High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints

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Abstract

The aim of this study was to determine the rate of carriage of ESBL-producing Enterobacteriaceae (ESBL-E) in the community in the Netherlands and to gain understanding of the epidemiology of these resistant strains. Faecal samples from 720 consecutive patients presenting to their general practitioner, obtained in May 2010, and between December 2010 and January 2011, were analysed for presence of ESBL-E. Species identification and antibiotic susceptibility testing were performed according to the Dutch national guidelines. PCR, sequencing and microarray were used to characterize the genes encoding for ESBL. Strain typing was performed with amplified fragment length polymorphism (AFLP) and multilocus sequence typing (MLST). Seventy-three of 720 (10.1%) samples yielded ESBL-producing organisms, predominantly *E. coli*. No carbapenemases were detected. The most frequent ESBL was CTX-M-15 (34/73, 47%). Co-resistance to gentamicin, ciprofloxacin and cotrimoxazole was found in (9/73) 12% of the ESBL-E strains. AFLP did not show any clusters, and MLST revealed that CTX-M-15-producing *E. coli* belonged to various clonal complexes. Clonal complex ST10 was predominant. This study showed a high prevalence of ESBL-producing Enterobacteriaceae in Dutch primary care patients with presumed gastrointestinal discomfort. Hence, also in the Netherlands, a country with a low rate of consumption of antibiotics in humans, resistance due to the expansion of CTX-M ESBLs, in particular CTX-M-15, is emerging. The majority of ESBL-producing strains do not appear to be related to the international clonal complex ST131.

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Introduction

Due to the extensive use of beta-lactam antibiotics in human medicine, beta-lactamases have co-evolved with them [1]. Extended-spectrum beta-lactamases (ESBLs) are the main source of acquired antibiotic resistance in Gram-negative bacteria and are of particular concern [2]. These enzymes have a broad spectrum of activity against almost all beta-lactam antibiotics. The genes that encode ESBLs are transferred very efficiently due to their location on plasmids. Furthermore, these ESBL-encoding plasmids frequently bear resistance genes for additional antibiotic classes, thereby posing a significant challenge to antimicrobial therapy [3,4].

Recently, a major increase in the prevalence of ESBL has been observed, mainly due to an increase of CTX-M–type ESBLs [2]. Today organisms producing these enzymes are

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the most common type of ESBL-producing bacteria found in most areas of the world [5]. The classic SHV and TEM enzymes, associated with nosocomial outbreaks, are substituted by CTX-M enzymes, principally in community-acquired infections caused by *Escherichia coli* [6]. This major shift in ESBL epidemiology is observed both in Europe and in other continents [5,6]. An increase in community-onset infections with ESBL-E due to CTX-M-producing *E. coli* is a large problem in many European countries, for example in Spain and France [3,5]. Especially, CTX-M-15 is predominant in community-acquired infections [2,7,8].

The Netherlands is well known for its low rate of resistance, and this also applies to resistance to third-generation cephalosporins, a surrogate marker for ESBL production (EARS-Net, http://wwwecdceuropaeu/en/activities/surveillance/EARS-Net/). Therefore it is interesting to gain insight into the prevalence of ESBLs in a country with a prudent use of antibiotics in human medicine (ESAC-Net. http://wwwecdc europaeu/en/activities/surveillance/ESAC-Net/).

The presence of ESBL-producing Gram-negative bacteria in Dutch retail meat found in recent studies is quite worrying [9,10]. To the best of our knowledge, no data are available on the prevalence of carriage of ESBL-producing Enterobacteriaceae (ESBL-E) in the Dutch community. The aim of this study was to determine the prevalence of ESBL carriage in the primary care population in the region of Amsterdam (a densely populated urban area) and Brabant (a more rural area), to assess the susceptibility of these isolates to common antibiotics that are important for treating community-acquired infections, to characterize the ESBL genes and plasmids involved, and to type the ESBL-positive strains to gain understanding of the epidemiology of this emerging resistance in the Dutch outpatient population.

Materials and Methods

Data collection/study design

Faecal samples, obtained between 12 April and 19 May 2010, and between 21 November 2010 and 9 January 2011, from patients presenting to their general practitioner (GP) with mild gastrointestinal discomfort and/or diarrhoea for more than 3 weeks were analysed. Samples were collected at the ATAL Medical Diagnostic Centre, a laboratory servicing GPs in Amsterdam, and the Microbiological Laboratory of Sint Elisabeth Hospital in Tilburg, a laboratory servicing GPs in the region of Brabant. Faecal samples were inoculated in trypticase soy enrichment broth. Screening for ESBL-producing Enterobacteriaceae (ESBL-E) was performed by inoculation onto a selective screening agar, the EbSA ESBL screening agar (Cepheid Benelux, Apeldoorn, the Netherlands) [11,12]. All broths and plates were incubated overnight at 37°C.

Antimicrobial susceptibility testing

Species identification and antibiotic susceptibility testing of colonies growing on the EbSA plates were performed with the Vitek 2 system (Vitek ID and Vitek AST; bioMérieux, Marcy l'Etoile, France). The MIC breakpoints used for interpreting the results were according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [13]. ESBL production was confirmed with a combination disk diffusion test (Rosco, Taastrup, Denmark) and the E-test on Mueller-Hinton agar, interpreted according to the Dutch national guidelines [14].

Molecular characterization and ESBL typing

The presence of ESBL genes was confirmed by molecular analysis of all phenotypically confirmed ESBL-positive strains. Bacterial DNA was isolated with the QIAamp DNA mini kit (Qiagen, Venlo, the Netherlands). Isolates obtained in Amsterdam were screened for ESBL resistance genes at the VUmc by Check-KPC ESBL microarray to identify CTX-M, TEM and SHV ESBL genes (Check-Points Health BV, Wageningen, the Netherlands) [15]. Isolates obtained at Amphia Hospital were screened with Check-MDR CT103 (Check-Points Health BV), a newly developed microarray that enables the detection of two commonly encountered ESBLs: CTX-M-1 and CTX-M-15. In isolates obtained in Amsterdam ESBL-encoding genes were characterized by polymerase chain reaction (PCR) at the VUmc, followed by sequencing (BaseClear, Leiden, the Netherlands), as described by Naiemi *et al.* [16]. Sequences were analysed with Bionumerics software (version 6.5; Applied Maths, Sint-Martens-Latem, Belgium) and compared with sequences in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST) and Lahey (http://www.lahey.org/studies/).

Characterization of plasmids

Identification of plasmids was performed by PCR-based replicon typing for the eight most prevalent plasmids [17]. This method allows the examination of plasmids conferring drug resistance by typing them by incompatibility groups in a multiplex PCR setting.

Epidemiological typing

Seventy ESBL-positive *E. coli* strains were analysed for genetic relatedness by amplified-fragment length polymorphism (AFLP). This DNA fingerprinting technique and the protocol used has been described by Savelkoul *et al.* [18]. AFLP banding patterns were analysed as described previously with Bionumerics software (Applied Maths).

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Multilocus sequencing typing (MLST) was performed on all the *E. coli* isolates by using seven conserved housekeeping genes (adkA, fumC, gyrB, icd, mdh, purA and recA) as described by Wirth *et al.* [19]. The MLST protocol is detailed at http://mlst.ucc.ie/mlst/dbs/Ecoli. Clonal complexes were determined by including whole *E. coli* MLST data using eBURST v3 (http://eburst.mlst.net).

Statistical analyses

Statistical analyses were performed with SPSS, version 15.0. Principal components analysis (PCA) was performed with Bionumerics version 6.5.

Results

In total, 720 faecal samples were obtained from 720 consecutive patients presenting to their GP with complaints of gastrointestinal discomfort. Analysis of the samples for diagnosis was performed separately in a routine setting. These samples were considered to be community based because the specimens were obtained from a laboratory serving only general practitioners. Data regarding the patients' history were not available. The median age of patients was 46 years (range, 2–87); 53% were female. Patients lived in different geographical areas and were not institutionalized.

In the region of Amsterdam, 50 out of 471 (10.6%, 9.7–11.5 95% CI) samples yielded ESBL-E: 49 *Escherichia coli* isolates and one *Shigella sonnei* isolate. In the region of Brabant 23 out of 249 (9.2%, 8.1–10.3 95% CI) samples yielded ESBL-E (Table 1). These included 21 *E. coli* and two *Klebsiella pneumoniae* isolates. Hence the frequency of ESBL-producing isolates was the same in both regions. No strains with reduced sensitivity to imipenem or meropenem were detected. The microarray revealed that both in Amsterdam and in Brabant the isolates contained genes belonging to the CTX-M family; $bla_{CTX\text{-}M\text{-}15}$ was predominant, found in 34/73 (47%) isolates (see also Table 2). We also performed PCR and sequencing on the 50 strains isolated in Amsterdam. This showed four $bla_{CTX\text{-}M\text{-}1}$, 24 $bla_{CTX\text{-}M\text{-}15}$, one $bla_{CTX\text{-}M\text{-}14}$, seven $bla_{CTX\text{-}M\text{-}14b}$, four $bla_{CTX\text{-}M\text{-}27}$, one $bla_{TEM\text{-}52}$, one $bla_{SHV\text{-}2a}$ and one $bla_{SHV\text{-}12}$ genes. One gene belonging to the CTX-M-1 family remained unidentified. No difference in the distribution of these genes in the two regions was seen.

The majority of the isolates showed co-resistance to co-trimoxazole, followed by ciprofloxacin and gentamicin. A summary of the co-resistances is shown in Table 3. Twelve per cent (9/73) of the ESBL-producing isolates were multiresistant (i.e. resistant to at least one agent in three or more antimicrobial categories (aminoglycosides, quinolones and co-trimoxazole)) [20]. Thirty per cent (22/73) of strains were intermediately susceptible to nitrofurantoin. All isolates were susceptible to meropenem and imipenem.

The ESBL-producing strains were genotyped by AFLP to assess their diversity. The AFLP-based dendogram showed a few pairs of isolates with identical AFLP patterns (Fig. 1). All patients with strains with related AFLP patterns lived in different geographical areas. Further analysis by PCA did not reveal any clusters (Figure not shown).

The results of MLST are shown in Fig. 2. Multilocus sequence typing revealed 43 different sequence types, and included nine new sequence types not present yet in the

TABLE 1. Number of patients and ESBL-producing bacterial isolates in the urban and rural communities

	Urban *n* (%)	Rural *n* (%)
Number of patients	471	249
ESBL-positive bacterial isolates	50 (10.6)	23 (9.2)

TABLE 3. Co-resistances in ESBL-producing isolates

Co-resistance ESBL	*n* = 73 (%)
cotrimoxazole	51 (70)
ciprofloxacin	31 (43)
gentamicin	21 (29)
gentamicin/ciprofloxacin/cotrimoxazole	9 (12)

TABLE 2. Distribution of ESBL genes and plasmids

		Plasmids							
ESBL group*	N	ColE	FrepB	FIB	ColEtp	IncI1	FIA	R	FIIs
CTX-M-1 group	40	20	16	16	14	14	14	5	1
CTX-M-2 group	2	1	0	1	0	0	1	0	0
CTX-M-9 group	18	10	14	9	8	4	7	1	0
SHV	10	6	6	3	4	7	2	1	0
TEM	2	0	0	0	1	2	0	1	0
Total	72*	37	36	29	27	27	24	8	1

* The genes belonging to the CTX-M-1 group were six $bla_{CTX\text{-}M\text{-}1}$ and 34 $bla_{CTX\text{-}M\text{-}15}$ genes. One gene belonging to the CTX-M-1 group remained unidentified. TEM and SHV were ESBL (no wild-type).

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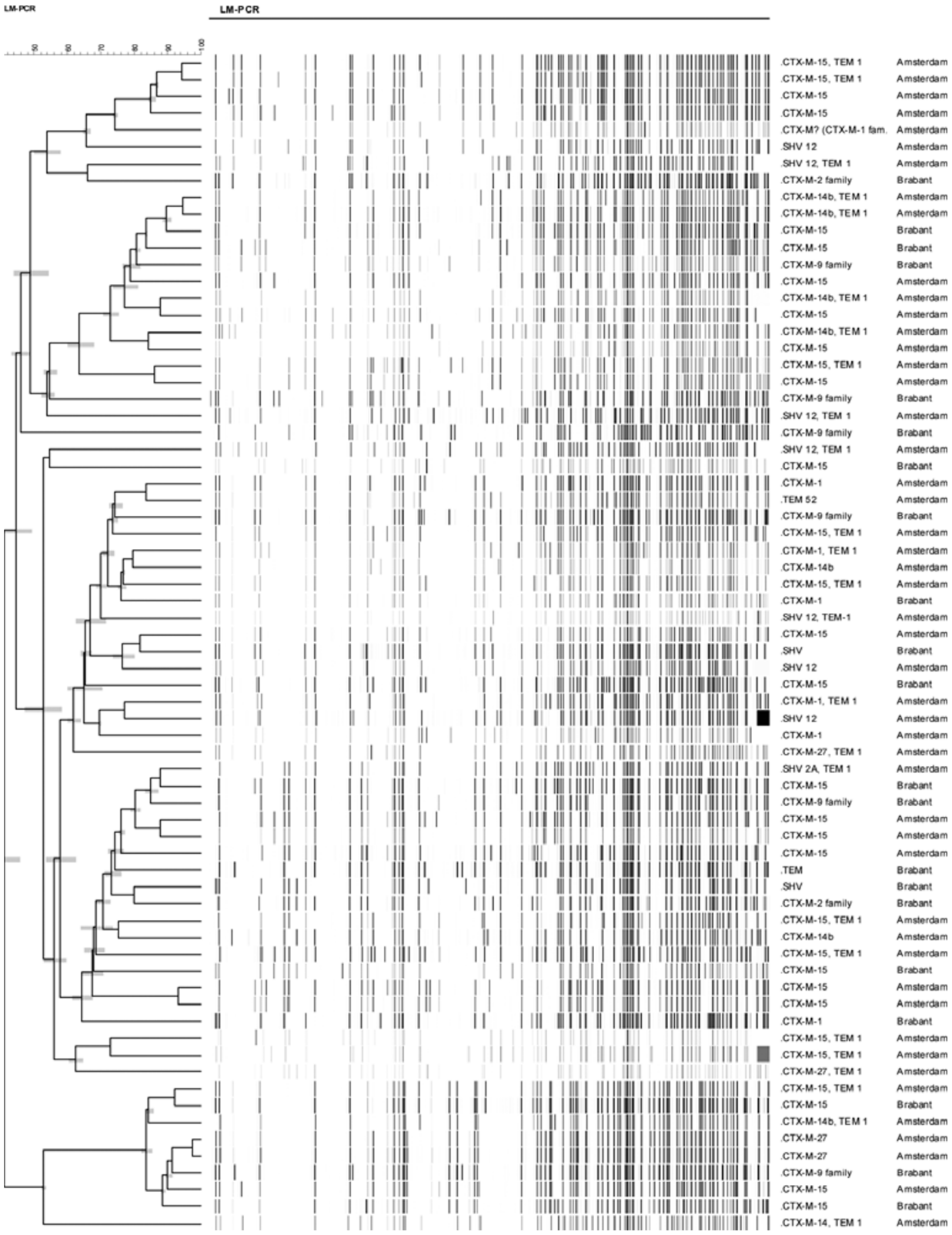

FIG. 1. Dendrogram showing the relatedness of AFLP patterns. Seventy ESBL-positive *E. coli* strains were analysed for genetic relatedness by amplified fragment length polymorphism (AFLP).

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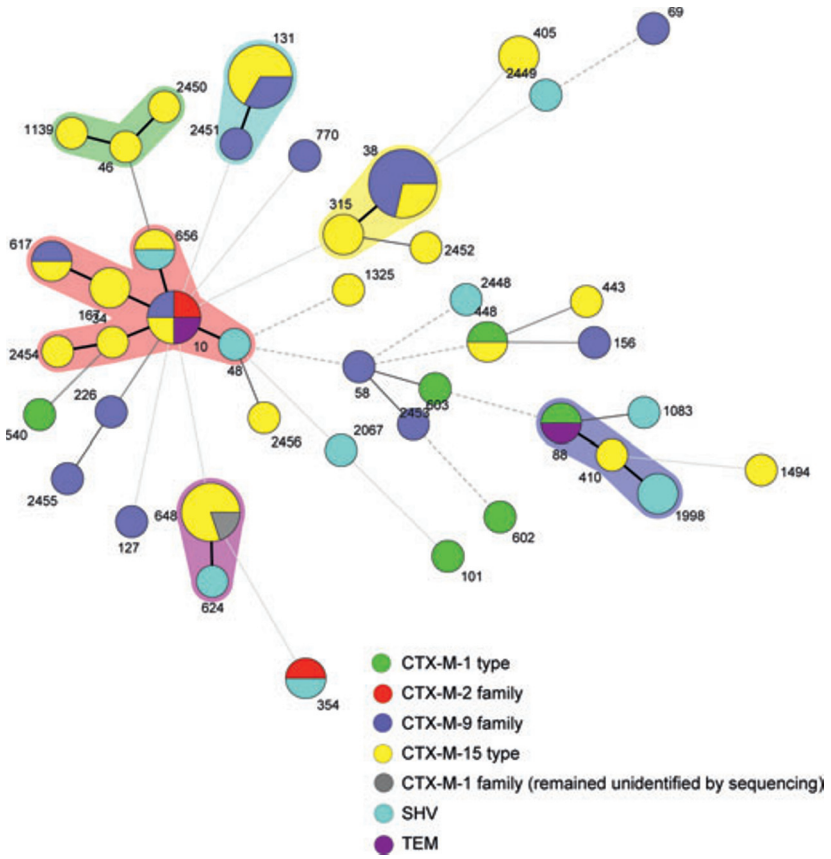

FIG. 2. Multilocus sequence typing of *E. coli* isolates (n = 70). The numbers indicate the different sequence types. Thick connecting lines indicate single-locus variants; thin connecting lines indicate variants with two or three loci difference; dashed connecting lines indicate variants with four loci difference; five loci differences are indicated by dotted connecting lines. Shadowing indicates that more than one sequence type belongs to the same complex.

E. coli MLST database. Most isolates belonged to sequence types ST38 (seven isolates; 10%), ST131 (six isolates; 8.6%), ST648 (five isolates; 7.1%) and ST10 (four isolates; 5.7%). The main clonal complexes according to the MLST database (including sequence types with one locus difference) were ST10 (12 isolates; 17.1%) and ST38 (nine isolates; 12.8%). All but one cluster harboured different ESBL genes. CTX-M-15 was scattered over all the ST types. There was no difference in MLST types between Amsterdam and Brabant (Fig. 3).

The distribution of ESBL genes and plasmids is described in Table 2. ColE, FIB and FIA were the most prevalent plasmids.

Discussion

This study showed that one out of ten Dutch outpatients with gastrointestinal complaints carried ESBL-producing Enterobacteriaceae in their faeces. This was an unexpected high prevalence because the Netherlands is a country well known for its prudent antimicrobial use in human clinical practice, both in the outpatient setting and in the hospital (ESAC-Net, http://wwwecdceuropaeu/en/activities/surveillance/ESAC-Net/). No carbapenemase-producing strains were detected. It is well-known that the prevalence of ESBL-E differs markedly between countries. To date a high prevalence is found in clinical isolates in southern European countries, in Turkey and in India while the prevalence is low in northern European countries, including the Netherlands (EARS-Net, http://wwwecdceuropaeu/en/activities/surveillance/EARS-Net/) [6,21]. Few studies have investigated the faecal carriage rate of ESBL-E in non-hospitalized patients. Valverde *et al.* [22] showed that the faecal carriage rate of ESBL-E in a Spanish community was 5.5% in 2003. In the same study a prevalence of 3.7% was seen in healthy volunteers [22]. Hence the prevalence we measured is high compared with surveys performed previously in surrounding European countries. Possibly, the high rate we measured in our more recent study is due to the steep increase in ESBL-producing

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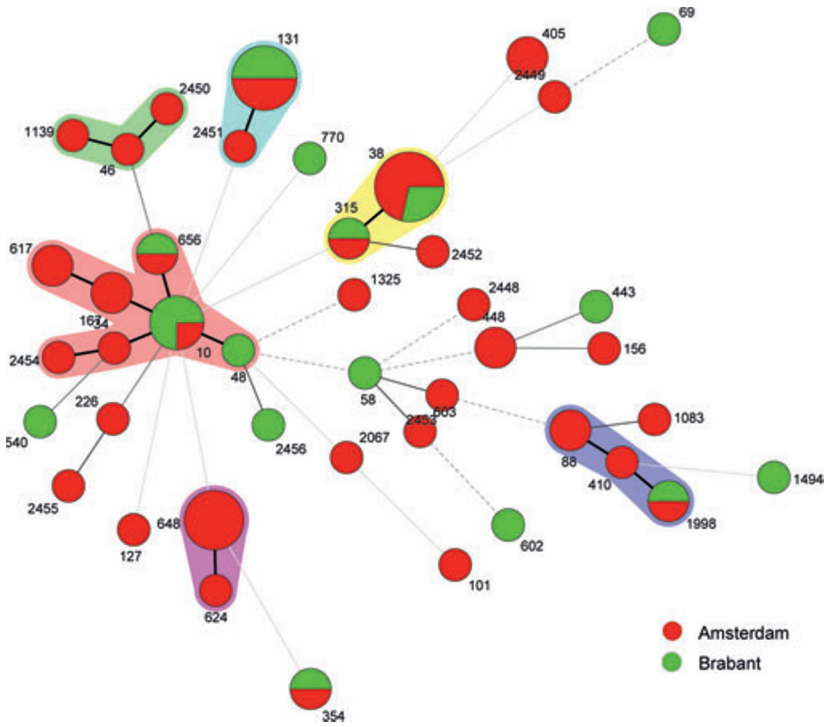

FIG. 3. Multilocus sequencing typing showing several clusters in *E. coli* isolates (n = 70) obtained from faecal samples: Amsterdam vs. Brabant.

strains that is being observed over the last few years all over the world.

The prevalence of rectal carriage among hospitalized patients in the Netherlands in previous years was lower. Before 2000 a prevalence of <1% was recorded in Dutch hospitals. This increased to 4–8% after 2005 [23,24]. Recently, Overdevest *et al.* [10] found a percentage of 6% in hospitalized patients and 4% in patients at time of admission to the hospital. This high percentage of carriage of ESBL-producing bacteria on admission already pointed towards a community reservoir. Initially, ESBL-producing Enterobacteriaceae were considered an in-hospital problem, but now this study also reveals an unexpected increase in the Dutch community. Therefore, our results confirm the worrisome element that a continuous influx from the community into the hospital might be possible [3,4].

In our study, the most prevalent ESBLs were CTX-M. This is consistent with the worldwide dissemination of this type of ESBL and is comparable with the CTX-M pandemic in the community in other European countries [3,6,8]. The most prevalent CTX-M ESBL in our survey was CTX-M-15, again as noticed elsewhere [6,7,21,25]. In several countries the expansion of CTX-M-15-producing *E. coli* is due to the worldwide pandemic clone ST131 [26]. In contrast, the *E. coli* strains that we identified belonged to multiple sequence type clonal complexes and the presence of CTX-M-15 in these community-acquired isolates was scattered over different clusters. AFLP and PCA confirmed the data obtained with MLST, and showed that there was no epidemiological relationship between the strains.

Next to clonal dispersion, the acquisition of multidrug-resistant plasmids plays a pivotal role in the dissemination of CTX-M-15-producing ESBLs. Various replicons, especially those widely distributed among *E. coli* strains, could be involved in part of this dissemination process. It has been proposed that most of the CTX-M-15 enzymes are encoded on IncF replicons (FIA, FIB and FII) [27]. Indeed, also in our study IncF replicons, FIB and FIA-type plasmids, were associated with the presence of $bla_{\text{CTX-M-15}}$. Taken together, MLST and plasmid replicon typing point to both dispersion of several different clones and to spread of mobile genetic elements as drivers of the dissemination of ESBL genes in the Dutch community.

The distribution of ESBL genes and plasmids in carriers of ESBL-positive *E. coli* isolates in this outpatient population differs from the distribution described recently in *E. coli* strains recovered from patients from hospitals and long-term care facilities in the Netherlands in 2009 and 2010. In these studies $bla_{\text{CTX-M-1}}$ and IncI1 were the most frequent genes and plasmids [9,10]. It has been postulated that these are acquired through contaminated poultry, because Dutch chicken meat has been shown to be heavily colonized with

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E. coli strains containing $bla_{CTX\text{-}M\text{-}1}$ and IncI1: 94% of chickens are colonized with these strains [9,10,28]. In human isolates from other countries $bla_{CTX\text{-}M\text{-}15}$ is the most frequent gene [7]. In our patient population, strains producing CTX-M-15 were predominant. Possibly, the difference between our study and the previous Dutch studies is due to the difference in patient populations; we analysed faecal samples from outpatients presenting to their GP with complaints of gastrointestinal discomfort. In Dutch general practice faeces cultures are only requested for patients with gastrointestinal complaints that last for more than 10 days or gastrointestinal complaints after travel to foreign countries, especially to the (sub)tropics [29]. Various studies show that foreign travel, especially to countries with a high prevalence of ESBL-E, is a risk factor for colonization with ESBL-E [30–32]. A high prevalence of faecal carriage of ESBL-producing strains is observed in particular in patients with travellers' diarrhoea [30,32]. We have no data on travel history, previous use of antibiotics or recent hospitalization for the individual patients in our study, but Dutch general practitioners very seldom prescribe antibiotics for treatment of gastrointestinal complaints, according to the algorithms laid down in their own professional standards [29]. Thus, knowing that diagnostics for diarrhoea is mainly performed after foreign travel, and that use of antibiotics in the treatment of diarrhoea is very unusual in Dutch general practice, it seems likely that foreign travel might be responsible for at least part of the prevalence of ESBL-E in Dutch outpatients. Whatever the source of the resistance, however, the prevalence of ESBL-E in this specific patient population was worryingly high. At the same time, it is reassuring that carbapenemase-producing strains were still absent in the community.

The association of ESBL production with multidrug resistance adds to the magnitude of the problem [5,22]. In this study we noted that nearly half of the ESBL-producing strains were resistant to ciprofloxacin, and nearly three-quarters were resistant to cotrimoxazole. None of the isolates were resistant to nitrofurantoin, the drug currently recommended for uncomplicated urinary tract infections in the Netherlands.

In conclusion, this study showed an unexpected high prevalence of ESBL-E in Dutch outpatients presenting to their GP with gastrointestinal complaints. The present study emphasizes that multidrug-resistant CTX-M-producing (in particular CTX-M-15) *E. coli* are present in the community even in the Netherlands, a country well known for its prudent antimicrobial use in human medicine. Therefore it is important to monitor systematically the epidemiology of ESBL-E in hospitals, in the community and in other reservoirs such as food and the environment.

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Transparency Declaration

Nothing to declare.

References

1. Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin Infect Dis* 1997; 24 (suppl 1): S19–S45.
2. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 2008; 8: 159–166.
3. Rodriguez-Bano J, Navarro MD, Romero L, *et al.* Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 2004; 42: 1089–1094.
4. Ben-Ami R, Schwaber MJ, Navon-Venezia S *et al.* Influx of extended-spectrum beta-lactamase-producing Enterobacteriaceae into the hospital. *Clin Infect Dis* 2006; 42: 925–934.
5. Rossolini GM, D'Andrea MM, Mugnaioli C. The spread of CTX-M-type extended-spectrum beta-lactamases. *Clin Microbiol Infect* 2008; 14 (suppl 1): 33–41.
6. Livermore DM, Canton R, Gniadkowski M *et al.* CTX-M: changing the face of ESBLs in europe. *J Antimicrob Chemother* 2007; 59: 165–174.
7. Coque TM, Novais A, Carattoli A *et al.* Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* 2008; 14: 195–200.
8. Arpin C, Quentin C, Grobost F *et al.* Nationwide survey of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the French community setting. *J Antimicrob Chemother* 2009; 63: 1205–1214.
9. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J *et al.* Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin Microbiol Infect* 2011; 17: 873–880.
10. Overdevest I, Willemsen I, Rijnsburger M *et al.* Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg Infect Dis* 2011; 17: 1216–1222.
11. Overdevest IT, Willemsen I, Elberts S, Verhulst C, Kluytmans JA. Laboratory detection of extended-spectrum beta-lactamase-producing

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Enterobacteriaceae: evaluation of two screening agar plates and two confirmation techniques. *J Clin Microbiol* 2011; 49: 519–522.
12. Naiemi NA, Murk JL, Savelkoul PH, Vandenbroucke-Grauls CM, Debets-Ossenkopp YJ. Extended-spectrum beta-lactamases screening agar with AmpC inhibition. *Eur J Clin Microbiol Infect Dis* 2009; 28: 989–990.
13. Clinical and Laboratory Standard Institute. *Performance standards for antimicrobial susceptibility testing*. CLSI M100-S18, Wayne, PA, USA: CLSI, 2008.
14. al Naiemi N, Cohen Stuart J, Leverstein van Hall M. NVMM guideline of the Dutch society for medical microbiology for screening and confirmation of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae. *Ned Tijdschr Med Microbiol* 2008; 16: 23–28.
15. Cohen Stuart J, Dierikx C, Al Naiemi N *et al.* Rapid detection of TEM, SHV and CTX-M extended-spectrum beta-lactamases in Enterobacteriaceae using ligation-mediated amplification with microarray analysis. *J Antimicrob Chemother* 2010; 65: 1377–1381.
16. Naiemi NA, Duim B, Savelkoul PH *et al.* Widespread transfer of resistance genes between bacterial species in an intensive care unit: implications for hospital epidemiology. *J Clin Microbiol* 2005; 43: 4862–4864.
17. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; 63: 219–228.
18. Savelkoul PH, Aarts HJ, de Haas J *et al.* Amplified-fragment length polymorphism analysis: the state of an art. *J Clin Microbiol* 1999; 37: 3083–3091.
19. Wirth T, Falush D, Lan R *et al.* Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol* 2006; 60: 1136–1151.
20. Magiorakos AP, Srinivasan A, Carey RB *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268–281.
21. Hawkey PM. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. *Clin Microbiol Infect* 2008; 14 (suppl 1): 159–165.
22. Valverde A, Coque TM, Sanchez-Moreno MP, Rollan A, Baquero F, Canton R. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. *J Clin Microbiol* 2004; 42: 4769–4775.
23. Mouton J, Voss A, Arends J, Bernards S, on behalf of the ONE study group. Prevalence of ESBL in The Netherlands: the ONE study. 17th ESCMID 2007, ICC, Munich, Germany.
24. al Naiemi N, Bart A, de Jong MD *et al.* Widely distributed and predominant CTX-M extended-spectrum beta-lactamases in Amsterdam, The Netherlands. *J Clin Microbiol* 2006; 44: 3012–3014.
25. Pitout JD. Infections with extended-spectrum beta-lactamase-producing Enterobacteriaceae: changing epidemiology and drug treatment choices. *Drugs* 2010; 70: 313–333.
26. Peirano G, Pitout JD. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. *Int J Antimicrob Agents* 2010; 35: 316–321.
27. Marcade G, Deschamps C, Boyd A *et al.* Replicon typing of plasmids in *Escherichia coli* producing extended-spectrum beta-lactamases. *J Antimicrob Chemother* 2009; 63: 67–71.
28. Overdevest ITMA, Kluytmans J. Extended-spectrum beta-lactamase producing Enterobacteriaceae in retail meat. *Clin Microbiol Infect* 2010; 16 (suppl 2): S372.
29. NHG-Standaarden. Standards of the Dutch college of general practitioners [in Dutch]. 2011.
30. Tangden T, Cars O, Melhus A, Lowdin E. Foreign travel is a major risk factor for colonization with *Escherichia coli* producing CTX-M-type extended-spectrum beta-lactamases: a prospective study with Swedish volunteers. *Antimicrob Agents Chemother* 2010; 54: 3564–3568.
31. Laupland KB, Church DL, Vidakovich J, Mucenski M, Pitout JD. Community-onset extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*: importance of international travel. *J Infect* 2008; 57: 441–448.
32. Tham J, Odenholt I, Walder M, Brolund A, Ahl J, Melander E. Extended-spectrum beta-lactamase-producing *Escherichia coli* in patients with travellers' diarrhoea. *Scand J Infect Dis* 2010; 42: 275–280.

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