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Widely Distributed and Predominant CTX-M Extended-Spectrum β-Lactamases in Amsterdam, The Netherlands

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Three hundred sixty *Enterobacteriaceae* and nonfermenting gram-negative bacilli, isolated during one week in May 2004 at five hospitals in Amsterdam, The Netherlands, were evaluated for the presence of extended-spectrum beta-lactamases (ESBLs). A prevalence of 7.8% was found, in contrast to the 1% observed in 1997. CTX-M ESBLs dominated, and four types were identified in 18 isolates.

Extended-spectrum beta-lactamases (ESBLs) are of growing concern. More than 200 different natural ESBL variants are known at present, and their evolution has been so fast that a website (http://www.lahey.org/studies) was initiated to report new progress in this field (18). While ESBLs were initially found mainly in *Klebsiella pneumoniae* and *Escherichia coli*, an increasing variety of gram-negative species have been shown to harbor such resistance mechanisms (7, 8). In recent years, ESBLs have become more prevalent in AmpC-producing species (1, 16). In spite of the worldwide use of β-lactam antimicrobial agents, which is considered the main reason for the emergence of ESBLs (2), the distributions of ESBLs are far from uniform. There are considerable geographical differences in prevalence of ESBLs in Europe; in general, the prevalence is lower in northern European countries than in eastern and southern European countries (5). In a French multicenter study (1996 to 2000), the prevalence of ESBLs was found to be 11.4% in *K. pneumoniae* isolates and 47.7% in *Enterobacter aerogenes* isolates (1). The prevalence of ESBL-producing *E. coli* and *Klebsiella* spp. isolates in Dutch hospitals was less than 1% in 1997 (17).

(Some of the information in this report was presented at the annual meeting of the NVMM/NVvM, April 2005.)

The aim of the present study was to evaluate the prevalence and the molecular epidemiology of ESBLs in Amsterdam, The Netherlands. During one week in 2004, five microbiological laboratories of five hospitals (Academic Medical Center [AMC, 1,002 beds], Vrije Universiteit Medical Center [VUMC, 733 beds], Onze Lieve Vrouwe Gasthuis [OLVG, 530 beds], Slotervaartziekenhuis [SLZ, 490 beds], and Sint Lucas Andreas Ziekenhuis [SLAZ, 551 beds]) in Amsterdam participated. *Enterobacteriaceae* and nonfermenting gram-negative bacilli cultured from clinical specimens were obtained, and one isolate per species per patient was included in the study. Three hundred sixty strains were collected, comprising 14 species (Table 1): 144 *E. coli* strains, 47 *Klebsiella* spp. strains, 94 non-*E. coli* non-*Klebsiella Enterobacteriaceae*, and 75 nonfermenters (Table 1). The following numbers of strains were isolated from each hospital: 123 from AMC, 70 from VUMC, 78 from OLVG, 63 from SLZ, and 26 from SLAZ. The strains were considered nonclonal according to the information from the participating hospitals, the antimicrobial susceptibility pattern, and common biochemical reactions.

Screening for ESBL production was performed with the cefpodoxime disk diffusion method (3). When a bacterial strain showed a zone diameter of ≤20 mm for cefpodoxime, we considered the isolate a suspected ESBL producer. The phenotypic confirmation for ESBL production was determined with a double and combined disk test (N. al Naiemi et al., submitted for publication), which includes disks of ceftazidime, cefotaxime, cefpodoxime, and cefepime placed around a disk containing amoxicillin plus clavulanate. A disk of ceftazidime plus clavulanate was also included. Susceptibility tests for gentamicin, tobramycin, ciprofloxacin, cotrimoxazole, imipenem, and piperacillin plus tazobactam were performed with Neo-Sensitabs from Rosco (Taastrup, Denmark), according to CLSI guidelines (3).

Fifty-two strains showed decreased susceptibility for cefpodoxime. Of these strains, 28 produced ESBLs and showed decreased susceptibility to several other antimicrobial agents (Table 2). The prevalence of ESBL-producing strains was 7.8% (Table 1). The prevalence for each hospital was as follows: 9.8% (12/123) for AMC, 7.1% (5/70) for VUMC, 6.4% (5/78) for OLVG, 6.3% (4/63) for SLZ, and 7.7% (2/26) for SLAZ.

The presence of bla_{SHV}, bla_{TEM}, and bla_{CTX-M} genes was confirmed by PCR and sequence analysis as described in a previous study (9). To prevent carryover contamination, the uracil system was applied. Well-characterized plasmid DNA was used as a positive control and ultra-high-purity water as a negative control. Eighteen isolates contained bla_{CTX-M} and 10 isolates contained bla_{SHV} ESBL genes. No bla_{TEM} ESBL genes were detected (Table 2).

The present survey shows that the overall prevalence of ESBL-producing gram-negative organisms in hospitals in the Amsterdam region in 2004 was still below 10% but was much higher than the prevalence as determined in a nationwide

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TABLE 1. Species distribution of gram-negative isolates

Species	No. of strains	Total no. of strains	No. of ESBL-positive strains	% ESBL positivity
Escherichia coli	144		8	
Total (*E. coli*)		144	8	5.6
Klebsiella pneumoniae	32		1	
Klebsiella oxytoca	15			
Total (*Klebsiella* spp.)		47	1	2.1
Enterobacter cloacae	37		8	
Enterobacter aerogenes	4		1	
Enterobacter agglomerans group	1			
Citrobacter spp.	13		2	
Morganella morganii	9		2	
Serratia spp.	6			
Proteus mirabilis	23		1	
Proteus vulgaris	1			
Total (non-*E. coli* non-*Klebsiella Enterobacteriaceae*)		94	14	14.9
Stenotrophomonas maltophilia	5		2	
Acinetobacter spp.	10		2	
Pseudomonas aeruginosa	60		1	
Total (nonfermenters)		75	5	6.7
Total (all species)		360	28	7.8

survey in 1997. The previous survey was performed in eight university hospitals, including the Academic Medical Center (AMC) which participated in the current study, and three large regional laboratories and showed that the prevalence of ESBL among isolates of *E. coli* and *Klebsiella* spp. was <1% (17). During the 1997 study, no ESBLs were found in the AMC. However, ESBL-producing *Enterobacteriaceae*, including *E. coli* and *Klebsiella* spp. organisms, have been isolated on several occasions during the past 5 years (9). In this study, the highest prevalence of ESBL producers, which amounted to 15%, was observed with non-*E. coli* non-*Klebsiella Enterobacteriaceae* (Table 1). This is of concern, since spread of resistance among these species is known to occur through plasmids and these species are therefore particularly prone to spread and cause outbreaks (4, 9, 10, 11, 15). To date, SHV-type ESBLs dominated in surveys of resistant clinical isolates in Europe and North America (12, 20). This was also the case in one of the participating hospitals in Amsterdam (AMC) in 2000 (9). In 2003, CTX-M ESBL genes were detected in the AMC for the first time, and the present study shows the dis-

TABLE 2. Phenotypic and genotypic characteristics of the ESBL-positive isolates

Hospital and organism	DCDT result[a]	ESBL gene	Antimicrobial susceptibility[b]							
			CAZ	CTX	GEN	TOB	CIP	COT	IMI	PIP-TAZ
SLAZ										
E. coli	+	CTX-M-2	S	R	S	S	S	R	S	S
E. coli	+	CTX-M-2	S	R	R	S	R	R	S	S
SLZ										
E. coli	+	SHV-12	I	I	R	R	R	R	S	R
Enterobacter cloacae	+	SHV-12	R	R	S	S	S	S	S	R
E. cloacae	+	CTX-M-2	S	R	S	S	S	S	S	R
Stenotrophomonas maltophilia	+	SHV-12	I	I	S	S	S	S	R	S
OLVG										
Citrobacter freundii	+	SHV-12	R	I	I	I	R	R	S	R
Morganella morganii	+	SHV-12	R	R	S	S	S	S	S	R
E. coli	+	SHV-12	I	S	S	S	R	R	S	S
E. cloacae	+	SHV-12	R	R	S	S	S	S	S	R
E. cloacae	+	SHV-12	R	R	S	S	S	S	S	R
VUMC										
E. cloacae	+	CTX-M-1	S	R	S	R	S	S	S	R
E. cloacae	+	CTX-M-9	S	R	S	R	S	S	S	R
Citrobacter koseri	+	CTX-M-2	I	R	S	S	S	S	S	S
Proteus mirabilis	+	CTX-M-1	S	I	S	S	S	R	S	R
E. cloacae	+	CTX-M-15	R	R	S	S	I	S	S	I
AMC										
E. coli	+	CTX-M-15	R	R	R	R	R	R	S	R
M. morganii	+	CTX-M-15	R	R	R	R	R	R	S	R
E. cloacae	+	CTX-M-2	I	R	S	S	S	S	S	R
Acinetobacter spp.	+	CTX-M-2	I	R	S	S	S	S	S	R
E. aerogenes	+	CTX-M-15	R	R	S	S	S	S	S	R
Acinetobacter spp.	+	CTX-M-15	R	R	S	S	S	S	S	S
E. coli	+	CTX-M-2	I	R	S	S	S	S	S	R
E. coli	+	SHV-2	I	I	S	S	S	R	S	R
Klebsiella pneumoniae	+	SHV-12	I	I	R	R	R	R	S	R
E. coli	+	CTX-M-1	S	I	S	S	S	S	S	S
Pseudomonas aeruginosa	+	CTX-M-1	R	R	I	S	S	S	S	S
S. maltophilia	+	CTX-M-1	R	R	S	S	S	S	R	R

[a] DCDT, double and combined disk test; +, positive.
[b] CAZ, ceftazidime; CTX, cefotaxime; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; COT, cotrimoxazole; IMI, imipenem; PIP-TAZ, piperacillin-tazobactam; S, susceptible; R, resistant; I, intermediate susceptible.

semination of four different CTX-M ESBLs in nine different species, including the nonfermenting gram-negative species (Table 1). This wide occurrence is remarkable. Pitout et al. (14) reported the spread of *Enterobacteriaceae* carrying different CTX-M ESBLs, originating from the community. Possibly, a similar phenomenon is occurring in Amsterdam. However, we cannot exclude the possibility of the spread of these different groups of CTX-M enzymes between the medical centers or through other routes, e.g., patient transfer.

The emergence and the increase in the prevalence of CTX-M ESBLs is remarkable and may be correlated with the increased use of cefotaxime, which has been suggested to play an important role in the selection for CTX-M ESBLs (13, 19). The gentamicin resistance rate in ESBL producers in this study was high compared to that found among all gram-negative isolates from patients in Dutch hospitals (6) and confirms that the use of non-β-lactam antibiotics for treatment of infections caused by ESBL-producing pathogens is limited and that only a few agents, such as carbapenems, provide solid coverage. More rational analysis of antibiotic policies and of risk factors for carriage of ESBL-producing pathogens among patients is required.

The present study suggests that ESBLs are a growing problem in Amsterdam and that the establishment of these baseline data is crucial for the evaluation of the epidemiology of ESBLs and the antibiotic policy in this region.

N. al Naiemi participated in the phenotypic and molecular typing of strains and genes. N. al Naiemi, A. Bart, M. D. de Jong, C. M. Vandenbroucke-Grauls, and B. Duim participated in the development of a research strategy, directed the analyses, and led the writing of the manuscript.

P. C. Wever, Y. J. Debets-Ossenkopp, P. J. G. M. Rietra, L. Spanjaard, and A. J. Bos participated in collection and identification of the bacterial isolates. All authors appraised and approved the final report.

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