Journal of Infection (2007) **55**, e125–e128

www.elsevierhealth.com/journals/jinf

CASE REPORT

Detection of CTX-M-14 extended-spectrum β-lactamase in *Shigella sonnei* isolates from China

Zizhong Xiong [a,b], Tao Li [c], Yuanhong Xu [c], Jun Li [b,*]

[a] *Department of Infectious Diseases, The First Affiliated Hospital, Anhui Medical University, Hefei 230022, China*
[b] *School of Pharmacy, Anhui Medical University, Hefei 230032, China*
[c] *Clinical Laboratory, The First Affiliated Hospital, Anhui Medical University, Hefei 230022, China*

Accepted 19 July 2007
Available online 4 September 2007

KEYWORDS
Shigella sonnei;
Resistance;
Antimicrobial agents;
Extended-spectrum β-lactamases

Summary Shigellosis is an important cause of acute diarrheal disease and multidrug-resistant phenotype has been reported in *S. sonnei*. In this study, we investigate the resistance and identify extended-spectrum β-lactamases (ESBLs) gene in 37 *S. sonnei* isolates by agar dilution procedure and the modified three-dimensional test, respectively. The *bla* genes of ESBL-producing isolates were detected by polymerase chain reaction (PCR) and sequencing. More than 50% of these strains were resistant to tetracycline, sulfamethoxazole-trimethoprim, ampicillin, ampicillin-sulbactam, or gentamicin. However, they were still susceptible to third generation cephalosporins, fluoroquinolones, and chloramphenicol. A total of 8.1% (3/37) of the isolates with intermediate susceptibility to ceftriaxone and cefotaxime were ESBL-producers, which produced CTX-M-14 ESBLs and TEM-1 β-lactamases. This is the first report of CTX-M-14 in *S. sonnei* isolates from China and it is important to closely monitor such strains.

Introduction

Shigellosis is an important cause of acute diarrheal disease in both developing and industrialized countries.[1] *Shigella flexneri* was the most predominant *Shigella* species in China, but *Shigella sonnei* has been more and more common, recently. Antimicrobial resistance of *Shigella* species is increasing because of antibacterial agents used widely in clinical medicine.[2–4] Multidrug-resistant (MDR) phenotype has been reported in *S. sonnei*, and a few of those strains were resistant to third generation cephalosporins, such as ceftriaxone, cefotaxime, in which antimicrobial therapy against shigellosis has become very limited.[5] In the study described here, we have investigated the antimicrobial susceptibility of *S. sonnei* isolated from Outpatient Clinic of The First Affiliated Hospital, Anhui Medical University, Hefei, China from May to October, 2004. The extended-spectrum β-lactamase (ESBL), CTX-M-14, was determined in those strains.

* Corresponding author. Tel./fax: +86 551 516 1001.
E-mail address: aydyxylj@126.com (J. Li).

0163-4453/$30
doi:10.1016/j.jinf.2007.07.017

Table 1 Primers for PCR

Primer	Sequence
TEM universal primers	P1 5′-TGCGGTATTATCCCGTGTTG-3′
	P2 5′-TCGTCGTTTGGTATGGCTTC-3′
SHV universal primers	P1 5′-TCTCCCTGTTAGCCACCCTG-3′
	P2 5′-CCACTGCAGCAGCTGC(A/C)GTT-3′
CTX-M-1group universal primers	P1 5′-ACAGCGATAACGTGGCGATG-3′
	P2 5′-TCGCCCAATGCTTTACCCAG-3′
CTX-M-2group universal primers	P1 5′-TGGAAGCCCTGGAGAAAAGT-3′
	P2 5′-CTTATCGCTCTCGCTCTGTT-3′
CTX-M-9group universal primers	P1 5′-CTGCTTAATCAGCCTGTCGA-3′
	P2 5′-TCAGTGCGATCCAGACGAAA-3′
	P2 5′-TCTTACTTCGCCAACTTCT-3′
TEM entire gene primers	P1 5′-CCCTGGTAAATGCTTC-3′
	P2 5′-GAGTAAACTTGGTCTG-3′
CTX-M-9group entire gene primers	P1 5′-CGAAGCAGTCTAAATTCTTCGTGAAATAG-3′
	P2 5′-GGGCCAGTTGGTGATTTGA-3′

Case report

Thirty-seven *S. sonnei* isolates were collected from patients in Outpatient Clinic of The First Affiliated Hospital, Anhui Medical University, Hefei, China from May to October, 2004. There were no replicate strains in this study. All patients came from the region of Hefei, China and have community acquired diarrheal disease. Antibiotic susceptibility of *S. sonnei* isolates were determined by standard agar dilution procedure[6] on Mueller-Hinton agar (Oxoid, Basingstoke, UK), and *Escherichia coli* ATCC 25922 (susceptible strain), *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain) were included for quality control. Results were interpreted according to NCCLS standards.[6] Antimicrobial agents for susceptibility test included tetracycline, chloramphenicol, sulfamethoxazole-trimethoprim, ciprofloxacin, gentamicin, ampicillin, cefotaxime, ceftriaxone, ceftazidime (National Institute for Control of Pharmaceutical and Biological Products, Beijing, China), ampicillin-sulbactam (Pfizer, USA), levofloxacin (Daiichi Seiyaku, Tokyo, Japan), norfloxacin (The Second Shanghai Pharmaceutical Company, Shanghai, China). ESBLs producing strains were confirmed by the modified three-dimensional test described previously,[7] and Ceftriaxone (30 μg), cefotaxime (30 μg), and cefoxitin (30 μg) disc (Oxoid, Basingstoke, UK) were used for the test. Partial *bla* genes of ESBL-producing isolates were detected by PCR using universal primers for TEM, SHV, CTX-M-1group, CTX-M-2group, CTX-M-9group, respectively.[8,9] If the partial genes were positive, entire genes were also tested by PCR.[9,10] All primers for PCR were shown in Table 1. The PCR was performed using a commercially available PCR kit (Takara, Dalian, China) and the Biometra PCR Thermal Cycler (Germany). Standard strains encoding different β-lactamase (TEM-1, SHV-18, CTX-M-3, Toho-1, CTX-M-24) employed as positive controls and *E. coli* ATCC25922 as the negative control. PCR product (5 μl) was subjected to electrophoresis on 1.2% agarose gel (Invitrogen, California, USA) to identify the amplified DNA fragment. The purified PCR products of entire genes from the isolates were sequenced with an ABI 3100 genetic analyzer (Shanghai Biotechnology Company, Shanghai, China) and continued by primers walking on both DNA strands. For sequence comparison, the NCBI BLAST program

Table 2 Susceptibility of *Shigella sonnei* isolates to antimicrobial agents

Antimicrobial agent	*Shigella sonnei* ($n = 37$)		
	R	I	S
Tetracycline	97.3% (36/37)	0% (0/37)	2.7% (1/37)
Sulfamethoxazole-trimethoprim	94.6% (35/37)	0% (0/37)	5.4% (2/37)
Chloramphenicol	2.7% (1/37)	0% (0/37)	97.3% (36/37)
Levofloxacin	0% (0/37)	2.7% (1/37)	97.3% (36/37)
Ciprofloxacin	0% (0/37)	2.7% (1/37)	97.3% (36/37)
Norfloxacin	0% (0/37)	2.7% (1/37)	97.3% (36/37)
Gentamicin	62.2% (23/37)	0% (0/37)	37.8% (14/37)
Ampicillin	64.9% (24/37)	0% (0/37)	35.1% (13/37)
Ampicillin-sulbactam	51.4% (19/37)	10.8% (4/37)	37.8% (14/37)
Ceftriaxone	0% (0/37)	8.1% (3/37)	91.9% (34/37)
Cefotaxime	0% (0/37)	8.1% (3/37)	91.9% (34/37)
Ceftazidime	0% (0/37)	0% (0/37)	100% (37/37)

R, resistant; I, intermediate; and S, susceptible.