

Multi-Drug-Resistant Diarrheagenic *Escherichia coli* Pathotypes in Pediatric Patients with Gastroenteritis from Central Iran

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Elnaz Abbasi ¹
Mahdieh Mondanizadeh ²
Alex van Belkum ³
Ehsanollah Ghaznavi-Rad ^{4,5}

¹Department of Microbiology & Immunology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran; ²Department of Biotechnology and Molecular Medicine, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran; ³Data Analytics Department, BioMérieux, La Balme les Grottes, France; ⁴Molecular and Medicine Research Center, Faculty of Medicine Arak University of Medical Sciences, Arak, Iran; ⁵Department of Medical Laboratory Sciences, Arak School of Paramedicine, Arak University of Medical Sciences, Arak, Iran

Background: Diarrheagenic *Escherichia coli* (DEC) is a significant cause of gastroenteritis and a major public health problem. This study investigates the prevalence and the antibiotic resistance patterns of DEC that were isolated from infectious diarrhea samples of pediatric patients from central Iran.

Patients and Methods: Pediatric diarrhea samples were collected from 230 pediatric patients visiting the hospital. *E. coli* pathotypes were diagnosed by using conventional culture methods and PCR. Antibiotic resistance profiles, the frequency of multi-drug resistance (MDR), and the phenotypic and genotypic characteristics of extended spectrum-β-lactamase (ESBL), AmpC and integron-associated genes were analyzed.

Results: Of the 230 samples of infectious diarrhea, 91 (39.5%) produced *E. coli* isolates. Of these, 32 cases (35.1%) were identified as DEC by culture and PCR. The frequency of the *E. coli* pathotypes obtained was as follows: EAEC 11/32 (34.3%), EPEC 9/32 (28.1%), ETEC 6/32 (18.7%), EIEC 3/32 (9.3%), and EHEC 3/32 (9.3%). The antibiotic resistance rates were greater for nalidixic acid (30/32; 93.7%), ampicillin (29/32; 90.6%), and tetracycline (25/32; 78.1%) than for any of the other tested antibiotics. High levels of MDR (25/32; 78.1%) and the presence of ESBL (18/32; 56.2%) and AmpC (9/32; 28.1%) were observed in the DEC isolates. The isolates showed a higher frequency of the ESBL genes [*bla*_{TEM} (18/18; 100%), *bla*_{CTX-M15} (17/18; 94.4%)], and AmpC [*bla*_{CT} (4/9; 44.4%) and *bla*_{DHA} (4/9; 44.4%)] than of the other ESBL and AmpC genes.

Conclusion: Compared to the previous study, DEC appeared to be the second-most abundant agent of diarrhea in pediatric patients after *Campylobacter jejuni*, with frequent MDR and ESBL presence.

Keywords: diarrheagenic *Escherichia coli* pathotypes, pediatrics, diarrhea, antibiotic resistance, MDR, Iran

Introduction

Gastroenteritis causes 525,000 deaths per year worldwide, especially in children younger than five years old.¹ It can cause serious growth retardation and malnutrition.² Diarrheagenic *Escherichia coli* (DEC) is one of the most abundant agents in pediatric gastroenteritis, particularly in developing countries.³ DEC can be divided into various pathogroups: enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC).⁴ Among these pathotypes, EAEC, EPEC and ETEC are the most significant enteric pathogens and cause 30–40% of the acute pediatric diarrhea cases in developing and developed countries.⁵ The

Correspondence: Ehsanollah Ghaznavi-Rad
Tel/Fax +98-8634173526
Email ghaznaviehs@yahoo.com

emergence of multi-drug-resistant (exhibiting resistance against two or more antibiotics, MDR) *E. coli* strains is a public health concern and is complicating the treatment of various serious infections, particularly in pediatrics.⁶ Lately, worldwide diffusion of extended spectrum- β -lactamase (ESBL)-producing *E. coli* has been most remarkable.⁷ Hence, the analysis of the frequency of diarrheagenic *E. coli* pathotypes to the total burden of diarrheal disease and the antimicrobial resistance pattern of these pathotypes is necessary for developing useful interventions that can effectively reduce the mortality and morbidity related to diarrhea.

The number of outbreaks of diarrhea and dysentery in central Iran, especially in the summer, is very high in pediatrics,⁸ but the prevalence of DEC and antibiotic resistance in this region is unknown. Therefore, the present study was conducted to investigate the abundance, the phenotypic antimicrobial resistance levels and the resistance gene content of the region's DEC by examining diarrhea samples from pediatric patients.

Patients and Methods

Sample Collection

For this cross-sectional, descriptive study, 230 samples of diarrhea were collected from children who were referred to the Amirkabir Educational-Referral Center from the beginning of May 2015 to the end of February 2016. Consent and questionnaire forms were supplied to the parents or guardians of each patient. The inclusion criteria of the study were 1- providing a signed consent form and completed questionnaire, either by the patient or the patient's parents or caregivers, 2- providing a stool sample containing more than five white blood cells per high-power field (HPF).⁸ 3- not having received antibiotics by the patient for at least a week before consultation at the hospital. This research obtained approval from the ethics committee of the Arak University of Medical Sciences (ARAKMU. REC. 93-176-30 and 1395.83).

Phenotypic Investigation

To identify *E. coli* strains and EHEC, diarrheal stool samples were cultured directly on MacConkey and Sorbitol MacConkey (SMAC) agar media (Merck, Hamburg, Germany), respectively. Next, colonies that grew on these media were identified by biochemical tests. API 20E test strips (bioMérieux, France) were used for final identification. Then, O157: H7 serological testing

(SSI, Copenhagen, Denmark) of isolated colorless colonies (due to lack of sorbitol fermentation) was done. Positive controls of different *E. coli* pathotypes were obtained from the microbiology department of the Arak University of Medical Sciences.

Antibiotic Resistance Determination

According to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines,⁹ a disk diffusion assay was performed on the isolated *E. coli* colonies. The antibiotic discs contained nalidixic acid (30 μ g), ampicillin (10 μ g), tetracycline (30 μ g), cotrimoxazole (25 μ g), chloramphenicol (30 μ g), ceftriaxone (30 μ g), cefixime (5 μ g), cefotaxime (30 μ g), ceftizoxime (30 μ g), cefoxitin (30 μ g), ceftazidime (30 μ g), azithromycin (15 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g) and imipenem (10 μ g) (Mast Diagnostics, United Kingdom).

Detection of ESBL and AmpC by Phenotypic Methods

To identify ESBL-positive isolates, the samples were subjected to combination disk diffusion, and double-disk synergy testing methods, and to identify AmpC-positive isolates, disk testing and phenol boronic acid methods according to the 2017 CLSI guidelines were used.⁹

DNA Extraction

DNA was extracted directly from the fecal samples and the reference *E. coli* isolates using the QIAamp DNA stool mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. The amount and purity of the extracted DNA were measured with a NanoDrop apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Genotypic Identification

The 25 μ L final volume of the PCR included 12.5 μ L of master mix (1X), 2 μ L of DNA template (5 ng), 1 μ L each of the forward and reverse primers (10 Pm), 0.5 μ L of Taq DNA polymerase (2.5 units), and 8 μ L of double-distilled water (all purchased from Yekta Tajhiz Company, Iran). PCR of the *uidA* gene was performed to confirm *E. coli* at the genus level.¹⁰ PCR was performed on the *pCVD432* gene for EAEC,¹¹ *eae* gene for EPEC,¹² *elt*, *est* genes for ETEC,¹³ *ial* gene for EIEC,⁴ and *stx* gene for EHEC.¹² The ESBL genes (*bla*_{TEM}, *bla*_{CTX-M-1, 2, 8, 14, 15}, and *bla*_{SHV}), AmpC genes (*bla*_{CMY-2}, *bla*_{CIT}, *bla*_{ACC}, *bla*_{FOX}, *bla*_{MOX} and

Table 1 The Primers Used in This Study

References	Annealing Temperature	Amplicon Size (bp)	Sequence 5'→3'	Primer	Target Gene Description
10	67	510	5-GCGTCTGTTGACTGGCAGGTGGTGG-3 5-GTTGCCCGCTTCGAAACCAATGCCT-3	<i>uidA</i> -F <i>uidA</i> -R	<i>E. coli</i>
11	57.5	630	5-CTGGCGAAAGACTGTATCAT-3 5-CAATGTATAGAAATCCGCTGTT-3	pCVD432-F pCVD432-R	EAEC
12	48	881	5-CCCGAATTCGGCACAAGCATAAGC-3 5-CCCGGATCCGTCTCGCCAGTATTTCG-3	<i>eae</i> -F <i>eae</i> -R	EPEC
13	55	274	5-ACGGCGTTACTATCCTCTC-3 5-TGGTCTCGGTCAGATATGTG-3	<i>elt</i> -F <i>elt</i> -R	ETEC
13	55	170	5-TCTTTCCCTCTTTTAGTCAGTC-3 5-CAGCACAGGCAGGATTAC-3	<i>st</i> -F <i>st</i> -R	ETEC
4	50	650	5-GGTATGATGATGATGAGTCCA-3 5- GGAGGCCAACAATTATTTCC-3	<i>ial</i> -F <i>ial</i> -R	EIEC
12	48	518	5-GAGCGAAATAATTTATATGTG-3 5-TGATGATGGCAATTCAGTAT-3	<i>stx</i> -F <i>stx</i> -R	EHEC
8	55	344	5-AAATCTGCCGTGTCGTTGGT-3 5-GCCATACCTACGGCGATACC -3	<i>gyrA</i> -F <i>gyrA</i> -R	Fluoroquinolone
8	55	168	5-CTGAATGCCAGCGCCAAATT-3 5-GCGAACGATTTTCGGATCGTC-3	<i>parC</i> -F <i>parC</i> -R	
8	60	656	5-TGGAACCTACAATCATAATATCG-3 5-TTAGTCAGGATAAACAACAATACCC-3	<i>qnrS</i> -F <i>qnrS</i> -R	
8	60	593	5-GATAAAGTTTTTCAGCAAGAGG-3 5-ATCCAGATCGGCAAAGGTTA-3	<i>qnrA</i> -F <i>qnrA</i> -R	
8	53	264	5-GTTGGCGAAAAAATTGACAGAA-3 5-ACTCCGAATTGGTCAGATCG-3	<i>qnrB</i> -F <i>qnrB</i> -R	
8	55	160	5-CAGTGGACATAAGCCTGTTC-3 5-CCCGAGGCATAGACTGTA-3	<i>Int1</i> -F <i>Int1</i> -R	Integrase1
8	55	288	5-TTGCGAGTATCCATAACCTG-3 5-TTACCTGCACTGGATTAAGC-3	<i>Int2</i> -F <i>Int2</i> -R	Integrase2
8	59	979	5-GCCTCCGGCAGCGACTTTCAG-3 5-ACGGATCTGCCAAACCTGACT-3	<i>Int3</i> -F <i>Int3</i> -R	Integrase3
8	65	331	5-TCACCGAGGACTCCTTCTTC-3 5-CAGTCCGCCTCAGCAATATC-3	<i>Sul1</i> -F <i>Sul1</i> -R	Sulfonamide resistance
8	58	435	5-CCTGTTTCGTCCGACACAGA-3 5-GAAGCGCAGCCGCAATTCAT-3	<i>Sul2</i> -F <i>Sul2</i> -R	
26	55	370	5-GCCCTACACAAATTGGGAGA-3 5-CTGCGGTACCACTGCCACAA-3	<i>qac</i> -F <i>qac</i> -R	Quaternary ammonium compounds

(Continued)

Table 1 (Continued).

References	Annealing Temperature	Amplicon Size (bp)	Sequence 5'→3'	Primer	Target Gene Description
8	44	425	5-AAAGATGCTGAAGATCA-3 5-TTTGGTATGGCTTCATTC-3	TEM-F TEM-R	β-Lactamase ESBL+
8	62	304	5-GCGAAAGCCAGCTGTCGGGC-3 5-GATTGGCGGGCGCTGTTATCGC-3	SHV-F SHV-R	
8	52	670	5-AAGACTGGGTGTGGCATTGA-3 5-AGGCTGGGTGAAGTAAGTGA-3	CTX-M1-F CTX-M1-R	
8	60	552	5-CGACGCTACCCCTGCTATT-3 5-CCAGCGTCAGATTTTTCAGG-3	CTX-M2-F CTX-M2-R	
8	55	307	5-CGCTTTGCCATGTGCAGCACC-3 5-GCTCAGTACGATCGAGCC-3	CTX-M8-F CTX-M8-R	
8	50	355	5-TACCGCAGATAATACGCAGGTG-3 5-CAGCGTAGGTTCAAGTGCATCC-3	CTX-M14-F CTX-M14-R	
8	55	955	5-CACACGTGGAATTTAGGGACT-3 5-GCCGTCTAAGGCGATAACA-3	CTX-M15-F CTX-M15-R	
8	58	758	5'-GCACTTAGCCACCTATACGGCAG-3' 5'-GCTTTTCAAGAATGCGCCAGG-3'	CMY-2-F CMY-2-R	β-Lactamase AmpC+
8	64	190	5-AACATGGGGTATCAGGGAGATG-3 5-CAAAGCGCGTAACCGGATTGG-3	Fox-F Fox-R	
8	64	520	5-GCTGCTCAAGGAGCACAGGAT-3 5-CACATTGACATAGGTGTGGTGC-3	Mox-F Mox-R	
8	64	405	5-AACTTTCACAGGTGTGCTGGGT-3 5-CCGTACGCATACTGGCTTTGC-3	DHA-F DHA-R	
8	64	346	5-AACAGCCTCAGCAGCCGGTTA-3 5-TTCGCCGAATCATCCCTAGC-3	ACC-F ACC-R	
8	64	462	5-TGGCCAGAAGTACAGGCAAA-3 5-TTTCTCCTGAACGTGGCTGGC-3	CIT-F CIT-R	

Abbreviations: EAEC, enteroaggregative *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; EIEC, entero-invasive *E. coli*; EHEC, enterohemorrhagic *E. coli*.

*bla*_{DHA}) and *sul*_{1,2} for sulfonamide resistance were identified by PCR as well.⁸ PCR of the *qnr* determinant genes *qnrS*, *qnrA*, and *qnrB* was performed to amplify the plasmid-mediated quinolone resistance (PMQR) targets. Mutations in the *gyrA* and *parC* genes of the quinolone-resistant *E. coli* isolates were identified using DNA sequencing.⁸ Quaternary ammonium compounds (*qac*) resistance genes were investigated using PCR (Table 1).⁸

Integron Detection

To investigate the presence of class 1, 2 and 3 integrons, PCR was performed as previously described (Table 1).⁸

Results and Discussion

Of the 230 analyzed samples, 91 (39.5%) produced *E. coli* isolates and 32 (35.1%) were identified as DEC, based on a combination of culture and PCR. All the culture-positive samples were confirmed as positive by PCR. The female-to-male DEC infection ratio was 1.1:1 (Table 2).

Considering a study which it was done in the past, we showed that DEC (n=32, 13.9%) was second only to *Campylobacter jejuni* (n=45, 19.5%; p = 0.003) as the most abundant agent of diarrhea in pediatric patients, whereas in Shiraz (Iran) and India, DEC was the most common agent found.¹⁴⁻¹⁶ Pediatricians should therefore

Table 2 Total Number, Sex, and Age Characteristics of the Patients with *E. coli* Pathotypes

E. coli Pathotypes	DEC	EAEC	EPEC	ETEC		EIEC	Stx Negative EHEC O157:H7
				LT	ST		
Total number (%)	32/91 (35.1%)	11/32 (34.3%)	9/32 (28.1%)	6/32 (18.7%)		3/32 (9.3%)	3/32 (9.3%)
				4/6 (66.6%)	2/6 (33.3%)		
Male	15/32 (46.8%)	4/11 (36.3%)	5/9 (55.5%)	4/6 (66.6%)		1/3 (33.3%)	1/3 (33.3%)
				3/4 (75%)	1/2 (50%)		
Female	17/32 (53.1%)	7/11 (63.6%)	4/9 (44.4%)	2/6 (33.3%)		2/3 (66.6%)	2/3 (66.6%)
				1/4 (25%)	1/2 (50%)		
Average age	4 years and 6 months	3 years and 1 month	2 years and 9 months	4 years and 6 months		5 years and 8 months	4 years and 1 month
				3 years and 8 months	6 years		
Youngest patient's age	5 months	5 months	8 months	9 months		3 years and 5 months	1 year
				9 months	3 years		
Oldest patient's age	10 years	10 years	10 years	9 years		9 years	6 years
				9 years	9 years		

Abbreviation: DEC, diarrheagenic *Escherichia coli*.

request standard microbiological methods for determination of the pathogens involved and for designing subsequent therapy.

Phenotypic and Genotypic Investigation

The isolates showed a greater frequency of the EAEC (11/32; 34.3%) and EPEC (9/32; 28.1%) pathotypes than of the other *E. coli* pathotypes. (Table 2). The PCR results were consistently in accordance with those obtained from culture. Table 3 shows the abundance of DEC and *E. coli* pathotypes in the present study and the pathotypes reported in previous studies. The differences in the frequencies of DEC and *E. coli* pathotypes may be related to a variety of factors, including differences in climate and other environmental conditions, the level of economic development, individual hygiene practices, lack of/improper medical health care, poor quality medical care, and contamination of food or of food manufacturing or processing facilities.¹⁷

The patients showed a greater frequency of mucus in the stool (28/32; 87.5%), abdominal pain (24/32; 75%), and vomiting (19/32; 59.3%) than of other clinical symptoms (Table 4). Table 3 shows the numerous clinical symptoms identified in this study and in other published papers. Our analysis showed differences in the clinical

symptoms between various bacterial infections, but many overlaps were also evident. Hence, a laboratory-based identification of DEC is still required for etiological verification of this diarrheal disease. Nevertheless, pediatricians should seriously consider EHEC as an etiological agent of diarrhea when blood is found in stool specimens.

Phenotypic and Genotypic Antibiotic Resistance Determination

The CLSI 2017 guidelines indicated greater antibiotic resistance rates for nalidixic acid (30/32; 93.7%), ampicillin (29/32; 90.6%), and tetracycline (25/32; 78.1%) than for any of the other antibiotics. All DEC isolates were susceptible to gentamicin and imipenem. DEC is a common agent in developing countries and is spread by contaminated water and food; therefore, its frequency and antimicrobial resistance are public health concerns.¹⁷ Full descriptions of the antibiotic resistance of DEC and *E. coli* pathotypes, from this study and others, are provided in Table 3. These results strongly suggest that nalidixic acid, ampicillin, tetracycline, and cotrimoxazole can no longer be empirically prescribed for the treatment of severe diarrhea and dysentery in central Iran. Since 1960, cotrimoxazole has been one of the World Health Organization's essential medicines, as it is widely

Table 3 Comparison of the Frequency, Clinical Symptoms, and Antibiotic Resistance of DEC and *E. coli* Pathotypes in This Study and in Other Studies

E. coli Pathotypes		Shiraz (Iran)	India	China (Throughout the Country)	Tehran (Iran)	Sudan	Iran's North and Northwest Provinces	China (Southeastern)
DEC		43.6%	30.7%	5%	58.9%	48%	27%	14.1%
EAEC		23.6%	6.9%	1.6%	16.6%	43%	0%	62.5%
EPEC		10.9%	21.5%	1.6%	12.6%	29%	10.5%	15%
ETEC		3.6%	10.7%	1%	10.8%	18%	5.8%	17.9%
EIEC		5.5%	–	0.5%	–	9%	0%	0.3%
EHEC		0%	4.6%	0.3%	18.9%	–	3.5%	4%
The most abundant <i>E. coli</i> pathotype		EAEC 84.6%	EPEC 21.5%	EAEC and EPEC 1.6%	EHEC 18.9%	EAEC 43%	EPEC 10.5%	EAEC 62.5%
Clinical symptoms in patients with DEC	Mucus in the stool	–	–	65%	51.5%	–	–	–
	Abdominal pain	–	–	3.4%	–	–	–	–
	Vomiting	–	–	5.1%	52.6%	–	–	–
Antibiotic resistance in patients with DEC	Nalidixic acid	–	–	–	–	34%	–	–
	Ampicillin	93.8%	–	–	–	47%	–	91.8%
	Tetracycline	–	–	–	–	24%	–	57%
	Cotrimoxazole	77.1%	–	–	–	–	–	52.3%
	Cefotaxime	66.7%	–	–	–	–	–	35.7%
	Ceftriaxone	66.7%	–	–	–	–	–	–
	ESBL in DEC isolates	66.7%	–	–	–	–	–	34.5%
The most abundant ESBL genes		–	–	–	–	–	–	<i>bla</i> _{CTX-M} 94.2%
References		16	14	27	11	28	29	30

Note: Not reviewed.

Table 4 Frequency of Clinical Symptoms in Pediatric Patients with *E. coli* Pathotypes

E. coli Pathotypes		Mucus in the Stool		Abdominal Pain		Vomiting		Fever		Blood in the Stool	
DEC		28/32 (87.5%)		24/32 (75%)		19/32 (59.3%)		17/32 (53.1%)		10/32 (31.2%)	
EAEC		10/11 (90.9%)		9/11 (81.8%)		8/11 (72.7%)		7/11 (63.6%)		4/11 (36.3%)	
EPEC		9/9 (100%)		4/9 (44.4%)		5/9 (55.5%)		4/9 (44.4%)		1/9 (11.1%)	
ETEC	LT	5/6 (83.3%)	3/4 (75%)	5/6 (83.3%)	3/4 (75%)	2/6 (33.3%)	1/4 (25%)	2/6 (33.3%)	1/4 (25%)	0%	0%
	ST		2/2 (100%)		2/2 (100%)		1/2 (50%)		1/2 (50%)		0%
EIEC		3/3 (100%)		3/3 (100%)		3/3 (100%)		3/3 (100%)		2/3 (66.6%)	
Stx negative		1/3 (33.3%)		3/3 (100%)		1/3 (33.3%)		1/3 (33.3%)		3/3 (100%)	
EHEC O157:H7											

available and easily affordable and shows a broad spectrum of activity against an extensive range of infections, including diarrhea.

High levels of MDR (25/32; 78.1%), ESBL (18/32; 56.2%), and AmpC (9/32; 28.1%) gene expression were observed for the DEC isolates (Table 5 and Figure 1). The most extensive MDR strains for our collection of DEC

showed combined resistance to nalidixic acid, ampicillin, tetracycline, and third-generation cephalosporins. The frequency of ESBL positivity was higher in the EAEC pathotype (81.8%) than in the other ESBL-positive *E. coli* pathotypes.

MDR DEC has quickly spread worldwide and represents a serious menace to the proper management of

Table 5 Phenotypic Antibiotic Resistance Rates in *E. coli* Pathotypes

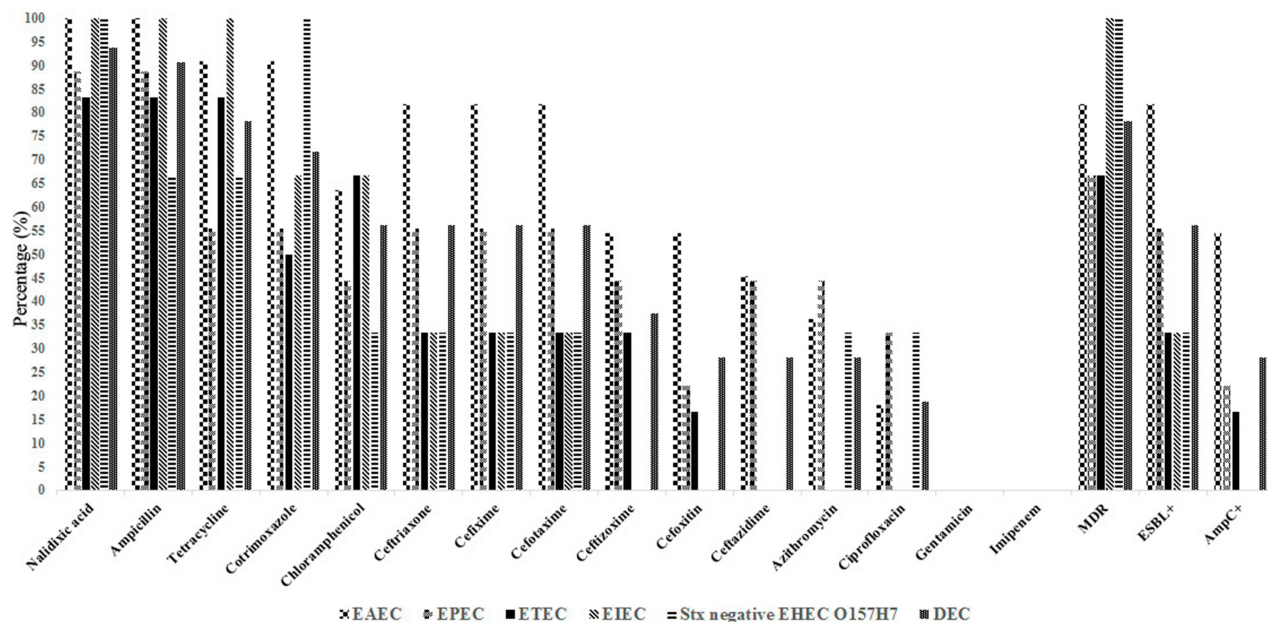
Antibiotic	DEC n:32	EAEC n:11	EPEC n:9	ETEC n:6	EIEC n:3	Stx Negative EHEC O157:H7 n:3
Nalidixic acid	30 (93.7%)	11 (100%)	8 (88.8%)	5 (83.3%)	3 (100%)	3 (100%)
Ampicillin	29 (90.6%)	11 (100%)	8 (88.8%)	5 (83.3%)	3 (100%)	2 (66.6%)
Tetracycline	25 (78.1%)	10 (90.9%)	5 (55.5%)	5 (83.3%)	3 (100%)	2 (66.6%)
Cotrimoxazole	23 (71.8%)	10 (90.9%)	5 (55.5%)	3 (50%)	2 (66.6%)	3 (100%)
Chloramphenicol	18 (56.2%)	7 (63.6%)	4 (44.4%)	4(66.6%)	2 (66.6%)	1 (33.3%)
Ceftriaxone	18 (56.2%)	9 (81.8%)	5 (55.5%)	2 (33.3%)	1 (33.3%)	1 (33.3%)
Cefixime	18 (56.2%)	9 (81.8%)	5 (55.5%)	2 (33.3%)	1 (33.3%)	1 (33.3%)
Cefotaxime	18(56.2%)	9 (81.8%)	5 (55.5%)	2 (33.3%)	1 (33.3%)	1 (33.3%)
Ceftizoxime	12 (37.5%)	6 (54.5%)	4 (44.4%)	2 (33.3%)	0 (0%)	0 (0%)
Cefoxitin	9 (28.1%)	6 (54.5%)	2 (22.2%)	1 (16.6%)	0 (0%)	0 (0%)
Ceftazidime	9 (28.1%)	5 (45.4%)	4 (44.4%)	0 (0%)	0 (0%)	0 (0%)
Azithromycin	9 (28.1%)	4 (36.3%)	4 (44.4%)	0 (0%)	0 (0%)	1 (33.3%)
Ciprofloxacin	6 (18.7%)	2 (18.1%)	3 (33.3%)	0 (0%)	0 (0%)	1 (33.3%)
Gentamicin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Imipenem	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
MDR	25 (78.1%)	9 (81.8%)	6 (66.6%)	4 (66.6%)	3 (100%)	3 (100%)
ESBL+	18 (56.2%)	9 (81.8%)	5 (55.5%)	2 (33.3%)	1 (33.3%)	1 (33.3%)
AmpC+	9 (28.1%)	6 (54.5%)	2 (22.2%)	1 (16.6%)	0 (0%)	0 (0%)

Abbreviations: MDR, multi-drug resistance; ESBL, extended spectrum- β -lactamase.

diarrhea and dysentery in developing countries.¹⁸ The frequencies of MDR DEC in the present study and in others are summarized in Table 3. The high rate of MDR-resistant isolates in pediatric patients with diarrhea can lead to more frequent treatment failures. In addition, resistant isolates demand the prescription of broad-spectrum

antibiotics for the empiric treatment of infections, adding tremendous costs to treatment of these infections.¹⁹

The isolates showed a higher frequency of the ESBL genes *bla*_{TEM} (18/18; 100%), *bla*_{CTX-M15} (17/18; 94.4%), and AmpC [*bla*_{CIT} (4/9; 44.4%) and *bla*_{DHA} (4/9; 44.4%)] than of the other ESBL and AmpC genes (Table 6 and Figure 2). Table 3

**Figure 1** Phenotypic antibiotic resistance rates in *E. coli* pathotypes.

Abbreviations: MDR, Multi-drug resistance; ESBL, Extended spectrum- β -lactamase.

Table 6 Frequency of Antibiotic Resistance Genes Among *E. coli* Pathotypes

Resistance	Target Gene	DEC	EAEC	EPEC	ETEC	EIEC	Stx Negative EHEC O157:H7
Sulfonamide	<i>Sul2</i>	18/23 (78.2%)	9/10 (90%)	5/5 (100%)	2/3 (66.6%)	1/2 (50%)	1/3 (33.3%)
	<i>Sul1</i>	14/23(60.8%)	6/10 (60%)	4/5 (80%)	1/3 (33.3%)	1/2 (50%)	2/3 (66.6%)
ESBL+	<i>bla_{TEM}</i>	18/18 (100%)	9/9 (100%)	5/5 (100%)	2/2 (100%)	1/1 (100%)	1/1 (100%)
	<i>bla_{CTX-M-15}</i>	17/18 (94.4%)	8/9 (88.8%)	5/5 (100%)	2/2 (100%)	1/1 (100%)	1/1 (100%)
	<i>bla_{CTX-M-1}</i>	12/18 (66.6%)	6/9 (66.6%)	4/5 (80%)	1/2 (50%)	1/1 (100%)	0%
	<i>bla_{CTX-M-14}</i>	12/18 (66.6%)	7/9 (77.7%)	4/5 (80%)	1/2 (50%)	0%	0%
	<i>bla_{SHV}</i>	6/18 (33.3%)	3/9 (33.3%)	3/5 (60%)	0%	0%	1/1 (100%)
	<i>bla_{CTX-M-2}</i>	6/18 (33.3%)	3/9 (33.3%)	2/5 (40%)	1/2 (50%)	0%	0%
	<i>bla_{CTX-M-8}</i>	0%	0%	0%	0%	0%	0%
	<i>bla_{CIT}</i>	4/9 (44.4%)	2/6 (33.3%)	1/2 (50%)	1/1 (100%)	–	–
	<i>bla_{DHA}</i>	4/9 (44.4%)	2/6 (33.3%)	1/2 (50%)	1/1 (100%)	–	–
	<i>bla_{CMY-2}</i>	3/9 (44.4%)	1/6 (100%)	1/2 (100%)	1/1 (100%)	–	–
	<i>bla_{ACC}</i>	1/9 (11.1%)	1/6 (16.6%)	0%	0%	–	–
	<i>bla_{FOX}</i>	0%	0%	0%	0%	–	–
	<i>bla_{MOX}</i>	0%	0%	0%	0%	–	–
Fluoroquinolone	<i>gyrA</i>	20/30 (66.6%)	8/11 (72.7%)	7/8 (87.5%)	2/5 (40%)	1/3 (33.3%)	2/3 (66.6%)
	<i>parC</i>	18/30 (60%)	7/11 (63.6%)	7/8 (87.5%)	2/5 (40%)	1/3 (33.3%)	1/3 (33.3%)
	<i>qnrS</i>	18/30 (60%)	7/11 (63.6%)	6/8 (75%)	2/5 (40%)	1/3 (33.3%)	2/3 (66.6%)
	<i>qnrA</i>	2/30 (6.6%)	1/11 (9%)	1/8 (12.5%)	0%	0%	0%
	<i>qnrB</i>	0%	0%	0%	0%	0%	0%
Integrase	<i>Int1</i>	19/32 (59.3%)	6/11 (54.5%)	6/9 (66.6%)	4/6 (66.6%)	2/3 (66.6%)	1/3 (33.3%)
	<i>Int2</i>	13/32 (40.6%)	4/11 (36.3%)	7/9 (77.7%)	1/6 (16.6%)	0%	1/3 (33.3%)
	<i>Int3</i>	0%	0%	0%	0%	0%	0%
Quaternary Ammonium Compounds	<i>qac</i>	23/32 (71.8%)	8/11 (72.7%)	8/9 (88.8%)	3/6 (50%)	2/3 (66.6%)	2/3 (66.6%)

shows the frequency of ESBL and its encoding genes in DEC and the other *E. coli* pathotype isolates. The differences in the frequencies of MDR, ESBL, and related genes across various regions may reflect different antibiotic usage patterns and different DEC community compositions.²⁰

Among the PMQR determinants, *qnrS*, and *qnrA* were positive in 18/30 (60%) and 2/30 (6.6%) in nalidixic acid-resistant DEC strains, respectively and no case of *qnrB* was found. 20/30 (66.6%) isolates carrying PMQR contain similar mutations in *gyrA* at amino acid 83 (replacement of serine with leucine) and 18/30 (60%) *parC* at amino acid 80 (replacement of serine with isoleucine; GenBank accession no. HM068910). Although quinolones/fluoroquinolones are intended to be appropriate drugs against resistant isolates, the enhancement in antimicrobial resistance is a burden in controlling infections caused by DEC.²¹ In India, *qnrS*, *qnrB*, and *qnrA* were found at 46.6%, 40%, and 0% in the DEC strains, respectively.²² In Tehran (Iran), *qnr*

was found at 72% in the DEC strains.²³ These studies indicate that diffusion of *qnr* resistance genes among these DEC isolates is variable.

Class 1 and 2 integrons were observed in a total of 19/32 (59.3%) and 13/32 (40.6%) DEC strains: were observed. No Class 3 integrons were found (Table 6 and Figure 2). Of the three categories of integrons pertinent to antimicrobial resistance, the class I integron is the one most frequently obtained from Gram-negative bacteria.²⁴ The prevalence of integrons in the enterobacteriaceae family has been shown to vary and has played a significant role in the spread of drug-resistance.²⁵ In India *Int1* had a prevalence of 41.6%, and no cases of *Int2* or *Int3* were found from DEC isolates.²² The class 1 integron is overcoming in outspreading the MDR genes among these DEC isolates despite the advent of class 2 and 3 integrons. In this study, 16/25 (64%) and 36% of MDR isolates of DEC contained *Int1* and *Int2* genes, respectively. In Iran (Southwest) MDR in intestinal pathogenic bacteria such as DEC is known to be related with presence.²⁰

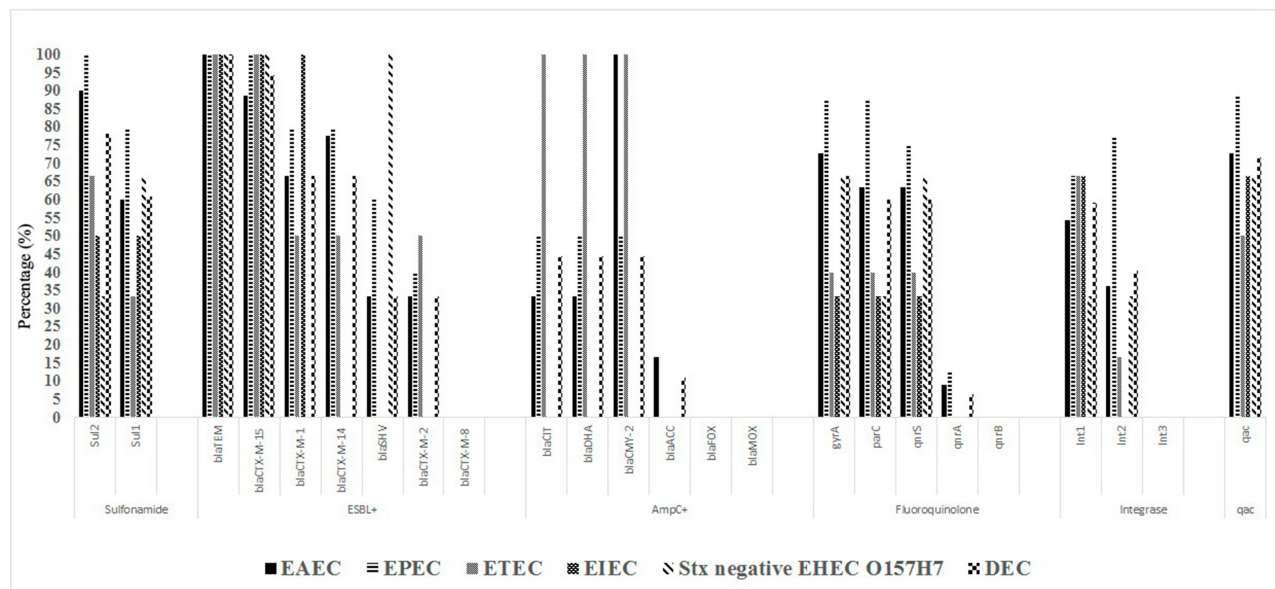


Figure 2 Frequency of antibiotic resistance genes among *E. coli* pathotypes.

Conclusion

According to our previous study, DEC is second only to *Campylobacter jejuni* in frequency when compared with other enteropathogens in pediatric patients with diarrhea from central Iran, and the abundance is greater for EAEC than for any other pathotype.¹⁵ Thus, pediatricians and, even more importantly, experts in laboratory diagnostics should pay special attention to the identification of these *E. coli* pathotypes. Pediatricians should keep these resistant bacteria in mind when treating pediatric patients with infectious diarrhea, and empiric antibiotic therapy should be adapted appropriately. Necessary measures must be taken to control the drug resistance of these bacteria.

Ethics Approval and Consent to Participate

This study received ethical approval from the Arak University of Medical Sciences. Informed consent was obtained from a parent and/or guardian for participants under 16 years old. There was no access to any information that enabled authors to identify individual patient.

Acknowledgments

The authors gratefully acknowledge the educational assistance of Arak University of Medical Sciences due to its financial contributions to and support of this study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

AvB is an employee of bioMérieux, a company designing, developing and selling infectious disease tests. The company had no influence on the design and execution, either of the study or in the writing of the manuscript. The authors report no other conflicts of interest in this work.

References

- Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. *Lancet*. 2016;388(10051):1291–1301. doi:10.1016/S0140-6736(16)31529-X
- Troeger C, Colombara DV, Rao PC, et al. Global disability-adjusted life-year estimates of long-term health burden and undernutrition attributable to diarrhoeal diseases in children younger than 5 years. *Lancet Glob Health*. 2018;6(3):e255–e269. doi:10.1016/S2214-109X(18)30045-7
- Canizalez-Roman A, Flores-Villaseñor HM, Gonzalez-Nuñez E, et al. Surveillance of diarrheagenic *Escherichia coli* strains isolated from diarrhea cases from children, adults and elderly at Northwest of Mexico. *Front Microbiol*. 2016;7:1924. doi:10.3389/fmicb.2016.01924
- Lee GY, Jang HI, Hwang IG, Rhee MS. Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. *Int J Food Microbiol*. 2009;134(3):196–200. doi:10.1016/j.ijfoodmicro.2009.06.013

5. Ifeanyi CIC, Ikeneche NF, Basse BE, Al-Gallas N, Aissa RB, Boudabous A. Diarrheagenic *Escherichia coli* pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria. *J Infect Dev Ctries*. 2015;9(02):165–174. doi:10.3855/jidc.5528
6. Mansour Amin MS, Javaherizadeh H, Motamedifar M, et al. Antibiotic resistance pattern and molecular characterization of extended-spectrum β -lactamase producing enteroaggregative *Escherichia coli* isolates in children from southwest Iran. *Infect Drug Resist*. 2018;11:1097. doi:10.2147/IDR.S167271
7. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution and clinical impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect Genet Evol*. 2018;61:185–188. doi:10.1016/j.meegid.2018.04.005
8. Abbasi E, Abtahi H, van Belkum A, Ghaznavi-Rad E. Multidrug-resistant *Shigella* infection in pediatric patients with diarrhea from central Iran. *Infect Drug Resist*. 2019;12:1535. doi:10.2147/IDR.S203654
9. Patel J, Weinstein M, Eliopoulos G, Jenkins S, Lewis J, Limbago B. M100 Performance standards for antimicrobial susceptibility testing. CLSI; 2017:240.
10. Mohammadzadeh M, Goudarzi H, Dabiri H, Fallah F. Characterization of enteropathogenic *Escherichia coli* associated with diarrhea among Iranian infants. *Arch Pediatr Infect Dis*. 2017;5(1).
11. Jafari F, Garcia-Gil L, Salmanzadeh-Ahrabi S, et al. Diagnosis and prevalence of enteropathogenic bacteria in children less than 5 years of age with acute diarrhea in Tehran children's hospitals. *J Infect*. 2009;58(1):21–27. doi:10.1016/j.jinf.2008.10.013
12. Ndlovu T, Le Roux M, Khan W, Khan S, van Overbeek LS. Co-detection of virulent *Escherichia coli* genes in surface water sources. *PLoS One*. 2015;10(2):e0116808. doi:10.1371/journal.pone.0116808
13. Xia X, Meng J, McDermott PF, et al. Presence and characterization of Shiga toxin-producing *Escherichia coli* and other potentially diarrheagenic *E. coli* strains in retail meats. *Appl Environ Microbiol*. 2010;76(6):1709–1717. doi:10.1128/AEM.01968-09
14. Shrivastava AK, Kumar S, Mohakud NK, Suar M, Sahu PS. Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. *Gut Pathog*. 2017;9(1):16. doi:10.1186/s13099-017-0166-0
15. Abbasi E, Belkum A, Ghaznavi-Rad E. Quinolone and macrolide-resistant campylobacter jejuni in pediatric gastroenteritis patients from Central Iran. *Microb Drug Resist*. 2019;25(7):1080–1086. doi:10.1089/mdr.2018.0455
16. Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F. Diarrheagenic *Escherichia coli* and *Shigella* with high rate of extended-spectrum Beta-lactamase production: two predominant etiological agents of acute diarrhea in Shiraz, Iran. *Microb Drug Resist*. 2017;23(8):1037–1044. doi:10.1089/mdr.2017.0204
17. Gomes TA, Elias WP, Scaletsky IC, et al. Diarrheagenic *Escherichia coli*. *Braz J Microbiol*. 2016;47:3–30.
18. Zeighami H, Haghi F, Hajiahmadi F, Kashefieh M, Memariani M. Multi-drug-resistant enterotoxigenic and enterohemorrhagic *Escherichia coli* isolated from children with diarrhea. *J Chemother*. 2015;27(3):152–155. doi:10.1179/1973947813Y.0000000161
19. Van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: trends and lessons learned. *Infect Dis Clin*. 2016;30(2):377–390. doi:10.1016/j.idc.2016.02.004
20. Kargar M, Mohammadalipour Z, Doosti A, Lorzadeh S, Japoni-Nejad A. High prevalence of class 1 to 3 integrons among multidrug-resistant diarrheagenic *Escherichia coli* in southwest of Iran. *Osong Public Health Res Perspect*. 2014;5(4):193–198. doi:10.1016/j.phrp.2014.06.003
21. Namboodiri SS, Opintan JA, Lijek RS, Newman MJ, Okeke IN. Quinolone resistance in *Escherichia coli* from Accra, Ghana. *BMC Microbiol*. 2011;11(1):44. doi:10.1186/1471-2180-11-44
22. Natarajan M, Kumar D, Mandal J, Biswal N, Stephen S. A study of virulence and antimicrobial resistance pattern in diarrhoeagenic *Escherichia coli* isolated from diarrhoeal stool specimens from children and adults in a tertiary hospital, Puducherry, India. *J Health Popul Nutr*. 2018;37(1):17. doi:10.1186/s41043-018-0147-z
23. 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;16(4). doi:10.5812/ircmj.12329
24. Kang HY, Jeong YS, Oh JY, et al. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J Antimicrob Chemother*. 2005;55(5):639–644. doi:10.1093/jac/dki076
25. Deng Y, Bao X, Ji L, et al. Resistance integrons: class 1, 2 and 3 integrons. *Ann Clin Microbiol Antimicrob*. 2015;14(1):45. doi:10.1186/s12941-015-0100-6
26. Guo W, Shan K, Xu B, Li J. Determining the resistance of carbapenem-resistant *Klebsiella pneumoniae* to common disinfectants and elucidating the underlying resistance mechanisms. *Pathog Glob Health*. 2015;109(4):184–192. doi:10.1179/2047773215Y.0000000022
27. Yu J, Jing H, Lai S, et al. Etiology of diarrhea among children under the age five in China: results from a five-year surveillance. *J Infect*. 2015;71(1):19–27. doi:10.1016/j.jinf.2015.03.001
28. Saeed A, Abd H, Sandstrom G. Microbial aetiology of acute diarrhoea in children under five years of age in Khartoum, Sudan. *J Med Microbiol*. 2015;64(4):432. doi:10.1099/jmm.0.000043
29. Miri ST, Dashti A, Mostaan S, Kazemi F, Bouzari S. Identification of different *Escherichia coli* pathotypes in north and north-west provinces of Iran. *Iran J Microbiol*. 2017;9(1):33.
30. Chen Y, Chen X, Zheng S, et al. Serotypes, genotypes and antimicrobial resistance patterns of human diarrhoeagenic *Escherichia coli* isolates circulating in southeastern China. *Clin Microbiol Infect*. 2014;20(1):52–58. doi:10.1111/1469-0691.12188

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>