25 μM of primer; and 1U of *Thermus aquaticus* (*Taq*) DNA polymerase (AB-gene products). Amplification was performed using a DNA thermal cycler (Bio-Rad) for 35 cycles for 3 min at 93 °C, 30 s at 55 °C, and 2 min at 72 °C, and a final extension at 75 °C for 10 min. Negative controls (no DNA template) were included with every PCR assays. After the PCR, 10-μl aliquots were analyzed through submarine electrophoresis with 0.1% agarose gel containing 0.25 μg ethidium bromide per ml. The DNA samples were analyzed through agarose gel electrophoresis, visualized through UV transmission, and photographed.

2.5. Antimicrobial susceptibility testing

Characterized strains were tested for their susceptibility to nine antimicrobials, using the disk diffusion method as set by the National Committee for Clinical Laboratory standards (1997) (NCCLS). Briefly, organisms were grown in a shaking water bath at 37 °C until a 0.5 McFarland turbidity standard was obtained. A volume of 0.1 ml of the culture was then spread over Brain Heart Infusion agar (BHIA, Oxoid) plates. Antimicrobial disks impregnated with either of the following antimicrobials; Oxacillin (1 μg), Teicoplanin (30 μg), Trimethoprim/sulfamethoxazole (1.25+23.75 μg), Gentamicin (10 μg), Clindamycin (2 μg), Cefotaxime (30 μg), Cefuroxime (30 μg), Erythromycin (15 μg) and Vancomycin (30 μg) were placed on the surface of inoculated agar plates. Zones of inhibition around each antimicrobial disk were measured after an incubation period of 24 h at 37 °C. Using NCCLS guidelines, each organism was classified either resistant or susceptible to the antimicrobials. Intermediate-resistant and resistant strains were grouped together. Antimicrobial disks were purchased from BioMerieux, France.

2.6. Plasmid extraction and transformation

Plasmid DNA was extracted using Qiagen Plasmid Mini Kit according to the manufacture's guideline (Qiagen, 40724 Hilden, Germany), and DNA was run using agarose gel electrophoresis, visualized through UV transmission, and photographed. To decipher the role of plasmids in conferring antimicrobial resistance, a number of 21 different plasmid preparations from *E. coli* and *Salmonella* spp. were transformed into wild type *E. coli* MG1655 strain (Provided by Dr. Khattar, Biology Department, AUB), and transformants were selected on BHI agar plates supplemented with different antimicrobials of specific concentrations: Ampicillin (50 μg/ml), Chloramphenicol (20 μg/ml), Naladixic acid (15 μg/ml), Neomycin (50 μg/ml), and Tetracycline (15 μg/ml). Transformation procedure was carried out as described by Sambrook (1989). In brief, 200-μl competent cells, prepared in the presence CaCl2, were mixed with 5–10 ng plasmids, incubated on ice for 30 min, and then heat-shocked at 42 °C for 90 s. Then, 1 ml of Lauria Broth (LB) was added to the mixture and incubated at 37 °C to enable bacterial growth. A volume of 100-μl aliquot was then spread on BHI agar plates supplemented with antimicrobials of specific concentrations. Plates were then incubated at 37 °C for 24 h, and transformants were detected by colony formations.

3. Results

3.1. Bacteriological counts and molecular characterization of Salmonella paratyphi and STX-EC using PCR

Regarding the distribution of microbial population in LBA, *E. coli* and *Salmonella* spp. were not detected at all. On the other hand, 55% of the samples was contaminated with *E. coli*, whereas contamination with suspected *Salmonella* spp. was detected in 47.5% of the samples.

Random isolates from the different samples were subjected to biochemical tests. A number of those tested positive for *Salmonella* spp. Ten of those *Salmonella* spp. isolates with different morphologies were then analyzed by PCR for the *rfb* gene cluster molecular marker. Isolates were classified according to the size of band visualized on agarose gel electrophoresis (Table 3). It was shown that 50% of tested isolates was positive using PCR performed with primers *rfb*(D), yielding a band of approximately 700 bp in size (Fig. 1a and b). Therefore, those

Table 3
Suspected PCR Fragments produced by *Salmonella* spp. primers specific for serogroups: B, C1, C2, D and A

Organism	*rfb*(B)	*rfb*(C1)	*rfb*(C2)	*rfb*(D)	Serogroup
S. typhimurium	851 bp[a]	–[b]	–	–	B
S. choleraesuis	–	781 bp	–	–	C1
S. newport	–	–	795 bp	–	C2
S. enteriditis	–	410 bp	–	703 bp	D
S. paratyphi A	–	–	–	703 bp	A

[a] bp: base pairs.
[b] –: no fragment detected.

isolates could be classified as *S. paratyphi* A, Serogroup A. Two of the tested isolates gave a very high intense band of 550 bp with *rfb*(C2), whereas the expected size is 795 bp (data not shown; Table 4).

Concerning *E. coli*, 12 isolates were analyzed by PCR for the *stx1*, *stx2*, *stx1+stx2*, *eaeA*, and *ehlyA* virulence markers. Those isolates were also tested for *fliC* genetic marker, which encodes for the production of *E. coli* O157:H flagellar protein. Taking into consideration the fact that mutations could occur spontaneously in nature, alteration in the sequence reading chain may result. To be on the safe side and to avoid missing any strains from the different isolates, we opted to use two sets of primers for the detection of the *Stx* genes (Table 2). It was found that 75% of the tested isolates was *stx1*-positive, using PCR performed with *stx1* primer (Table 5; Fig. 2a–c), one of which was positive with *eaeA* primer, giving a product of approximately 380 bp in size (Fig. 3c). On the other hand, two of the tested strains showed a very intense band of approximately 1100 bp in PCR performed with *fliC* primer, whereas the expected band has a size of 984 bp (Fig. 2b).

Table 4
Strains positive in PCR performed with different *Salmonella* spp. genetic markers

Salmonella spp.	Total tested isolates	*rfb*(B)	*rfb*(C1)	*rfb*(C2)	*rfb*(D)
Data	10	–	–	1	5

Hence, those isolates, which showed a 348-bp band with *stx1* primer, could be classified as enterohaemorrhagic *E. coli* (EHEC) or more specifically *STX-EC*, two of which could be classified as O157:H7. It is noteworthy that none of the tested strain has shown positive results using PCR performed with the rest of the primers.

3.2. Antimicrobial susceptibility assays

A total number of 16 bacterial isolates were tested for their antimicrobials susceptibility, using the agar disk diffusion method tables (Tables 6 and 7). In the case of *E. coli*, 9 out of 12 were confirmed positive by PCR and thus included in the study. On the other hand, out of the 10 *Salmonella* spp. isolates, five were *rfb*(D)-positive and two were *rfb*(C2)-positive, and those were included in the study. It was found that 77.8% of the tested *Salmonella* spp. isolates was resistant to at least one of the tested antimicrobials. Frequencies of 100% of the tested *Salmonella* spp.

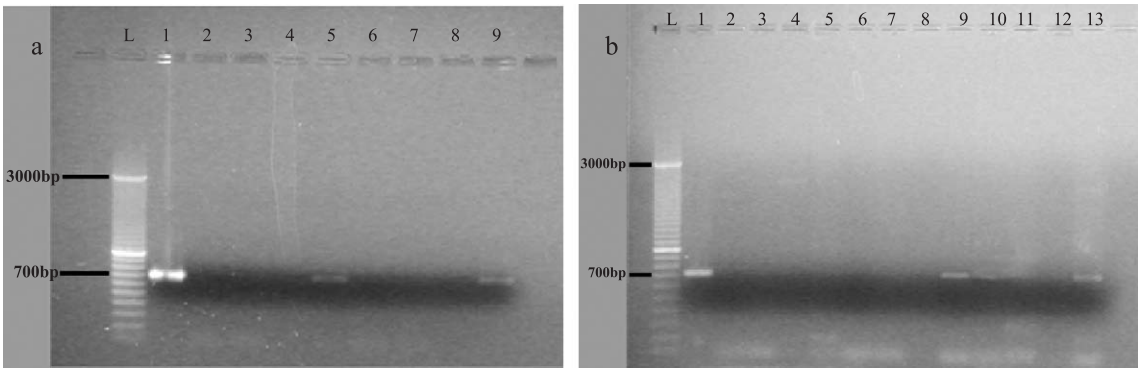

Fig. 1. DNA fragments observed with specific primers for *Salmonella* serogroups following PCR. (a) L: DNA ladder; 1: *S. paratyphi* [*rfb*(D)]; 2–5 and 6–9: BSA.1D and BSA.S1 [*rfb*(B), *rfb*(C1), *rfb*(C2), and *rfb*(D) primers], respectively. (b) L: DNA ladder; 1—*S. paratyphi* [*rfb*(D)]; 2–5, 6–9, and 10–13: BSA.D1, BSA.S3, and BSA.T1 [*rfb*(B), *rfb*(C1), *rfb*(C2), and *rfb*(D) primers], respectively. BSA—Bismuth Sulfate Agar; S3, T1, D1—represents samples symbols from which the isolates were chosen.

Table 5
Strains positive in PCR performed with different *STX-EC* genetic markers

E. coli	Total tested isolates	*stx1* positives	*stx2* positives	*slt1* positives	*slt2* positives	*STEC* Positives	*eaeA* positives	*ehlyA* positives
Data	12	9	–	–	–	–	1	2

strains were resistant to Oxacillin, Teicoplanin, Clindamycin, Erythromycin, and Vancomycin, and 86% was resistant to Trimethoprim/sulfamethoxazole. *Salmonella* spp. tested strains were least resistant to Cefotaxime (25.9%) and with moderate susceptibility of 57.1% against both Cefuroxime and Gentamicin.

Regarding *E. coli*, 69.1% of all the tested isolates was resistant to at least one of the tested antimicrobials. One hundred percent resistance was noted against Teicoplanin, while 88.9% resistance was seen in response to those four antimicrobials: Oxacillin, Clindamycin, Erythromycin, and Vancomycin. All tested *E. coli* strains were susceptible to Gentamicin.

3.3. Transformable plasmid transfer of antimicrobial resistance

Our studies revealed that the *Salmonella* spp. and *E. coli* isolates were highly resistant to various antimicrobials, as mentioned above. In order to determine whether some antimicrobial resistance is plasmid-encoded or not, plasmids were extracted from different isolates (nine preparations were extracted from *E. coli* and six from *Salmonella*

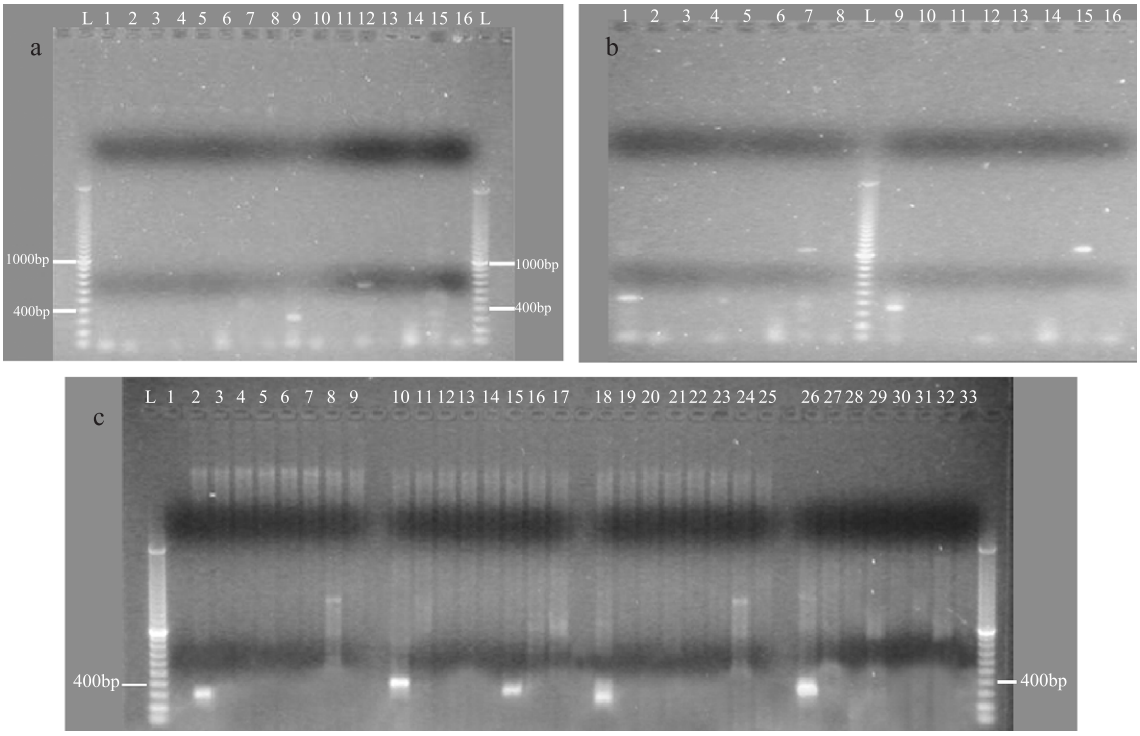

Fig. 2. DNA fragments observed with specific primers for *E. coli* serogroups following PCR. (a) L: DNA ladder; 1–8 and 9–16: SMAC.MT2 and SMAC.T1 [*stx1*, *stx2*, *slt1*, *slt2*, *STEC*, *eaeA*, *fliC*, and *ehlyA*], respectively. (b) SMAC.Sa and SMAC.MT1 [*stx1*, *stx2*, *slt1*, *slt2*, *STEC*, *eaeA*, *fliC*, and *ehlyA*], respectively. (c) MCA.Tn, MCA.Sa, MCA.T1, and MCA.MT1 [*stx1*, *stx2*, *slt1*, *slt2*, *STEC*, *eaeA*, *fliC*, and *ehlyA*], respectively. SMAC—Sorbitol MacCankoky Agar.

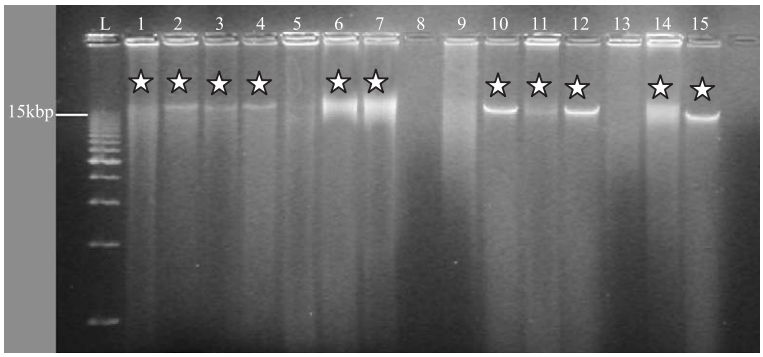

Fig. 3. Plasmid preparations form *E. coli* and *Salmonella* spp. isolates. L: DNA ladder; 1–6: Plasmids preparations from *Salmonella* spp. isolates; 7–15: plasmid preparations from *E. coli* isolates; ☆: positive plasmid preparations.

strains). Plasmid preparations were run on 1% agarose gels. The results showed that 74% of the tested isolates had a plasmid which exceeds 15 kbp in size (Fig. 3). Such big plasmids could harbor a lot of genes including the antimicrobial-resistant ones. Five different plasmid preparations (three from *E. coli* strains and two from *Salmonella* ones) that carried the large plasmids were then transformed into the wild type *E. coli* MG16155. Transformants were grown on media containing different antimicrobials for selection. The antimicrobials that were incorporated in the media included Tetracycline, Ampicillin, Neomycin, Naladixic acid, or Chloramphenicol. Interestingly, Neomycin and Naladixic acid resistances were associated with all successful transformations. However, only two of the transformants were Ampicillin-resistant, and none of them was Tetracycline- or Chloramphenicol-resis-

Table 6
Antimicrobial resistance patterns of *Salmonella* spp.

			Resistance of bacteria to antibiotic			Total
			Susceptible	Intermediate	Resistant	
Antibiotic used	Oxacillin	Count			7	7
		% within antibiotic used			100.0%	100.0%
	Teicoplanin	Count			7	7
		% within antibiotic used			100.0%	100.0%
	Trimethoprim–Sulfamethoxazole	Count	1	3	3	7
		% within antibiotic used	14.3%	42.9%	42.9%	100.0%
	Gentamicin	Count	4	3		7
		% within antibiotic used	57.1%	42.9%		100.0%
	Clindamycin	Count			7	7
		% within antibiotic used			100.0%	100.0%
	Cefotaxime	Count	5	1	1	7
		% within antibiotic used	71.4%	14.3%	14.3%	100.0%
	Cefuroxime	Count	4	2	1	7
		% within antibiotic used	57.1%	28.6%	14.3%	100.0%
	Erythromycin	Count			7	7
		% within antibiotic used			100.0%	100.0%
	Vancomycin	Count			7	7
		% within antibiotic used			100.0%	100.0%
Total		Count	14	9	40	63
		% within antibiotic used	22.2%	14.3%	63.5%	100.0%

Seven isolates were tested; five characterized by PCR and two with biochemical reactions.

Table 7
Antimicrobial resistance patterns of nine characterized *E. coli* strains

			Resistance of bacteria to antibiotic			Total
			Susceptible	Intermediate	Resistant	
Antibiotic used	Oxacillin	Count	1		8	9
		% within antibiotic used	11.1%		88.9%	100.0%
	Teicoplanin	Count			9	9
		% within antibiotic used			100.0%	100.0%
	Trimethoprim–Sulfamethoxazole	Count		2	7	9
		% within antibiotic used		22.2%	77.8%	100.0%
	Gentamicin	Count	9			9
		% within antibiotic used	100.0%			100.0%
	Clindamycin	Count	1		8	9
		% within antibiotic used	11.1%		88.9%	100.0%
	Cefotaxime	Count	7	2		9
		% within antibiotic used	77.8%	22.2%		100.0%
	Cefuroxime	Count	5	3	1	9
		% within antibiotic used	55.6%	33.3%	11.1%	100.0%
	Erythromycin	Count	1		8	9
		% within antibiotic used	11.1%		88.9%	100.0%
	Vancomycin	Count	1		8	9
		% within antibiotic used	11.1%		88.9%	100.0%
Total		Count	25	7	49	81
		% within antibiotic used	30.9%	8.6%	60.5%	100.0%

tant. Interestingly, the plasmids that conferred Ampicillin resistance were extracted from *Salmonella* strains.

4. Discussion

In 2001, the Lebanese Ministry of Health reported 3497 cases attributed to food/water-borne diseases (http://www.public-health.gov.lb, 2003–2004). However, there is little information concerning the molecular characterization of pathogenic bacterial strains in Lebanon. Considering the marked importance of *E. coli* and *Salmonella* infection organisms as food-borne pathogens, we aimed in this study to evaluate the levels of contamination by those organisms in meat-based fast food in Lebanon. The isolated strains were characterized at the molecular level using PCR and evaluated their antimicrobial resistance patterns to different antimicrobials. Lastly, the involvement of plasmids in antimicrobial resistance exhibited by the isolated bacteria was investigated.

Bacteriological analysis performed showed a differential distribution of bacteria between Shawarma and LBA. Shawarma showed a higher level of contamination than Lahm-bi-Ajeen (LBA). The fact that Shawarma had a higher level of contamination than Lahm-bi-Ajeen can be attributed to many reasons. LBA is usually cooked in the oven at 500 °C for around 5 min, which possibly eliminates all kinds of bacteria present. LBA is often served with pressed lemon that increases its acidity and likely reduces bacterial growth. Moreover, there is less handling in the case of LBA as compared to that of Shawarma. In the case of Shawarma, after cutting the meat, most of the food handlers adds vegetables and sauce with their bare hands.

Some factors that may attribute to bacterial contamination of Shawarma could be due to the way Shawarma is cooked. It is usually sliced meat on a rotating stick, which leads to cooking of the outer meat while the inside remains rare. Also, the addition of accessory ingredients to Shawarma, such as vegetables and Tahina sauce (made from sesame seeds oil), could be another source of contamination. Vegetables could be contaminated with soil (Okafo et al., 2003), and they reduce the temperature of the meat thus providing a conducive environment for bacterial contamination (Raiden et al., 2003). Another source of contamination may be attributed to the Tahina

sauce used, which was recently found to be contaminated with *Salmonella* spp.

After the microbiological examination, rapid and sensitive methods were used to characterize bacterial strains in meat-based fast food. DNA-based assays were followed for the identification of pathogens because these methods rely on nucleic acid composition of bacterium instead of the phenotypic expression that might vary under culture conditions.

For *Salmonella* species identification, four sets of primers were used to detect the *rfb*(B), *rfb*(C1), *rfb*(C2), and *rfb*(D) genetic markers. A ratio of 50% of the tested samples was positive in PCR performed with *rfb*(D) primer, indicating the presence of *S. paratyphi* A, serogroup A, in the contaminated food. The negative results showed by the rest of the tested strains could be attributed to the high number of *Salmonella* species that we did not test for or due to the existence of unknown mutations in the *rfb* genes that might interfere with the detection method.

As for *E. coli* serotyping, the results showed different gene profiles of the isolated strains. Seventy-five percent of the tested strains were *stx1*-positive. However, most of those were lacking the H7 flagellar protein gene as tested by PCR amplification of the *fliC* operon. Such H7-negative strains have recently been identified in Germany, Czech Republic, and Poland, and it was demonstrated that they represent a distinct clone within the *E. coli* O157 serogroup. However, it shares several virulence characteristics with other *STX-EC* of the O157:H7 serotype (Bielaszewska et al., 1998, 2000; Ammon et al., 1999; Osek and Gallein, 2002). Moreover, only one strain was positive in PCR performed with *eaeA* primers, and none of the tested strains were *ehlyA* positive.

Considering the significant rise in the annual consumption of antimicrobials as a medication or its use in various products, it is extremely important to document the level of antimicrobial resistance of bacteria isolated from different foods for the public health safety. Our findings clearly indicated that two-third of the tested bacteria, both for *E. coli* and *Salmonella* spp., were resistant to more than five antimicrobials. In contrast to previously reported results (Araj and Zaatari, 2002 medical report by the American University of Beirut Medical Center) that showed 100% susceptibility of clinical *Salmonella* spp. isolates to Trimethoprim/sulfamethoxazole, only 14.3% was found to be susceptible in our study.

A 30% reduction frequency was observed in *Salmonella* spp. susceptibility patterns to Cefotaxime, as compared to the previously mentioned report. *E. coli* on the other hand showed a reduction of more than 40% in susceptibility to Cefuroxime, where it showed 100% susceptibility in the clinical isolates (Araj and Zaatari, 2002) compared to 55.6% in our isolates. The three previously mentioned antimicrobials are commonly used for the treatment of bacterial infections in Lebanon (Araj and Zaatari, 2002), which may explain the difference in the susceptibility patterns between the clinical and food isolates. The high percentage of Erythromycin and Oxacillin resistances also refers to the frequent use of these antimicrobials for treatment of infectious diseases in Lebanon. Such resistance may not only be a direct concern to human health, but also, it is important because it could be transferred to other important pathogenic serotypes (Dzidic and Bedekovic, 2003).

Interestingly, both organisms showed a similar resistance profile where the only difference was observed in the resistance patterns to Gentamicin. This result indicates a high possibility of horizontal gene transfer between bacteria, via plasmids or transposons, and therefore contributing in the increase of the resistant genes in the environment (Kruse and Sørum, 1994).

Transfer of plasmid-encoded resistance to antimicrobial agents is a significant public health concern. The possibility of transfer of resistance genes between bacteria in natural habitats has recently attracted a lot of attention. Within this framework, we evaluated the association of plasmid existence with antimicrobial resistance in the isolated strains. Both *E. coli* and *Salmonella* spp. harbored the same size of plasmid that exceeds 15 kbp in size. Plasmids with Neomycin and Naladixic acid were most frequently transformed; however, few were with ampicillin, and none were transformed with Tetracycline or Chloramphenicol. Interestingly, plasmids that conferred ampicillin resistance were all isolated from *Salmonella* species. Such results cannot give a clear-cut picture on whether the two bacteria are carrying the same kind of plasmid or not due to the differential expression of antimicrobial resistance by plasmids isolated from both bacteria.

Several antimicrobials earlier used as growth promoters have been banned in several countries. In Lebanon, the usage of antimicrobials is not well controlled. In our study, we presented some data for antimicrobial resistance patterns of *E. coli* and *Salmonella* spp. in two kinds of fast food in Lebanon. Obviously, the controlled use of antimicrobial agents is a prerequisite to limit the emergence of drug-resistant bacteria, but such prudence in itself is not enough to control the emerging public health concern. Additional research is certainly needed to better understand the mechanisms behind bacterial resistance to antimicrobials and the underlying mechanisms such as the transfer of genetic material between bacteria in the environment.

Finally, the data obtained indicated that meat-based fast foods in Lebanon, especially Shawarma, are potential reservoir for many pathogenic organisms, which were shown to be resistant to many antimicrobials, suggesting a potential public health hazard. Improper hygienic standards and the indiscriminate use of antimicrobials are the main reasons behind the emergence of antimicrobial-resistant strains in Lebanon. These results will emphasize the need to implement proactive measures, and more emphasis will be placed on the application of hygienic practices and the use of Hazard Analysis and Critical Control Point (HACCP) in the preparation and processing of foods to reduce the risk of infection.

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