REVIEW ARTICLE

An overview of diarrheagenic *Escherichia coli* in Iran: A systematic review and meta-analysis

Hesam Alizade[1,2], Saeed Hosseini Teshnizi[3], Mohsen Azad[1], Saeed Shojae[1], Hamed Gouklani[1], Parivash Davoodian[1], Reza Ghanbarpour[4]

[1]Infectious and Tropical Disease Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, [2]Student Research Committee, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, [3]Department of Biostatistics, Shiraz University of Medical Sciences, Shiraz, [4]Molecular Microbiology Research Group, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Background: Diarrheagenic *Escherichia coli* (DEC) is a common enteric pathogen that causes a wide spectrum of gastrointestinal infections, particularly in developing countries. This is a systematic review and meta-analysis to determine the prevalence of DEC in various geographical regions in Iran. **Materials and Methods:** English (PubMed, Web of Science, Scopus, Embase, Cochrane Library, and Google Scholar) and Persian (IranMedex, SID, Magiran, and Iran Doc) databases were comprehensively searched from January 1990 to April 2017. Study selection and data extraction were performed by two independent reviewers. After assessing heterogeneity among studies, a random effects model was applied to estimate pooled prevalence. Data analyses were done with the Stata software (version 12.0). This meta-analysis was registered with PROSPERO, number CRD42017070411. **Results:** A total of 73 studies with 18068 isolates were eligible for inclusion within the meta-analysis. The results of random effects model showed that the most prevalent DEC pathotypes were enterotoxigenic *E. coli* (ETEC) (16%; 95% confidence interval [CI]: 11%–23%), enteroaggregative *E. coli* (11%; 95% CI: 8%–15%), atypical enteropathogenic *E. coli* (EPEC) (11%; 95% CI: 8%–14%), Shiga toxin-producing *E. coli* (9%; 95% CI: 6%–13%), diffuse adherent *E. coli* (6%; 95% CI: 6%–12%), enteroinvasive *E. coli* (4%; 95% CI: 2%–6%), and typical EPEC (3%; 95% CI: 1%–5%). **Conclusion:** This study showed that DEC infections in the Iranian population have low frequency. Our data suggest that the ETEC pathotype can be regarded as one of the most important etiological agents of diarrhea in this country. However, the prevalence of DEC pathotypes is diverse in different regions of Iran.

Keywords: Diarrhea, *Escherichia coli*, Iran, meta-analysis, systematic review

How to cite this article: Alizade H, Hosseini Teshnizi S, Azad M, Shojae S, Gouklani H, Davoodian P, *et al.* An overview of diarrheagenic *Escherichia coli* in Iran: A systematic review and meta-analysis. J Res Med Sci 2019;24:23.

INTRODUCTION

Escherichia coli is a ubiquitous commensal inhabitant bacterium present in the gastrointestinal tracts of humans and animals, although some strains have acquired several putative virulence factors that enable it to adapt to new niches and cause a broad spectrum of infections.[1,2] Pathogenic *E. coli* is one of the major causes of infectious diseases that span from the gastrointestinal tract to extraintestinal sites. Extraintestinal diseases include the urinary tract infection (UTI), septicemia, newborn meningitis, central nervous system, and respiratory system infections.[3,4]

Pathogenic *E. coli* agents of gastrointestinal infections are significant causes of sporadic outbreaks of diarrhea worldwide, especially in developing countries.[5] Based on virulence factors and phenotypic traits, diarrheagenic *E. coli* (DEC) strains are generally classified into eight pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC)/verotoxin-producing

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_256_18

Address for correspondence: Prof. Reza Ghanbarpour, Molecular Microbiology Research Group, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
E-mail: ghanbar@uk.ac.ir
Received: 16-04-2018; **Revised:** 26-11-2018; **Accepted:** 03-12-2018

E. coli (VTEC), enteroaggregative *E. coli* (EAEC or EAggEC), diffuse adherent *E. coli* (DAEC), and adherent-invasive *E. coli* (AIEC).[6-8]

The prevalence of pathogenic *E. coli* which causes gastrointestinal infections has been reported in the literature from different regions of Iran; therefore, it is necessary to conduct a comprehensive analysis. This study presents a systematic review and meta-analysis of the literature on the DEC pathotypes in the Iranian population.

MATERIALS AND METHODS

Search strategy

This study is a systematic review and a meta-analysis study on original research articles that have been published in English or Persian which present the prevalence of DEC in Iran. The databases of PubMed, Web of Science, Scopus, Embase, Cochrane Library, Google Scholar, Iranmedex, SID, and Magiran were searched for studies published from January 1990 to April 2017. The following keywords or medical subject headings in titles or abstracts were used with the help of Boolean operators ("and" or "or"): "diarrheagenic *Escherichia coli*," "enterotoxigenic *Escherichia coli*," "ETEC," "enteropathogenic *Escherichia coli*," "EPEC," "enterohemorrhagic *Escherichia coli*," "EHEC," "enteroinvasive *Escherichia coli*," "EIEC," "enteroaggregative *Escherichia coli*," "EAEC," "shiga-toxigenic *Escherichia coli*," "shiga toxin-producing *Escherichia coli*," "STEC," "verotoxin-producing *Escherichia coli*," "verotoxigenic *Escherichia coli*," "VTEC," "Diffusely Adherent *Escherichia coli*," "DAEC," "Adherent-invasive *E. coli*," "AIEC," and "Iran." This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement with the PRISMA 2015 checklist. The planned systematic review and meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number CRD42017070411.[9]

Selection studies

To select studies, two authors scrutinized titles and abstracts of all studies returned by the search strategy. Then, they independently evaluated the full text of potentially relevant nonduplicated articles. Any disagreement was resolved by discussion between the two reviewers. When no agreement was reached, a third reviewer was consulted.

Eligibility criteria

The manual revision was conducted on all displayed articles, and the first levels of screening were based on information in the titles and/or abstracts. The final level was applied to the original publications. The included articles were confirmed by bacteriological standard methods for identification of *E. coli* isolates (e.g., the biochemical API 20E identification system or conventional biochemical tests) and molecular method for the detection of DEC genes (e.g., PCR). Studies were excluded from the analysis that did not focus on the prevalence of DEC from human specimens in various region of Iran.

Data extraction

For all articles, the compiled information contains age (e.g., young children, adults, and the elderly), year of study, year of publication, place of study, the source of samples, number of cases analyzed, and the prevalence of *E. coli* pathotypes. The isolates were divided into five groups depending on the sample status: diarrheic, UTI, bloody diarrhea, healthy feces, and mixed (diarrheic and healthy). To assess the quality of each included study in the meta-analysis, we used the 22-item STROBE checklist (https://strobestatement.org). We categorized all studies in three categories in which obtained based on total score of checklists: >80% of the total score of checklists yes = high, 60%–80% of total score yes - medium, and <60% yes = low.

Statistical analysis

At the beginning of the analysis, we undertook an initial descriptive analysis for describing the essential characteristics of studies. Then, for each study, the prevalence (the number of current positive cases divided into a total number of the sample) and standard error were calculated. When the estimated prevalence for a study tends toward either 0% or 100%, the variance for that study moves toward zero, and as a result, its weight is overestimated in the meta-analysis. Therefore, we conducted the meta-analysis with prevalence estimates that had been transformed using the double arcsine method.[10] To investigate the influence of each individual study on the overall estimate, a sensitivity analysis was performed. Before the quantitative pooling of the results, heterogeneity among studies was assessed using the Cochran's Q-statistic ($P < 0.1$ as significant) and the I^2 index (25%, 50%, and 75% as low, moderate, and high heterogeneity; respectively).[11] When heterogeneity was present, we used a random effects model (DerSimonian–Laird method); otherwise, we applied a fixed effects model (Mantel–Haenszel method) to estimate the pooled prevalence. The point prevalence of pathotypes and its 95% confidence interval (CI) were estimated for pooled effect size and each study. Forest plots illustrated the proportion of *E. coli* pathotypes, along with 95% CI. We used subgroup analysis to assess sources of heterogeneity using categorical variables as type of DEC, location which study performed, the source of sample and also using meta-regression for sample size of studies, quality of studies and year of study publication. The funnel plot (visual method) and

Egger's test ($P < 0.1$ as significant) were used to evaluate the possibility of publication bias among studies. In the case with significant publication bias, a nonparametric trim and fill method was performed to rectify the bias. All statistical analyses were conducted using Stata MP Software version 14.0 (Stata Corp, College Station, TX, USA) with metaprop command.

RESULTS

Study characteristics

In the initial database searches, 786 potential studies were identified. After the primary screening of titles and abstracts, 254 articles were selected for full-text search. Assessment of title and abstract resulted in the identification of 158 duplicate studies. Among them, 96 full-text articles were retrieved to check the eligibility, of which 73 were included. A total of 73 studies with 86 datasets (18068 isolates) were included in the final review and meta-analysis. Figure 1 presents the PRISMA flowchart, which describes the articles identified from the search strategy. These 86 data consisted of diarrhea samples (53), feces of healthy samples (13), acute diarrhea samples (9), UTI samples (6), bloody diarrhea samples (2), persistent diarrhea samples (1), and mixed (diarrheic and healthy feces) samples (1). The exclusion of articles based on the title and abstract of studies was mainly because of the following reasons: duplicated population groups, the articles were based on case report, assessment of specific methods on DEC pathotypes diagnosis, reported DEC pathotypes from animal and environmental samples, narrative reviews studies with inadequate data and generally, and studies that did not focus on the prevalence of even one of the DEC pathotypes from human samples in Iran. Table 1 presents a summary of the characteristics of the 86 included data.

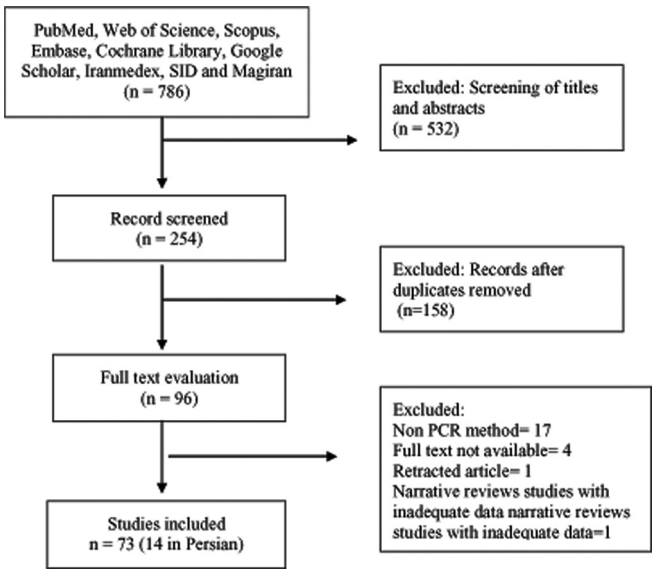

Figure 1: Flowchart of study selection

A description of the characteristics of each study, reporting the prevalence of DEC pathotypes in the Iranian population, is presented in "Appendix 1." Quality of study for five studies was moderate and for 68 studies was high; the results of subgroup analysis showed that the prevalence of DEC in the two groups (moderate and high quality) was statistically not significant ($P = 0.13$).

Sensitivity analysis

At first, the sensitivity analysis was conducted to evaluate whether individual studies influenced pooled prevalence. The result indicated that no study substantially influenced the pooled prevalence of pathotypes of DEC, which indicates that all studies need to be included for further research.

Heterogeneity

We performed a fixed effects model to estimate the pooled prevalence of pathotypes of DEC, 17.0% (95% CI: 16.9%–18.0%; $I^2 = 98.9$, $P < 0.001$). The results showed that there was substantial statistical heterogeneity among the effect size of studies. To assess the causes of the heterogeneity, five subgroup analyses were undertaken. The results showed that characteristics such as sample size, quality of studies, and year of publication are possible sources of heterogeneity [Table 1]; however, after using these variables in multiple meta-regression, the results indicated that sample size ($P = 0.031$) can be one of the causes of heterogeneity [Table 2]; so that, studies with small sample size were affected than studies with large sample [Figure 2].

Shiga toxin-producing *Escherichia coli*

The presence of STEC was examined in 9185 isolates in 38 publications. Overall, 953 of 9185 *E. coli* isolates were positive for the STEC pathotype. According to a random effects model ($I^2 = 95.14$, $P < 0.001$), the pooled prevalence was estimated to be 9% (95% CI: 6%–13%) [Table 1]. The

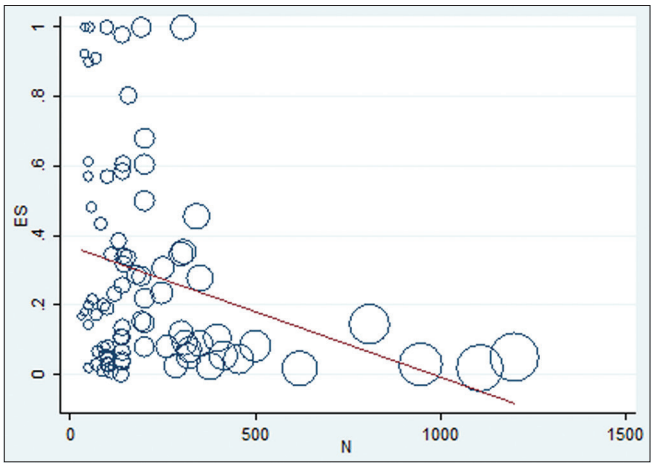

Figure 2: Relationship between sample size and effect size for included studies

Table 1: Subgroup meta-analysis and publication bias for diarrheagenic *Escherichia coli* pathotypes

Characteristics	Subgroup	Number of study	Isolates positive	Isolates tested	Prevalence of pathotype (95% CI)	Heterogeneity		Publication bias test	
						I^2 (%)	*Q*-test	Bias	Egger's
DEC pathotypes	STEC	38	953	9185	9 (6-13)	95.14	<0.001	2.48	0.04
	ETEC	28	878	5669	16 (11-23)	97.29	<0.001	0.64	0.31
	aEPEC	38	923	9459	11 (8-14)	95.91	<0.001	1.97	0.002
	tEPEC	12	144	4240	3 (1-5)	92.45	<0.001	1.08	0.06
	EAEC	27	815	6526	11 (8-15)	94.91	<0.001	0.54	0.17
	EIEC	17	142	3184	4 (2-6)	88.42	<0.001	0.54	0.46
	DAEC	2	35	401	6 (6-12)	NES	NES	NES	NES
	AIEC	-	-	-	-	-	-	-	-
Type of sample	Diarrhea	53	2398	12,234	19 (8-38)	98.74	<0.001	4.35	0.21
	UTI	6	104	866	7 (4-16)	97.11	<0.001	3.41	0.16
	Stool	13	329	2416	12 (7-21)	97.99	<0.001	2.12	0.77
	Acute Dia	9	959	2296	14 (5-31)	99.56	<0.001	2.57	0.435
Quality of studies	High	68	3692	17,426	11 (9.0, 14.0)	96.97	<0.001	5.11	0.021
	Moderate	5	179	642	19 (14.0, 31.0)	89.77	<0.001	NES	NES
Year of publication	<2010	13	1191	5491	11 (8-14)	98.5	<0.001	4.82	0.055
	2010-2015	50	1912	10,095	3 (1-5)	91.3	<0.001	3.57	0.001
	>2015	10	768	2482	11 (8-15)	84.7	<0.001	6.31	0.054
Location*	Shiraz	7	215	943	30 (0.8-58)	98.32	<0.001	2.21	0.69
	Tabriz	5	321	824	36 (12-65)	90.11	<0.001	7.17	0.02
	Tehran	31	1801	10,501	19 (11-28)	98.45	<0.001	2.45	0.47
	Hamadan	4	181	575	47 (17-78)	81.01	<0.001	3.68	0.02
	Kerman	6	278	1558	33 (12-58)	98.61	<0.001	1.70	0.52
	Zanjan	3	203	920	19 (13-27)	73.70	0.003	2.96	0.74
Sample size	<100	23	589	1445	11 (7-25)	98.17	<0.001	8.13	0.091
	100-500	45	3046	11,940	9 (3-21)	98.7	<0.001	4.02	0.001
	>500	5	236	4683	4 (2-9)	88.7	<0.001	3.0	0.031

*Cities which not included in analysis had not enough sample. *E. coli=Escherichia coli*; EPEC=Enteropathogenic *E. coli*; aEPEC=Atypical EPEC; tEPEC=Typical EPEC; ETEC=Enterotoxigenic *E. coli*; STEC=Shiga toxin-producing *E. coli*; EAEC=Enteroaggregative *E. coli*; EIEC=Enteroinvasive *E. coli*; DAEC=Diffuse adherent *E. coli*; AIEC=Adherent-invasive *E. coli*; UTI=Urinary tract infection; DEC=Diarrheagenic *E. coli*; CI=Confidence interval; NES=No enough sample size

Table 2: Results of multiple meta-regression to assess the source of heterogeneity

Variables	Coefficient	SE	*t*	*P*
Sample size	−0.0004	0.0014	−2.94	0.004
Quality of studies	−0.002	0.003	−0.70	0.484
Year of publication	−0.006	0.003	−0.62	0.540
Location	−0.002	0.006	−0.33	0.745

SE=Standard error

prevalence of STEC isolated from diarrheic samples was 21/43 (48.8%), from acute diarrhea samples was 9/43 (20.9%), from healthy stool samples was 6/43 (13.9%), from UTI samples was 3/43 (6.9%), from bloody diarrhea samples was 2/43 (4.6%), and from persistent diarrhea and mixed samples each one was 1/43 (2.3%). Based on previous reports from various regions in Iran, a high prevalence of the STEC pathotype of *E. coli* was detected in Shahrekord (central; 48.3%);[12] central, western, and northern (44.5%);[13] and Shiraz (south; 35.3%)[14] [Appendix 1 and Figure 3].

Enterotoxigenic *Escherichia coli*

The presence of ETEC was investigated in 5669 isolates in 28 publications. A random effects model ($I^2 = 97.21$, $P < 0.001$) showed that the pooled prevalence of ETEC was 16% (95% CI: 11%–23%) [Table 1]. The prevalence of ETEC in dependent of diarrheic sample status was 17/34 (50%) with 8/34 (23.5%) in acute diarrhea samples, 7/34 (20.6%) in healthy samples, 2/34 (5.9%) in UTI samples, and 1/34 (2.9%) in the persistent diarrhea sample. The highest report of the ETEC was 64.1%,[15] 58.3%,[16] and 46.4%[17] of *E. coli* isolates from Kerman (south-east), Tehran and Sanandaj (capital and west), and Tabriz (north-west), respectively [Appendix 1 and Figure 3].

Enteropathogenic *Escherichia coli*

The presence of atypical EPEC (aEPEC) was investigated in 9459 isolates in 38 publications. In total, the pooled prevalence of aEPEC (923 out of 9459) based on a random effects model ($I^2 = 95.9$, $P < 0.001$) was estimated to be 11% (95% CI: 8%–14%) [Table 1]. aEPECs were found more often in diarrheic (25/44; 56.8%) samples than in acute diarrhea (10/44; 22.7%), feces of healthy (6/44; 13.6%), UTI (1/44; 2.3%), mixed (1/44; 2.3%), and persistent diarrhea (1/44; 2.3%) samples. The prevalence of aEPEC ranged from 0.00% among *E. coli* isolated from children (Tehran, capital)[18] to 59.6% among *E. coli* isolated from acute diarrhea (Sanandaj, west).[19] Egger's

test and funnel plot showed a significant publication bias for STEC (bias = 2.48, P = 0.04) and aEPEC (bias = 1.97, P = 0.002), but for other pathotypes, there was no publication bias [Appendix 1 and Figure 3].

The presence of typical EPEC (tEPEC) was examined in 4240 isolates in 12 publications. The results of a random effects mode showed that (I^2 = 92.45, P < 0.001) the pooled effect prevalence of tEPEC was estimated to be 3% (95% CI: 1%–5%) from 144 *E. coli* isolates which were positive for tEPEC pathotype [Table 1]. This pathotype was identified in diarrheic (10/14; 71.4%) samples than in the feces of healthy (3/14; 21.4%) and acute diarrhea (1/14; 7.1%) samples. The percentage of tEPEC is higher in Tehran (central; 21.5%)[20] and Tehran, Ilam, and Mazandaran (central, west, and north of Iran; 14.2%)[21] [Appendix 1 and Figure 3].

Enteroaggregative *Escherichia coli*

The presence of EAEC was investigated in 6526 isolates in 27 publications. The EAEC was present in 815 isolates. Based on a random effects model (I^2 = 92.45, P < 0.001), the pooled estimate of prevalence was obtained 11% (95% CI: 8%–15%) [Table 1]. The prevalence of EAEC in diarrheic samples was 42.8% (15/35), in healthy stool was 25.7% (9/35), in acute diarrhea was 20% (7/35), in UTI was 8.6% (3/35), and in persistent diarrhea was 2.8% (1/35). The percentages of EAEC vary with the geographical location of the patients: 28.3% in Tabriz,[22] 25.6% in Zanjan,[23] and 20% in Tehran[24] [Appendix 1 and Figure 3].

Enteroinvasive *Escherichia coli*

The presence of EIEC was examined in 3184 isolates in 17 publications. A random effects model showed that the pooled prevalence of EIEC was 4% (95% CI: 2%–6%) from 142 *E. coli* isolates which were positive for the EIEC pathotype [Table 1]. The prevalence of the EIEC pathotype isolated from diarrheic samples was 12/21 (57.1%), and it was 3/21 (14.3%) from acute diarrhea samples, 3/21 (14.3%) from healthy stool samples, 2/21 (9.5%) from UTI samples, and 1/21 (4.8%) from persistent diarrhea. EIEC was found in 22.2%, 14.3%, and 8.5% of the isolates in Sanandaj (west),[19] Shiraz (south),[25] and Tehran (central),[26] respectively [Appendix 1 and Figure 3].

Diffusely adherent *Escherichia coli*

The presence of DAEC was evaluated in 401 isolates in two publications. To calculation of heterogeneity, there were not enough studies (n = 2). According to a fixed effects model, the pooled prevalence of DAEC was estimated at 6% (95% CI: 6%–12%) based on 35 isolates [Table 1]. DAEC was found more often in isolates from diarrheic isolates (29/35; 82.8%) as compared to isolates from UTI isolates (6/35; 17.1%). The prevalence of DAEC ranged from about 8% (Shiraz)[27] to 9% (Tehran and Sanandaj)[16] in Iran [Appendix 1 and Figure 3].

Adherent-invasive *Escherichia coli*

There are no data about the prevalence of the AIEC pathotype until April 2017 in Iran.

Publication bias

Funnel plot depicted for 73 studies. It seems that there is an asymmetric in funnel plot and small studies (smaller precision) trending to have large effect t size (prevalence) [Figure 4], this was cross-checked by Egger's regression test in which the results of this test confirm that a publication bias among studies (t = 4.05, P < 0.001). Furthermore, publication bias was assessed in each of subgroup using Egger's test; the results indicated that there was a considerable publication bias in some variables such as quality of studies, year of publication, and

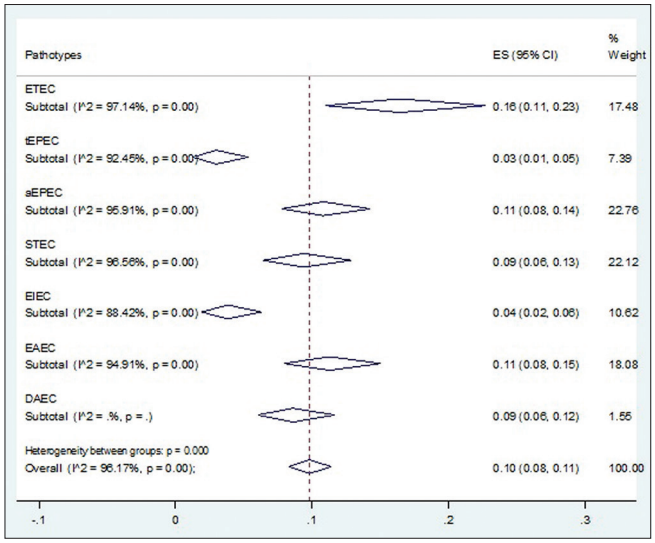

Figure 3: Forest plot of prevalence for seven pathotypes of diarrheagenic *Escherichia coli* in Iran. Diamonds indicate the 95% confidence interval for each pathotypes

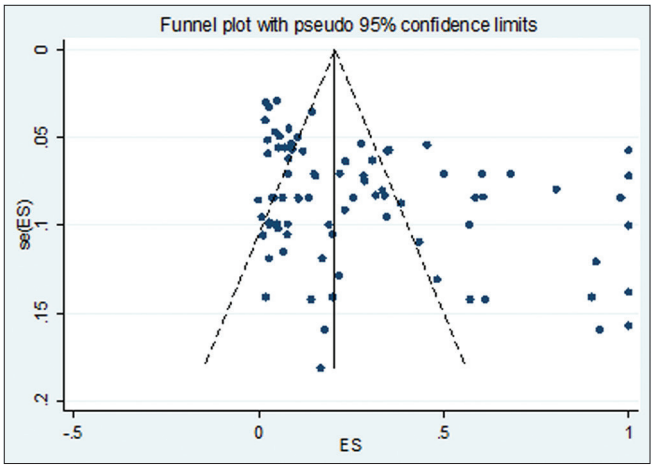

Figure 4: Funnel plot to display publication bias among 73 studies included in meta-analysis

sample size [Table 1]. A trim and fill method was performed to rectify the detected publication bias and then the pooled effect size for each subgroup (with $P < 0.1$) was filled.

DISCUSSION

Gastrointestinal infections due to pathogenic *E. coli* are significant causes of outbreaks and sporadic cases worldwide, particularly in children from countries in South Asia and Sub-Saharan Africa.[3,28] The present systematic review and meta-analysis was designed to estimate the prevalence of DEC pathotypes in the Iranian population according to available data from articles collected from various regions of the country by Iranian researchers. The findings of this study highlight the low frequency of DEC pathotypes in various geographical areas of Iran. The ETEC was identified as one of the most common pathotypes (16%; 95% CI: 11%–23%) among the studied data. ETEC strains are the significant cause of diarrhea in travelers, and there is major morbidity and mortality in children residing in developing countries.[29,30] Annually, this pathogen causes 280–400 million diarrheal episodes in children aged <5 years and causes substantial disease in adults in developing countries, with an estimated 400 million cases per year in people aged over 15 years.[31] Several studies conducted in Iran have also revealed the high prevalence rate of *E. coli* isolates to ETEC in different parts.[15-17,32,33] According to the report of Gupta *et al.*, ETEC was the etiological agent isolated in a median of 13% of diarrheal cases in children of developing countries.[34] Previous studies showed that ETEC is usually a frequent cause of diarrhea in infants younger than 2 years of age.[35,36] In Iran, it was found to be the most common cause of diarrhea in children younger than 5 years of age.[17,28,33,37] This variation may be because children under 3 months became susceptible to a primary infection; ETEC strains to produce STh and LT were most common, whereas at 6–7 months, ETEC strains to produce STp, STpLT, and SThLT were dominant.[35] The susceptibility of young children has also been reported in other settings which have poor hygiene conditions.

VTEC, also known as STEC infections, causes a wide spectrum of clinical manifestations ranging from symptom-free carriage forms of intestinal illnesses to bloody diarrhea. EHEC is a subset of STEC capable of causing hemorrhagic colitis and hemolytic uremic syndrome in humans.[38,39] In earlier studies in Iran, the prevalence of STEC isolates ranged from 0.0% among *E. coli* isolated from children <5 years of age[28,40-42] to 30%–48% among *E. coli* isolated from patients of unknown age.[12-15,43] In developing countries, where studies typically focus solely on children, STEC has also been detected as a pathogen of concern in this age group.[44,45] However, in developed countries, studies investigating all ages typically identified STEC more frequently and with greater severity in young children.[46,47] Unfortunately, despite the existence of many data of aEPEC in Iran (39 publications), there is not sufficient data about the incidence of tEPEC (12 publications). The results of our study indicated that the incidence of aEPEC in the investigated studies varies in different provinces, such that the highest rate of incidence was reported from Sanandaj with 59.6% (west of Iran);[19] central, western, and northern Iran with 38.8%;[13] Khuzestan with 31% (south west of Iran);[43] and Kashan with 28.6% (central of Iran).[48] Previous studies in Northeast India,[49] Ghana,[50] and Kenya[51] showed that EPEC was the most frequently identified bacterial pathogen.

In Iran, moderately high reports are available on the occurrence of EIEC (17 publications). In the present study, 4.5% (95% CI: 2%–6%) of *E. coli* isolates were confirmed as EIEC, of which the most prevalent were from Sanandaj (west)[19] and Tehran (central)[26]. Other studies in the world also reported less percentage of EIEC (0%–1.5%).[52,53]

EAEC is well known as a diarrhea-causing agent in people of all ages in developing and industrialized countries, most prominently in association with persistent diarrhea.[54] According to our results, the total prevalence of EAEC strains was 11.1% (95% CI: 8%–15%). It is consistent with some studies conducted in Switzerland (10.2%) and Kenya (8.9%).[51,55] Another study in urban and rural South Korea showed that the prevalence of EAEC was 0.3%–3.7% of acute diarrhea samples.[56]

There are very few reports on the epidemiology of DAEC infections in Iran (two publications). However, the frequency of this pathotype was 8.7%. Both studies were conducted in Brazil, and DAEC was significantly associated with diarrhea in children.[57,58] In a study in Iran, DAEC strains were isolated in 6% of patients with UTI.[16] DAEC strains may represent the reservoir for uropathogenic *E. coli*, since several virulence factors of DAEC, such as adhesins of the Afa/Dr family, are found in uropathogenic *E. coli* strains.[59] Four *E. coli* isolates presenting mixed characteristics (mixed EAEC/DAEC genes) were identified.[15] Several studies showed the presence of diarrheagenic isolates presenting mixed characteristics of two different pathotypes.[60,61]

CONCLUSION

By combining the results from 73 studies, this systematic review and meta-analysis identified the lower frequency of DEC infections in Iran. Our data suggest that the ETEC pathotype is one of the most important etiological agents of disease in this country. However, the prevalence of DEC pathotypes is diverse in different regions of Iran. The limitation of the study was associated with high heterogenicity, especially in sample size subgroup. We used

subgroup analysis to find the source of heterogeneity, but in all moderator variables, there is a high heterogeneity. Maybe there is a possibility that a moderator variable is missing that does, in fact, explain the heterogeneity (i.e., age and sex). Furthermore, nonuniform study design and various types of publication bias may be the other causes of heterogeneity in these studies. Our study included other limitations such as it cannot fully represent the prevalence of DEC infections in Iran because the extent of DEC has not yet been examined in some regions of the country.

Financial support and sponsorship

The authors would like to thank the Infectious and Tropical Disease Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran, for funding assistance with this project. This study was funded by grant number (960112) and ethical number (1396.65).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, *et al*. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. Genet Mol Res 2011;10:4114-25.
2. Kim HJ, Koo M, Jeong AR, Baek SY, Cho JI, Lee SH, *et al*. Occurrence of pathogenic *Escherichia coli* in commercially available fresh vegetable products in Korea. J Korean Soc Appl Biol Chem 2014;57:367-70.
3. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB, *et al*. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev 2013;26:822-80.
4. Banu A, Kabbin JS, Anand M. Extraintestinal infections due to *Escherichia coli*: An emerging issue. J Clin Diagn Res 2011;5:486-90.
5. Allocati N, Masulli M, Alexeyev MF, Di Ilio C. *Escherichia coli* in Europe: An overview. Int J Environ Res Public Health 2013;10:6235-54.
6. Darfeuille-Michaud A. Adherent-invasive *Escherichia coli*: A putative new *E. coli* pathotype associated with Crohn's disease. Int J Med Microbiol 2002;292:185-93.
7. Kaper JB. Pathogenic *Escherichia coli*. Int J Med Microbiol 2005;295:355-6.
8. Askari Badouei M, Jajarmi M, Mirsalehian A. Virulence profiling and genetic relatedness of Shiga toxin-producing *Escherichia coli* isolated from humans and ruminants. Comp Immunol Microbiol Infect Dis 2015;38:15-20.
9. Alizade H, Ghanbarpour R, Azad M, Shoja S, Davoodian P. Prevalence of diarrheagenic *Escherichia coli* pathotypes in humans of any age in Iran: Systematic review and meta-analysis. Prospero 2017:CRD42017070411. Available from: http://www.crd.york.ac.uk/PROSPERO_REBRANDING/display_record.asp?ID=CRD42017070411.
10. Mansfield KE, Sim J, Jordan JL, Jordan KP. A systematic review and meta-analysis of the prevalence of chronic widespread pain in the general population. Pain 2016;157:55-64.
11. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60.
12. Bonyadian M, Momtaz H, Rahimi E, Habibian R, Yazdani A, Zamani M, *et al*. Identification and characterization of Shiga toxin-producing *Escherichia coli* isolates from patients with diarrhoea in Iran. Indian J Med Res 2010;132:328-31.
13. Aslani MM, Ahrabi SS, Alikhani YM, Jafari F, Zali RM, Mani M, *et al*. Molecular detection and antimicrobial resistance of diarrheagenic *Escherichia coli* strains isolated from diarrheal cases. Saudi Med J 2008;29:388-92.
14. Rajaee M, Emami A, Bazargani A, Pirbonyeh N, Moattari A. Identification of serotypes and virulence markers (stx) of *Escherichia coli* isolated from patients with diarrhea in Shiraz, Iran. Gene Rep 2017;6:49-53.
15. Alizade H, Sharifi H, Naderi Z, Ghanbarpour R, Bamorovat M, Aflatoonian MR, *et al*. High frequency of diarrheagenic *Escherichia coli* in HIV-infected patients and patients with thalassemia in Kerman, Iran. J Int Assoc Provid AIDS Care 2017;16:353-8.
16. 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;9:e28331.
17. Zeighami H, Haghi F, Hajiahmadi F, Kashefiyeh M, Memariani M. Multi-drug-resistant enterotoxigenic and enterohemorrhagic *Escherichia coli* isolated from children with diarrhea. J Chemother 2015;27:152-5.
18. Pourakbari B, Heydari H, Mahmoudi S, Sabouni F, Teymuri M, Ferdosian F, *et al*. Diarrhoeagenic *E. coli* pathotypes in children with and without diarrhoea in an Iranian referral paediatrics centre. East Mediterr Health J 2013;19:617-21.
19. Kalantar E, Solatni J, Khosravi B, Salehi A. Frequency of *E. coli* pathotypes in acute diarrhea of children and its related factors at Beassat hospital, Sanandaj. Asian Pac J Trop Med 2009;2:64-6.
20. Mohammadzadeh M, Oloomi M, Bouzari S. Genetic evaluation of locus of enterocyte effacement pathogenicity island (LEE) in enteropathogenic *Escherichia coli* isolates (EPEC). Iran J Microbiol 2013;5:345-9.
21. Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. J Med Microbiol 2006;55:1159-63.
22. Ahangarzadeh-Rezaee M, Shahbazi GH, Hasani A, Asgharzadeh M, Rahim-Rahimi AA, Alebouyeh M. Prevalence of enteroaggregative *Escherichia coli* among children with gastroenteritis in the Northwest of Iran. J Anal Res Clin Med 2015;3:183-9.
23. Bafandeh S, Haghi F, Zeighami H. Prevalence and virulence characteristics of enteroaggregative *Escherichia coli* in a case-control study among patients from Iran. J Med Microbiol 2015;64:519-24.
24. Bouzari S, Jafari A, Zarepoor M. Distribution of genes encoding toxins and antibiotic resistance patterns in diarrhoeagenic *Escherichia coli* isolates in Tehran. East Mediterr Health J 2007;13:287-93.
25. Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani Dalini S, Dehyadegari MA. Real time PCR for characterization of enteroinvasive *E. coli* (EIEC) in children with diarrhea in Shiraz. Ann Colorectal Res 2015;2:e22721.
26. Kermani NA, Jafari F, Mojarad HN, Hoseinkhan N, Zali R. Prevalence and associated factors of persistent diarrhoea in Iranian children admitted to a paediatric hospital. East Mediterr Health J 2010;16:831-6.
27. Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani-Dalini S, Dehyadegari MA. Molecular detection of diffusely adherent *Escherichia coli* strains associated with diarrhea in Shiraz, Iran. Arch Pediatr Infect Dis 2017;5:e37629.
28. Alizade H, Ghanbarpour R, Aflatoonian MR. Molecular study on diarrheagenic *Escherichia coli* pathotypes isolated from under

5 years old children in Southeast of Iran. Asian Pac J Trop Dis 2014a; 4:S813-7.
29. Qadri F, Saha A, Ahmed T, Al Tarique A, Begum YA, Svennerholm AM, *et al*. Disease burden due to enterotoxigenic *Escherichia coli* in the first 2 years of life in an urban community in Bangladesh. Infect Immun 2007;75:3961-8.
30. Jafari A, Aslani MM, Bouzari S. *Escherichia coli*: A brief review of diarrheagenic pathotypes and their role in diarrheal diseases in Iran. Iran J Microbiol 2012;4:102-17.
31. World Health Organization. Future directions for research on enterotoxigenic *Escherichia coli* vaccines for developing countries. Wkly Epidmiological Rec 2006;11:97-104.
32. Alizade H, Ghanbarpour R, Aflatoonian M, Sobhanipour M. Determination of phylogenetic groups and antibiotic resistance pattern of enterotoxigenic *Escherishia coli* isolates from diarrheoic cases in Bam city by PCR. Iran J Med Microbiol 2015;9:6-13.
33. Haghi F, Zeighami H, Hajiahmadi F, Khoshvaght H, Bayat M. Frequency and antimicrobial resistance of diarrhoeagenic *Escherichia coli* from young children in Iran. J Med Microbiol 2014;63:427-32.
34. Gupta SK, Keck J, Ram PK, Crump JA, Miller MA, Mintz ED, *et al*. Part III. Analysis of data gaps pertaining to enterotoxigenic *Escherichia coli* infections in low and medium human development index countries, 1984-2005. Epidemiol Infect 2008;136:721-38.
35. Steinsland H, Valentiner-Branth P, Perch M, Dias F, Fischer TK, Aaby P, *et al*. Enterotoxigenic *Escherichia coli* infections and diarrhea in a cohort of young children in Guinea-Bissau. J Infect Dis 2002;186:1740-7.
36. Rao MR, Abu-Elyazeed R, Savarino SJ, Naficy AB, Wierzba TF, Abdel-Messih I, *et al*. High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. J Clin Microbiol 2003;41:4862-4.
37. Kashefiyeh M, Zeighami H, Haghi F. Frequency of enterotoxigenic *Escherichia coli* isolates harboring eltb and elta genes in diarrheal specimens among children younger than 5 years in Tabriz hospitals. J Zanjan Univ Med Sci 2014;21:120-7.
38. Alizade H, Ghanbarpour R, Nekoubin M. Phylogenetic of shiga toxin-producing *Escherichia coli* and atypical enteropathogenic *Escherichia coli* strains isolated from human and cattle in Kerman, Iran. Int J Entric Pathog 2014b; 1:e15195.
39. Bagheri M, Ghanbarpour R, Alizade H. Shiga toxin and beta-lactamases genes in *Escherichia coli* phylotypes isolated from carcasses of broiler chickens slaughtered in Iran. Int J Food Microbiol 2014;177:16-20.
40. Asadi Karam M, Bouzari S, Oloomi M, Aslani M, Jafari A. Phenotypic and genotypic characterization of enteropathogenic *Escherichia coli* (EPEC) strains in Tehran, Iran. Iran J Microbiol 2010;2:3-7.
41. Bakhshi B, Fallahzad S, Pourshafie MR. The occurrence of atypical enteropathogenic *Escherichia coli* strains among children with diarrhea in Iran. J Infect Chemother 2013;19:615-20.
42. Mahmoudi-Aznaveh A, Bakhshi B, Najar-Peerayeh S. The trend of enteropathogenic *Escherichia coli* towards atypical multidrug resistant genotypes. J Chemother 2017;29:1-7.
43. Darbandi A, Owlia P, Bouzari S, Saderi H. Diarrheagenic *Escherichia coli* pathotypes frequency in Khuzestan province of Iran. Iran J Microbiol 2016;8:352-8.
44. Bonkoungou IJ, Haukka K, Österblad M, Hakanen AJ, Traoré AS, Barro N, *et al*. Bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso. BMC Pediatr 2013;13:36.
45. Lozer DM, Souza TB, Monfardini MV, Vicentini F, Kitagawa SS, Scaletsky IC, *et al*. Genotypic and phenotypic analysis of diarrheagenic *Escherichia coli* strains isolated from Brazilian children living in low socioeconomic level communities. BMC Infect Dis 2013;13:418.
46. Ruzante JM, Majowicz SE, Fazil A, Davidson VJ. Hospitalization and deaths for select enteric illnesses and associated Sequelae in Canada, 2001-2004. Epidemiol Infect 2011;139:937-45.
47. Buvens G, De Gheldre Y, Dediste A, de Moreau AI, Mascart G, Simon A, *et al*. Incidence and virulence determinants of verocytotoxin-producing *Escherichia coli* infections in the Brussels-capital region, Belgium, in 2008-2010. J Clin Microbiol 2012;50:1336-45.
48. Motallebi M, Piroozmand A, Rohani M, Akbari H, Khorshidi A. Multiple drug resistance of enteropathogenic *Escherichia coli* isolated from children with diarrhea in Kashan, Iran. Afr J Microbiol Res 2011;5:3305-9.
49. Chellapandi K, Dutta TK, Sharma I, De Mandal S, Kumar NS, Ralte L, *et al*. Prevalence of multi drug resistant enteropathogenic and enteroinvasive *Escherichia coli* isolated from children with and without diarrhea in Northeast Indian population. Ann Clin Microbiol Antimicrob 2017;16:49.
50. Addy PA, Antepim G, Frimpong EH. Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. East Afr Med J 2004;81:353-7.
51. Sang WK, Oundo V, Schnabel D. Prevalence and antibiotic resistance of bacterial pathogens isolated from childhood diarrhoea in four provinces of Kenya. J Infect Dev Ctries 2012;6:572-8.
52. Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic *Escherichia coli* by multiplex PCR. Indian J Med Microbiol 2012;30:279-84.
53. Ali MM, Mohamed ZK, Klena JD, Ahmed SF, Moussa TA, Ghenghesh KS, *et al*. Molecular characterization of diarrheagenic *Escherichia coli* from Libya. Am J Trop Med Hyg 2012;86:866-71.
54. Weintraub A. Enteroaggregative *Escherichia coli*: Epidemiology, virulence and detection. J Med Microbiol 2007;56:4-8.
55. Pabst WL, Altwegg M, Kind C, Mirjanic S, Hardegger D, Nadal D, *et al*. Prevalence of enteroaggregative *Escherichia coli* among children with and without diarrhea in Switzerland. J Clin Microbiol 2003;41:2289-93.
56. Cho SH, Shin HH, Choi YH, Park MS, Lee BK. Enteric bacteria isolated from acute diarrheal patients in the republic of Korea between the year 2004 and 2006. J Microbiol 2008;46:325-30.
57. Scaletsky IC, Fabbricotti SH, Carvalho RL, Nunes CR, Maranhão HS, Morais MB, *et al*. Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: A case-control study. J Clin Microbiol 2002;40:645-8.
58. Spano LC, Sadovsky AD, Segui PN, Saick KW, Kitagawa SM, Pereira FE, *et al*. Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. J Med Microbiol 2008;57:359-63.
59. Blanc-Potard AB, Tinsley C, Scaletsky I, Le Bouguenec C, Guignot J, Servin AL, *et al*. Representational difference analysis between Afa/Dr diffusely adhering *Escherichia coli* and nonpathogenic *E. coli* K-12. Infect Immun 2002;70:5503-11.
60. 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-culturable state in response to various stresses and resuscitates upon stress relief. Environ Microbiol 2011;13:3139-48.
61. Riveros M, García W, García C, Durand D, Mercado E, Ruiz J, *et al*. Molecular and Phenotypic Characterization of Diarrheagenic Escherichia coli Strains Isolated from Bacteremic Children. Am J Trop Med Hyg 2017;97:1329-36.
62. Abbasi P, Kargar M, Doosti A, Mardaneh J, Dehyadegari MA, Ghorbani-Dalini S. Multiplex real-time PCR assay for the detection of LT, STIa and STIb genes in enterotoxigenic *Escherichia coli*. Int J Entric Pathog 2014a; 2:e16431.
63. Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani-Dalini S, Dehyadegari MA, *et al*. Characterization of Shiga-toxin producing

E. coli (STEC) and enteropathogenic *E. coli* (EPEC) using multiplex real-time PCR assays for Stx1, Stx2, EaeA. Iran J Microbiol 2014;6:169-74.
64. Abbasi P, Kargar M, Doosti A, Mardaneh J, Ghorbani-Dalini S, Dehyadegari MA. Enteroaggregative *Escherichia coli* (EAEC): An emerging enteric pathogen in South of Iran. J Med Bacterial 2014;3:40-6.
65. Abri R, Razavilar V, Zahraei Salehi T, Ahangarzadeh Rezaee M, Javadi A. Differentiation of enterotoxigenic and enteropathogenic *Escherichia coli* isolated from humans and calves by rep-PCR. Biomed Pharmacol J 2015;8:1363-8.
66. Akhi MT, Ostadgavahi AT, Ghotaslou R, Asgharzadeh M, Pirzadeh T, Sorayaei Sowmesarayi V, *et al.* Detection, virulence gene assessment and antibiotic resistance pattern of O157 enterohemorrhagic *Escherichia coli* in Tabriz, Iran. Jundishapur J Microbiol 2015;8:e25317.
67. Alikhani MY, Sedighi I, Zamani A, Aslani MM, Sadrosadat T. Incidence of diarrhoeagenic *Escherichia coli* isolated from young children with diarrhoea in the West of Iran. Acta Microbiol Immunol Hung 2012;59:367-74.
68. Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic *Escherichia coli* isolated from adolescents and adults in Hamedan, Western Iran. Iran J Microbiol 2013;5:42-7.
69. Alizadeh AH, Behrouz N, Salmanzadeh S, Ranjbar M, Azimian MH, Habibi E, *et al. Escherichia coli*, Shigella and salmonella species in acute diarrhoea in Hamedan, Islamic republic of Iran. East Mediterr Health J 2007;13:243-9.
70. Aslani MM, Alikhani MY, Zavari A, Yousefi R, Zamani AR. Characterization of enteroaggregative *Escherichia coli* (EAEC) clinical isolates and their antibiotic resistance pattern. Int J Infect Dis 2011;15:e136-9.
71. Bakhshi B, Eftekhari N, Pourshafie MR. Genetic elements associated with antimicrobial resistance among intestinal bacteria. Jundishapur J Microbiol 2014;7:e9924.
72. Bouzari S, Aslani MM, Oloomi M, Jafari A, Dashti A. Comparison of multiplex PCR with serogrouping and PCR-RFLP of fliC gene for the detection of enteropathogenic *Escherichia coli* (EPEC). Braz J Infect Dis 2011;15:365-9.
73. Hoseinzadeh T, Ghanbarpour R, Rokhbakhsh-Zamin F. Phylogenetic background of enterotoxigenic and enteroinvasive *Escherichia coli* from patients with diarrhea in Sirjan, Iran. Iran J Microbiol 2016;8:187-92.
74. Hosseini Nave H, Mansouri S, Taati Moghadam M, Moradi M. Virulence gene profile and multilocus variable-number tandem-repeat analysis (MLVA) of enteroinvasive *Escherichia coli* (EIEC) isolates from patients with diarrhea in Kerman, Iran. Jundishapur J Microbiol 2016;9:e33529.
75. Jafari A, Katouli M, Shokouhi F, Bouzari S. Detection and characterization of verotoxin-producing strains among sorbitol non-fermenting *Escherichia coli*. East Mediterr Health J 2001;7:756-62.
76. Jafari F, Shokrzadeh L, Hamidian M, Salmanzadeh-Ahrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. Jpn J Infect Dis 2008;61:269-73.
77. Jafari F, Hamidian M, Rezadehbashi M, Doyle M, Salmanzadeh-Ahrabi S, Derakhshan F, *et al.* Prevalence and antimicrobial resistance of diarrheagenic *Escherichia coli* and Shigella species associated with acute diarrhea in Tehran, Iran. Can J Infect Dis Med Microbiol 2009;20:e56-62.
78. Kargar M, Aein V, Doosti A, Gholami M, Homayoon M. Molecular identification and antibiotic resistance of shigatoxigenic *Escherichia coli* strains in children of the age of under 5-years with diarrhea in Yasuj, Iran. J Isfahan Med Sch 2014;32:67-78.
79. Kargar M, Abbasi P, Doosti A, Ghorbani Dalini S. Identification of enterotoxigenic *Escherichia coli* strains by real-time PCR method in children of the age of under 2-years in Shiraz. Sci J Kurdistan Univ Med Sci 2013;18:29-38.
80. Khorshidi A, Motallebi M, Rohani M, Piroozmand A. Characterization of virulence genes in typical and atypical enteropathogenic *Escherichia coli.* Sci Res Essays 2011;6:6600-5.
81. Khoshvaght H, Haghi F, Zeighami H. Extended spectrum betalactamase producing enteroaggregative *Escherichia coli* from young children in Iran. Gastroenterol Hepatol Bed Bench 2014;7:131-6.
82. Memariani M, Najar Peerayeh S, Zahraei Salehi T, Shokouhi Mostafavi SK. Occurrence of SHV, TEM and CTX-M β-lactamase genes among enteropathogenic *Escherichia coli* strains isolated from children with diarrhea. Jundishapur J Microbiol 2015;8:e15620.
83. 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;9:33-7.
84. Mirzarazi M, Rezatofighi SE, Pourmahdi M, Mohajeri MR. Occurrence of genes encoding enterotoxins in uropathogenic *Escherichia coli* isolates. Braz J Microbiol 2015;46:155-9.
85. Mohammadzadeh M, Goudarzi H, Dabiri H, Fallah F. Molecular detection of lactose fermenting enteroinvasive *Escherichia coli* from patients with diarrhea in Tehran-Iran. Iran J Microbiol 2015;7:198-202.
86. Mohammadzadeh M, Goudarzi H, Dabiri H, Fallah F. Distribution of enterotoxigenic *Escherichia coli* among *E. coli* isolates from diarrheal samples referred to educational hospitals in Tehran-Iran. Novel Biomed 2015b; 3:144-7.
87. Moshtagian F, Alipour M, Yahyapour Y. Prevalence of *Escherichia coli* pathotypes among children with diarrhea in Babol, Northern Iran. Int J Enteric Pathog 2016;4:e36326.
88. Nakhjavani FA, Emaneini M, Hosseini H, Iman-Eini H, Aligholi M, Jabalameli F, *et al.* Molecular analysis of typical and atypical enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhoea. J Med Microbiol 2013;62:191-5.
89. Navidinia M, Karimi A, Rahbar M, Fallah F, Ahsani RR, Malekan MA, *et al.* Study prevalence of verotoxigenic *E. coli* isolated from urinary tract infections (UTIs) in an Iranian children hospital. Open Microbiol J 2012;6:1-4.
90. Nazarian S, Gargari SL, Rasooli I, Alerasol M, Bagheri S, Alipoor SD, *et al.* Prevalent phenotypic and genotypic profile of enterotoxigenic *Escherichia coli* among Iranian children. Jpn J Infect Dis 2014;67:78-85.
91. Nazemi A, Mirinargasi M, Khataminezhad MR, Mostafavi SK, Sharifi SH. Detection of stx(1), stx(2), LT and ST toxin genes and O-157 and H-7 antigen genes among uropathogenic *Escherichia coli* isolates from Iran. Afr J Microbiol Res 2012;6:867-9.
92. Nezarieh R, Shakibaie MR, Hosseini Nave H, Norouzi A, Salajegheh G, Hayatbakhsh M. Distribution of virulence genes, enterotoxin and biofilm formation among enteroaggregative *Escherichia coli* (EAEC) strains isolated from stools of children with diarrhea in South East Iran. Arch Pediatr Infect Dis 2015;3:e29745.
93. Salmanzadeh-Ahrabi S, Habibi E, Jaafari F, Zali MR. Molecular epidemiology of *Escherichia coli* diarrhoea in children in Tehran. Ann Trop Paediatr 2005;25:35-9.
94. Shams S, Haghi-Ashtiani MT, Nasrollahi L, Shahsiah R, Monajemzadeh M, Tahbaz-Lahafi B, *et al.* Frequency of Shiga toxin-producing genes of *Escherichia coli* isolated from diarrheic stools of Iranian children by PCR. Iran J Pediatr 2013;23:637-42.
95. Sharifi Yazdi MK, Akbari A, Soltan Dallal MM. Multiplex polymerase chain reaction (PCR) assay for simultaneous detection of Shiga-like toxin (stx1 and stx2), intimin (eae) and invasive plasmid antigen H (ipaH) genes in diarrheagenic *Escherichia coli.* Afr J Biotechnol 2011;10:1522-6.

96. Aslani M, Shush Poli AS, Sadeghian S, Alikhani MS. Prevalence of shiha toxin-producing *Escherichia coli* in patients with hemorrhagic colitis refered to Tehran hospitals. J Gorgan Univ Med Sci 2008b; 9:17-23.
97. Akbari A, Pourmand MR, Sharifi Yazdi MK, Hosseini M, Soltan Dallal MM. Isolation of enteroinvasive *Escherichia coli* (EIEC) from diarrhea in children by polymerase chain reaction (PCR) technique. Tehran Univ Med J 2010;3:25-30.
98. Aein V, Kargar M, Doosti A, Gholami M. Detection of Enteroinvasive *Escherichia coli* by PCR technique in children with diarrhea. Armaghane Danesh 2014;19:727-36.
99. Bagheri H, Ghaemi E, Aslani MM, Mozafari NA, Livani S. Prevalence of enteroaggregative *Escherichia coli* in diarrhea cases Gorgan city. Med Lab J 2009;2:8-13.
100. Bazzar Z, Bouzari S, Aslani MM, Asmar M, Oloomi M, Jafari A, *et al*. Molecular identification of enteropathogenic *Escherichia coli* (EPEC) in children with diarrheal in the Ardebil. Iran J Infect Dis Trop Med 2011;16:9-12.
101. Bayat M, Haghi F, Zeighami H. Antimicrobial resistance of enteropathogenic *Escherichia coli* isolated from diarrheal children in Zanjan during 2012-13. J Sabzevar Univ Med Sci 2014;21:61-8.
102. Soltan Dallal MM, Shirazi MH, Akbari A, Sharifi Yazdi MK. Identification of enteropathogenic *E. coli* (EPEC) pathotype using PCR technique in under 5- years old children in Tehran and evaluation of their antibiotic resistance patterns. Razi J Med Sci 2015;22:54-62.
103. Soleimanifard N, Amini K, Moradli G. Molecular identification of enteroaggregative and enteropathogenic *Escherichia coli* pathotypes (EPEC and EAEC) strains isolated from urinary tract infections and their antibiotic susceptibility pattern. J Isfahan Med Sch 2015;32:1954-64.
104. Adeli Z, Firoozeh F, Zibaei M, Shakib P. Prevalence of shiga toxin and intimine genes in shiga toxin-producing *Escherichia coli* isolated from urine samples in Lorestan, Iran. Feyz 2013;17:188-94.
105. Kargar M, Homayoon M. Survey of enterohemorrhagic *Escherichia coli* (EHEC) and its antibiotic resistance among children less than 5 Years in Marvdasht. Med Sci J Islamic Azad Univ Tehran Med Branch 2009;19:268-73.
106. Mohammadzadeh M, Goudarzi H, Dabiri H, Fallah F. Frequency of enteroaggregative *Escherichia coli* isolates among patients with diarrhea referred to Tehran hospitals. Res Med 2016;40:24-9.
107. Mirsalehian A, Kamal Khani R, Kazemi B, Fatolahzadeh B. Prevalence of Shiga toxin-producing strains in patients with bloody diarrhea by multiplex PCR method. Tehran Univ Med J 2004;62:1008-15.

Appendix 1: Characteristics of studies included in the meta-analysis

Study	Study period	Published time	Age	Location	Source of sample	Number of isolates/samples	Prevalence of DEC pathotype	Study quality
Abbasi *et al*.[62]	2012	2014a	3-61 years	Shiraz	Diarrhea	52	ETEC=52	20
Abbasi *et al*.[63]	2012	2014b	1-26 months	Shiraz	Diarrhea	49	aEPEC=13 EHEC=15	20
Abbasi *et al*.[64]	2012	2014c	2 months-63 years	Shiraz	Diarrhea	101	EAEC=5	20
Abbasi *et al*.[25]	2012	2015	1-24 months	Shiraz	Diarrhea	49	EIEC=7	20
Abbasi *et al*.[27]	2012-2013	2017	2 months-63 years	Shiraz	Diarrhea	101	DAEC=8	20
Abri *et al*.[65]	-	2015	-	Tabriz	Diarrhea	60	ETEC=10 aEPEC=2 tEPEC=1	20
Aein *et al*.[98]	2011-2012	2014	<5 years	Yasuj	Diarrhea	200	EIEC=16	16
Adeli *et al*.[104]	2011-2012	2013	-	Lorestan	UTI	100	EHEC=3	16
Ahangarzadeh-Rezaee *et al*.[22]	2013	2015	<10 years	Tabriz	Diarrhea	194	EAEC=55	21
Akbari *et al*.[97]	2009	2010	<5 years	Tehran	Diarrhea	39	EIEC=7	18
Akhi *et al*.[66]	2014	2015	-	Tabriz	Diarrhea, stool	200	EHEC=11 aEPEC=5	21
Alikhani *et al*.[21]	-	2006	0-10 years	Tehran, Ilam, Mazandaran	Diarrhea	247	aEPEC=23 tEPEC=35	22
Alikhani *et al*.[21]	-	2006	0-10 years	Tehran, Ilam, Mazandaran	Stool	1108	aEPEC=13 tEPEC=8	
Alikhani *et al*.[67]	2007	2012	Children	Hamadan	Diarrhea	251	ETEC=13 aEPEC=41 EHEC=7 EIEC=0 EAEC=16	20
Alikhani *et al*.[68]	2009	2013	12-65 years	Hamadan	Acute diarrhea	40	ETEC=7 aEPEC=19 EHEC=6 EAEC=8	20
Alizade *et al*.[28]	2010-2012	2014a	<5 years	Kerman	Diarrhea	142	ETEC=52 aEPEC=9 EHEC=0 EIEC=2 EAEC=23	20
Alizade *et al*.[38]	2010-2011	2014b	-	Kerman	Diarrhea	96	aEPEC=5 EHEC=0	20
Alizade *et al*.[15]	2014	2017	-	Kerman	Stool, HIV	49	ETEC=29 aEPEC=8 EHEC=13	20
Alizade *et al*.[15]	2014	2017	-	Kerman	Stool, thalassemia	68	ETEC=46 aEPEC=13 EHEC=23	
Alizade *et al*.[32]	2010	2015	-	Bam	Diarrhea	155	ETEC=52	19
Alizadeh *et al*.[69]	2003	2007	5 months-90 years	Hamadan	Acute diarrhea	144	ETEC=22 aEPEC=2 EHEC=15 EAEC=10	20
Asadi Karam *et al*.[40]	-	2010	<5 years	Tehran	Diarrhea	321	aEPEC=17 EHEC=0	19
Askari Badouei *et al*.[8]	-	2014	2-10 years	Garmsar	Diarrhea	75	aEPEC=1 EHEC=4	21
Aslani *et al*.[13]	-	2008a	-	Central, western, northern	Diarrhea	193	ETEC=14 aEPEC=74 EHEC=86 EAEC=19	19
Aslani *et al*.[70]	2007-2008	2011	<12 years	Hamadan	Stool	140	EAEC=15	21

Contd...

Appendix 1: Contd...

Study	Study period	Published time	Age	Location	Source of sample	Number of isolates/samples	Prevalence of DEC pathotype	Study quality
Aslani *et al*.[96]	2005	2008b	Children	Tehran	Diarrhea	70	EHEC=12	20
Aslani *et al*.[96]	2005	2008b	Children	Tehran	Hemorrhagic colitis	70	EHEC=2	
Bafandeh *et al*.[23]	-	2015	<30->45 years	Zanjan	Diarrhea	350	EAEC=97	21
Bafandeh *et al*.[23]	-	2015	<30->45 years	Zanjan	Stool	200	EAEC=44	
Bagheri *et al*.[99]	2006-2007	2009	1 month-66 years	Gorgan	Diarrhea	455	EAEC=20	19
Bakhshi *et al*.[41]	2009-2010	2012	<5 years	Tehran	Acute diarrhea	309	aEPEC=17 tEPEC=11 EHEC=0	21
Bakhshi *et al*.[71]	-	2014	-	Tehran	Diarrhea	120	aEPEC=28	20
Bazzar *et al*.[100]	-	2011	<5 years	Ardabil	Diarrhea	194	aEPEC=28 tEPEC=2	19
Bayat *et al*.[101]	2012-2013	2014	<5 years	Zanjan	Diarrhea	140	aEPEC=19	18
Bonyadian *et al*.[12]	2007-2008	2010	-	Shahrekord	Diarrhea	58	EHEC=28	20
Bouzari *et al*.[24]	2000	2007	Children	Tehran	Diarrhea	200	ETEC=22 EHEC=38 EAEC=40	20
Bouzari *et al*.[72]	-	2011	-	Tehran	Diarrhea	500	aEPEC=14 tEPEC=27	19
Darbandi *et al*.[43]	2012-2013	2016	-	Khuzestan	Diarrhea	158	ETEC=21 aEPEC=49 tEPEC=1 EHEC=50 EIEC=1 EAEC=5	20
Haghi *et al*.[33]	2011-2012	2014	<5 years	Tabriz	Diarrhea	140	ETEC=65 aEPEC=13 tEPEC=6 EHEC=17 EAEC=36	21
Haghi *et al*.[33]	2011-2012	2014	<5 years	Tabriz	Stool	90	ETEC=8 aEPEC=3 tEPEC=0 EHEC=0 EAEC=7	
Hoseinzadeh *et al*.[73]	2014	2016	-	Sirjan	Diarrhea	110	ETEC=32 EIEC=6	19
Hosseini Nave *et al*.[74]	2013-2014	2016	-	Kerman	Diarrhea	620	EIEC=11	20
Jafari *et al*.[75]	-	2001	Children	Tehran	Diarrhea	1200	EHEC=60	21
Jafari *et al*.[76]	2004-2005	2008	5-60 years	Tehran	Acute diarrhea	808	ETEC=41 aEPEC=11 EHEC=52 EAEC=13	21
Jafari *et al*.[77]	2003-2005	2009	<5 years	Tehran	Acute diarrhea	305	ETEC=38 aEPEC=70 EHEC=105 EAEC=92	21
Kalantar *et al*.[19]	2008	2009	<12 years	Sanandaj	Acute diarrhea	99	ETEC=7 aEPEC=59 EHEC=11 EIEC=22	21
Kargar *et al*.[78]	2011-2012	2014	<5 years	Yasuj	Diarrhea	300	EHEC=104	20
Kargar *et al*.[79]	2010	2013	<2 years	Shiraz	Diarrhea	285	ETEC=7	19
Kargar and Homayoon *et al*.[105]	2006-2007	2009	<5 years	Marvdasht	Diarrhea	89	EHEC=1	20

Contd...

Appendix 1: Contd...

Study	Study period	Published time	Age	Location	Source of sample	Number of isolates/samples	Prevalence of DEC pathotype	Study quality
Kermani *et al*.[26]	-	2010	<5 years	Tehran	Acute diarrhea	341	ETEC=21 aEPEC=18 EHEC=25 EIEC=31 EAEC=60	20
Kermani *et al*.[26]	-	2010	<5 years	Tehran	Persist diarrhea	83	ETEC=5 aEPEC=5 EHEC=6 EIEC=5 EAEC=15	
Khorshidi *et al*.[80]	2009-2010	2011	<5 years	Kashan	Diarrhea	178	EPEC=51	19
Khoshvaght *et al*.[81]	2011-2012	2014	0-60 months	Zanjan	Diarrhea	140	EAEC=36	21
Khoshvaght *et al*.[81]	2011-2012	2014	0-60 months	Zanjan	Stool	90	EAEC=7	
Mahmoudi-Aznaveh *et al*.[42]	2012-2013	2016	<5 years	Tehran	Diarrhea	349	aEPEC=30 tEPEC=1 EHEC=0	21
Memariani *et al*.[82]	2011-2013	2015	<10 years	Tehran	Diarrhea	398	aEPEC=26 tEPEC=16	21
Miri *et al*.[83]	2012-2013	2017	-	East-Azerbaijan, Gilan, Zanjan, Kurdistan, Tehran, Hamedan	Stool	145	ETEC=10 aEPEC=17 tEPEC=1 EHEC=18 EIEC=0 EAEC=0	19
Mirzarazi *et al*.[84]	-	2015	-	Isfahan	UTI	138	EIEC=2 EAEC=13	19
Mirzarazi *et al*.[84]	-	2015	-	Isfahan	Stool	30	EIEC=0 EAEC=5	
Mirsalehian *et al*.[107]	2003	2004	-	-	Bloody diarrhea	103	EHEC=3	17
Mohammadzadeh *et al*.[20]	-	2013	-	Tehran	Diarrhea	130	aEPEC=22 tEPEC=28 EHEC=0	20
Mohammadzadeh *et al*.[85]	2013	2015a	-	Tehran	Diarrhea	140	EIEC=5	20
Mohammadzadeh *et al*.[86]	2013	2015b	Mean age 38 years	Tehran	Diarrhea	140	ETEC=9	20
Mohammadzadeh *et al*.[86]	2013	2015b	Mean age 34 years	Tehran	Stool	110	ETEC=1	
Mohammadzadeh *et al*.[106]	-	2016	-	Tehran	Diarrhea	140	EAEC=6	19
Mohammadzadeh *et al*.[106]	-	2016	-	Tehran	Stool	136	EAEC=0	
Motallebi *et al*.[48]	2009-2010	2011	<5 years	Kashan	Diarrhea	178	aEPEC=51	20
Nakhjavani *et al*.[88]	2009-2010	2013	<10 years	Tehran	Diarrhea	412	aEPEC=16 tEPEC=7	21
Navidinia *et al*.[89]	2008-2009	2012	1-12 years	Tehran	UTI	378	EHEC=9	19
Nazarian *et al*.[90]	2010-2011	2014	<5 years	Tehran, Kerman, Golestan	Diarrhea	261	ETEC=21	21
Nazemi *et al*.[91]	2010-2011	2012	-	Tehran	UTI	100	ETEC=2 EHEC=17	18
Nezarieh *et al*.[92]	2013-2014	2015	1-10 years	Kerman	Diarrhea	322	EAEC=23	18
Pourakbari *et al*.[18]	2010-2011	2013	1 month-12 years	Tehran	Acute diarrhea	50	ETEC=21 aEPEC=0 EHEC=14 EIEC=0 EAEC=10	18

Contd...

Appendix 1: Contd...

Study	Study period	Published time	Age	Location	Source of sample	Number of isolates/samples	Prevalence of DEC pathotype	Study quality
Pourakbari *et al*.[18]	2010-2011	2013	1 month-12 years	Tehran	Stool	50	ETEC=5 aEPEC=0 EHEC=3 EIEC=0 EAEC=2	
Rajaee *et al*.[14]	2014-2015	2017	-	Shiraz	Diarrhea	306	EHEC=108	20
Salmani *et al*.[16]	-	2016	-	Tehran, Sanandaj	Diarrhea	200	ETEC=153 aEPEC=10 EIEC=13 EAEC=39 DAEC=21	20
Salmani *et al*.[16]	-	2016	-	Tehran, Sanandaj	UTI	100	ETEC=22 aEPEC=0 EIEC=6 EAEC=23 DAEC=6	
Salmanzadeh-Ahrabi *et al*.[93]	2003	2005	<5 years	Tehran	Acute diarrhea	200	ETEC=31 aEPEC=12 EHEC=30 EAEC=48	16
Salmanzadeh-Ahrabi *et al*.[93]	2003	2005	<5 years	Tehran	Stool	200	ETEC=0 aEPEC=10 EHEC=4 EAEC=16	
Shams *et al*.[94]	2008-2009	2013	<14 years	Tehran	Diarrhea	947	EHEC=27	19
Sharifi Yazdi *et al*.[95]	2008	2011	<5 years	Tehran	Diarrhea	39	aEPEC=20 EHEC=9 EIEC=7	15
Soleimanifard *et al*.[103]	-	2015	-	Saveh	UTI	50	EAEC=1	20
Soltan Dallal *et al*.[102]	2013	2015	<5 years	Tehran	Diarrhea	300	aEPEC=36	20
Zeighami *et al*.[17]	2011-2012	2014	<5 years	Tabriz	Diarrhea	140	ETEC=65 EHEC=17	20

EHEC=Enterohemorrhagic *E. coli; E. coli=Escherichia coli*; EPEC=Enteropathogenic *E. coli*; aEPEC=Atypical EPEC; tEPEC=Typical EPEC; ETEC=Enterotoxigenic *E. coli*; STEC=Shiga toxin-producing *E. coli*; EAEC=Enteroaggregative *E. coli*; EIEC=Enteroinvasive *E. coli*; DAEC=Diffuse adherent *E. coli*; UTI=Urinary tract infection; DEC=Diarrheagenic *E. coli*