iMedPub Journals
Our Site: http://www.imedpub.com/

THE INTERNATIONAL ARABIC JOURNAL OF ANTIMICROBIAL AGENTS

2013
Vol. 3 No. 2:3
doi: 10.3823/730

Fresh leafy green vegetables associated with multidrug resistant *E.coli*

Shereen Z. Burjaq and Asem A. Shehabi*

Department of Pathology-Microbiology, Faculty of Medicine, University of Jordan, Amman, Jordan.

Correspondence:

ashehabi@ju.edu.jo

*Prof. Dr. Asem Shehabi

Abstract

Objective: To detect the occurrence of diarrheagenic *E. coli*, contamination and antibiotic resistance of *E. coli* isolates from fresh leafy vegetables presented in Jordanian retail markets.

Materials and Methods: A total 150 fresh leafy green vegetable samples were collected from different markets in Amman and Al-Zarqa in Jordan over the five months; June through October, 2010. All samples were prepared in sterile normal saline and cultured on MacConkey agar at 37°C for 24 hours. *E. coli* isolates were later identified by biochemical tests and the isolates were investigated for presence of diarrheagenic *E. coli* types and antimicrobial resistance genes, integrons and plasmids.

Results: A total of 61/150 (40.6%) of fresh leafy green vegetable samples were found contaminated with *E. coli*. The highest contamination with *E. coli* occurred in parsley and lowest in Lettuce. A range of 3-41% of *E. coli* isolates were resistant to 7 common antibiotics used often in Jordan. A total of 17 (27.8%) *E. coli* isolates were resistant to three or more antimicrobial agents and considered to be multidrug resistant. All these 17 (100%) carry at least one plasmid with a common sizes (7.3 and 54.3 Kb), 14 (82%) isolates were positive for class 1 integrons, 12 (70.6%), and 2 (11.8%) isolates were positive for *Tet* A and *Tet* B genes, respectively. A total of 15 (88.2%) isolates were positive for *sul 2* and 4 (23.5%) isolates for *sul1genes.* Conclusion: This study demonstrates the absence of common diarrheagenic *E. coli*, but indicates widespread of antimicrobial resistance in *E. coli* contaminating fresh green produce which may increase the reservoir of antimicrobial resistance in the intestinal tract of Jordanian population.

Key words: *E. coli*, Fresh produce, Antimicrobial resistance, Plasmids, Integron.

This article is available from:
www.iajaa.org

Introduction

Recent studies showed that fresh fruits and vegetables are important contributors for transmission of human pathogens, and represent the second leading cause of food borne illnesses in the USA and other western countries [1-3]. It has been also reported that *Salmonella* and *E. coli* can be found in vegetables if they are fertilized with contaminated manure or irrigated with contaminated waste water [4-5].

A recent massive outbreak of bloody diarrhea caused by enterohemolytic *E. coli* serotype strain (O104:H4) has been detected in Germany, 2011. The outbreak was caused by contamination of fresh sprout and has infected about 4000 persons and caused 845 (20%) cases of hemolytic uremic syndrome (HUS) including 54 deaths due to the deadly complication HUS [6-7]. The isolated strain was resistant to more than a dozen of antibiotics in eight classes: penicillins; streptomycin; tetracycline; quinolones; the sulfa drug combimation

trimethoprim-sulfamethoxazol; three generations of cephalosporins; and the combination drugs amoxicillin-clavulanic acid, piperacillin-sulbactam, and piperacillin-tazobactam [7].

Currently, multidrug resistance is commonly observed in *E. coli* isolates from human clinical cases world-wide, and this feature has an increasing impact on empirical treatment of community acquired infections by *E. coli* [8-10]. Previous studies have reported that *E. coli* isolates from animals and food products carried resistance determinants to many classes of antimicrobial agents, constituting an important reservoir for transmissible resistance genes [11]. These resistant bacteria could enter the food chain, representing a problem for food safety because they can transfer resistance genes to opportunistic pathogenic bacteria [12-13].

In addition, previous studies from Jordan showed that high percentage of *E. coli* isolates from human feces and water are multidrug resistance and carried class 1 integrons and conjugative plasmids [8, 14-15].

It is well known that lettuce, peppermint and parsley are major ingredients of many fresh cut salads in Jordan. These fresh produce are available all year long in Jordan and consumed widely by Jordanian people. Lettuce and parsley are leafy vegetables and have large surfaces; therefore, they are at stake for microbial contamination [16].

This study investigated contamination of fresh leafy green vegetable with potential diarrheagenic *E. coli* and its association with antimicrobial resistance determinates.

Materials and Method

Sampling culture

A total of 150 samples from green leafy vegetables were collected from different markets in Amman and Al-Zarqa regions in Jordan. Each 50 samples of parsley, lettuce and peppermint were collected over five-months (June-October 2010). The fresh samples were collected in clean plastic bags. Lettuce samples were cut into 4-6 cm^2 using sterilized scissors.

Bacteriological analysis

Each 5 grams of leafy vegetable sample were transferred to 50 ml sterile normal saline, using sterilized forceps, and shacked for 2 minutes by hand. One ml from each suspension was transferred into 9 ml of Lauryl sulphate tryptose (LST) broth for enrichment. The LST tubes were incubated for 24 hours in 37°C, then were subcultured on MacConkey agar and incubated for 24 hr in 37°C. All used media were obtained from Oxoid, England. *E. coli* isolates were identified first by biochemical standard tests, including citrate utilization; Kligler iron agar, indole and urease production; and later confirmed by commercial Remel RapID ONE test (Remel INC, USA). All positive cultures of *E. coli* were inoculated into 5 ml of LST tubes that contained inverted fermentation tubes (Durham) and incubated from 24-48 hr in 44.5°C to confirm the fecal origin of these isolates [17]. Five colonies of each pure growth *E. coli* on MacConkey agar were picked up and inoculated in 1 ml brain heart infusion broth (Oxoid, England) containing 25% glycerol and then stored at -70 for further investigation.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed according to the recommendation of the Clinical Laboratory and Standards Institute/CLSI [18]. The results were interpreted according to the guidelines of CLSI.

E. coli ATCC 25922 strain was included for quality control. The antimicrobials (µg/disk) used in our study (Oxoid, England) were: Augmentin (amoxicillin/clavulanic acid 20/10), cefuroxime (30), cotrimoxazole (trimethoprim- sulfamethoxazole 1.25/23.75), gentamicin (10), nalidxic acid (30), nitrofurantoin (300), norfloxacin (10) and tetracycline (30).

Minimal inhibitory concentration (MIC) were determined by broth microdilution technique for those multiresistant *E. coli* isolates (resistance for ≥ 3 antibiotic classes). Sstandard antibiotic powders were obtained from Hikma pharmaceuticals, Amman, Jordan. DNA extraction of *E. coli* isolates from fresh green leafy vegetables, positive controls for diarrhoeagenic *E. coli* (EHEC ATCC 43894, and ETEC35401- *eltB*, *estA*) and positive *E. coli* controls for *int* 1 *and int* 2 genes (obtained from Dr. A. Shehabi) were all done by Wizard Genomic DNA purification kit (Promega, USA) according to the manufacturer instructions. All DNA preparations were tested by spectrophotometer and electrophoresis to ensure contained DNA of sufficient quality and quantity for PCR amplification.
Detection of Diarrhoeagenic E. coli

All *E. coli* isolates were tested for the presence of virulence genes of diarrhoeagenic *E. coli.* The PCR master mix (Promega, USA) was included with specific primers (Invitrogen, USA) to detect the virulence genes of ETEC (Human *estA*, Porcine *estA*, *eltA*) , EHEC (*vtx1*, *vtx2*, *eae*), EPEC (*eae*) and EIEC (*ipa*H) and of the universal 16S rDNA as a positive internal PCR control as shown in **Table 1.** [19]. In addition to the primers, the mixture contain 50 units/ ml *Taq* DNA polymerase, 400 uM of each deoxyribonucleoside triphosphate, 3mM $MgCl_2$ and 4 µl of purified DNA as a DNA template. PCR reactions were performed in a total reaction volume of 50 µl.

The optimum amplification conditions at which best separation, resolution of amplicons and reduction of non-specific amplification were performed by using programmable thermocycler as follows: 94°C for 6 min, followed by 35 cycles of 94°C for 50 s, 57°C for 40 s and 72°C for 50 s, and finally 72°C for 3 min. Tubes were hold at 4°C when the cycles ended [19]. Agarose gel at a concentration of (2%) was prepared by dissolving 4.0 gram of agarose in 200 ml of 1x TBE buffer and boiling until the agarose was completely dissolved. Agarose gel electrophoresis was run for 1:15 hours at 110 V using horizontal electrophoresis apparatus. After the electrophoresis was completed the gel was visualized under ultraviolet light.

PCR Detection of Integrons

Detection of class 1, 2 and 3 integrons in multidrug resistant *E. coli* isolates was done using PCR and primer pairs targeting *Int 1*, *Int 2* and *Int 3* genes [20]. Pure DNA which was extracted from *E. coli* isolates was used as a DNA template in all PCR reactions. PCR was performed in a final volume of 25 µl containing 0.1 units/µl Taq DNA polymerase, 5.5 mM $MgCl_2$ and 0.4 mM of each dNTPs, 200 nM of each primer (Invitrogen, USA) and 2 µl of purified DNA. The PCR conditions were as follow: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min; primer annealing at 59°C for 1 min; extension 72°C for 1 min and final extension at 72°C for 7 min. Tubes were kept at 4°C when the cycles ended. The PCR product was analyzed by performing 1.5 % agarose gel electrophoresis for 80 min at 120 V using horizontal electrophoresis apparatus. The gels were visualized under UV light.

PCR Detection of Tetracycline Resistance Genes and sulphonamide resistance genes

All multidrug resistant *E. coli* isolates were screened for tetracycline resistance genes: *tetA* and *tetB* [21] and sulphonamide

Table 1. Specific primer sequences used in the detection of diarrhoeagenic *E. coli* (Ref. 19), class 1, 2 and 3 integrons (Ref. 20, 2005), *tetA* and *tetB* gene cassettes (Ref. 21) and *sul*1 and *sul*2 (Ref. 22).

Amplicon size (bp)	Primer sequence (5'-3')	Virulence gene	Gene Target
151	F TTTCGCTCAGGATGCTAAACCAG R CAGGATTACAACACAATTCACAGCAGTA	STSh	Human estA
160	F CTTTCCCCTCTTTTAGTCAGTCAACTG R CAGGATTACAACAAAGTTCACAGCAG	STSp	Porcine estA
479	F AAACCGGCTTTGTCAGATATGATGA R TGTGCTCAGATTCTGGGTCTCCT	LTS	eltA
260	F GTTTGCAGTTGATGTCAGAGGGA R CAACGAATGGCGATTTATCTGC	VT1	Vtx1
420	F GCCTGTCGCCAGTTATCTGACA R GGAATGCAAATCAGTCGTCACTC	VT2	Vtx2
377	F GGYCAGCGTTTTTTCCTTCCTG R TCGTCACCAAAGGAATCGGAG	Intimin	Eae
647	F TTGACCGCCTTTCCGATACC R ATCCGCATCACCGCTCAGAC	IpaH	ipaH
1062	F GGAGGCAGCAGTGGGGAATA R TGACGGGCGGTGTGTACAAG		16S rDNA
160	F 5' – CAGTGGACATAAGCCTGTTC-3' R 5'-CCCGAGGCATAGACTGTA-3'		Int1
788	F 5'- CACGGATATGCGACAAAAAGGT-3' R 5'- GTAGCAAACGAGTGACGAAATG -3'		Int2
977	F 5'- GCCTCCGGCAGCGACTTTCAG-3' R 5'-ACGGATCTGCCAAACCTGACT-3'		Int3
956	F 5'-GTAATTCTGAGCACTGTCGC-3' R 5'-CTGCCTGGACAACATTGCTT-3'		tetA
414	F 5'-CTCAGTATTCCAAGCCTTTG-3' R 5'-ACTCCCCTGAGCTTGAGGGG-3'		tetB
660	F 5'- GTGACGGTGTTCGGCATTCT-3' R 5'- TTTACAGGAAGGCCAACGGT-3'		sul1
393	F 5'- GGCAGATGTGATCGACCTCG-3' R 5'- ATGCCGGGATCAAGGACAAG-3'		sul2

resistance genes *sul*1 and *sul*2 [22]. Those genes were detected using PCR specific primers (Invitrogen, USA) as shown in **Table 1.**

PCR reactions were performed in a final volume of 25 µl containing 2 µl of extracted DNA, 50 units/ ml Taq DNA polymerase, 400 uM of each DNTP, 3mM $MgCl_2$ and 5pmol from each primer. The PCR product was analyzed by performing agarose gel electrophoresis using horizontal electrophoresis apparatus. The gels were visualized under U.V light.

Screening of Plasmids

Sample preparation was performed by culturing multidrug resistant *E. coli* isolates on LB-agar containing 50µg/ml ampicillin. Selected pure colonies were grown in 5 ml of LB-Broth (Biolife, Italy) containing 50µg/ml ampicillin and incubated at 37°C for 18 hours. The plasmid DNA was extracted by using PureYield ^{TM} Plasmid Miniprep System A1223 (Promega, USA). Agarose gel electrophoresis (0.8%) was run for 70 min at 120 V using horizontal gel electrophoresis apparatus. The plasmid fragments were visualized with ultraviolet light and their sizes calculated in relation to reference plasmid DNAs included in *E. coli* V517 (sizes: 54, 5.6, 5.1, 3.9, 3.0, 2.7, and 2.1 kb) [16].

Statistical Analysis

Data were entered and analyzed using Statistical Package of Social Sciences (SPSS, 2011) version 16 to make descriptive statistics. Chi-square test was used to calculate resistance frequencies of antimicrobial agents and p-value (to test association between fecal *E. coli* contamination and type of fresh green produce). A p-value < 0.05 was considered as statistically significant.

Results

Incidence of fecal E. coli

A total of 61/150 (40.7%) green leafy vegetable samples were contaminated with fecal *E. coli* isolates and non of the isolates were positive as diarrhoeagenic *E. coli* (ETEC, EHEC and EPEC). The highest contamination with fecal *E. coli* was associated with parsley (24/50; 48%) and lowest found in romaine lettuce samples (16/50; 32%). Statistically, there is no association between the type of leafy vegetables and degree of contamination with *E. coli* since all have p-value >0.05 (**Table 1**).

Antimicrobial resistance of fecal E. coli isolates

The majority of *E. coli* isolates were resistant to tetracycline (41%), augmentin (36%) and co-trimoxazole (31%). A total of 17/61 (27.8%) *E.coli* isolates were recorded as multidrug resistant (resistant to ≥ 3 drugs) using disk diffusion and MIC methods (**Table 3**).

Table 2. Incidence of fecal *E. coli* in parsley, romaine lettuce and peppermint.

Type of the samples (No.)	No. (%) positive *E. coli* samples	p-value
Parsley (50)	24(48)	0.258
Peppermint (50)	21(42)	0.543
Romaine lettuce (50)	16(32)	0.255
Total (150)	61(40.7)	

Table 3. Antimicrobial resistance pattern of 61 *E. coli* isolates and MICs of 17 multidrug resistant *E.coli* isolates.

Antimicrobial agent	No. (%) resistant isolates	No. (%) multiresistant isolates	MIC(mg/L) 50%	MIC(mg/L) 50%
Tetracycline (Te)	25 (41)	16	48	86
Augmentin (Aug)	22(36)	17	64*	115
Cotrimoxazole (Ts)	19(31)	15	241**	434
Nalidixic acid (NA)	10 (16)	9	33	59
Gentamicin (Gm)	4(6.6)	4	8	14
Norfloxacin) NOR	4(6.6)	5	11	20
Cefuroxime (Cxm)	2(3.3)	0	Not done	Not done

*MIC of ampicillin, **of sulphamethoxaz.

Table 4. Distribution of class 1 integron associated with plasmid of 17 multidrug resistant *E. coli* isolates from green leafy vegetables

No. of Isolates*	Resistance profile	No. of Plasmid	Plasmid profile (Kb)	Presence of class 1integron
2	Aug, Ts, Te	2	54.3, 7.3	+
1	Aug, Ts,NA, Te	2	54.3, 7.3	-
3	Aug, Ts,NA Te	2	54.3, 7.3	+
1	Aug, Ts,GM, Te	3	54.3, 7.3, 2.9	+
2	Aug, Ts, Te	3	54.3, 7.3, 3.9	+
1	Aug, Ts, NA, NOR, Te	4	54.3, 7.3, 5.2, 5.0	+
1	Aug, Ts, Te	4	54.3, 7.3, 2.9, 2.0	+
1**	Aug, Ts, GM, NA, NOR, Te	4	7.3,6.0, 5.8, 5.6	+
1	Aug, Ts, GM, NA,NOR	4	54.3, 7.3, 5.8, 5.6	-
1	Aug, Cxm, Ts, NA, Te	6	53.0, 7.3, 6.0,5.8, 5.6,5.0	+
1**	Aug, Ts, GM, NA, NOR, Te	3	7.3,5.2,5.1	+
1	Aug, Ts, Cxm,	2	54.3, 7.3	+
1	Aug, Ts, NA, NOR, Te	3	54.3, 7.3, 5.0	-

*All 17 *E. coli* isolates carried at least *sul1* or *sul2* gene, whereas only 14 *E. coli* isolates harbored *tetA* gene and were also positive for class 1 integrons and 2 *E. coli* isolates (**) carried both *tetA and tetB* genes.

Detection of Class 1, 2 and 3 Integrons, tetracycline and sulphonamide resistance genes in Multidrug resistant E. coli isolates

Class 1 integrons was detected in 14/17 (82%) of multiresistant *E. coli* isolates, while class 2 and 3 integrons were negative in all isolates (Table 4). A total of 15 (88.2%) multidrug resistant *E.coli* isolates were positive for *sul2* and 4 (23.5%) for *sul1* gene. Also, 12/17 (70.6%) of multidrug resistant *E.coli* isolates were positive for *tetA* gene and 2/17 (11.8%) for *tetB*, respectively. Plasmid Profile of Multidrug resistant E. coli isolates

E. coli isolates carried various numbers of plasmids (1-6) and their sizes ranged between 2.0 - 54.3 Kb. Two common plasmid sizes of 7.3 and 54.3Kb were found in 17/17 (100%) and 11/17 (65%) of *E. coli* isolates, respectively (Table 3).

Discussion

The prevalence of food borne infection outbreaks due to contamination of fruit and leafy green vegetables with microorganisms are not well documented in developing countries [3]. This study demonstrates that fresh leafy green vegetable samples collected from local markets in Jordan can be highly contaminated with *E. coli* (41%), especially fresh parsley. However, none of the *E. coli* isolates were a diarrheagenic *E. coli*. This high load of *E. coli* in fresh produce may be due to the fact that these vegetables have flat leaves which can be easily contaminated with soil or irrigation water [3]. In addition, these leafy vegetables are more prone to be contaminated with microorganisms because they are collected with hands during harvesting and packaging. Few studies from Arab countries have reported on the prevalence of intestinal pathogens or *E. coli* in particular as contaminates of fresh green produce [24, 25].

This study demonstrates that *E. coli* isolates from fresh green leafy vegetables were resistant in the range between 3% - 41% to commonly used antibiotics in the treatment of urinary tract infection in Jordan. A total of (27.8%) *E. coli* isolates were resistant to three or more antimicrobial classes. A previous study carried out during 2000-2001, reported similar antimicrobial resistance patterns in uropathogenic *E. coli* isolates from inpatients and outpatients at the Jordan University Hospital [26]. *E. coli* isolates in this study showed similar susceptibility rates to nitrofurantoin, cefuroxime, tetracycline, co-trimoxazole and nalidixic acidt compared with fecal *E. coli* obtained from one family over a 6-month period in Jordan [14].

A recent study from Saudi Arabia found that bacterial isolates from fresh vegetables exhibited higher resistance rates than our study to ampicillin, cephalothin, trimethoprime-sulfamethoxazole, aminoglycosides, tetracycline, fluoroquinolones, amoxycillin-clavulanic acid, and chloramphenicol [24].

Previous studies reported that *E. coli* contaminating leafy green vegetables do not contribute substantially to increase levels of bacterial resistance in human fecal flora [1, 27], while one study indicated that sprouts contaminated with multi-resistant Gram-negative enteric bacteria may contribute to common gene pool of human commensal and pathogenic bacteria [4]. It is well established that the human intestinal tract play a major role in transfer of antibiotic resistance genes by conjugation between various enteric bacterial species [28].

The present study shows that class 1 integrons was highly prevalent among multidrug resistant *E. coli* isolates (82%), and it was almost associated with presence of one or more plasmids. In addition, the results demonstrated that all *E. coli* isolates carried 1-6 different plasmids sizes (2.0 Kb to 54.3 Kb), and two of these plasmids (7.3 and 54.3 Kb) were found in the majority of isolates (100-65%). Previous studies carried out few years ago in Jordan indicated also high incidence rates of class 1 integrons in *E. coli* isolated from water and stool of normal population with a range of 48% to 74% [14-15]. Since there is a significant relationship between the presences of class 1 integrons and the presence of multidrug resistant strain [8, 15]. The occurrence of integrons in association with antibiotic resistance genes is often carried by mobile elements such as transposons and conjugative plasmids, and both contribute extensively to the rapid transfer resistance genes among enteric bacterial species [14, 15, 29].

The present study also shows that co-trimoxazole resistance in *E. coli* isolates (31%) was mostly associated with the presence of *sul I / sul II* gene or both. The high incidence rate of *sul II* gene (88.2%) is much more than observed in a previous study [15]. It is known that sulphonamide resistance in Gram-negative bacilli generally arises from the acquisition of *sul1*, *sul2* or *sul3* genes. The *sul I* gene has been detected as part of the 3 'conserved segment of class1 integrons, while *sul 2* gene is frequently detected in *Enterobacteriaceae* on small non-conjugative or large conjugative plasmid [30]. Moreover, high levels of sulphonamide resistance genes in *E. coli* would ultimately stop treatment of urinary tract infections with co-trimoxazole which is still widely used in many countries [10, 14]. This study shows that the prevalence of tetracycline resistance was 41% and it is mostly associated with *Tet* A gene (64.7%) and less with *Tet* B gene (5.9%). These results are similar to a previously published study [14], and similar to a recent study reported from Tunisia [12]. In general, the rapid spread of tetracycline resistance among bacteria is due to the localization of *tet* genes on plasmids, transposons, and integrons [21]. In addition, *Tet* A and *Tet* B genes were commonly found and preserved in soil and water environments for long time [31]. Therefore, this study may suggest that most *E. coli* resistant isolates from contaminated fresh green produce were acquired through soil or irrigated used water.

A recent study found that *E. coli* isolates from stools of normal Jordanian population carried a plasmid size of 54.3 Kb, and these were always associated with tetracycline and co-trimoxazole resistance [14]. Also, a similar study from Saudi Arabia showed that coliforms with multiple resistances to four or more antimicrobial agents were contaminating raw vegetables, and they were also associated with the presence of plasmids of varied sizes [24].

In conclusion, this study suggests that *E. coli* isolates from fresh produce have a common antimicrobial resistance features with other *E. coli* isolates from healthy human and clinical cases in Jordan, and this fact may contribute to increase the reservoir ofantimicrobial resistance in the intestinal tract of Jordanian population.

References

1. Berger, CN., Sodha, SV., Shaw, RK., Griffin, PM., Pink, D., Hand, P., Frankel, G. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology 2010; 12: 2385-2397.
2. López-Gálvez, F., Allende, A., Selma, M. Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. International Journal of Food Microbiology 2009; 133: 167-171.
3. Abadias, M., Usall, J., Anguera, M. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. International Journal of Food Microbiology 2008; 123: 121-129.
4. Keskinen, L., Burke, A., Annous, B. Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate Escherichia coli O157:H7 from lettuce leaves. International Journal of Food Microbiology 2009; 132: 134-140.
5. Islam, M., Doyle, M., Phatak, Sc., Millner, P., Jiang, X. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. Journal of food protection 2004; 67: 1365-70.
6. Frank, C., Werber, D., Cramer, JP., Askar, M., Faber, M., van der Heiden, M., et al. (HUS Investigation Team). Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. New England Journal of Medicine 2011; 365: 1771-82.
7. Aurass, P., Prager, R., Flieger, A. EHEC/EAEC O104:H4 strain linked with the 2011 German outbreak of haemolytic uremic syndrome enters into the viable but non-cultural state in response to various stresses and resuscitates upon stress relief. Environmental Microbiology 2011; 13: 3139-48.
8. Asem, A., Shehabi, A., Haider, M., Fayyad, K. Frequency of antimicrobial resistance markers among *Pseudomonas aeruginosa* and *Escherichia coli* isolates from municipal sewage effluent water and patients in Jordan. The International Arabic journal of antimicrobial agents 2011; 11): 1-5.

9. Garza-Gonz lez, E., Mendoza Ibarra, S., Llaca-´ıaz, J., Gonaález, G. Molecular characterization and antimicrobial susceptibility of extended spectrum b-lactamaseproducing Enterobacteriaceae isolates at a tertiary care centre in Monterrey, Mexico. Journal of Medical Microbiology 2011; 60: 8-90.
10. Yüksel, S., Oztürk, B., Kavaz, A., Ozçakar, ZB., Acar, B., Güriz, H., Aysev, D., Ekim, M., Yalçinkaya, F. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections. *Int J Antimicrob Agents* 2006; 28: 413-6.
11. eáenz, Y., Bri˜as, L., Domínguez, E., Ruiz, J., Zarazaga, M., Vila, J., Torres, C. Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. Antimicrobial Agents and Chemotherapy 2004; 48: 399-4001.
12. Slama, K., Jouini, A., Sallem, R., Somalo, S., Sáenz, Y., Estepa, V., Boudabous, A., Torres, C. Prevalence of broad-spectrum cephalosporin-resistant *Escherichia coli* isolates in food samples in Tunisia, and characterization of integrons an antimicrobial resistance mechanisms implicated. International Journal of Food Microbiology 2010; 137: 28–-286.
13. Sunde, M. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. Journal of Antimicrobial Chemotherapy 2005; 56: 101–-1024.
14. Al-Dweik, M., Shehabi, A.A. Common antimicrobial resistance phenotypes and genotypes of fecal *Escherichia coli* isolates from a single family over a six month period. Microbial Drug Resistance 2009; 15: 103-107.
15. Shehabi, AA., Odeh, JF., Fayyad, M. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from Jordanian Human Stools and Drinking Water Sources. Journal of Chemotherapy 2006; 18 5): 468-47,.
16. Aycicek, H., Oguz, U., Karci, K. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. International Journal of Hygiene and Environmental Health 2005; 209: 19–-201.
17. Anderson, MA., Whitlock, JE., Harwood, VJ. Diversity and distribution of *Escherichia coli* genotypes and antibiotic resistance phenotypes in feces of humans, cattle, and horses. Applied Environmental Microbiolgy 2006; 72: 691–-6922.
18. CLSI. Clinical Laboratory and Standards Institute Method for dilution antimicrobial susceptibity tests for bacteria that grow aerobically. CLSI, Villanova, PA, USA.
19. Persson, S., Olsen, K., Scheutz, F., Krogfelt, K., Gerner-Smidt, P. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. Clincal Microbiology and Infection 2007; 13: 51-524.
20. Dillon, B., Thomas, L., Mohmand, G., Zelynski, A., Iredell, J. Multiplex PCR for screening of integrons in bacterial lysates. Journal of Microbiological Methods 2005; 6,: 22 - 232.
21. Sengeløv, G., Agresø, Y., Halling-Sørensen, B., Baloda, SB., Andersen, J., Jensen, LB. Bacterial antibiotic resistance levels in Danish farmland as a result of treatment with pig manure slurry. Environment International 2003, 2 : 587-595.
22. Leverstein-van Hall, MA., Paauw, A., Donders, RT., Blok, HEM., Verhoef, J., Fluit, AC. Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolates origin. The Journal of Infectious Disease 2003; 86: 49-56.
23. Wang, M., Tran, J., Jacoby, G., Zhang, Y., Wang, F., Hooper, D. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrobial Agents and Chemotherapy 2003; 47: 224-2248.
24. Hassan, S., Altalhi, A., Gherbawy, Y., El-Deeb, B. Bacterial load of fresh vegetables and their resistance to the currently used antibiotics in Saudi Arabia. Food borne pathogens and Disease 2011; 8: 1011-8.
25. Ibenyassine, K., Mhand, RA., Karamoko, Y., Anajjar, B., Chouibani, MM., Ennaji, M. Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. Joumal of Environmental Health 2007; 69: 47-51.
26. Shehabi, A., Mahafzah, A., Al-Khalili, K. Antimicrobial resistance and plasmid profiles of urinary *Escherichia coli* isolates from Jordanian patients. Eastern Mediterranean Health Journal 2004; 6: 322-328.
27. Österblad, M., Pensala, O., Peterzéns, M., Heleniusc, H., Huovinen, P. Antimicrobial susceptibility of Enterobacteriaceae isolated from vegetables. Journal of Antimicrobial Chemotherapy 1999; 43: 50-509.
28. Mazel, D. Integrons: agents of bacterial evolution. Nature Reviews Microbiology 2006; 60–-620.
29. Soufi, L., Sáenz, Y., Vinué, L., Abbassi, MS., Ruiz, E., Zarazaga, M., Hassen, AB., Hammami, S., Torres, C. *Escherichia coli* of poultry food origin as reservoir of sulphonamide resistance genes and integrons. International Journal of Food Microbiology 2011; 144: 49–-502.
30. Hammerum, A., Sandvang, D., Andersen, S., Seyfarth, AM., Porsbo, LJ., Frimodt-Møller, N., Heuer, OE. Detection of sul1, sul2 and sul3 in sulphonamide resistant *Escherichia coli* isolates obtained from healthy humans, pork and pigs in Denmark. International Journal of Food Microbiolog, 2006; 106: 23 -237.
31. BÖrjesson, S., Mattsson, A., Lindgren, P. Genes encoding tetracycline resistance in a full-scale municipal wastewater treatment plant investigated during one year. Journal of Water and Health 2010; 8: 247-256.

Follow us: