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.

Keywords: Antibiotic resistance, community-acquired, ESBL-producing Enterobacteriaceae, gastrointestinal complaints, outpatient population
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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

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).