USING O SEROGROUPING ALONE TO ASCERTAIN THE DIARRHEAGENIC *ESCHERICHIA COLI* SHOULD BE DISCOURAGED

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ABSTRACT

Introduction: In many of our clinical laboratories, the detection of diarrheagenic *Escherichia coli* (DEC) strains is performed by an O-serogrouping method with commercially available antiserum pools containing the antisera against O serogroups traditionally associated with enteropathogenic (EPEC) and typical verocytotoxin-producing *E. coli* (VTEC) categories.

Objectives: The aim of this study was to correlate O serogrouping with DEC virulence gene detection.

Methods: Seventy-six *E.coli* strains isolated during 2016 and 2017 in different laboratories of Romania were characterized for serogroup and virulence markers using commercially available monosera and a PCR-based assay for the routine diagnostic identification of DEC infections.

Results: Overall, the strains were assigned to the following serogroups: O26 (24 strains), O55 (5 strains), O86 (1 strain), O103 (2 strains), O111 (8 strains), O119 (1 strain), O121 (1 strain), O124 (1 strain), O125 (3 strains), O126 (4 strains), O127 (7 strains), O128 (7 strains), O145 (2 strains), and O157 (10 strains). Only 66% of the strains displayed virulence markers and qualified as EPEC (25 strains), VTEC (24 strains), and enteroinvasive *E. coli* (EIEC, 1 strain). PCR-positive and PCR-negative strains were present in most of the frequently identified serogroups, but the rates of DEC (EPEC and/or VTEC) positivity decreased in the following order: O26 (22/24 strains), O127 (6/7 strains), O128 (5/7 strains), O157 (6/10 strains), O111 (4/8 strains).

Conclusion: The use of commercially available O antisera may still be considered when screening for presumptive DEC strains but the laboratory tendency to limit the microbiological diagnostic to this procedure should be discouraged.

Keywords: diarrheagenic *Escherichia coli*, serotyping, enteropathogenic *E. coli*, verocytotoxin-producing *E. coli*, O serogrouping, PCR, virulence gene markers

REZUMAT

Introducere: În multe dintre laboratoarele clinice de la noi detecția tulpinilor de *Escherichia coli* producătoare de diaree (ECD) se realizează prin serogrupare cu amestecuri de anticorpi disponibile comercial, care recunosc antigenele O corespunzătoare unor serogrupuri tradiționale de *E. coli* enteropatogen (EPEC) și *E. coli* producător de verocitotoxine (VTEC).

Obiective: Acest studiu a urmărit sa coreleze serogrupul cu detecția genelor de virulență caracteristice tulpinilor ECD.

Metode: Au fost analizate 76 de tulpini izolate în diverse laboratoare din România în anii 2016 și 2017, serogrupate cu monoseruri comerciale și testate pentru markeri de virulență cu o trusă bazată pe tehnica PCR, utilizată în diagnosticul curent al infecțiilor cu ECD.

Rezultate: Tulpinile au fost distribuite în următoarele serogrupuri: O26 (24 tulpini), O55 (5 tulpini), O86 (1 tulpină), O103 (2 tulpini), O111 (8 tulpini), O119 (1 tulpină), O121 (1 tulpină), O124 (1 tulpină), O125 (3 tulpini), O126 (4 tulpini), O127 (7 tulpini), O128 (7 tulpini), O145 (2 tulpini) și O157 (10 tulpini). Numai 66% dintre acestea au prezentat markeri de virulență care le clasificau în patotipurile EPEC (25 tulpini), VTEC (24 tulpini) și *E. coli* enteroinvaziv (EIEC, 1 tulpină). Marea majoritate a serogrupurilor identificate au fost reprezentate atât de tulpini PCR pozitive cât și negative, dar procentul de pozitivitate pentru ECD (EPEC și/sau VTEC) s-a redus după cum urmează: O26 (22/24 tulpini), O127 (6/7 tulpini), O128 (5/7 tulpini), O157 (6/10 tulpini), O111 (4/8 tulpini).

Concluzie. Utilizarea serurilor anti O prezente pe piață poate fi încă o opțiune de selectare a posibilelor tulpini ECD mai ales când este vorba de unele serogrupuri, dar tendința laboratorului de a limita diagnosticul microbiologic la această procedură trebuie descurajată.

Cuvinte-cheie: *Escherichia coli* cauzator de diaree, serotipizare, *E. coli* enteropatogen, *E. coli* producătoare de verocitotoxină, serogrupare O, PCR, gene de virulență markeri.

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INTRODUCTION

Escherichia coli can be easily isolated from fecal samples in laboratory but in order to decide if the isolate is the cause of a patient's diarrhea, the microbiologist has to bring evidence of its virulence potential, as the species is equally a commensal member of the human healthy gastrointestinal tract [1]. The *E. coli* strains that are capable to cause diarrheal disease are diverse and unlike the commensal strains possess means to affect a wide range of cellular processes in the host. They are collectively named diarrheagenic *E. coli* (DEC) strains and are grouped in the following pathotypes based on the mechanisms of pathogenicity and clinical syndromes: enteropathogenic *E. coli* (EPEC), verocytotoxin-producing *E. coli* (VTEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [2]. Currently, there is no standardized protocol and no single method that can be used to enrich, isolate or select for these various *E. coli* intestinal pathotypes and the laboratory diagnostic of clinically significant strains in humans is still difficult despite the wealth of data gathered on DEC. Generally, two approaches are used in the microbiology laboratories. One of them targets specific serogroups in which a higher concentration of strains with virulence properties was documented [3-5] in order to use serotyping results as an indication of which isolates should be further selected for specific virulence testing. The other approach is relying on the primary screening of virulence indicators by PCR-based assays and subsequent isolation of the DEC strain [6]. Efforts are made to improve the detection of pathogenic *E. coli* as each of these approaches has limitations that lead to false-positive or false-negative results.

In Romania, the laboratory diagnostic of DEC is mainly performed for infants with diarrhea and the serological screening for traditional EPEC type O-serogroups is still the method of choice in most clinical and diagnostic laboratories. Aiming to gather more laboratory-based data in order to understand the true incidence of DEC-associated diarrhea at least across autochthonous pediatric population, we report here information about the virulence markers found in *E. coli* strains presumed to be EPEC based only on the serotyping markers. This study focused on the fecal *E. coli* isolates referred to the National Reference Laboratory (NRL) for *E. coli* for confirmation in the last couple of years.

MATERIAL AND METHODS

E. coli strains

The 76 *E. coli* strains reported in this study were isolated in microbiology laboratories from all over the country (Bucharest metropolitan area and 19 counties) from children with acute diarrhea and/or hemolytic uremic syndrome (HUS) during a two-year period (2016-2017).

They were selected from the *E. coli* strains referred to the National Reference Laboratory for Bacterial Enteric Infections in Cantacuzino National Institute of Research (current name Cantacuzino National Medico-Military Institute for Research and Development) for confirmation and were presumptive DEC based on the positive reactivity with commercially available O antisera recommended for clinical use.

Their accompanying information indicated positive agglutination results with pooled and/or single antisera for known EPEC and/or VTEC serogroups. At the level of the reference laboratory the isolates were first confirmed as *E. coli* by standard biochemical tests and then retested with commercial O antisera (SSI Diagnostica, BioRad) against the following serogroups: O26, O45, O55, O86, O103, O111, O113, O114, O119, O121, O124, O125, O126, O127, O128, O142, O145, and O157. Fifteen O26 *E. coli* strains, confirmed as VTEC, were previously published strains in association with the HUS outbreak that occurred in Romania in the year 2016 [7].

Analysis of virulence genetic markers

DEC-specific virulence markers were searched in all the strains assigned to one of the targeted DEC serogroups using a commercial multiplex PCR-based kit (DEC Primer Mix, SSI Diagnostica).

The bacterial DNA was prepared from 200 µl of bacterial suspension in sterile water (Promega) boiled for 15 minutes and used as indicated by the kit's manufacturer in order to detect the following virulence genes: *eae* (intimin), *vtx1* (verocytotoxin 1), *vtx2*

(verocytotoxin 2), *elt* (heat-labile enterotoxin), *est* (heat-stable enterotoxin), *ipaH* (invasive plasmid antigen).

According to the virulence genotype, the strains were clasified as EPEC (*eae*), VTEC (*vtx1* and/or *vtx2*; *eae* and *vtx1* and/or *vtx2*), ETEC (*elt* and/or *est*), EIEC (*ipa*H). In this study, the strains that did not harbour any of the mentioned virulence genes were considered non-DEC.

RESULTS

Identification of EPEC and VTEC strains by serogrouping

The 76 *E. coli* strains confirmed for their O-antigen group with the commercial antisera available were distributed into 14 serogroups of which six were represented by ≥ 5 strains: O26 (24 strains), O157 (10 strains), O111 (8 strains), O127 (7 strains), O128 (7 strains), and O55 (5 strains), respectively (Table 1).

Identification of EPEC and VTEC strains by PCR for virulence gene markers of DEC

Fifty *E. coli* strains (50/76 strains, 66%) possessed at least one of the virulence genes investigated in this study while the remaining 26 strains (34%) lacked any of them.

According to their virulence genotypes, the *E. coli* strains qualified as EPEC (25 strains), VTEC (24 strains), EIEC (1 strain) or non-DEC (26 strains). EPEC strains belonged to the following serogroups: O26, O55, O111, O119, O125, O127, O128, O145, and O157. VTEC strains belonged to serogroups O26, O126, O128, and O157. The EIEC strain was assigned

Table 1. Associations between the serotypic and genotypic markers found across the 76 E. coli strains investigated in this study

Serogroup	No. of strains	PCR result	Virulence gene markers (no. of strains)
O26	24	negative	none (2)
		positive	*eae* (4)
			eae+vtx1(5)
			eae+vtx2 (7)
			eae+vtx1+vtx2 (5)
			vtx1+vtx2 (1)
O55	5	negative	none (4)
		positive	*eae* (1)
O86	1	negative	none (1)
O103	2	negative	none (2)
O111	8	negative	none (4)
		positive	*eae* (4)
O119	1	positive	*eae* (1)
O121	1	negative	none (1)
O124	1	positive	*ipaH* (1)
O125	3	negative	none (2)
		positive	*eae* (1)
O126	4	negative	none (3)
		positive	*vtx1*(1)
O127	7	negative	none (1)
		positive	*eae* (6)
128	7	negative	none (2)
		positive	*eae* (4)
			eae+vtx2 (1)
O145	2	positive	*eae* (2)
O157	10	negative	none (4)
		positive	*eae* (2)
			eae+vtx2 (3)
			eae+vtx1+vtx2(1)

to serogroup O124. PCR-negative strains were detected in all but two of the 14 serogroups.

The highest percentage of PCR positive strains was found in serogroup O26 (22/24 strains), followed in descending order by serogroups O127 (6/7 strains), O128 (5/7 strains), O157 (6/10 strains), and O111 (4/8 strains). Moreover, serogroups O26, O128, and O157 were more heterogeneous than the rest with respect to the pathogenic potential of the strains.

The overview of the associations between the serotypic and genotypic markers found across the whole *E. coli* strain collection investigated in this study is presented in Table 1.

DISCUSSION

The most important issue when considering implementation of a new protocol in the laboratory is clinical justification. However, it is usually most difficult to address this issue in an objective manner and considerable laboratory data gathering is necessary to decide which is the most adequate procedure to satisfy the clinical need.

When it comes to *E. coli* as a cause of diarrhea, although there is enough laboratory evidence that serotyping is an imperfect predictor of the virulence properties of the strains isolated from the gut, many laboratories from Romania still use it as the sole diagnostic procedure. Besides, because of cost constraints, many of them cannot afford further screening with more discriminatory methods meant to confirm the bacterial intrinsic virulence.

Therefore, in order to raise awareness of the diagnostic errors that could be made if relying exclusively on an O-serogrouping method, we evaluated the virulence gene markers displayed by strains of classical EPEC and VTEC serogroups. This approach showed that only 66% of the *E. coli* strains investigated qualified as true DEC, the clinical significance of the rest being arguable.

As expected, taking into account previous studies that addressed the same subject, serogrouping procedure overestimated the incidence of EPEC and displayed a rather weak correlation with the molecular markers of pathogenicity [8-12]. Thus, there were less strains confirmed as EPEC based on their virulence genotype than initially presumed and at the same time there were much more strains that possessed the virulence markers specific of VTEC pathotype than expected taking into account the strains derived from the O157 reservoir. The finding that VTEC non-O157 strains outnumbered the O157 ones was not a surprise as international reports indicate the increasing importance of the former in Europe [13].

For the two years considered in our report, the dominant sources of autochthonous VTEC strains was serogroup O26, the most common non-O157 VTEC group associated with severe diarrhea and HUS in Europe [14].

It is possible that the prevalence of such strains might be overestimated because the *E. coli* strains reported in our study were collected during a period that coincided with an outbreak of infections generated by VTEC O26 *E. coli*.

However, what is more important to note are the high rates of DEC positivity identified for this serogroup and its diversity which was previously observed [15, 16].

Additionally, when considering the rest of the serogroups with a higher frequency in our collection, at least half of the strains of serogroups O111, O127, O128, and O157 were positive for virulence markers. All these serogroups were already reported in the literature as commonly associated with infections [5, 17-19].

Taking into consideration the results of this study, we conclude that the use of commercially available O antisera may still be considered when screening for presumptive DEC strains but the laboratory tendency to limit the microbiological diagnostic to this procedure should be discouraged. Besides, extensive studies of autochthonous pathogenic *E. coli* strains are needed to understand how the laboratory should adapt the diagnostic strategy to meet the local needs.

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Conflict of interests. None to declare.

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