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Emergence of extended-spectrum beta-lactamase CTX-M-27 in *Shigella sonnei* clinical isolate from croatia

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Abstract

The patient developed bacillary dysentery with frequent stools mixed with blood and mucus and was hospitalized in Sestre Milosrdnice Hospital in Zagreb, Croatia. *Shigella sonnei* was isolated from the stool specimen. The antibiotic susceptibility testing was performed by disk-diffusion and broth microdilution method. Double disk synergy test and combined disk test with clavulanic acid were done to determine the production of an Extended-Spectrum Beta-Lactamase (ESBL). The transferability of cefotaxime resistance was done by broth mating method. PCR was applied to detect genes encoding ESBLs. Whole genome sequencing was performed to analyse the whole resistome of the strain.

The strain exhibited resistance to amoxicillin, cephalexin, cefuroxime, azithromycin, tetracycline, cefotaxime, ceftriaxone, and ciprofloxacin but remained susceptible to ceftazidime, cefepime, amoxicillin/clavulanic acid, piperacillin/tazobactam, imipenem, meropenem and gentamicin. Phenotypic tests confirmed production of an ESBL. PCR identified bla_{CTX-M} gene belonging to the cluster 1. WGS demonstrated a variety of aminoglycoside, β-lactam macrolide sulphonamide (trimethoprim and tetracycline resistance determinants. Four different plasmids were found: IncFIB, IncFII, IncI1 and Inc Col. The isolate was found to belong to the widespread ST152 which is dominant in Europe and North America and usually associate with MSM population. To our best knowledge, this is the first report of an ESBL in Shigella spp. from Croatia. This report emphasizes the ability of S. sonnei belonging to the widespread ST 152 to accumulate various resistance determinants.

Keywords: *Shigella sonnei*; Antibacterial resistance; Extended-Spectrum Beta-Lactamase (ESBL); CTX-M beta-lactamase.

Introduction

Recently, it was observed that *shigellae* can acquire a wide variety of different resistance traits including genes encoding broad and extended-spectrum β-lactamases and carbapenemases [1]. Resistance to fluoroquinolones is usually due to Plasmid-Mediated Fluoroquinolone-Resistance Region (PMQRs), including *qnr*A, *qnr*B, qnrC, *qnr* D and *qnr* S genes [2].

Case report

The patient was a 30 year old male, who inhaled sewage during work due to the rupture of the sewage pipeline. He developed bacillary dysentery with frequent stools mixed with blood and mucus and was hospitalized in Sestre Milosrdnice Hospital in Zagreb, Croatia. He was released after 1 day without antibiotic therapy.

The antimicrobial susceptibility of the strain 27789 to amoxicillin alone and combined with clavulanate, expanded- spectrum cephalosporins (ceftazidime, cefotaxime, ceftriaxone), cefepime, imipenem, meropenem, ertapenem, gentamicin, ciprofloxacin, tetracycline and azithromycin was determined by broth microdilution method according to Clinical & Laboratory Standards Institute standards [3]. *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603 were used as quality control strains for Minimum Inhibitory Concentration (MIC) determination. The susceptibility Totrimethoprim/Sulfamethoxazole (SXT/TMP) and chloramphenicol was determined by disk-diffusion test. MDR was defined as resistance to >3 antimicrobial drug classes and clinical resistance as resistance to >1 of the major antimicrobial drug classes (penicillins, cephalosporins, folate-pathway inhibitors, fluoroquinolones, tetracyclines and macrolides [4].

The Double Disk Synergy Test (DDST) [5] was carried out in the frames of routine laboratory analysis of the isolates. ESBL production was confirmed by CLSI combined disk test using disks with expanded-spectrum cephalosporins (ceftazidime, cefotaxime, ceftriaxone) or ESC alone and with addition of clavulanic acid [3]. Plasmid-Mediated Ampc B-Lactamases (p-AmpC) were detected by combined disk test using cephalosporin disks with 3-Aminophenylboronic Acid (PBA) [6]. To confirm cefotaxime hydrolysis a modified method described for carbapenem inactivation was done [7]. The transferability of cefotaxime resistance was determined by conjugation (broth mating method) at 35°C employing *E. coli* J 65 recipient strain resistant to sodium-azide [8]. The transconjugants were selected on Mac Conkey agar containing either ampicillin (0.5 mg/L) or ciprofloxacin (0.5 mg/L) and sodium azide (100 mg/L).

The genes conferring resistance to β-lactam antibiotics including (*blaSHV, blaTEM, blaCTX-M, blaOXA-9, blaOXA-1 and blaPER-1)* [9-12], p-AmpC β-lactamases [13], and fluoroquinolone resistance genes *qnr*A, *qnr*B, *qnr*S [14] were determined by PCR using protocols and conditions as described previously. The CTX-M β-lactamase cluster was detected with multiplex PCR including five primer pairs: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 [15]. Amplicons were visualised after electrophoresis in a 1% agarose gel by staining it with ethidium bromide. The genetic context of bla_{CTX-M} genes was determined by PCR mapping with forward primer for insertion sequences ISEcp1 and IS26 combined with primer MA-3 (universal reverse primer for bla_{CTX-M} genes) [16].

Plasmids were extracted from ampicillin resistant donor strains and their respective transconjugants with Qiagen plasmid mini kit according to the manufacturer's instructions (Hilden, Germany). After staining with ethidium bromide, the DNA was visualised by ultraviolet light. PCR-Based Replicon Typing (PBRT) was applied to determine the plasmid content of the tested strains [17,18]. WGS was done using the Ion Torrent PGM platform (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions [19].

The isolate was genotyped by MLST according to the protocol of University of Warwick/EnteroBase website: https://enterobase.warwick.ac.uk/warwick_mlst_legacy).

The strain exhibited resistance to amoxicillin, cephalexin, cefuroxime, azithromycin, tetracycline, cefotaxime, ceftriaxone, and ciprofloxacin with MIC values of ≥128, ≥128, ≥128, ≥128, ≥128, 32, 8 and 8 mg/L, respectively, but remained susceptible to were ceftazidime, cefepime, amoxicillin/clavulanic acid, piperacillin/tazobactam, imipenem, meropenem and gentamicin with MIC of 8, 0.5, 1, 0.25, 0.06, 0.06, and 1 mg/L. Cotrimoxazole and cefoxitin tested resistant and susceptible in disk-diffusion test with the inhibition zones of 6 and 25 mm, respectively. DDST and inhibitor based test with clavulanic acid tested positive, indicating production of an ESBL. Inhibitor based test with PBA exhibited negative result. PCR identified blaCTX-M gene belonging to the cluster 1, but PCR for insertion sequences displayed negative result. WGS demonstrated a variety of aminoglycoside (aadA, aph(6), aph(3), β-lactam (blaCTX-M-27), macrolide (Erm (B,) Mph (A)), sulphonamide (sul 1), trimethoprim (dfrA17,dfrA)and tetracycline (tet A) and a variety of virulence determinants (Table 1). Four different plasmids were found: IncFIB, IncFII, IncI1 and IncCol. The gene sequences were deposited in the Gene bank with accession number JAMKCB000000000.

Discussion

The isolate was found to belong to the widespread ST152 which is dominant in Europe and North America and usually associate with MSM population [20]. ESBLs were reported in Shigella spp. in developing countries almost two decades ago [21], but they are still rare in Europe [22]. The majority of other reports identified CTX-M-3 and CTX-M-15 allelic variants whereas our study found CTX-M-27. This allelic variant was recently described in S. sonnei in UK and Australia [23,24]. Plasmid-mediated resistance determinants to fluoroquinolones were not found and thus it is very likely that ciprofloxacin resistance was associated with mutations of gyrA and parC genes. IncFIB and IncFII plasmids were previously identified in CTX-M producing E. coli from Croatia [25] where as IncI1 was carried by CTX-M positive E. coli strains from chicken [26].

Table 1: Whole genome sequencing results of *Shigellasonnei* isolate 27789.

Isolate	AG	β-lactam	FQ	ML	SUL	TET	TMP	Plasmid inc groups
*Shigellasonnei*27789	*aadA5* *aph(6)* *aph(3)*	$bla_{CTX-M-27}$	*aac(6')Ib-cr* *oqxB* *oqxA*	*Erm(B)* *Mph(A)*	*Sul1*	*tetA*	*dfrA17, dfrA1*	Col, IncFIB, IncFII IncI1
	Virulence traits							
	ipaD invasion protein	*lucA* aerobactin	*senB* plasmid encoded enterotoxin	*traT* complement resistance	*virF* transcriptional activator	*sitA* iron transport protein	*sigA* IgA protease	*celB* colicin

Abbreviations: FQ: Fluoroquinolones; ML: Macrolides; SUL: Sulphonamides; TET: Tetracyclines; TMP: Trimethoprim.

Conclusion

To our best knowledge, this is the first report of an ESBL in Shigella spp. from Croatia. Intestinal carriage of ESBL positive coliform bacteria is increasing in the outpatient setting which leads to the conclusion that blaESBL gene may have been transferred from K.pneumoniae or E. coli by in vivo conjugation. This report emphasizes the ability of S. sonnei belonging to the widespread ST 152 to accumulate various resistance determinants.

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