

Prevalence and risk factors for carriage of ESBL-producing Enterobacteriaceae in Amsterdam

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Objectives: The objectives of this study were to determine the prevalence of carriage of ESBL-producing Enterobacteriaceae (ESBL-E) in a representative sample of the general adult Dutch community, to identify risk factors and to gain understanding of the epidemiology of these resistant strains.

Methods: Adults enrolled in five general practices in Amsterdam were approached by postal mail and asked to fill in a questionnaire and to collect a faecal sample. Samples were analysed for the presence of ESBL-E. ESBL genes were characterized by PCR and sequencing. Strains were typed using MLST and amplified fragment length polymorphism (AFLP) and plasmids were identified by PCR-based replicon typing. Risk factors for carriage were investigated by multivariate analysis.

Results: ESBL-E were found in 145/1695 (8.6%) samples; 91% were *Escherichia coli*. Most ESBL genes were of the CTX-M group (*bla*_{CTX-M-1} and *bla*_{CTX-M-15}). MLST ST131 was predominant and mainly associated with CTX-M-15-producing *E. coli*. One isolate with reduced susceptibility to ertapenem produced OXA-48. In multivariate analyses, use of antimicrobial agents, use of antacids and travel to Africa, Asia and Northern America were associated with carriage of ESBL-E, in particular strains with *bla*_{CTX-M-14/15}.

Conclusions: This study showed a high prevalence of ESBL-E carriage in the general Dutch community. Also, outside hospitals, the use of antibiotics was a risk factor; interestingly, use of antacids increased the risk of carriage. A major risk factor in the general population was travel to countries outside Europe, in particular to Asia, Africa and Northern America.

Introduction

Resistance to β -lactam antibiotics due to ESBLs has become a common problem worldwide.¹ The prevalence of this resistance mechanism has increased rapidly, even in countries known for prudent antibiotic use.²

In a previous study we showed that over 10% of Dutch community patients with gastrointestinal complaints carry ESBL-producing Enterobacteriaceae (ESBL-E) in their gastrointestinal tract.³ This is remarkable, because the Netherlands is a country with low antibiotic use in humans and has among the lowest

resistance rates in clinical isolates in Europe.² This triggered us to perform the present study, which focused on the prevalence and molecular epidemiology of carriage of ESBL-E in the general population and on risk factors for carriage.

Methods

Study design and data collection

For this cross-sectional study we approached all adult persons (individuals aged ≥ 18 years), excepting those who were terminally ill, present in the

databases of five general practices (~10000 persons), affiliated to the Academic General Practice Network (AGPN), VU University Medical Center, Amsterdam. In the Netherlands, citizens are registered with a general practitioner, regardless of health status. The database therefore is a representative sample of the general population. Individuals were approached by postal mail with a questionnaire, an informed consent form and a container for a faecal sample or perineal swab (according to their preference). Samples were returned in transport medium (Copan Italia, Brescia, Italy) between June 2011 and November 2011.

The questionnaire asked about sampling date, sample type (perineal swab or faecal sample), age, gender, profession, country of birth of the participant and his/her parents, years living in the Netherlands, admission to a (foreign) hospital, healthcare institution or long-term care facility and travel to foreign countries, all in the previous 12 months. Data on antimicrobial, antacid and corticosteroid use and comorbid conditions in the past 12 months were extracted from the database of the AGPN. Please see the Supplementary data (available at JAC Online) for items included in the questionnaire and data extracted from the AGPN database. Fifty ESBL-positive participants were asked for participation of their household members, with the same questionnaires and request for samples.

The medical ethics committee (METc ID NL29769.029.09) of the VU University Medical Center approved the study (NTR Trial ID NTR2453).

ESBL detection

Samples were inoculated in selective enrichment broth (Trypticase soy broth with ampicillin). After overnight incubation (37°C) an aliquot was inoculated on EbSA-ESBL screening agar (Cepheid Benelux, Apeldoorn, The Netherlands) and on blood agar.^{4,5} Growth on the blood agar plate indicated the sample was suitable for analysis. Three colonies of each distinct morphotype on the EbSA-ESBL agar were characterized. ESBL production was confirmed by combination disc diffusion test with both cefotaxime and ceftazidime, with and without clavulanic acid (Rosco, Taastrup, Denmark), interpreted according to the Dutch national guideline.⁶ Species identification and antibiotic susceptibility testing were performed with Vitek 2 (bioMérieux, Marcy-l'Étoile, France). MIC breakpoints were according to EUCAST.⁷ Reduced susceptibility (MIC ≥0.25 mg/L) to ertapenem (Etest, bioMérieux) indicated the possible presence of a carbapenemase.

ESBL- and carbapenemase-encoding genes were characterized by PCR and sequencing (BaseClear, Leiden, The Netherlands).^{8–11} Sequences

were analysed with Bionumerics software (version 6.6; Applied Maths, Sint-Martens-Latem, Belgium) and compared with sequences in the NCBI (<http://www.ncbi.nlm.nih.gov/BLAST>) and Lahey database (<http://www.lahey.org/studies/>).

Molecular typing

E. coli strains were typed by MLST (<http://mlst.warwick.ac.uk/mlst/mlst/dbs/Ecoli>). Clonal complexes were assigned using eBURST v3 (<http://eburst.mlst.net/>).

A subset of *E. coli* strains was typed by amplified fragment length polymorphism (AFLP).¹²

Plasmids were identified by PCR-based replicon typing, as described by Carattoli and adapted by Boot *et al.*¹³

Analysis of risk factors and statistical methods

For a case-control analysis of risk factors, cases were carriers of ESBL-E and controls were persons free of ESBL-E. Statistical analyses were performed with Statistical Package for the Social Sciences, version 20.0 (SPSS, Chicago, IL, USA). Possible risk factors were analysed by univariate and multivariate logistic regression. ORs and 95% CIs were calculated.

Results

Participants

Of 7000 persons approached, 1695 (24.2%) returned the questionnaire with a completed consent form and a specimen. Participants lived in the region of Amsterdam. Age and gender characteristics are given in Table 1.

Prevalence of carriage of ESBL-E and ESBL gene characterization

ESBL-E were detected in 145 of 1695 samples (8.6%, 95% CI 7.3%–10.0%): 132 (91.0%) *Escherichia coli*, 11 (7.6%) *Klebsiella pneumoniae*, 1 *Enterobacter cloacae* (0.7%) and 1 *Serratia plymuthica* (0.7%). The presence of genes encoding ESBL was confirmed in all phenotypically ESBL-producing strains (Table 2); these genes comprised mainly *bla*_{CTX-M-15} and

Table 1. Participant characteristics and main risk factors for ESBL-E carriage in univariate analysis

Risk factor	Cases	Controls	OR	95% CI
Age, median (range); N=129 and 1393	48 (20–90)	50 (18–95)	NA	NA
Female, n (%); N=129 and 1393	75 (58.1)	852 (61.2)	0.9	0.6–1.3
Use of antibiotics, n (%); N=129 and 1393	33 (25.6)	195 (14.0)	2.1	1.4–3.2
PPIs or H2 blockers, n (%); N=129 and 1393	28 (21.7)	166 (11.9)	2.0	1.3–3.2
Travel to, n (%) ^a				
Africa; N=111 and 1245	19 (17.1)	88 (7.1)	2.7	1.6–4.7
Latin America/Caribbean; N=109 and 1264	12 (11.0)	112 (8.9)	1.3	0.7–2.4
Northern America; N=106 and 1255	23 (21.7)	133 (10.6)	2.3	1.4–3.8
Asia; N=118 and 1310	39 (33.1)	247 (18.9)	2.1	1.4–3.2
Australia/New Zealand; N=102 and 1222	0 (0)	18 (1.5)	NA	NA

NA, not applicable; PPIs, proton-pump inhibitors.

^aNumber of patients who travelled to WHO region/total number of patients with exclusion of those patients that also travelled to one of the other WHO regions or did not travel outside the Netherlands.

*bla*_{CTX-M-1}. Co-resistance to other antibiotics was common: 33% of strains were multiresistant as defined by Magiorakos et al.¹⁴ (Table S1, available as Supplementary data at JAC Online). No strains with reduced susceptibility to imipenem or meropenem were found; one *E. coli* strain had reduced susceptibility to ertapenem (MIC 0.75 mg/L); this strain carried *bla*_{OXA-48} and *bla*_{CTX-M-14}. No difference was found in detection rate for faecal samples compared with perineal swabs (OR 1.0, 95% CI 0.7–1.4).

The prevalence of carriage of ESBL-E in participants not using antibiotics (*N* = 1294) was 7.4% (95% CI 6.0%–8.9%).

MLST

MLST showed 47 different STs, 6 of which represented new types. Types ST131 (21 isolates; 15.9%), ST10 (18 isolates;

13.6%) and ST38 (9 isolates; 6.8%) were most frequent. ST10 was the main clonal complex, including 26 isolates (19.7%) (Figure S1). MLST ST131 was mainly associated with CTX-M-15-producing *E. coli*.

Household members

Fifty carriers volunteered 41 household members, of which five (12.2%, 95% CI 4.9%–26.0%) were carriers of ESBL-E. Figure 1 and Table 3 present the distribution of ESBL genes and plasmids within households. Three of five clusters of isolates from single households had identical AFLP patterns and shared the same ESBL genes and plasmids. In cluster A, two *E. coli* strains shared an ESBL gene and an Inc1 plasmid, but each contained an additional plasmid, resulting in one band difference in the AFLP pattern. *E. coli* strains in cluster E also belonged to the same CTX-M-1 family, however had different ESBL genes, did not share plasmids, and the AFLP pattern was different.

Risk factors

For the case–control analysis, we included 1522 (129 ESBL-E carriers and 1393 non-carriers) of the 1695 participants who sent in a sample with the questionnaire and for whom data from the electronic database of the AGPN were available. Table 1 shows the main risk factors, with their univariate ORs and 95% CIs. Table S2 shows the full list of potential risk factors with univariate ORs. Countries were classified according to the format of the United Nations Department of Economic and Social Affairs into regions and major areas.¹⁵ Europe was chosen as the reference category.

Table 4 shows the multivariate analysis of the main potential risk factors. Travel to the different continents, antimicrobial use and antacid use (use of proton-pump inhibitors or H2 blockers) were identified as relevant factors and were therefore included in the multivariate analysis. The full list of factors with multivariate ORs and 95% CIs can be found in Table S3.

Travel

In the multivariate analysis, travel to Northern America, Africa and Asia remained associated with an increased risk of acquisition of ESBL-E relative to travel in Europe (Table 4). Detailed analysis by

Table 2. ESBL-encoding genes

ESBL family	ESBL gene/type	<i>n</i>
CTX-M-1	<i>bla</i> _{CTX-M-15}	59
	<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM-52}	1
	<i>bla</i> _{CTX-M-1}	25
	<i>bla</i> _{CTX-M-1} + <i>bla</i> _{SHV-12}	1
	<i>bla</i> _{CTX-M-3}	4
CTX-M-2	<i>bla</i> _{CTX-M-2}	2
CTX-M-9	CTX-M-9 group	1
	<i>bla</i> _{CTX-M-14} ^a	19
	<i>bla</i> _{CTX-M-9}	4
	<i>bla</i> _{CTX-M-27}	5
CTX-M ^b	<i>bla</i> _{CTX-M}	2
TEM and SHV	<i>bla</i> _{SHV-12}	5
	<i>bla</i> _{TEM-52}	6
	<i>bla</i> _{TEM-52} + <i>bla</i> _{SHV-12}	1
Other	<i>bla</i> _{CTX-M-21}	1
	<i>bla</i> _{CTX-M-22}	3
	<i>bla</i> _{CTX-M-32}	3
	<i>bla</i> _{CTX-M-55}	3
Total		145

^aOne isolate also encoded OXA-48.
^bExact subtype of two CTX-M genes remained unresolved by sequencing.

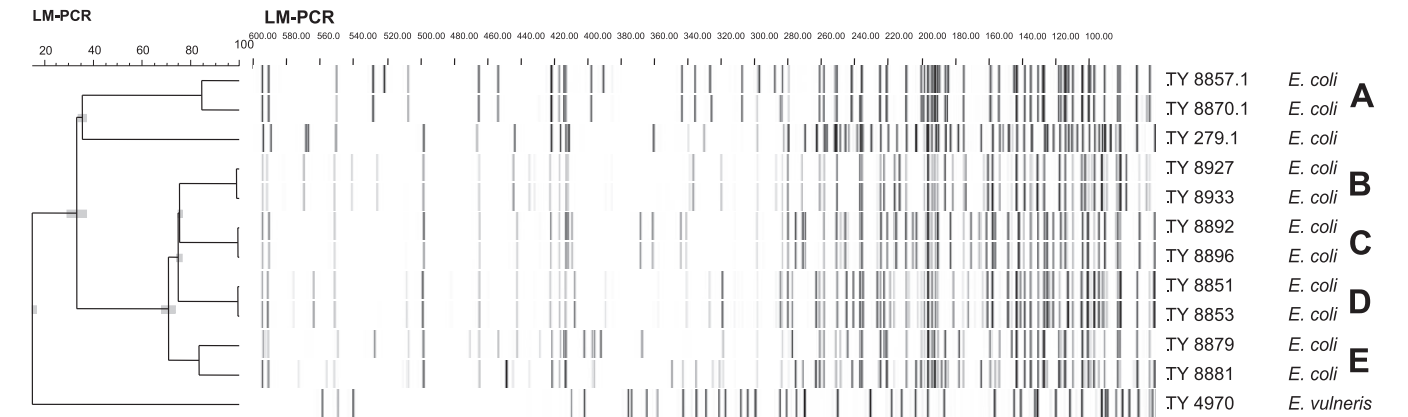


Figure 1. AFLP household members. TY numbers are used for numbering laboratory strains; *Escherichia vulniferus* (DSM 4564) and TY 279.1 (ATCC 35218) are reference strains used for AFLP. LM-PCR, ligation-mediated PCR.

Table 3. Household members: strains and plasmids

Cluster	Strain ^a	Gene(s)	Plasmid ^b					
			Inc I1	FrepB	ColE	FIA	Y	B/O
A	TY 8857.1	<i>bla</i> _{CTX-M-1}	+	–	–	–	+	–
	TY 8870.1	<i>bla</i> _{CTX-M-1}	+	+	–	–	–	–
B	TY 8927	<i>bla</i> _{CTX-M-14/18}	–	+	+	–	–	–
	TY 8933	<i>bla</i> _{CTX-M-14/18}	–	+	+	–	–	–
C	TY 8892	<i>bla</i> _{CTX-M-15}	–	+	–	+	+	–
	TY 8896	<i>bla</i> _{CTX-M-15}	–	+	–	+	+	–
D	TY 8851	<i>bla</i> _{CTX-M-15}	–	+	+	+	–	–
	TY 8853	<i>bla</i> _{CTX-M-15}	–	+	+	+	–	–
E	TY 8879	<i>bla</i> _{CTX-M-3/TEM-1}	+	+	–	+	+	+
	TY 8881	<i>bla</i> _{CTX-M-1/TEM-1}	–	–	–	–	–	–

^aLaboratory strain numbers.^bPlasmids R, ColEtp, FIIIs, FIB, P, A/C, U, HI1, L/M, HI2, W, T, N, X, F/C and K were not detected.**Table 4.** Main risk factors included in multivariate analysis

Risk factor	Multivariate OR	95% CI
Age (continuous variable)	1.0	1.0–1.0
Female	0.9	0.6–1.5
Use of antibiotics	2.2	1.4–3.7
PPIs or H2 blockers	1.9	1.1–3.3
Travel to		
Africa ^a	2.2	1.1–4.6
Latin America/Caribbean ^a	0.7	0.3–1.9
Northern America ^a	2.7	1.6–4.8
Asia ^a	2.1	1.3–3.6
Australia/New Zealand ^a	NA	NA

NA, not applicable; PPIs, proton-pump inhibitors.

^aCountries grouped according to WHO major area codes, reference=Europe (inclusive of persons who only travelled in the Netherlands or did not travel).

region, sub-region and country is given in Table S3. These analyses showed a statistically robust increase in the risk for Northern Africa (OR 2.9, 95% CI 1.1–7.7) and Eastern Africa (OR 5.5, 95% CI 1.1–27.4) and that the risk of travelling to Northern America was increased more than 3-fold and limited to the USA (OR 3.1, 95% CI 1.7–5.7). The risk associated with travel to Asia was highest for South-Central Asia (OR 5.5, 95% CI 2.2–14.2), for India in particular (OR 4.7, 95% CI 1.4–16.0).

Antimicrobial and antacid use

Both in univariate (Table 1) and multivariate (Table 4) analysis the use of antibiotics or antacids increased the risk of carriage of ESBL-E ~2-fold.

Other factors

We explored other potential risk factors (Table S2) by adding them separately, i.e. one at a time, into the multivariate analysis shown

in Table 4. Factors that stood out in the multivariate analysis were all travel related: working as airline cabin crew, admission to a foreign hospital, being born in Africa or having a father or mother born in Africa or Asia. We also performed an analysis for the risk associated with these travel-related factors, restricted to those participants who did travel outside the Netherlands. These were 1270 persons: 112 cases and 1158 controls. This restricted analysis suggested that working for an airline (multivariate OR 4.3, 95% CI 0.5–34.3) may pose an extra risk, since the OR in the restricted analysis did not change substantially. The OR associated with admission to a foreign hospital was halved in the restricted analysis, with a wide CI (multivariate OR 3.0, 95% CI 0.3–27.1). The OR associated with being born outside the Netherlands or having a father or a mother born outside the Netherlands was slightly different in the restricted analysis; the highest risk was having a mother born in Asia (multivariate OR 2.4, 95% CI 1.0–5.8), indicating that this risk was independent of the possible association with travel.

Association of travel with specific ESBL genes

The association of travel with carriage of strains with specific ESBL genes is shown in Table 5. *bla*_{CTX-M-1} is typically found in poultry in the Netherlands, while *bla*_{CTX-M-14} and *bla*_{CTX-M-15} are found in humans worldwide. *bla*_{CTX-M-15} and *bla*_{CTX-M-14} were associated with travel (Africa, Asia and Northern America); *bla*_{CTX-M-1} was not. The carbapenemase OXA-48 was found in an *E. coli* strain from a participant who visited Egypt and the USA; he was born in the Netherlands and the country of origin of both parents was Southern Asia. He had no other risk factors.

Discussion

We showed a faecal carriage rate of ESBL-E of >8% in the general adult population in Amsterdam. This confirms and extends our previous finding of a 10% carriage rate in patients who visit their general practitioner with gastrointestinal complaints.³ Main risk factors were antibiotic use, use of gastric acid-suppressing medication and travel to Africa, Asia or the USA. Additional

Table 5. Association of genes with travel to different regions (univariate)

	ESBL gene(s)	No ESBL	OR	95% CI
ESBL <i>bla</i> _{CTX-M-1} (N = 26)				
Europe (reference)	20	901		
Africa	0	88	NA	
Latin America/Caribbean	1	112	0.5	0.1–3.7
Northern America	1	133	0.4	0.1–3.2
Asia	1	247	0.2	0.0–1.5
Australia/New Zealand	0	19	NA	
ESBL <i>bla</i> _{CTX-M-14 and -15} (N = 79) ^a				
Europe (reference)	19	901		
Africa	18	88	5.5	3.0–9.9
Latin America/Caribbean	8	112	1.7	0.8–3.6
Northern America	15	133	3.1	1.7–5.7
Asia	25	247	3.0	1.8–5.1
Australia/New Zealand	0	18	NA	

NA, not applicable.

^aCTX-M-14 and -15 are grouped together because of their similar epidemiological distribution.

risk factors were having a mother born in Asia and possibly working as cabin crew for an airline. While the findings that antibiotic use and travel to Asia and Africa increase the risk of carriage of ESBL-E are not unexpected, the association with antacid use and the >3-fold increased risk associated with travel to the USA have, to the best of our knowledge, not been clearly shown before.¹⁶ An interesting finding was that carriage of Enterobacteriaceae producing CTX-M-14 or CTX-M-15 was associated with travel to Africa, Asia or Northern America/the USA, while carriage of strains producing CTX-M-1 was not.

A strength of our study is that it aimed at the carriage rate in the general adult population, because we did not select patients upon a visit to their general practitioner or on admission to hospital, but used the general practitioner's databases to draw a sample from the general population. This was possible because in the Netherlands health insurance is obligatory and inhabitants are registered with a general practitioner. A second advantage of our approach is that we did not select for persons attending a travel clinic, which introduces strong bias towards countries that require vaccination or malaria prophylaxis. The weakness of our study was the participation rate of ~25%. Participants had been informed that we were screening for resistant strains and of a possible relation with antibiotic use. This could have introduced self-selection bias for those participants that were concerned, because of previous antibiotic use, and could have affected the prevalence rate, rendering it higher. Therefore, we also determined the prevalence of carriage of resistant strains in participants who had not used antibiotics. This was also high, nearly 7.5%, and confirmed the high prevalence of ESBL-E in the Dutch population. Participants were unaware of other interests, such as types of ESBL, travel, ethnicity or acid-suppressing medication. Finally, our study was restricted to Amsterdam, a large, cosmopolitan city with inhabitants from many different origins and possibly a high propensity for international travel. Such selection does not invalidate the analysis of risk factors, because this selection is likely to be the same in all participants; numerically, it may have the effect of making the ORs closer to unity.

Several reports describe increasing rates of faecal carriage of ESBL-E in the community (reviewed by Woerther *et al.*¹⁶). The review by Woerther *et al.*¹⁶ shows that in Europe percentages of carriage of ESBL increased between 2002 and 2011, with the highest figures in Spain, where carriage rates of >7% were already noted in 2007.¹⁶ Overall, rates are quite similar to those we measured, albeit that our carriage rate of 8.6% is the highest determined so far in Europe. Possibly, this is due to our sensitive detection method, with an enrichment step.¹⁷ In other regions of the world, especially in South-East Asia and China, ESBL-E carriage rates can be as high as nearly 70%.¹⁶ The majority of ESBL-positive isolates in our study were *E. coli* and the predominant CTX-M allele was *bla*_{CTX-M-15}, although a substantial proportion, almost one-fifth, of strains produced CTX-M-1. The predominance of CTX-M-15 is in concordance with the epidemiology in the community worldwide and comparable to what we found in our study in patients with gastrointestinal complaints.^{3,16} In the present study, carriage of CTX-M-15- and 14-producing ESBL-E was associated with visiting a foreign country, while carriage of CTX-M-1-producing strains was not. Possibly, Enterobacteriaceae producing ESBLs of this allele are acquired in the Netherlands, since CTX-M-1 is the main ESBL type found in *E. coli* on chicken meat.^{18,19} A large proportion of the ESBL-producing *E. coli* appeared to be related to ST131. This more virulent clone could lead to more adverse outcomes in case of infection.²⁰

Risk factors for faecal carriage of ESBL-E in Europe have been investigated especially in healthcare settings. Because of our focus on the community we will limit our discussion to studies in community settings. Only a few European studies are available.^{16,21,22} With the exception of a study in Germany and one in the Netherlands, no risk factors for carriage were identified, possibly due to the limited size of the studies. The German study showed an association of ESBL-E carriage with travel to Greece and Africa and with ownership of a pet, while antibiotic use was not a risk factor.²¹ The Dutch study found ownership of a horse to be the only risk factor.²² In the present study, previous antimicrobial use increased the risk of ESBL-E carriage ~2-fold. This finding

is interesting because it is biologically plausible, but has not been shown in other European community studies. Possibly, the low level of antibiotic use, compared with other countries, makes this risk factor stand out in our country. Noteworthy is the relationship between use of acid-suppressing medication and ESBL-E carriage. Two clinical studies showed an association between antacid use and colonization with ESBL-E, one conducted among hospitalized patients in Israel and the other in the USA.^{23,24} The authors of these studies noticed the role of acid suppression, but did not discuss it. Our study indicates that antacids play a role as risk factors for acquisition of ESBL-E in the community too. Gastric acid suppression by bicarbonate has been shown to lower the infective dose of *Vibrio cholerae* in seminal studies conducted in the 1960s on inmates in correctional facilities that received oral doses of *V. cholerae*.²⁵ An association has been shown between gastric pH and non-typhoidal salmonellosis.^{26,27} The risk of antacid use points to ingestion as a main route of acquisition of ESBL-E. Possibly, the use of antacids or antibiotics while abroad may pose an additive risk for acquisition of ESBL. Our study, however, did not have the statistical power to test this hypothesis.

Several studies have shown that travellers to foreign countries can be colonized with ESBL-E upon return.¹⁶ Travel, especially to Asia, but also to Africa, may be the most important risk factor also in the general population. An intriguing finding is the risk associated with travel to the USA, which was not identified before. Studies on travel so far, however, have used travel clinics as sources of participants. This means that participants mainly travelled to countries for which vaccinations or malaria prophylaxis is needed and not to countries in Northern America. Such studies, therefore, cannot detect risk associated with travel to the USA. It would be interesting to investigate the prevalence of ESBL-E in the community in the USA. Like in the Netherlands, ESBL-E might be present in the food chain. Different studies reported that >90% of chicken meat in our country is contaminated with ESBLs and we showed that raw vegetables may be contaminated as well.^{19,28–30} Also in the USA the use of antimicrobials is high in the food industry.³¹ Our finding that carriage of strains producing CTX-M-1 was not associated with travel, and therefore was probably acquired in the Netherlands, further points to the possibility of contaminated food as a source of ESBL. It would be interesting to investigate whether the decrease in antibiotic use in animals that was noted recently will reflect in a decrease in CTX-M-1 carriage in humans in the future.³² Like Leistner *et al.*,³³ we found that having an Asian mother is a risk factor for ESBL-E. Admission to a foreign hospital did not stand out as a risk for ESBL-E carriage after adjustment for travel. Hence, in the general population travel seems to be the major risk factor, irrespective of hospitalization.

Several studies describe person-to-person transmission of resistant strains. In three households in our study, the ESBL-carrying *E. coli* strains were genetically identical and carried the same plasmids and ESBL genes, pointing to person-to-person transmission. The presence of different strains and plasmids in two households suggests that acquisition of ESBL-E within households is not due only to strain transmission.

In summary, this study shows that ESBL-E carriage is prevalent in the Dutch community, a worrying finding in a country with low resistance rates in healthcare facilities. Risk factors included use of antimicrobial agents, use of antacids and visits to foreign countries, in particular Asia, Africa and, surprisingly, the USA. That

the use of antacids posed a risk points to ingestion as a mode of acquisition of ESBL-E. Our findings, combined with previous studies that show an abundant presence of ESBL-E in the food chain, warrant more attention to the potential risk to public health of resistant microorganisms in food and water.

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Transparency declarations

None to declare.

Author contributions

N. a. N. and C. M. J. E. V.-G. conceived the study. A. M. K., M. H., J. A. J. W. K., P. H. M. S. and P. J. M. E. critically reviewed the study design and participated in the study. C. M. J. E. V.-G., N. a. N. and E. A. R. did the literature review. E. A. R. collected the data. C. M. J. E. V.-G., A. M. K. and E. A. R. analysed the data. E. A. R. wrote the first draft of the article. C. M. J. E. V.-G., N. a. N., M. H., J. A. J. W. K., P. H. M. S. and P. J. M. E. interpreted data, contributed to report writing and critically reviewed the article. All authors read and approved the final draft of the article.

Supplementary data

Items included in the questionnaire, data extracted from the AGPN database, Tables S1 to S3 and Figure S1 are available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

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