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Original article

Risk of acquisition of human diarrhoeagenic *Escherichia coli* virulence genes in intercontinental travellers: A prospective, multi-centre study

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ABSTRACT

Background: We studied geographic distribution of diarrhoeagenic *Escherichia coli* virulence genes (DEC VGs) acquisition in travellers and investigated if they acquired highly virulent EAEC/STEC hybrid strains.
Methods: From the prospective, multicentre COMBAT study among 2001 Dutch travellers, 491 travellers were selected based on travel destination to 7 subregions. Faecal samples taken directly before and after travel were screened for nine DEC VGs with real-time PCR. Incidence proportions and rates were calculated for each gene and subregion.
Results: 479 travellers were analysed. 21.8% acquired *aggR* (EAEC), with highest acquisition rates in Northern and Western Africa and 15.3% acquired *eae* (STEC/EPEC) with highest rates in travellers to Western and Eastern Africa. ETEC (*elt* or *est* gene) was acquired by 4.2% of travellers and acquisition of *est* was associated with traveller's diarrhoea. Overall, the risk of acquiring DEC VGs was low in Southern Africa and South America. Although the combination of *aggR* (EAEC) and *stx*1/2 (STEC) was acquired by 3 travellers, these genes could not be detected together in a single *E. coli* strain.
Conclusions: The risk of acquisition of DEC VGs strongly depends on the travel destination, with those travelling to Africa - except Southern Africa - having a higher risk.

1. Introduction

Traveller's diarrhoea (TD) is caused by a variety of agents including bacterial, viral and parasitic enteropathogens. Enterotoxigenic *E. coli* (ETEC) is generally viewed as the leading bacterial cause of traveller's diarrhoea (TD) [1–3]. In addition, the Enteroaggregative *E. coli* (EAEC)

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can cause diarrhoea, including TD, in both developing and industrialized countries [4,5].

ETEC and EAEC belong to the well-known diarrhoeagenic *Escherichia coli* (DEC) group, which comprises another four intestinal pathotypes, including Shigatoxin-producing *E. coli* (STEC/EHEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC) and Diffusely Adherent *E. coli* (DAEC) [6]. All these pathotypes possess diverse virulence factors (VFs), such as toxins, fimbriae or adhesins, which are responsible for their pathogenicity and are encoded by virulence genes (VGs), mainly located on plasmids [7]. Although a large number of putative VFs have been identified, no single factor appears to be consistently present in all pathogenic strains, and the presence of a VG does not necessarily lead to expression of the VF, thus complicating the evaluation of the significance of their detection [8].

In addition to the typical pathotypes, hybrid pathotypes carrying VGs from more than one pathotype can exist, as seen during the EHEC O104:H4 outbreak in Germany in 2011. The unusual combination of VGs of EAEC and EHEC pathotypes made this outbreak strain extremely virulent [9]. Fenugreek sprouts imported from Egypt were identified as the most likely source of this strain, although still debatable given the lack of isolated colonies from this matrix [10,11].

Travel-acquired pathogenic *E. coli* strains can potentially be further transmitted within households after return as previously shown for travel-acquired multiresistant *E. coli* isolates [12,13] and VGs, especially shiga toxin genes (*stx*), can spread by horizontal transmission [14,15]. Therefore, international travel could play a substantial role in the emergence of outbreak strains. Previous studies have mainly focused on the association between acquisition of DEC and (persisting) TD, but only few have prospectively studied geographic distribution of DEC VGs acquisition and carriage in healthy individuals [16–19]. Assessment of the risk of acquisition per geographic region may give an indication of the likelihood of outbreak strains to emerge elsewhere.

We and others have previously shown that travel destination is the most important risk factor for acquisition of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) during international travel [13,20]. We hypothesized that the risk of acquiring DEC VGs during travel also depends on the sub-region visited and we studied the acquisition of *E. coli* VGs in travellers with a focus on geographic distribution. Secondly, we investigated whether travellers acquired the combination of EAEC and STEC VGs in single *E. coli* strains.

2. Material and methods

2.1. Study population

Within the prospective, multicentre COMBAT study, 2001 Dutch travellers were included from November 2012 until November 2013. Faecal swabs in modified Cary Blair transport medium (Fecal Swab®; Copan, Brescia, Italy) and questionnaires were collected before travel and immediately after return as previously described [13,21]. Samples were processed directly upon arrival at the laboratory, where they were inoculated on McConkey agar plates and incubated overnight. A scrape of the growth on this agar from all faecal samples was suspended in glycerol and together with the residuals in Cary Blair medium stored at −80 °C for future research. Only participants providing written informed consent were enrolled. Ethical approval was obtained by the Medical Ethical Committee of Maastricht University Medical Center (study number: METC 12-4-093). A full description of the study design has been published elsewhere [21].

2.2. Selection of travellers

For the present study, we focused on the seven United Nations' defined subregions that were most frequently visited by the participants in our study [Table 1]. After excluding subjects that had travelled to multiple subregions, we randomly selected 70 travellers for six and 71 travellers for the remaining of these seven subregions, resulting in a study population of 491 travellers.

Table 1
Characteristics of included travellers (n = 479).

Sex	Female	263	54.9%
	Male	216	45.1%
Age (median, range in years)		52	19–81
Chronic illness	Yes	105	21.9%
	No	374	78.1%
Antibiotic use within 3 months before travel	Yes	45	9.4%
	No	434	90.6%
Use antacids	Yes	73	15.2%
	No	406	84.8%
Median duration of travel in days (IQR)		18	(14–23)
Purpose of travel	Holiday	395	82.5%
	Work or internship	38	7.9%
	Visiting friends or relatives	19	4.0%
	Other	27	5.6%
Subregion visited during travel	South Eastern Asia	69	14.4%
	Eastern Africa	68	14.2%
	Northern Africa	68	14.2%
	Southern Africa	70	14.6%
	Western Africa	67	14.0%
	South America	67	14.0%
	Southern Asia	70	14.6%
Accommodation during travel	Hotel or apartment	121	25.3%
	Luxury	109	22.8%
	Low budget	53	11.1%
	Family or local people	20	4.2%
	Tent	12	2.5%
	Several	146	30.5%
	Other	18	3.7%
Traveller's diarrhoea	Yes	183	38.2%
	No	296	61.8%
Antibiotic use during travel	Yes	28	5.8%
	No	451	94.2%
Medical care during travel	Visited doctor or hospital	16	3.3%
	No medical care	463	96.7%

Legend: IQR, interquartile range.

2.3. Detection of VGs directly on stool samples

Fecal genomic DNA of pre- and post-travel samples was extracted using the MagNA Pure 96 System (Roche Diagnostics, the Netherlands) with inclusion of Phocine Herpes Virus (PhoHV) DNA as an internal control (IC) for extraction and amplification efficiency. Real-time PCRs were performed to detect the presence of nine VGs characteristic for intestinal *E. coli* pathotypes [Table 2]. Primers for amplification of the VGs have been published previously [22].

Table 2
Pathotypes of diarrhoeagenic *E. coli* and corresponding virulence genes (VGs) tested in this study.

Pathotype	VGs
STEC/EHEC	*stx*1
	*stx*2
	eae
	*ehx*A
ETEC	*elt*
	est
EIEC/Shigella	*inv*A
EAEC	*agg*R
EPEC	*eae*
	*bfp*A[a]

[a] Typical EPEC: *eae* + *bfp*A + ; atypical EPEC; *eae* + *bfp*A-.

Five *E. coli* reference strains (STEC, EPEC, ETEC, EIEC and EAEC/EHEC) were included as positive controls for each of the targets and PCRs were performed following a previously described and validated protocol [23]. In each PCR assay, three negative controls and three positive controls were included. PCRs with Cq values under or equal to 38 were considered positive. Subjects with one or more samples with negative internal controls were not analysed.

2.4. Detection of possible hybrid EAEC/STEC strains on cultured colonies

From the travellers who acquired simultaneously both *aggR* (EAEC) and *stx* (STEC) genes, the stored growth on McConkey agar plates was analysed for the presence of hybrid EAEC/STEC strains. First, samples were defrosted and inoculated on Columbia agar supplemented with 5% sheep blood (Biomérieux) and McConkey agar. After overnight incubation, twelve morphologically different colonies were selected and confirmed to be *E. coli* species by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Bruker Microflex LT, Bruker, London, UK). All *E. coli* isolates were subsequently subjected to PCR specific to the *aggR* (EAEC) and *stx*1 and *stx*2 (STEC) genes.

2.5. Definitions

The dynamics of DEC VGs were defined as follows:

1) Non-carrier: negative PCR pre- and post-travel
2) Acquisition: negative PCR pre-travel, positive PCR post-travel
3) Loss: positive PCR pre-travel, negative PCR post-travel
4) Persistent carrier: positive PCR pre- and post-travel.

Acquisition rates (incidence proportions and incidence rates) were calculated for those who were negative for the given gene before travel (the 'at risk population'). TD was defined as three or more unformed stools within a 24-h period during travel, with or without accompanying symptoms.

2.6. Statistical analysis

Incidence proportions (IP) and incidence rates (IR) per 100 person-days of travel (100 pdt) and accompanying 95% CIs for acquisition were calculated for each sub-region and VG. IR per 100 pdt was calculated with a maximum likelihood method that was based on a constant acquisition rate with right-censored and interval-censored data. If 95% CIs between sub-regions did not overlap, the difference in acquisition was considered significant.

The association between acquisition of VGs and TD was calculated with the Chi-squared test ($\chi 2$) using MedCalc [24]. P-values below 0.05 were considered statistically significant.

3. Results

Of the 491 selected travellers, one or more samples were missing from 7 subjects and samples of 5 subjects had negative IC-PCR's. Therefore 479 travellers were included in further analyses. Median travel duration was 18 days (IQR 14–23) and leisure (82.5%) was the main purpose of travel. Travellers to Northern Africa travelled for shorter periods than those to other regions with median durations of 12 days (IQR 8–14) and 19 days (IQR 15–24) respectively. Travellers' diarrhoea was reported by 183 travellers (38.2%) [Table 1].

In [Table 3] the dynamics of DEC VGs are summarized. Briefly, twenty-nine (6.1%) travellers carried one or more VGs before travel (*eae* (STEC/EPEC), n = 14; *aggR* (EAEC), n = 11; *ehx*A (STEC), n = 6; *bfp*A (EPEC), n = 3; *stx*1 (STEC), n = 2; *stx*2 (STEC), n = 1; *inv*A (EIEC), n = 1 and *est* (ETEC), n = 1). During travel, 164 subjects acquired a total of 234 genes. Of the travellers at risk (negative before travel), substantial IPs of 21.8% (95%CI 18.2–26.5) and 15.3% (12.1–19.3) were seen for the EAEC *agg*R and STEC/EPEC *eae* genes respectively, while acquisition of the other VGs under study was much lower ranging from 0.4% to 4.4% [Table 3].

In addition, there were 28/456 (6.1%) travellers at risk that acquired both *eae* (STEC/EPEC) and *aggR* (EAEC) VGs while 11/468 (2.4%) travellers at risk acquired both *aggR* (EAEC) and ETEC VGs (5 *est*, 4 *elt* and 2 both). Of those travellers, 8/11 (72.7%) had TD of which 3 travellers were still symptomatic on return. Nine travellers acquired the combination of STEC/EPEC *eae* and STEC *ehx*A, three acquired the combination of *stx1* and *eae*, but none of them acquired the combination of *stx/eae/ehx*A (typical EHEC). Another three travellers to Africa (one to Eastern, one to Northern, and one to Western Africa) acquired both *eae* and *bfp*A VGs, which are genes from typical EPEC (tEPEC) pathotype.

Of the travellers with acquisition of *est* (ETEC) 10/12 (83.3%) had TD compared to 173/467 (37.0%) of travellers without acquisition of *est* (ETEC) (p = 0.001). For the other VGs, no significant association between acquisition and TD was found.

Depending on the studied VG, large differences in acquisition rates between sub-regions were observed. For *aggR* (EAEC), incidence proportions (acquisition rates) where comparable for Western, Northern and Eastern Africa (33.3%, 31.3% and 29.2% respectively) and these acquisition rates did not differ significantly from South-Eastern Asia and Southern Asia. If we look at incidence rates per 100 pdt however, significant differences arefound between Northern Africa and South-Eastern and Southern Asia. Also, incidence rates per 100 pdt are [3.13/1.71 =] 1.83 times higher in Northern Africa than in Eastern Africa, although this difference is not significant as 95CI's overlap [Fig. 1, Supplementary Table].

For *eae* (STEC/EPEC), similar acquisition rates - i.e. not significantly different - were found in Western, Northern and Eastern Africa, South-Eastern Asia, Southern Asia and South America. Significant differences in acquisition rates were found between Eastern and Southern Africa only. When looking at incidence rates of per 100 pdt of *eae,* additionally, significant differences were found between Southeastern-/Southern-Asia and Southern Africa and between Western and Southern Africa [Fig. 1, Supplementary Table].

The third most frequently acquired gene, STEC *ehx*A, was most frequently acquired in Eastern Africa (7/67; 10.4% 95CI 5.0–21.9%) and only by one of the travellers to Western Africa, South America and Southern Asia, although this difference was not statistically significant [Supplementary Table].

A total of 20 travellers acquired ETEC genes: 9 acquired *elt*, 8 *est* and 3 travellers acquired both genes. Incidence rates ranged from 0.00 per 100 pdt (94%CI 0.00–0.28) in Southern Asia to 0.38 per 100 pdt (0.10–1.00) in Northern Africa [Supplementary Table].

EIEC *invA* was acquired by 4 travellers: two in Northern Africa and one in Western Africa and Southern Asia.

The combination of *aggR* (EAEC) and *stx2* (STEC) genes as harboured by the German EHEC outbreak strain was acquired by one of the travellers and the combination of *aggR* (EAEC) and *stx1 (*STEC) genes was acquired by two travellers. One of these three travellers acquired the combination of *aggR* (EAEC), *stx*2 (STEC) and *ehx*A (STEC) and had travelled to Gambia, in Western Africa. Another two acquired the combination of *aggR* (EAEC), *stx*1 (STEC) and *eae* (STEC/EPEC) and both of them had travelled to Eastern Africa, one to Zambia and the other to Tanzania.

From each of these 3 travellers, PCRs targeting *aggR* (EAEC) and *stx*1 and *stx*2 (STEC) were performed on 35 randomly selected *E. coli* colonies isolated from the stored McConkey agar. Although *stx* (STEC) and *aggR* (EAEC) genes were found in separate isolates, none of the tested strains presented the *agg*R/*stx* combination. Of the traveller that acquired *aggR* (EAEC) and *stx*2 (STEC), 7 isolates were *stx*2 (STEC) positive and 3 were *aggR* (EAEC) positive. Of the travellers that acquired *agg*R/*stx*1, one traveller had 7 *aggR* (EAEC) positive and 1 *stx*1 (STEC) positive isolate and the other traveller just 1 *aggR* (EAEC)

Table 3
Dynamics of nine diarrhoeagenic *E. coli* virulence genes (VGs) in the cohort of 479 travellers.

	***aggR* (EAEC)**						***stx*1 (STEC)**						***stx*2 (STEC)**		
	TD[a]		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Non-carrier	135	36.9%	231	63.1%	366	76.4%	179	37.9%	293	62.1%	472	98.7%	183	38.4%	293
Acquisition[b]	44	43.1%	58	56.9%	102	21.3%	4	80.0%	1	20.0%	5	0.8%	0	0.0%	2
Loss	1	20.0%	4	80.0%	5	1.0%	0	0.0%	2	100.0%	2	0.4%	0	0.0%	1
Persistent carrier	3	50.0%	3	50.0%	6	1.3%					0				
Total	**183**		**296**		**479**	**100%**	**183**		**296**		**479**	**100%**	**183**		**296**
Before travel[c]					11	2.3%					2	0.4%			
At risk[d]					468						477				
Acquisition among at risk population					102	21.8%					4	0.8%			

	***est* (ETEC)**						***elt* (ETEC)**						***eae* (STEC/EPEC)**		
	TD[a]		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N
Non-carrier	173	37.1%	293	62.9%	466	97.3%	179	38.2%	289	61.8%	468	97.7%	145	36.8%	249
Acquisition[b]	10	83.3%	2	16.7%	12	2.5%	4	36.4%	7	63.6%	11	2.3%	33	46.5%	38
Loss	0	0.0%	1	100.0%	1	0.2%	0		0		0		3	33.3%	6
Persistent carrier	0				0		0		0		0		2	40.0%	3
Total	**183**		**296**		**479**	**100%**	**183**		**296**		**479**	**100%**	**183**		**296**
Before travel[c]					1	0.2%					0	0.0%			
At risk[d]					478						479				
Acquisition among at risk population					12	2.5%					11	2.3%			

	***stx*2 (STEC)**			***invA* (EIEC)**						***bfp*A (EPEC)**					
	No TD[a]	**Total**		**TD[a]**		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**		**Total**	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Non-carrier	61.6%	476	99.4%	179	37.8%	295	62.2%	474	99.0%	178	38.0%	291	62.0%	469	97.9%
Acquisition[b]	100.0%	2	0.4%	3	75.0%	1	25.0%	4	0.8%	4	57.1%	3	42.9%	7	1.5%
Loss	100.0%	1	0.2%	1	100.0%	0	0.0%	1	0.2%	1	33.3%	2	66.7%	3	0.6%
Persistent carrier		0						0		0				0	
Total		**479**	**100%**	**183**		**296**		**479**	**100%**	**183**		**296**		**479**	**100%**

(*continued on next page*)

Table 3 (*continued*)

	***stx*2 (STEC)**			***inv*A (EIEC)**						***bfp*A (EPEC)**					
	No TD[a]	**Total**		**TD[a]**		**No TD[a]**		**Total**		**TD[a]**		**No TD[a]**		**Total**	
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Before travel[c]		1	0.2%					1	0.2%					3	0.6%
At risk[d]		478						478						476	
Acquisition among at risk population		2	0.4%					4	0.8%					7	1.5%

	***eae* (STEC/EPEC)**			***ehx*A (STEC)**				
	No TD[a]	**Total**		**TD[a]**		**No TD[a]**		**Total**
	%	N	%	N	%	N	%	N	%
Non-carrier	63.2%	394	82.3%	176	38.9%	276	61.1%	452	94.4%
Acquisition[b]	53.5%	71	14.8%	7	33.3%	14	66.7%	21	4.4%
Loss	66.7%	9	1.9%	0	0.0%	6	100.0%	6	1.3%
Persistent carrier	60.0%	5	1.0%	0				0	
Total		**479**	**100%**	**183**		**296**		**479**	**100%**
Before travel[c]		14	2.9%					6	1.3%
At risk[d]		465						473	
Acquisition among at risk population		71	15.3%					21	4.4%

[a] The denominators for percentages are the numbers of travellers in the 'Total' column.
[b] Acquisition was calculated over the complete study population of 479 travellers.
[c] Subjects that were positive before travel.
[d] Subjects that were negative before travel and therefore at risk of acquiring a VG.

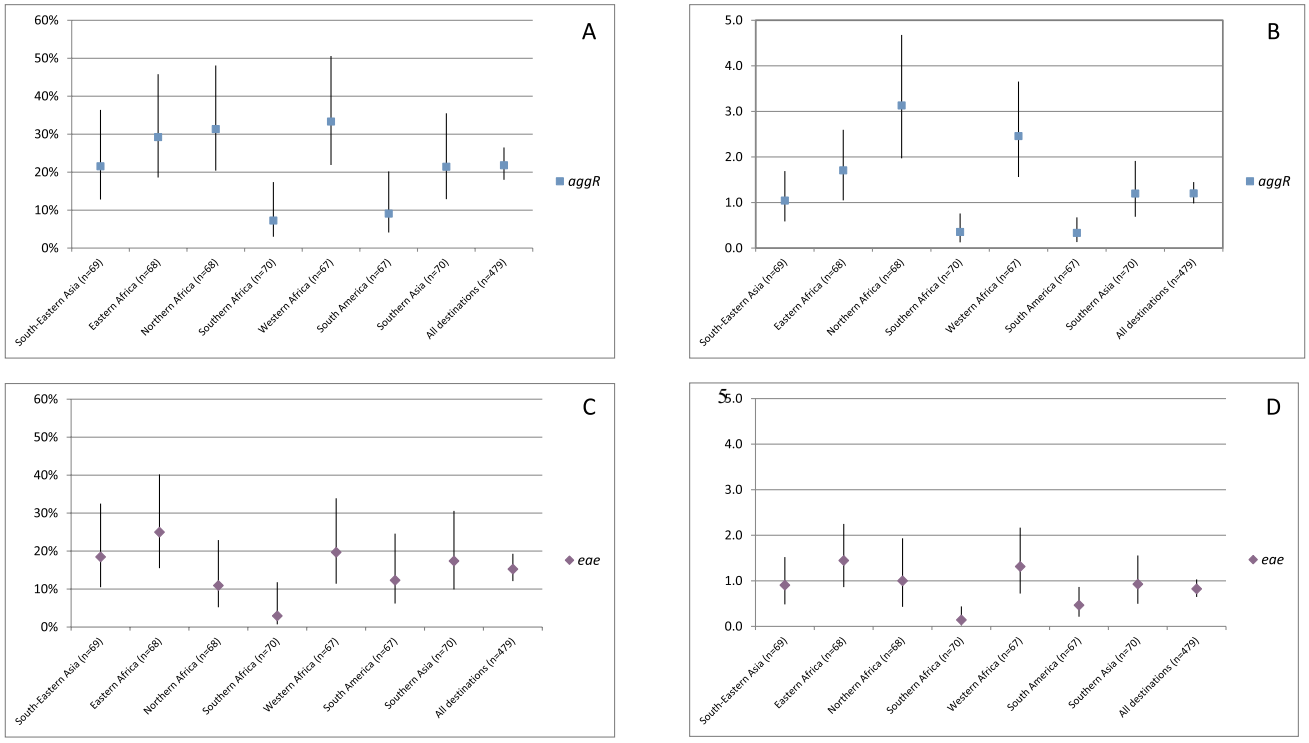

Fig. 1. Plots showing incidence proportions (acquisition rates, panel A and C) and incidence rates per 100 person-days of travel (panel B and D) of *aggR* (EAEC) and *eae* (STEC/EPEC) virulence genes, per subregion visited.
Black line: 95% confidence interval (95%CI). In case 95%CI's do not overlap, the difference in incidence rates between subregions is considered significant.

positive isolate.

4. Discussion

Substantial acquisition of EAEC (*agg*R), STEC and EPEC (*eae*) VGs was observed among international travellers, with the highest acquisition of *aggR* (EAEC) in Northern and Western Africa and of *eae* (STEC/EPEC) in Eastern and Western Africa. Overall, the risk of acquiring DEC VGs was low in Southern Africa and South America. Acquisition of ETEC, EIEC and STEC genes was relatively low.

The 'weighted selection' strategy and the large sample size made it possible to study acquisition in less visited sub regions with enough power to determine differences between sub-regions. For some genes however, acquisition rates were too low to detect statistical differences in acquisition between sub-regions.

The calculation of incidence rates per 100 pdt in addition of incidence proportions made it possible - as opposed to other studies - to evaluate the effect of time at risk on acquisition. Some significant differences in incidence rates between subregions were not found when comparing incidence proportions. As a shorter travel duration does not seem to lead to less acquistion, one could speculate that VGs are acquired relatively shortly after arrival.

Since qPCR was performed on DNA directly extracted from faecal samples and not on DNA from isolates, the possibility that two genes acquired by a single traveller are present in separate *E. coli* strains or in other bacterial species within the travellers' gut microbiome, cannot be excluded. The prospectively stored scrapings of McConkey agars of the faecal samples of all our travellers provided the opportunity to study the combined acquisition of EAEC/STEC. Although the combination of *aggR* (EAEC) and *stx* (STEC) genes was acquired by 3 travellers, these genes could not be detected in a single *E. coli* strain, indicating these genes may be present in different *E. coli* isolates or strains carrying both genes were present in a quantity that was too low to be cultured.

In agreement with our results, Lääveri and co-workers, who performed a similar study in 459 Finnish travellers, reported EPEC (n = 194; 42%) and EAEC (192; 42%) genes to be most frequently detected post-travel [25]. ETEC is considered to be an important stool pathogen in travellers and was found in stools of 30% of returned travellers with diarrhoea who visited health clinics in Mexico, Jamaica and India [2], whereas we found only 4% acquisition of ETEC (*elt* or *est* genes). The large difference in ETEC-positive travellers post travel can be explained by selection towards a more symptomatic population in the former study, as all included travellers visited health clinics. In the Finnish study, ETEC was detected in post-travel stools of 88/459 travellers (19%) [25]. Also, acquisition rates of EPEC, EAEC, and EHEC pathotypes were higher in the Finnish study than in the present study, although the definitions of these pathotypes were not fully specified. The differences in acquisition between these two studies could - at least partly - be explained by a difference in pathotype definition. In addition, the difference could be related to the travel period as the Finnish study included travellers between March 2009 and February 2010, whereas in our study travellers were included from November 2012 until November 2013. Another possible explanation is a difference in Ct-value cut-offs for positivity, although analytical sensitivity of both PCR methods are similar [23,26].

A study by the same Finnish study group in 45 travellers to Benin, West Africa, found high post-travel carriage of EPEC (77%), EAEC in 59% and ETEC in 56% [17]. A recently published prospective cohort study among French Hajj pilgrims in 2016, found acquisition of EPEC, EAEC, and STEC in 30%, 10%, and 7% of pilgrims, respectively and, in concordance with the present study, little acquisition of ETEC (4%) [19].

In a Dutch case-control study to study causes of diarrhoea in returned travellers, EAEC and EPEC were also frequently detected, although less frequently than diffuse adherent *E. coli* strains (DAEC). EAEC was found in 8.3% (23/277) and EPEC was found in 8/277 (2.9%) post-travel samples of all included travellers (cases and controls) [27].

The import of shigatoxin and *aggR* (EAEC) genes by single travellers could potentially lead to the emergence of hypervirulent EHEC strains,

through acquisition of *stx* phages by EAEC strains or by horizontal transmission of the (pAA) plasmid on which EAEC virulence genes are located [28] in the human gut or the environment.

In the present study the risk of acquiring diarrhoeagenic *E. coli* VGs during travel strongly depended on the subregion visited. The large difference in acquisition rates of *aggR* (EAEC) between travellers to Southern Africa and South America and the other subregions does not only reflect a difference in local prevalence and dissemination of VGs, since several studies show EAEC/*agg*R to be highly prevalent in humans and the environment in South America and South Africa [29–34]. Nevertheless, only little acquisition by travellers to these subregions is seen in this study. Hypothetically, this could be explained by less spread in the food chain or a difference in (hand) hygiene and food handling compared to subregions where acquisition was much higher, such as Northern, Eastern and Western Africa. Surprisingly, although not statistically significant, less acquisition of *aggR* (EAEC) VGs was seen in Southeastern and Southern Asia while these DEC are thought to be highly prevalent in the community in both regions [35].

The import of pathogenic *E. coli* could potentially lead to subsequent clonal spread or horizontal transmission of VGs. The underlying mechanism through which the risk of acquisition of DEC VGs during travel strongly varies between the visited subregions, needs to be further clarified.

Author contributions

All authors had an important role in initiating and designing the study. JvH and MA collected the samples and AC performed the laboratory analyses. JvH, AC and JP analysed the data. JvH and AC drafted the first version of the manuscript. All authors revised the manuscript critically and contributed to the final version.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2018.12.005.

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