Molecular characterization of virulence genes and antibiotic resistance among fecal *Escherichia coli* isolated from surface water of Wadi Shueib-Jordan

Shereen Z. Burjaq¹, Saeid M. Abu-Romman²,
Moawya A. Haddad³

- 1 Department of Medical Analysis, Faculty of Science, Al-Balqa' Applied University, Salt, Jordan.
- 2 Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa Applied University Salt, Jordan.
- 3 Department of Nutrition and Food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University Salt, Jordan.

Abstract

Objective: Contamination of surface water with pathogenic organism is highly dangerous for people who used it for drinking or for domestic activities. Detection of *Escherichia coli* in water can be used as a general important indicator of fecal contamination. This study investigated the occurrence of fecal *E. coli*, two important toxigenic types of *E. coli* isolates and their antimicrobial resistance in water samples collected from the surface running source of Wadi Shueib in Jordan.

Methods: A total of 51 water samples were collected from three different locations of wadi shueib over a three month, July through September, 2016. For each sample, 200 ml of water was collected in sterilized containers. All samples which were positive for fecal *E. coli* were subcultured on Eosin Methylelne Blue Agar and incubated at 37 °C for 24 hours. *E. coli* isolates were identified by API- 20 E test, and all isolates were tested for antimicrobial susceptibility and for the presence of virulence genes of enterotoxigenic *E.coli* (ETEC) and enterohaemorrhagic *E.coli* (EHEC).

Results: A total of 46/51 (90%) of water samples were contaminated with fecal *E. coli*. The 46 *E. coli* isolates were resistant in the range between 4%-76% to commonly used antibiotics in the treatment of infection in Jordan. Multidrug resistant isolates to at least three antibiotics accounted for 17/46 (37%) of the isolates. Out of 46 fecal *E. coli* isolates, 4 (8.7%) were ETEC and 2 (4.3%) were EHEC as detected using PCR.

Conclusion: This study indicated that the surface running water of Wadi Shueib is contaminated with potential enteropathogenic *E. coli*,

Contact information:

Shereen Z. Burjaq.

✉ shereen.burjaq@bau.edu.jo

and it can be a source of causing diarrheal diseases. Fecal *E. coli* isolates from water also showed high level of antibiotic resistance comparable to human *E. coli* isolates in Jordan.

Keywords

Fecal *E. coli*; ETEC; EHEC; Antimicrobial Resistance; Surface Water; Jordan.

Received 11-05-2017; accepted 14-05-2017

Introduction

The quality and availability of fresh safe water is an essential element in health and living for every human community [1-2]. Water borne infection are still a major public health concern in most developing countries [3].

Jordan is considered as one of the ten most water scarce countries in the world. High population growth and flow of hundred thousands of refugees from other Arab countries, have aggravated the shortness of ground water reserves in the country [4]. Therefore, every drop of water should be considered important for usage in human activities and agriculture [5].

Wadi Shueib represents a draining area of approximately 180 square kilometers (69 sq mi). Towns and villages along the wadi include Salt, fuheis and Mahis. These discharge treated and untreated sewage into wadi Shueib course. Therefore, high load of biological contaminants, especially water-borne microbial pathogens, would be expected.

E. coli is the most commonly used indicator of fecal contamination in drinking water distribution systems. *E. coli* of enteric origin can persist with other enteric bacteria and parasites for long time in water, and can be used as indicator of water contamination [6]. *E. coli* survives in drinking water between 4 and 12 weeks, depending on many environmental conditions [7].

Antimicrobial resistance genes are widely distributed in human intestinal bacterial flora, particu-

larly in gram negative enteric bacteria contaminated aquatic environment [8]. In water, bacteria of different human and animal origins are able to mix with environmental commensal bacteria and contribute for transfer of antibiotic resistance genes [9]. In recent years, infections caused by multidrug resistant (MDR) pathogenic bacteria have severe health implication in patients and offer limited treatment options [10]. Many diarrheagenic *E. coli* types can be also associated with contamination of water, particularly ETEC, EHEC and enteropathogenic *E. coli* (EPEC) [11]. The implementation of PCR methods can easily and rapidly identify diarrheagenic *E. coli* in water and human stool samples [12].

This study aimed to investigate the general occurrence of *E. coli* and particularly the presence of enterotoxigenic and enterohemorrhagic *E. coli* in water of Wadi Shueib and their antibiotic resistance profiles.

Material and Method

Water samples collection

Water samples were collected from three different locations of Wadi Shueib; water springs before Al-Salt wastewater treatment plant, treated water immediately after Al-Salt wastewater treatment plant (WWTP) and final region of surface water before Wadi Shuaib Dam. For each sample, 200 ml of water was collected in sterilized containers (glass bottles).

When the sample was collected, an ample air space was left in the bottle (2.5 cm) to facilitate mixing and shaking before examination. Bottles were fully submerged in the water, then immediately covered by the sterilized lid according to the guidelines of the American Public Health Association [13]. The samples were carried in ice box and brought to the laboratory within few hours for microbial analysis.

Isolation of fecal *E.coli*

Out each water sample, 1 ml of water was taken and delivered to Lauryl Tryptose broth (Neogen, USA) with inverted durhams tubes, then incubated at 35°C for 24 hours, and examined for gas formation or acidic growth. When no gas or acidic growth had formed, inoculated tubes were re-incubated for another 24 hours. To detect fecal *E. coli*, positive Lauryl Tryptose broth tubes were shaken to resuspend the organism, and with a sterile loop, one loopful of culture was transferred to EC Broth (HiMedia, India) and incubated at 44.5 °C for 24 hours and examined for growth and gas formation [13]. Test tubes which show positive results were subcultured on Eosin Methylene Blue (EMB) Agar (HiMedia, India), and were identified by API- 20 E test. *E. coli* isolates were stored in Brain- Heart Infusion broth (Oxoid, England) with 15% glycerol in freezer for further examination.

Antibiotic susceptibility test

E. coli isolates were tested by disk diffusion method against the following antimicrobials (Oxoid, England): Ampicillin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), cefotaxime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), tetracycline (30 µg) cotrimoxazol (25 µg) amikacin (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), azetreonam (30 µg), and ceftazidime (30 µg) [2,14]. Antimicrobial susceptibility testing was performed according to the recommendation of the Clinical Laboratory and Standards Institute/ CLSI [15]. The results were interpreted according to

the guidelines of CLSI. *E. coli* ATCC 25922 strain was included as a control was obtained from Dr. Asem Shehabi, The Jordan University, Amman, Jordan.

Detection and characterization using PCR

All DNA of *E. coli* isolates and control *E. coli* strains were extracted using Wizard® Genomic DNA Purification Kit (promega, USA). Control strains of EHEC (ATCC 43894) and ETEC (ATCC 35401) (Dr. Asem Shehabi) were included as positive controls. DNA templates of fecal *E. coli* strains were subjected to multiplex PCR using specific primer (Integrated DNA Technologies, USA) sequences for the detection of virulence genes in ETEC and EHEC as shown in **Table 1** [16].

Table 1. Primers used in the multiplex PCR for the detection of ETEC and EHEC [16].

Primer	Target gene	Primer sequence	Amplicon size (bp)
LT	eltB	5'-TCTCTATGTGCATACGGAGC-3' 5'-CCATACTGATTGCCGCAAT-3'	322
ST	estA	5'-GCTAAACCACTAGGGTCTTCAAAA-3' 5'-CCCGGTACAGGCAGGATTACAACA-3'	147
VT1	vt1	5'-GAAGAGTCCGTGGGATTACG-3' 5'-AGCGATGCAGCTATTAATAA-3'	130
VT2	vt2	5'-ACCGTTTTTCAGATTTTGCACATA-3' 5'-TACACAGGAGCAGTTTCAGACAGT-3'	298
eae	eaeA	5'-CACACGAATAAACTGACTAAAATG-3' 5'-AAAAACGCTGACCCGCACCTAAAT-3'	376

In addition to the primers, the mixture contain 12.5 µl master mix (Promega, USA), 2 µl purified DNA and a 0.2µM concentration of each primer except primer VT1 (which was used at a concentration of 0.4µM). PCR reactions were performed in a total reaction volume of 20 µl. The amplification conditions were as the following: 96°C for 4 min, 94°C for 20 s, 55°C for 20 s, and 72°C for 30 s for 30 cycles, with a final 7-min extension at 72°C. 10 µl of each PCR product was evaluated with a 1.5% (wt/vol) agarose gel (Bio Basic Inc., USA) stained with ethidium bromide. Agarose gel electrophoresis was run for 30 min at 120 V [16]. A 50 bp ladder (iNtRON Biotechnology, USA) was used as control.

After electrophoresis was completed the gel was visualized under ultraviolet light.

Results

Incidence of fecal *E. coli* in water

In order to evaluate the presence of fecal *E. coli* in water, a total of 51 water samples were collected from Wadi Shueib. The water samples were examined using a protocol for the presence of fecal *E. coli* and the results showed that 46 samples (90.2 %) showed contamination with fecal *E. coli* (Table 2).

Table 2. Distribution of fecal *E. coli* in water samples collected from Wadi Shueib.

Collection site of water sample	no. of samples	No. of fecal <i>E. coli</i> isolates	%
Water springs	17	15	88
Immediately after Al-Salt wwtp	17	16	94
Before Wadi Shuaib Dam	17	15	88

Antimicrobial resistance of 46 fecal *E. coli* isolates

Antimicrobial resistance rates of *E. coli* isolates are shown in Table 3. Out of 46 *E. coli* strains, 17 (37%) isolates were resistant to at least three antibiotic classes and considered multidrug resistant (MDR) isolates[17]. Most of these MDR isolates were isolated from water springs (Table 3, 4).

Incidence of ETEC and EHEC isolates

Out of 46 fecal *E. coli* isolates, 4 (8.7%) were ETEC and 2 (4.3%) were EHEC as detected using PCR (Table 5).

Discussion

This study demonstrates that the surface water of Wadi Shuaib is highly contaminated with fecal *E. coli*, since 46/51 (90.2%) of the water samples were found contaminated with fecal *E. coli*. The isolates were resistant in the range 4%-76% to commonly

Table 3. Resistance rates of 46 fecal *E. coli* isolates to antimicrobial agents.

Antimicrobial agent	No. of resistant isolates	%
Ampicillin (AM)	35	76.1
Tetracycline (TE)	19	41.3
Streptomycin (S)	10	21.7
Cotrimoxazol (STX)	12	26.1
Nalidixic acid (NA)	8	17.4
Azetreonam (ATM)	4	8.7
Ceftazidime (CAZ)	4	8.7
Ciprofloxacin (CIP)	4	8.7
Cefotaxime (CTX)	2	4.3
Gentamicin (CN)	2	4.3
Amikacin (AK)	0	0.0
Imipenem (IMI)	0	0.0
Meropenem (MEM)	0	0.0
Tazobactam (PTZ)	0	0.0

Table 4. Distribution of MDR *E. coli* isolates in water samples collected from different location along the water flow.

Collection site of water sample	no. of samples	No. of MDR <i>E. coli</i> isolates	%
Water springs	15	8	53.3
Immediately after Al-Salt wwtp	16	3	18.8
Before Wadi Shuaib Dam	15	6	40.4

Table 5. Distribution of virulence genes in 4 ETEC and 2 EHEC isolates from different location of the surface water of Wadi Shuaib.

Collection sites	no. of <i>E. coli</i> isolates	ST	LT		vt1		vt2		eaeA	
			No.*	%	No.*	%	No.*	%	No.*	%
Water springs	15	0	1	6.7	0	0.0	0	0	0	0.0
After Al-Salt wwtp	16	0	0		1	6.3	0	1	6.3	
Before Wadi Shuaib Dam	15	0	3	20.0	1	6.7	0	1	6.7	
Total	46	0	4	8.7	2	4.3	0	2	4.3	

*: Pathogenic *E. coli* strains carrying virulence genes.

used antibiotics in the treatment of infection in Jordan [8, 18]. A total of 17 (37%) *E. coli* isolates were multidrug resistant to three or more antimicrobial classes.

A previous study from Saudi Arabia [2] found that *E. coli* isolates from Al-Ahsa water springs exhibited high resistance rates as found in our study for the following antimicrobial agents: tetracycline (50%), cotrimoxazol (38.5%), streptomycin (65.4%), nalidixic acid (42.3%), and ciprofloxacin (34.6%). In addition, the percentage rate of multiresistant strains were higher (57.7%) than this study (37%). The important aspect of contamination of running surface water with opportunistic pathogenic bacteria constitutes the occurrence of antibiotic-resistant bacteria of human and animal sources into aquatic environment. Antibiotic resistance genes are introduced in natural bacterial ecosystems through mainly contamination of water with fecal bacteria. In such systems, commensal bacteria in water could acquire antibiotic resistance genes through natural genetic processes such as conjugation and transduction [19]. This study shows that *E. coli* isolates from water samples carried antibiotic resistance profiles comparable to *E. coli* isolates from human sources in Jordan [18, 19].

Diarrhea caused by pathogenic types of *E. coli* is still a health care problem in developing countries including Jordan, mostly found in children younger than five years old [11, 20, 21]. Among diarrheagenic bacteria, enterotoxigenic and enterohemorrhagic *E. coli* are important enteropathogens causing single and outbreaks of diarrhea following consumption of contamination of fresh food and drinks [22]. ETEC affects small intestine, and it is still a major cause of traveler diarrhea. It is responsible for 280 million diarrheal episodes and more than 400 thousand death annually [23]. ETEC strains produce one or two types of enterotoxins; heat-stable enterotoxin and heat-labile enterotoxin, encoded by the *estA* and *eltB* genes, respectively [24]. While EHEC are more associated

with severe outbreak of intestinal infections, and its enterotoxins affect large intestine and leads to severe abdominal pain, watery diarrhea followed by bloody diarrhea and may cause fetal hemolytic uremic syndrome in certain patients. EHEC strains produce verocytotoxins VT1 and/or VT2, which are encoded by the *vt1* and *vt2* genes, respectively [24]. This study demonstrates that ETEC (8.7%) is frequently more detected in the surface water than EHEC (4.3%). The higher incidence of ETEC isolates has also been reported previously in a study performed in Jordan and which has showed that children with diarrhea and without diarrhea carried in their feces mostly EPEC and ETEC [21]. On the other hand, another study in Jordan carried in 2012 showed that none of *E. coli* isolates which contaminated green leafy vegetables, belonged to diarrheagenic *E. coli* [14]. The higher occurrence of the ETEC type than EHEC strains suggests that human fecal material is the main source of contamination of water sources with fecal *E. coli*, since EHEC found mostly in feces of animals [25]. Additionally, heat-stable toxin gene (ST gene) is generally found in ETEC of animal origin, while the heat-labile toxin gene (LT gene) is more detected in human isolates (8.7%) as the case in our study [21].

Conclusion

This study shows that the surface running water of Wadi Shueib is contaminated with potential enteropathogenic strains of *E. coli*, and this organism can be associated with other potential pathogenic organisms and a source of causing diarrheal and enteric diseases. In addition, fecal *E. coli* isolates from this Jordanian surface water has showed high percentage of antibiotic resistance.

Acknowledgements

The authors thank Dr. Asem Shehabi/The Jordan University, for providing *E.coli* control strains.

References

1. Adekunle LV, Sridhar M, Ajayi AA, Oluwade PA, Olawuyi JF. An Assessment of the Health and Social Economic Implications of Satchet Water in Ibadan Nigeria: A Public Health Challenge. *Afr J Biomed Res.* 2004; 7: 5-8.
2. Alzahrani A, Gherbawy Y. Antibiotic resistance in *Escherichia coli* strains isolated from water springs in Al-Ahsa Region. *Afr. J. Microbiol. Res.* 2011 Jan; 5 (2):123-130.
3. Oyediji O, Olutiola P, Moninuola M. Microbiological quality of packaged drinking water brands marketed in Ibadan metropolis and Ile-Ife city in South Western Nigeria. *Afr. J. Microbiol. Res.* 2010; 4 (1): 96-102.
4. Lahn G, Grafham O, Sparr A. Refugees and Energy Resilience in Jordan. Retrieved from Reliefweb, UN Office for the Coordination of Humanitarian Affairs (OCHA) 2016. <https://www.chathamhouse.org/>.
5. Abu-Sharar T, Battikhi A. Water Resources Management under Competitive Sectoral Demand A Case Study from Jordan. *Water International* 2002; 27:364-378.
6. Abberton CL, Bereschenko L, van der Wielen PW, Smith CJ. Survival, Biofilm Formation, and Growth Potential of Environmental and Enteric *Escherichia coli* Strains in Drinking Water Microcosms. *Appl Environ Microbiol.* 2016 Aug; 82 (17):5320-31.
7. Edberg SC, Rice EW, Karlin RJ, Allen MJ. *Escherichia coli*: the best biological drinking water indicator for public health protection. *Symp Ser Soc Appl Microbiol.* 2000; (29):106S-116S.
8. Shehabi AA, Masoud H, Maslamani FA. Common antimicrobial resistance patterns, biotypes and serotypes found among *Pseudomonas aeruginosa* isolates from patient's stools and drinking water sources in Jordan. *J Chemother.* 2005 Apr; 17 (2):179-83.
9. Baquero F, Martínez JL, Cantón R. Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol.* 2008 Jun; 19 (3):260-5.
10. Barza M, Travers K. Excess infections due to antimicrobial resistance: the "Attributable Fraction". *Clin. Infect. Dis.* 2002 Jun 1; 34 Suppl 3:S126-30
11. Ramadan H, Awad A, Ateya A. Detection of phenotypes, virulence genes and phylotypes of avian pathogenic and human diarrheagenic *Escherichia coli* in Egypt. *J Infect Dev Ctries.* 2016 Jun 30; 10 (6):584-91.
12. Salmani H, Azarnezhad A, Fayazi MR, Hosseini A. Pathotypic and Phylogenetic Study of Diarrheagenic *Escherichia coli* and Uropathogenic *E. coli* Using Multiplex Polymerase Chain Reaction. *Jundishapur J Microbiol.* 2016 Feb 2; 9 (2):e28331.
13. American Public Health Association, Water Environment Federation, and American Water Works Association (AWWA). 2015. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, Washington DC.
14. Burjaq SZ, Shehabi AA. Fresh leafy green vegetables associated with multidrug resistant *E. coli*. *The International Arabic Journal of Antimicrobial Agents* 2013; 3:3.
15. CLSI. Clinical Laboratory and Standards Institute Disk Diffusion: Quality Control Ranges for Nonfastidious Organisms (Unsupplemented Mueller-Hinton Medium), and Zone Diameter and MIC Interpretive Standards for *Enterobacteriaceae*. CLSI, Villanova, PA, USA.
16. Nguyen TV, Le Van P, Le Huy C, Gia KN, Weintraub A.. Detection and Characterization of Diarrheagenic *Escherichia coli* from Young Children in Hanoi, Vietnam. *J Clin Microbiol.* 2005 Feb; 43 (2):755-60.
17. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012 Mar; 18 (3):268-81
18. Al-Mardeni RI, Batarseh A, Omaish L, Shraideh M, Batarseh B, Unis N. Empirical treatment for pediatric urinary tract infection and resistance patterns of uropathogens, in Queen Alia hospital and prince A'isha military center Jordan. *Saudi J Kidney Dis Transpl.* 2009 Jan; 20 (1):135-9.
19. 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. *J Chemother.* 2006; 18, 5: 468-47.
20. Heidary M, Momtaz H, Madani M. Characterization of Diarrheagenic Antimicrobial Resistant *Escherichia coli* Isolated From Pediatric Patients in Tehran, Iran. *Iran Red Crescent Med J.* 2014 Apr; 16 (4):e12329.
21. Shehabi AA, Bulos NK, Hajjaj KG. Characterization of diarrhoeagenic *Escherichia coli* isolates in Jordanian children. *Scand J Infect Dis.* 2003; 35 (6-7):368-71.

22. Kazemi R, Akhavian A, Amani J, Salimian J, Motamedi MJ, Mousavi A, Jafari M, Salmanian AH. Immunogenic properties of trivalent recombinant protein composed of B-subunits of LT, STX-2, and CT toxins. *Microbes Infect.* 2016 Jun; 18 (6):421-9.
23. Steffen R, Castelli F, Dieter Nothdurft H, Rombo L, Jane Zuckerman N. Vaccination against enterotoxigenic *Escherichia coli*, a cause of travelers' diarrhea. *J Travel Med.* 2005 Mar-Apr; 12 (2):102-7.
24. Persson S, Olsen KE, Scheutz F, Krogfelt KA, Gerner-Smidt P. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. *Clin Microbiol Infect.* 2007 May; 13 (5):516-24.
25. Sidhu JP, Ahmed W, Hodgers L, Toze S. Occurrence of Virulence Genes Associated with Diarrheagenic Pathotypes in *Escherichia coli* Isolates from Surface Water. *Appl Environ Microbiol.* 2013 Jan; 79 (1):328-35.

**Publish in The International
Arabic Journal of Antimicrobial Agents**

The Journal is an open access peer-reviewed journal that publishes scientific papers about all aspects of antimicrobials. The journal will publish original research articles, reviews, brief reports and case reports dealing with basic and clinical antibacterial agents, antiviral, antiprotozoals, antituberculous, antifungal and antihelminthes agents. All manuscripts must be prepared in English, and are subject to a rigorous and fair peer-review process. Accepted papers will immediately appear online. The journal aims to advance the knowledge, attitude and the research of chemotherapy in the Arabic world in cooperation with international, national scientific and public societies as well as research centers with similar aims and objectives.