Antibiotic resistance and molecular characterization of enteroaggregative
*Escherichia coli* isolated from patients with diarrhea in the Eastern Province of Saudi Arabia

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Aim: To investigate presence of Enteroaggregative *Escherichia coli* (EAEC) in patients suffering with diarrhea by targeting the pCVD432 (pAA) gene using PCR.

Methods: There were 63 non-duplicate isolates of *E. coli* isolated from diarrheal cases in teaching hospital in Eastern Province of Saudi Arabia between May 2013 to July 2014. All *E. coli* strains were examined for antibiotic susceptibility testing and polymerase chain reaction (PCR) for detection of virulence gene markers for EAEC.

Results: Of the 63 *E. coli* strains that were reported with diarrheal cases, 35 (55.6%) EAEC were tested positive for pCVD432 gene and *aggR* gene was present in 19 (54.3%) strains. All strains tested positive for pCVD432 and *aggR* genes were classified as typical EAEC (tEAEC). EAEC revealed resistance to tetracycline, ampicillin, nalidixic acid, trimethoprim sulfamethoxazole, ciprofloxacin, streptomycin, noroxin, and pipercillin.

Conclusion: EAEC was detected for the first time, among Saudi patients with diarrhea in this region of Saudi Arabia. The reported antibiotic resistance in this study is considered high among isolated EAEC strains to routinely prescribed antibiotics in our area.

1. Introduction

Enteroaggregative *Escherichia coli* (EAEC) is among the most remarkable heterogeneous strains of emerging *E. coli* that are responsible from causing persistent watery diarrheal cases in children and adults worldwide [1]. EAEC was determined to be a diarrheal enteric pathogen due the distinctive features of aggregative adherence (AA) pattern to HEp-2 cells in culture as well as its ability to form a "stacked-brick" adherence pattern, which is harboring a 60-MDa plasmid (pAA) [2]. However, the HEp-2 cell assay test is remaining as the gold standard technique for the confirmation of EAEC [3, 4, 5]. Therefore, this test is a diagnostoc assay that is not suitable for most laboratories pattern as it is too laborious and need to be carried out only in reference research laboratories with cell culture, it requires cell culture setup, [6]. At the molecular level, polymerase chain reaction (PCR) is most suitable and reliable techniques for identification of virulence gene markers of EAEC in suspected diarrheal cases.

The pAA plasmid in EAEC is encoding AA fimbriae (AAF) from I to IV genes [7]; anitaggregating protein dispersin (app); entroaggregative heat stable enterotoxin 1 (EAST-1) also known as astA) [8] and the gene encoding the transcriptional activator of virulence genes (AggR) [9]. The *aggR* gene is playing significant role in adherence and pathogenesis of EAEC, however, any strain harboring *aggR* gene is considered typical EAEC (tEAEC) strain [9]. The AggR promotes the expression of plasmid harboring the virulence factors, since its role as a transcriptional activator [7]. The surveillance of antibiotic resistance is very important in bacterial isolates due to continuous application of antibiotics in treatment of enteropathogenic infection and cephalosporins and fluoroquinolones are the most common used. Continuous surveillance and documentation of antibiograms resistance patterns will provide useful information to treatment and control the spread of antibiotic resistance.
In Saudi Arabia, there is no information on the prevalence of EAEC and other diarrheagenic E. coli (DEC) pathotypes isolates and their antimicrobial resistance pattern. Therefore, the aims of this study were as follows: 1) to investigate the pAA plasmid gene in E. coli strains isolated from inpatients and outpatients with cases of diarrhea by using PCR; 2) to analyze the strains harboring pAA plasmid for the prevalence of virulence genes that are associated with EAEC; and 3) to carry out anti-biotic susceptibility to find out the resistance pattern in all E. coli strains, and to analyze all strains using enterobacterial repetitive intergenic consensus (ERIC-PCR) to track clonal relationship.

2. Materials and methods

2.1. Fecal samples strains

There were 63 strains of E. coli isolated and collected from children and adults patients with diarrheal episodes in King Fahd teaching hospital in Al-Khobar between May 2013 and July 2014. Among the 63 E. coli strains included in this study, 52 and 11 strains were isolated from adults and children, respectively. All data concerning the patients age, gender, and month of isolation were recorded in this study. The collected strains of E. coli isolated from diarrheal specimens were identified using the VITEK 2 system (Bio-Merieux) and standard methods [16]. PCR was used to screen all E. coli strains for EAEC virulence gene markers.

2.2. EAEC assay by PCR

The obtained strains of E. coli isolated from patients with diarrheal episodes were further cultured in on McConkey agar. The lactose-fermenting Gram-negative colonies of obtained E. coli were tested using standard biochemical tests for confirmation of E. coli. The isolated purified colonies on selective agar were cultured onto Tryptic soy agar plates (TSA, Oxoid) and tested for indole production using API 20 E strips and other biochemical tests. Bacterial genomic DNA was extracted by using boiling method as described elsewhere [11]. Three colonies of each E. coli strain from the TSA plates were mixed with 300 μl of nuclease free water in micro-centrifuge tubes and boiled in water bath for 10 min. The boiled bacterial suspensions were centrifuged at 10,000 g for 5 min and the supernatants were used as DNA templates for PCR [11]. Primers to detect the strains harboring pAA to analyze the strains harboring pAA plasmid for the prevalence of virulence genes that are associated with EAEC; and 3) to carry out anti-biotic susceptibility to find out the resistance pattern in all E. coli strains, and to analyze all strains using enterobacterial repetitive intergenic consensus (ERIC-PCR) to track clonal relationship.

2.3. Molecular typing

The ERIC-PCR DNA fingerprinting method with repetitive primer sequence and amplification conditions, as described elsewhere [17, 18] was used to analyze E. coli strains for clonal relationship. The obtained ERIC fingerprint profiles were clustered by using Dice coefficients and the unweighted average pair group method (UPGMA) with position tolerance of 1%. The ERIC electrophoresis agarose gels images of DNA fingerprints were constructed with the use of Gel Compar II software (Applied Maths, St-Martens-Latem, Belgium).

2.4. Antibiotic susceptibility testing

All 63 strains of E. coli were examined by using disc diffusion method for 20 antimicrobial agents (Oxoid, England) [19]. The tested antibiotic agents were: ampicillin (AM, 25 μg); amikacin (AK, 30 μg); augmentin (AUG, 30 μg); aztreonam (ATM, 30 μg); cephalothin (KF, 30 μg); ceftaxime (CTX, 30 μg); cefazidime (CAZ, 30 μg); cephefim (FEP, 30 μg); cefoxitin (FOX, 30 μg); chloramphenicol (C, 30 μg); colistin (CT: 30 μg); ciproflaxacin (CIP, 5 μg); gentamicin (GM, 5 μg); nalidixic acid (NA, 30 μg); neomycin (N, 30 μg); noroxin (NOR, 10 μg); piperacillin (PRL, 10 μg); streptomycin (S, 10 μg); trimethoprim sulfa methoxazole (SXT, 25 μg); and tetracycline (TE, 30 μg). The reference control strain of E. coli ATCC25922 was used while doing antibiotic susceptibility testing.

2.5. Statistical analysis

A chi-square test was performed to analysis the difference in proportion between antimicrobial resistance results of EAEC and Non-EAEC strains isolated from patients with diarrhea. All calculations were done by using MedCalc software version 17.9.2 (MedCalc Software, BVBA, Ostend, Belgium).

3. Results

There were 63 patients who were included in the present study; 11 (17.5%) samples were from children under 15 years of age and 52 (82.5%) samples were from adults aged 21—85 years. Out of 63 examined isolates of E. coli by using PCR, 35 (55.6%) were harboring pCVD432 (pAA) and 82.9% of these infections occurred during warmer months from May through October, as shown in Table 2. All isolates of E. coli were tested positive for the pCVD432 gene were examined using PCR to detect aggR, aap, aap/II, and astA virulence genes of EAEC. Among the 35 patients with pCVD432 gene, only 19 (54.3%) isolates were positive for the aggR gene and were classified as tEAEC. In this study, all the 35 isolates were found positive for the astA gene, and the aap gene was found in 33 (94.3%) patients; no single isolate was found positive for the aap gene, as shown in Tables 2 and 3. The most prevalent combination of the virulence factor profiles in strains of EAEC were (aap, astA) and (aap, aggR, astA) as shown in Table 3.

All the 63 diarrheagenic E. coli (DEC) isolates that were analyzed using ERIC-PCR were typeable and displayed a unique genotypic pattern, as presented in Figures 1 and 2 (see also Figures S1, S2 and S3). Using the cluster cutoff method, six distinct clusters with 60 strains that were closely related to ERIC fingerprints were identified while a similarity cutoff value of 90% was applied, except for three strains (Non-EAEC6, EAEC15 and EAEC75) that were present in a single cluster (SC), as shown in Table 2.

Table 1. Primers used for detection of virulence markers in this study.

| Target gene | Properties of target | Primer sequence | Size of product (bp) | Ref. |
|-------------|----------------------|-----------------|---------------------|-----|
| PCDV432 (pAA) | Aggregative adherence plasmid | 5′-CTGGCGGAAAAGACTGTATCAATG-3′<br>5′-CAATGATGAAATAGCCGCTT-3′ | 629 | (Schmidt et al., 1995) |
| aggR | Transcriptional activator | 5′-CTAATGTCAATCACTGTATCAATG-3′<br>5′-AGATGTCATCCTTCTGTAAGG-3′ | 457 | (Yamaya et al., 2002) |
| aap | Anti-aggregation protein dispersin | 5′-CCCGGCGGACGGCTTGAAAGG-3′<br>5′-AACCCATTGTCATCCGAC-3′ | 310 | (Cerna et al., 2003) |
| astA | Heat stable enterotoxin EAST1 | 5′-TGCATCAACAGCACTGATCGTCCG-3′<br>5′-AGCCCTTCTGATGCTCCATCT-3′ | 102 | (Müller et al., 2007) |
| aap/II | Aggregative adherence fimbriae | 5′-CAGAGGACACCTAATGAATGCTG-3′<br>5′-ATCCCCATGATGTCGACACTTC-3′ | 378 | (Boisen et al., 2008) |
in Figure 1 (see also Figure S1). Grouping of DNA fingerprints similarities revealed by ERIC-PCR for all E. coli isolates were analyzed constructed and calculated according to algorithm for the unweighted pair group method with arithmetic mean (UPGMA). As shown in Figure 2 (see also Figures S2 and S3 for full images), 12 strains of tEAEC fingerprint profiles were grouped together in clusters 2 and 3 compared with the other clusters. Subsequently, 13 strains of tEAEC were isolated from patients’ fecal specimens during 2013 while only three strains of tEAEC were isolated during 2014, as shown in Figure 1. Two strains of tEAEC (EAEC71 and EAEC74) were isolated during June and September 2013 and were grouped in cluster ET-6 with strains that were isolated during 2014. Of the 63 strains of E. coli, 21 (33.3%) with identical fingerprints were grouped in cluster 6 and most of these strains were isolated in June 2014.

The results of antimicrobial susceptibility testing of all 63 strains were presented in Table 4. EAEC revealed the following high percentages of resistance: tetracycline, 68.6%; ampicillin, 60%; nalidixic acid, 60%; trimethoprim sulfamethoxazole, 46.4%; cefotaxime, 28.6%; nalidixic acid, 28.6%; piperacillin, 28.6%; and cefalothin, 25%. All EAEC isolates were susceptible to amikacin, while all non-EAEC isolates were susceptible to amikacin and colistin. Overall, 68.3% of tested EAEC and non-EAEC isolates were multidrug resistant (MDR) and EAEC showed greater resistance than non-EAEC isolates. As presented in Table 4, most of the p-values were more than 0.05 meaning that there is no statistical difference between the proportions. The only exceptions are for cefepime for sensitive (S) category. The two other significant p-values are very small with only one case and this is not meaningful. The highest antibiotic resistance patterns were noticed among 68.6% of EAEC isolates and were grouped together in cluster ET-2 and ET-3 while all 63 isolates of EAEC and non-EAEC were typed using ERIC-PCR DNA fingerprinting, as shown in Figure 2.

4. Discussion

The EAEC pathotype is one of subgroup of diarrheagenic E. coli (DEC) and its well-known worldwide for causing severe diarrhea in infected children and adults [2]. Nevertheless, EAEC its pathogenicity is

Table 2. Characterization of virulence factors of EAEC strains isolated from patients with diarrhea.

| Isolate | Month of isolation | Sex | Age | virulence factors | ERIC-PCR pattern type |
|---------|--------------------|-----|-----|-------------------|----------------------|
|         |                    |     |     | aafII | Aap | aggR | AstA |         |
| EAEC3   | December 2013      | F   | 19  | -     | +   | -    | +    | ET-3    |
| EAEC12  | May 2013           | F   | 21  | -     | +   | -    | +    | ET-3    |
| EAEC13  | June 2013          | M   | 44  | -     | +   | +    | +    | ET-3    |
| EAEC14  | December 2013      | M   | 2   | -     | +   | -    | +    | ET-5    |
| EAEC15  | December 2013      | M   | 68  | -     | +   | -    | +    | *SC     |
| EAEC20  | September 2013     | F   | 55  | -     | +   | +    | +    | ET-3    |
| EAEC21  | September 2013     | M   | 29  | -     | +   | -    | +    | ET-3    |
| EAEC22  | September 2013     | F   | 26  | -     | +   | -    | +    | ET-3    |
| EAEC25  | October 2013       | M   | 25  | -     | +   | -    | +    | ET-4    |
| EAEC27  | December 2013      | F   | 71  | -     | +   | +    | +    | ET-3    |
| EAEC28  | December 2013      | M   | 69  | -     | +   | +    | +    | ET-3    |
| EAEC29  | October 2013       | M   | 71  | -     | +   | +    | +    | ET-3    |
| EAEC31  | July 2013          | M   | 22  | -     | +   | -    | +    | ET-4    |
| EAEC33  | June 2013          | M   | 85  | -     | +   | -    | +    | ET-1    |
| EAEC41  | May 2013           | F   | 2   | -     | +   | -    | +    | ET-2    |
| EAEC45  | June 2013          | M   | 47  | -     | +   | +    | +    | ET-1    |
| EAEC47  | January 2014       | F   | 66  | -     | +   | +    | +    | ET-5    |
| EAEC55  | September 2013     | M   | 61  | -     | +   | -    | +    | ET-2    |
| EAEC60  | May 2013           | F   | 41  | -     | +   | +    | +    | ET-2    |
| EAEC61  | May 2013           | M   | 21  | -     | +   | +    | +    | ET-2    |
| EAEC62  | June 2013          | M   | 45  | -     | +   | +    | +    | ET-2    |
| EAEC63  | October 2013       | M   | 62  | -     | +   | +    | +    | ET-2    |
| EAEC64  | August 2013        | F   | 12  | -     | +   | +    | +    | ET-2    |
| EAEC65  | October 2013       | M   | 39  | -     | +   | +    | +    | ET-2    |
| EAEC67  | May 2013           | F   | 21  | -     | +   | +    | +    | ET-2    |
| EAEC71  | June 2013          | F   | 48  | -     | +   | +    | +    | ET-6    |
| EAEC74  | September 2013     | M   | 31  | -     | +   | +    | +    | ET-6    |
| EAEC75  | May 2013           | F   | 22  | -     | +   | +    | +    | SC      |
| EAEC80  | May 2013           | F   | 30  | -     | +   | +    | +    | ET-2    |
| EAEC85  | May 2014           | F   | 39  | -     | +   | +    | +    | ET-6    |
| EAEC86  | June 2014          | F   | 12  | -     | +   | -    | +    | ET-6    |
| EAEC87  | June 2014          | F   | 27  | -     | +   | +    | +    | ET-6    |
| EAEC89  | June 2014          | M   | 21  | -     | +   | +    | +    | ET-6    |
| EAEC92  | June 2014          | F   | 30  | -     | +   | -    | +    | ET-6    |
| EAEC95  | June 2014          | M   | 4   | -     | -   | -    | +    | ET-6    |

No. of positive: 0, 33, 19, 35
% of positive: 0%, 94%, 54%, 100%

* SC, single cluster.
remaining controversial and that due to heterogeneity and clinical relevance of \textit{E. coli} strains \cite{1}. In meta-analysis study conducted by Huang et al., (2006) \cite{20} was reported that EAEC is responsible of causing persistent and chronic diarrhea in different group of populations in developing and industrialized countries.

In meta-analysis study conducted by Huang et al. (2006) \cite{20} was reported that EAEC is responsible of causing acute and persistent diarrhea in different group of populations in developing and industrialized countries, in addition to that, this study confirmed the heterogenicity of EAEC strains and inadequate studies were reported the role of emerging EAEC strains in acute diarrheal illness. The present study also documented the EAEC causes sporadic cases of diarrhea in the Eastern Province of Saudi Arabia. Globally, EAEC shows an alarming increase in resistance to different ranges of antibiotics \cite{21} (Kong et al., 2015). In Saudi Arabia, no published study has reported the situation of EAEC that is associated with diarrhea in terms of molecular characterization and antimicrobial resistance. This study is the first study to report the detection of virulence factors and high rate of antibiotic resistance among the isolated EAEC strains in Saudi patients infected with diarrhea in Saudi Arabia and to provide insight on the current DEC situation.

In this study, 63 fecal specimens from outpatients and inpatients with diarrhea were examined for EAEC. Thirty-five (55.6\%) were positive for the \textit{pCVD432} gene and were identified as EAEC. The DNA probe \textit{pCVD432} has been developed from the 60-MDa plasmid \textit{pAA} to detect EAEC using PCR \cite{22}. Several studies have reported a high specificity of the \textit{pCVD432} probe for identification of EAEC \cite{22, 23, 24, 25}. In this study, the HEp-2 cell adherence assay was not used for confirmation of EAEC because this assay experiment is currently performed only in reference research laboratories and it is also laborious to perform. However, in this study, the PCR assay targeting the \textit{pCVD432} gene was shown to be a more sensitive and reliable molecular assay for the identification of EAEC. Several epidemiological studies have shown that

| Virulence gene profile | No (%) of EAEC |
|------------------------|................|
| \textit{aafII}         | 0             |
| \textit{Aap}          | 33 (94.3)     |
| \textit{aggR}         | 19 (54.3)     |
| \textit{aap}          | 35 (100)      |
| \textit{aap}, \textit{astA} | 33 (94.3)     |
| \textit{aap}, \textit{aggR}, \textit{astA} | 19 (54.3)     |

Table 3. Prevalence of virulence genes among EAEC strains isolated from patients with diarrhea.

Figure 1. ERIC-PCR profiles and antibiotic resistance patterns of 63 DEC strains isolated from patients with diarrhea in KFHU (See supplementary Figure 1 for full image).
strains that are positive for the pCVD432 gene-positive strains and harboring the aggR regulon are characterized as tEAEC pathogens [26]. In this study, 54.3% of strains were identified as tEAEC and they showed different variations to other virulence genes.

In this study, there are different combinations of virulence gene markers were detected in examined strains of EAEC, as presented in Table 3. Among the virulence gene markers, the astA gene was detected in all 63 strains of EAEC and non-EAEC. A recent and similar study in China reported a high prevalence rate of the astA virulence gene with a detection rate (88%) among EAEC strains that were isolated from clinical fecal specimens [27]. However, our results disagreed with some other studies, which reported that the astA gene is rarely detectable in EAEC strains [24, 28]. The astA gene that encodes the EAST-1 toxin is responsible for causing diarrhea and chloride secretion [29], and also is playing a significant role it was found to be important in the development of prolonged acute and persistent diarrhea [30]. However, the EAST-1 is associated with EAEC and it has been also detected in enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC) and enterohaemorrhagic E. coli (EHEC) strains [28]. In this study, 19 (54.3%) tEAEC were found to be positive for three distinct combinations of virulence genes (aap, aggR, and astA), and most of the strains were isolated from adult patients, as shown in Table 3. However, our results agreed with those of Huang et al. (2006) [20] who reported and proven that EAEC strains harboring aggR, aap, and astA virulence genes are definitely responsible of causing acute diarrhea in adults.

The adherence of EAEC to the intestinal epithelium is the first step in gut colonization, which requires fimbrial structures that are known as AAf factors (AAF) [31, 32]. There are four major variants of the AAF struc-tures in pilin subunits (AAF/I to AAF/IV), and all of them are regulated by the transcriptional activator aggR, which is situated on the EAEC virulence plasmid pAA [16]. In the present study, the aaf/II gene was not detected among all strains of EAEC isolates. The current study results are

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**Table 4. Antimicrobial susceptibility of EAEC and Non-EAEC strains isolated from patients with diarrhea.**

| Antimicrobial agent | Isolates no. (%) for | dR p-value | iI p-value | iS p-value |
|---------------------|----------------------|------------|------------|------------|
|                     | EAEC | Non-EAEC | EAEC | Non-EAEC | EAEC | Non-EAEC |
| Ampicillin | 21 (60) | 18 (64.3) | 0.9561 | 0 | 0 | - |
| Amikacin | 0 | 0 | - | 0 | 0 | - |
| Augmentin | 2 (5.7) | 5 (17.9) | 0.4378 | 0 | 0 | 0.6 |
| Aztreonam | 4 (11.4) | 3 (10.7) | 0.2354 | 1 (2.9) | 4 (14.3) | 0.1599 |
| Cephalothin | 7 (20) | 7 (25) | 0.6774 | 1 (2.9) | 3 (10.7) | 0.0712 |
| Cefotaxime | 5 (14.3) | 8 (28.6) | 0.9353 | 0 | 0 | - |
| Ceftriaxone | 1 (2.9) | 5 (17.9) | 0.2551 | 1 (2.9) | 3 (10.7) | 0.0712 |
| Cefepime | 2 (5.7) | 3 (10.7) | 0.1541 | 1 (2.9) | 2 (7.1) | 0.0127 |
| Cefotaxime | 1 (2.9) | 4 (14.3) | 0.1599 | 0 | 0 | - |
| Chloramphenicol | 16 (47.9) | 12 (38.7) | 0.4183 | 3 (8.6) | 0 | - |
| Colistin | 2 (5.7) | 0 | - | 0 | 0 | - |
| Ciprofloxacin | 14 (40) | 5 (17.9) | 0.73 | 1 (2.9) | 0 | - |
| Gentamicin | 4 (11.4) | 2 (7.1) | 0.2006 | 0 | 0 | - |
| Nalidixic Acid | 21 (60) | 8 (28.6) | 0.2729 | 0 | 0 | - |
| Neomycin | 1 (2.9) | 1 (3.6) | 0.0001 | 2 (5.7) | 0 | - |
| Noroxin | 13 (37.1) | 5 (17.9) | 0.827 | 1 (2.9) | 0 | - |
| Piperacillin | 8 (22.9) | 8 (28.6) | 0.7558 | 1 (2.9) | 0 | - |
| Streptomycin | 14 (40) | 9 (22.9) | 0.9525 | 0 | 0 | - |
| Tetracycline | 14 (40) | 9 (22.9) | 0.5489 | 1 (2.9) | 0 | - |
| Trimethoprim sulfamethoxazole | 15 (42.9) | 13 (46.4) | 0.8451 | 0 | 0 | - |

dR, resistance; iI, intermediate; iS, sensitive.

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Figure 2. Representative ERIC profiles (See supplementary Figures 2 and 3 for full images). Lanes: M, molecular mass marker (GelPilot 1kb Plus ladder ranging from 100 to 10000 bp): 1, EAEC71; 2, EAEC74; 3, EAEC75; 4, Non-EAEC; 5, EAEC80; 6, Non-EAEC81; 7, Non-EAEC82; 8, Non-EAEC84; 9, EAEC85; 10, EAEC86; 11, EAEC87; 12, Non-EAEC88; 13, EAEC92; 14, Non-EAEC93; 15, Non-EAEC94; 16, EAEC95; 17, Non-EAEC106; 18, Non-EAEC107; 19, Non-EAEC108; 20, Non-EAEC109.
consistent with other published epidemiological studies, which reported that most EAEC strains do not express any of the four known AAF variants [33, 34]. This suggests that strains of EAEC with a “stacked-brick” AA lack AAF and might have other adhesion mechanisms [2]. Boisen et al. (2008) [16] demonstrated that a novel aggregative adhesion pilin that was regulated by the aggR regulon in the EAEC strains lacks a known AAF and that might be distantly linked to adhesins of Dr family. Several studies have suggested that other additional undiscovered AAF variants may exist and need to be explored [16]. From this study and other results that were reported worldwide, and because of the EAEC heterogeneity, there are no specific common combinations of virulence factor sets that have been found to identify all EAEC strains using the PCR assay [4, 24, 35]. However, the pathogenicity of these bacteria remains controversial [1].

ERIC is a useful chromosomal DNA fingerprinting PCR-based technique for molecular epidemiological characterization of several bacterial pathogens of medical importance. However, the obtained ERIC patterns are easy to evaluate. In this study, the ERIC-PCR typing revealed six clusters patterns, as shown in Figure 2 and Table 3. Our results indicate that ERIC is useful as a molecular tool for typing microbial outbreaks. In this study, we detected high levels of antibiotic resistant EAEC strains that were isolated from Saudi patients with diarrhea, but the percentages of MDR patterns among EAEC and Non-EAEC were 68.6% and 67.9%, respectively, as presented in Figure 2 and Table 4. Overall, EAEC isolates showed greater antibiotic resistance than non-EAEC. Having knowledge about the antimicrobial susceptibility of enteric bacteria that are associated with causing frequent diarrhea such as EAEC and other E. coli pathotypes will help to develop useful information for treatment. Our study documented a high level of quinolone resistance among EAEC isolates toward nalidixic (60%) and ciprofloxacin (40%) (Table 4). Therefore, our results are in agreement with a recent study that was performed in Denmark, which reported that 34% of ciprofloxacin resistance in adult patients with diarrhea was caused by EAEC [30]. In this study, the investigated strains of EAEC showed a high rate of resistance to most of the commonly used antibiotics such as tetracycline (68.6%), ampicillin (60%), nalidixic acid (60%), trimethoprim sulfamethoxazole (42.9%), streptomycin (40%), ciprofloxacin (40%), and noroxin (37.1%). Additionally, 68.3% of EAEC and DEC strains showed MDR resistance patterns to more than three antibiotics, as shown in Figure 1. Therefore, our study results are consistent with the findings of similar studies that reported the recent dramatic increase in antibiotic resistance towards most commonly used antibiotics that are used to treat EAEC [30, 36, 37, 38].

5. Conclusion

The high number of MDR EAEC strains that were detected in our study is a cause for concern, and therefore, the establishment of long-term surveillance programs are required to find changes in the spectrum of antimicrobial resistance patterns of EAEC in Saudi Arabia. In this study, resistance of EAEC strains to ampicillin (60%), nalidixic acid (60%), trimethoprim sulfamethoxazole (42.9%), and ciprofloxacin (40%) were extremely high in Saudi patients with diarrhea. Therefore, antibiotics should be prescribed only for patients with severe and persistent diarrhea. In conclusion, the findings of this study confirm that EAEC was detected in children and adult patients with diarrhea and highlights the importance of continuous monitoring for antibiotic resistance in EAEC and another DEC pathotypes.

Declarations

Author contribution statement

Nasreldin Elhadi: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Reem Aljindan: Performed the experiments; Analyzed and interpreted the data.
Khaldoon Alsammam: Performed the experiments.
Amer Alomar, Mohammed Aljeldah: Analyzed and interpreted the data.

Funding statement

This work was supported by the Deanship of Scientific Research, Imam Abdulrahman Bin Faisal University, Dammam (grant No. 2013263).

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e03721.

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