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Development and clinical validation of a molecular diagnostic assay to detect CTX-M-type β-lactamases in Enterobacteriaceae

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ABSTRACT

Enterobacterial isolates producing CTX-M β-lactamases have recently emerged worldwide in the community and hospital settings. Because of the significant public health implications, the spread of organisms producing CTX-M enzymes merits close monitoring with enhanced surveillance efforts. A molecular diagnostic assay using two different sets of primers simultaneously for the detection of all $bla_{\text{CTX-M}}$-like β-lactamase genes was developed. This assay repeatedly demonstrated 100% sensitivity, specificity and positive and negative predictive values for detecting different CTX-M enzymes in well-characterised strains that included producers of VEB-, TEM- and SHV-type extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC enzymes. The majority (132/240; 55%) of ESBL-producing enterobacterial isolates recovered in the Calgary Health Region during 2003 and 2004 were positive for $bla_{\text{CTX-M}}$ genes, including 81 (61%) positive for the CTX-M-9 group, 49 (37%) for the CTX-M-1 group, and two (2%) for the CTX-M-2 group. The CTX-M-specific PCR assay was reproducible and easy to use. It can be introduced in a clinical or reference laboratory to track and monitor the spread of organisms producing CTX-M enzymes in the community and hospital settings.

Keywords β-Lactamase, CTX-M enzymes, detection, Enterobacteriaceae, PCR, surveillance

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INTRODUCTION

Enterobacteriaceae, especially *Klebsiella* spp. producing extended-spectrum β-lactamases (ESBLs) belonging to the SHV and TEM groups, have been recognised since the 1980s as a major cause of hospital-acquired infections [1]. More recently, Enterobacteriaceae (mostly *Escherichia coli*) producing novel ESBLs, e.g., the CTX-M enzymes, have emerged within the hospital and community settings as an important cause of urinary tract infection [2,3]. The CTX-M β-lactamases, of which there are now more than 40 types, can be divided into five groups based on their amino-acid identities [4]: the CTX-M-1 group (including CTX-M-1, -3, -10, -12, -15, -28 and -30, and FEC-1); the CTX-M-2 group (including CTX-M-2, -4, -5, -6, -7 and -20, and Toho-1); the CTX-M-8 group (including CTX-M-8); the CTX-M-9 group (including CTX-M-9, -13, -14, -16, -17, -19, -21, -24 and -27, and Toho-2); and the CTX-M-25 group (including CTX-M-25 and -26).

Surveys from several countries, e.g., Canada [5,6], Spain [7–9], France [10], Korea [11] and the UK [12], have shown that CTX-M-producing *Esch. coli* strains, isolated from hospital and community settings, often exhibit co-resistance to trimethoprim–sulphamethoxazole, tetracycline, gentamicin and ciprofloxacin. Woodford *et al.* [12] identified an epidemic clone of *Esch. coli* producing CTX-M-15 that had become widely distributed throughout the UK [12], while a Canadian study described a CTX-M-14-producing strain of *Esch. coli* that was the cause of a community-wide clonal outbreak of urinary tract

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infection during 2000 and 2001 [13]. Similar findings involving *Esch. coli* strains producing CTX-M-9 and CTX-M-14 have been reported from Spain [14,15]. The significant public health implications, including the treatment of community-acquired urinary tract infection, mean that the spread of organisms producing ESBLs (particularly CTX-M enzymes) merits close monitoring with enhanced surveillance, and efforts should be made to prevent the spread of organisms producing these enzymes within the hospital and community settings.

Several studies have described molecular approaches that allow screening of ESBL-positive organisms for the presence of different CTX-M genes. Amplification and sequencing of $bla_{\text{CTX-M}}$ genes is time-consuming and expensive, and involves multiple reactions with different primers for each specific gene [16,17]. Some studies have utilised amplification of a universal DNA fragment specific for most of the different groups of CTX-M β-lactamases [18,19], and a multiplex PCR that differentiates between the five CTX-M phylogenetic groups has also been described [20]. Unfortunately, none of these methods has been verified extensively or validated in routine use. Therefore, the present study was designed to develop, validate and verify a molecular detection assay for the simultaneous detection of strains carrying different types of $bla_{\text{CTX-M}}$ β-lactamase genes, and to investigate the extent to which genes encoding these enzymes were present among ESBL-producing isolates of Enterobacteriaceae from clinical specimens in the Calgary Health Region, Canada, during 2003 and 2004.

MATERIALS AND METHODS

Bacteria

Consecutive non-duplicate isolates of all Enterobacteriaceae (one per patient; $n = 240$) collected at Calgary Laboratory Services during January 2003 and December 2004 were used in this study. These organisms were screened for ESBL production and then investigated for the presence of CTX-M β-lactamases. Isolates were identified to the species level with the Vitek AMS (bioMérieux Vitek Systems Inc., Hazelwood, MO, USA). Strains ($n = 28$) with well-characterised β-lactamases were used as positive and negative controls for the verification and validation of the PCR assay (Table 1). An additional group ($n = 14$) of AmpC-producing *Esch. coli* strains, isolated recently in the Calgary Health Region, were also included; these isolates were resistant to cefoxitin, cefotaxime and ceftazidime, and tested positive for AmpC cephalosporinases using the combination of the AmpC

Table 1. Control strains producing well-characterised β-lactamases

Strain	Organism	β-Lactamase	Group[a]	Primers		Reference or source
				CTXMA1/A2[b]	CTX825F/R[b]	
CF2	*Enterobacter cloacae*	CTX-M-1	1	+	–	[31]
Rio-4	*Proteus mirabilis*	CTX-M-2	2	+	–	[32]
VER-1	*Ent. cloacae*	CTX-M-3	1	+	–	[31]
Cfr2525/96	*Citrobacter freundii*	CTX-M-3	1	+	–	[33]
34	*Salmonella typhimurium*	CTX-M-5	2	+	–	[34]
Rio-3	*Enterobacter aerogenes*	CTX-M-8	8	–	+	[32]
785D	*Escherichia coli*	CTX-M-9	9	+	–	[35]
EC97/38582	*Esch. coli*	CTX-M-10	1	+	–	[36]
EC984167	*Esch. coli*	CTX-M-14	9	+	–	[37]
CF1	*Esch. coli*	CTX-M-14	9	+	–	[31]
Eco3553/98	*Esch. coli*	CTX-M-15	1	+	–	[38]
N00-0666	*Esch. coli*	CTX-M-15	1	+	–	[39]
Rio-6	*Esch. coli*	CTX-M-16	9	+	–	[40]
BM4493	*Klebsiella pneumoniae*	CTX-M-17	9	+	–	[41]
ILT-2	*K. pneumoniae*	CTX-M-18	9	+	–	[42]
ILT-3	*K. pneumoniae*	CTX-M-19	9	+	–	[42]
ESBL530	*Esch. coli*	CTX-M-25	25	–	+	[43]
Cf29	*C. freundii*	CTX-M-30	1	+	–	[44]
CT1	*Esch. coli*	Toho-1	2	+	–	[45]
1A	*Esch. coli*	VEB-1	–	–	–	[46]
S1	*Esch. coli*	SHV-2	–	–	–	[47]
S2	*Esch. coli*	SHV-7	–	–	–	[47]
T1	*Esch. coli*	TEM-3	–	–	–	[47]
T4	*Esch. coli*	TEM-10	–	–	–	[47]
T5	*Esch. coli*	TEM-50	–	–	–	[47]
A1	*Esch. coli*	MOX-1	–	–	–	[47]
A2	*Esch. coli*	FOX-1	–	–	–	[47]
A3	*Salmonella* spp.	CMY-2	–	–	–	[48]
C1	*Ent. cloacae*	NMC-A	–	–	–	[47]
C2	*K. pneumoniae*	KPC-1	–	–	–	[47]

[a]Group 1 includes CTX-M-1, -3, -10, -11, -12, -15, -22, -23, -28, -29 and -30; group 2 includes CTX-M-2, -4, -5, -6, -7 and -20, and Toho-1; group 8 includes CTX-M-8; group 9 includes CTX-M-9, -13, -14, -16, -17, -18, -19, -21 and -27, and Toho-2; and group 25 includes CTX-M-25 and -26.
[b]PCR primers for CTX-M groups (see Materials and methods).