The bla_{SHV-5} gene is encoded in a compound transposon duplicated in tandem in *Enterobacter cloacae*

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Abstract

The presence of bla_{SHV-5} is described in a compound transposon, duplicated in tandem and flanked by IS*26* copies on a 70-kb conjugative plasmid (pHNM1), in an *Enterobacter cloacae* strain associated with a nosocomial outbreak that occurred in Mexico.

Keywords: Compound transposon, *Enterobacter cloacae*, ESBL, IS*26*, outbreak, SHV-5, tandem

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Enterobacter cloacae has emerged in recent years as an important nosocomial pathogen in neonatal units, with several outbreaks involving strains resistant to expanded-spectrum cephalosporins [1]. Although overproduction of chromosomal AmpC β-lactamase is the mechanism usually observed, extended-spectrum β-lactamase (ESBL)-producing *E. cloacae* are currently increasing [2,3] and several SHV-type β-lactamases have been described [4].

Previous studies have demonstrated that SHV-5 is the most frequent ESBL in several enterobacterial species [5–7], including *E. cloacae* strain C1177-7 recovered from hospitalized patients in Mexico. The present study aimed to characterize the genetic context of SHV-5 in strain C1177-7, which was isolated during an outbreak in a neonatal unit [8].

Enterobacter cloacae C1177-7 was resistant, according to CLSI criteria [9], to cefotaxime (16 mg/L), ceftazidime (256 mg/L) and aztreonam (128 mg/L). This strain transferred the cefotaxime resistance gene bla_{SHV-5} on a 70-kb plasmid (named pHNM1) that contained also bla_{TEM-1} [8], with a frequency of 10^{-3} transconjugants per donor cell.

To determine the genetic context of the SHV-5 gene in the transconjugant of strain C1177-7, two libraries (2–4 and 4–7 kb) of pHNM1 DNA were constructed and cloned into the pZErO vector. A total of 576 high-quality readings were collected and the sequences assembled using PHRED-PHRAP-CONSED software [10]. (The sequence data reported here are deposited in the GenBank/EMBL nucleotide database under accession no. AY532647.1.) The open reading frame (ORF) prediction and BLASTn and BLASTx analyses identified a compound transposon duplicated in tandem (CTDT), which included the bla_{SHV-5} gene. This structure was located in a large contig of 19 958 kb. The first two ORFs identified corresponded to the *mucA-mucB*Δ operon. The next 9520 bp corresponded to a compound transposon containing two IS*26* in the same direction with perfect identical terminal inverted repeats of 14-bp (GGCACTGTTGCAAA) on each side. These IS*26* flanked the *ygbM*Δ, *fucA*, *ygbK*, *ygbJ*, *deoR*, bla_{SHV-5}, *recF* and *lacY*Δ ORFs.

A third IS*26* flanked a duplication of all eight of these ORFs, which accounted for the remaining 8819 bp of the main contig generating the CTDT (Fig. 1). PCR fragments and sequencing obtained from the overlapping amplified segments, the entire CTDT, and flanking sequences, corresponded to those expected, and confirmed the structure (Table 1, Fig. 1). The stability of the CTDT was evaluated by PCR amplification using *lacYF-ygbMR*-specific primers (Table 1) in the C1177-7 strain and its transconjugant after at least ten bacterial generations in the absence of antibiotic pressure.

The enzyme SHV-5, a plasmid-encoded, broad-spectrum β-lactamase, was first identified in *Klebsiella pneumoniae* in 1989 [11]. However, the genetic context of SHV-type β-lactamase genes was in question for several years [12,13]. In 2004, bla_{SHV-5} was identified on a compound transposon flanked by IS*26* insertion sequences in the pACM1 plasmid (*Klebsiella oxytoca*) [14], which was obtained from an outbreak in 1993 [15]. Subsequently, the compound transposon

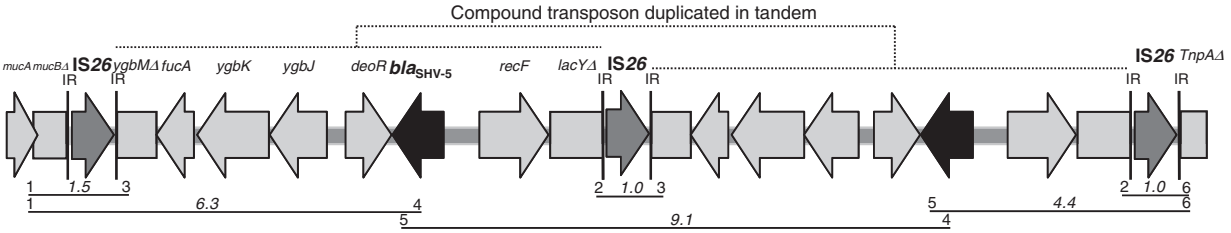

FIG. 1. Schematic representation of the compound transposon duplicated in tandem (CTDT) harbored on plasmid pHNM1 from the clinical isolate *Enterobacter cloacae* E1177-7. The CTDT carrying the bla_{SHV-5} gene and seven additional genes duplicated in tandem are flanked by IS*26*. Arrows indicate open reading frames; inverted repeats (IR) of 14-bp at the insertion sequence IS*26* boundaries are indicated. Approximate PCR product lengths (kb) are shown in italics. Numbers at both ends of each PCR product indicate the primer pairs used for PCR and sequencing.

TABLE 1. Sequences of primers used in the present study

Target	Primer[a]	Sequence (5′–3′)	References
mucB	mucBF (1)	CCCGTGTACGCCTTAATC	Present study
lacY	lacYF (2)	TGACGCGTTCGATATAAGC	Present study
ygbM	ygbMR (3)	CGGGCTATCTCTACCACAG	Present study
SHV-5	P1 (4)	ACTGAATGAGGCGCTTCC	[21]
bla_{SHV-5}	P2 (5)	TCCCGCAGATAAATCACC	[21]
bla_{TEM-1}	T1	CAACATTTTCGTGTCGCCC	Present study
bla_{TEM-1}	T2	GCTTAATCAGTGAGGCACC	Present study
TnpA	tnpAR (6)	GAGAAGCTTCAGCTGGCGGC	Present study

[a]Numbers in parentheses correspond to the primers used for PCR amplification and sequencing of the compound transposon duplicated in tandem.

was found on pSEM (*Salmonella enterica*) [16] and p1658/97 (*Escherichia coli*) [17] plasmids. Thus, this compound transposon has been identified on plasmids from several bacterial species from 1993 (pACM1) to 2007 (p1658/97). It is worth noting that these were isolated in different geographic locations (USA – pACM1; Poland – p1658/97; Albania – pSEM), demonstrating the wide spread of this compound transposon. It has now been identified as a tandem duplicate structure on a 70 kb plasmid (pHNM1) from an *E. cloacae* strain associated with a nosocomial outbreak that occurred in Mexico, a country with a record of earlier outbreaks of SHV-5-producing *Enterobacteriaceae* [5,6,18]. The possibility that this CTDT is related to outbreaks of different bacterial species, and/or that it is implicated in increased production of the SHV-5 β-lactamase, cannot be dismissed.

A tandem duplication of a composite transposon via the insertion sequences (IS600) is associated with increased toxin (Shiga toxin) production in *Shigella dysenteriae* [19]. In plasmid p1658/97, the compound transposon that contains the bla_{SHV-5} gene was identified as an active genetic element. In addition, under high concentrations of antibiotic, more copies of the amplified bla_{SHV-5} were detected compared to when lower antibiotic concentrations were used. The overproduction of this enzyme correlated with a compound transposon structure of 9521 bp flanked by the IS*26* insertion sequence [17]. Sequences flanking the SHV-5 gene in p1658/97 include one ORF of 260 bp (86 amino acids) that is not present on pHNM1 (AY532647.1) or pSEM (AJ245670) plasmids. This almost identical region encompasses the bla_{SHV-5} gene and is flanked by two IS*26* copies. The nucleotide sequence comparison between the copies of this compound transposon on plasmids p1658/97 and pSEM plasmids revealed high levels of identity (i.e. 99%).

The mechanism of the acquisition of CTDT by pHNM1 suggests recombination as a result of the lack of direct repeat sequences at the IS*26* ends, such as those identified in plasmid p1658/97. Duplication in tandem could be characteristic of the IS*26* element, which gives rise exclusively to replicon fusions in which the donor and target replicons are separated by two directly repeated IS copies, suggesting that this rearrangement occurred by homologous recombination between the flanking IS*26* sequences in the same orientation [20]. These rearrangements could comprise a potential alternative mechanism for generating several copies of the bla_{SHV-5} gene on the same plasmid, which can be maintained as a tandem duplicate for several bacterial generations, as in the case of pHNM1. The identification of the CTDT structure offers new insights into the mechanism implicated in the increase of SHV-type gene copy numbers. Future studies are needed to elucidate genetic rearrangements that generate tandem amplification of the *bla*-$_{SHV-5}$ gene carried on compound transposons.

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Transparency Declaration

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References

1. Dalben M, Varkulja G, Basso M *et al.* Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. *J Hosp Infect* 2008; 70: 7–14.
2. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18: 657–686.
3. Gaynes R, Edwards JR. Overview of nosocomial infections caused by Gram-negative bacilli. *Clin Infect Dis* 2005; 41: 848–854.
4. Szabo D, Bonomo RA, Silveira F *et al.* SHV-type extended-spectrum beta-lactamase production is associated with Reduced cefepime susceptibility in *Enterobacter cloacae*. *J Clin Microbiol* 2005; 43: 5058–5064.
5. Alcantar-Curiel D, Tinoco JC, Gayosso C *et al.* Nosocomial bacteremia and urinary tract infections caused by extended-spectrum beta - lactamase-producing *Klebsiella pneumoniae* with plasmids carrying both SHV-5 and TLA-1 genes. *Clin Infect Dis* 2004; 38: 1067–1074.
6. Miranda G, Castro N, Leanos B *et al.* Clonal and horizontal dissemination of *Klebsiella pneumoniae* expressing SHV-5 extended-spectrum beta-lactamase in a Mexican pediatric hospital. *J Clin Microbiol* 2004; 42: 30–35.
7. Silva J, Aguilar C, Becerra Z, Lopez-Antunano F, Garcia R. Extended-spectrum beta-lactamases in clinical isolates of enterobacteria in Mexico. *Microb Drug Resist* 1999; 5: 189–193.
8. Garza-Ramos U, Martinez-Romero E, Silva-Sanchez J. SHV-type Extended-spectrum ß-lactamase (ESBL) are encoded in related plasmids from enterobacteria clinical isolates from Mexico. *Salud Publica Mex* 2007; 49: 415–421.
9. Clinical and Laboratory Standards Institute. . *Performance standards for antimicrobial susceptibility testing*. Document M100-S18. Wayne, PA: CLSI, 2008.
10. Gordon D, Abajian C, Green P. Consed: a graphical tool for sequence finishing. *Genome Res* 1998; 8: 195–202.
11. Gutmann L, Ferre B, Goldstein FW *et al.* SHV-5, a novel SHV-type beta-lactamase that hydrolyzes broad-spectrum cephalosporins and monobactams. *Antimicrob Agents Chemother* 1989; 33: 951–956.
12. Levesque RC, Jacoby GA. Molecular structure and interrelationships of multiresistance beta-lactamase transposons. *Plasmid* 1988; 19: 21–29.
13. Nugent ME, Hedges RW. The nature of the genetic determinant for the SHV-1 beta-lactamase. *Mol Gen Genet* 1979; 175: 239–243.
14. Preston KE, Venezia RA, Stellrecht KA. The SHV-5 extended-spectrum beta-lactamase gene of pACM1 is located on the remnant of a compound transposon. *Plasmid* 2004; 51: 48–53.
15. Preston KE, Kacica MA, Limberger RJ, Archinal WA, Venezia RA. The resistance and integrase genes of pACM1, a conjugative multiple-resistance plasmid, from *Klebsiella oxytoca*. *Plasmid* 1997; 37: 105–118.
16. Miriagou V, Filip R, Coman G, Tzouvelekis LS. Expanded-spectrum cephalosporin-resistant salmonella strains in Romania. *J Clin Microbiol* 2002; 40: 4334–4336.
17. Zienkiewicz M, Kern-Zdanowicz I, Golebiewski M *et al.* Mosaic structure of p1658/97, a 125-kilobase plasmid harboring an active amplicon with the extended-spectrum beta-lactamase gene blaSHV-5. *Antimicrob Agents Chemother* 2007; 51: 1164–1171.
18. Martinez-Aguilar G, Alpuche-Aranda CM, Anaya C *et al.* Outbreak of nosocomial sepsis and pneumonia in a newborn intensive care unit by multiresistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*: high impact on mortality. *Infect Control Hosp Epidemiol* 2001; 22: 725–728.
19. McDonough MA, Butterton JR. Spontaneous tandem amplification and deletion of the shiga toxin operon in *Shigella dysenteriae* 1. *Mol Microbiol* 1999; 34: 1058–1069.
20. Mahillon J, Chandler M. Insertion sequences. *Microbiol Mol Biol Rev* 1998; 62: 725–774.
21. Prodinger W, Fille M, Bauernfeind A *et al.* Molecular epidemiology of Klebsiella pneumoniae producing SHV-5 β-lactamase: parallel outbreaks due to multiple plasmid transfer. *J Clin Microbiol* 1996; 3: 564–568.

International diagnostic accuracy study for the serological detection of chikungunya virus infection

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Abstract

External quality assurance for serological detection of chikungunya virus infection was performed to assess the diagnostic quality of expert laboratories. Of 30 participants, only six correctly analysed all reference samples with their respective tests. Thirteen laboratories gave at least 85% correct results, and 11 laboratories 75% or less. IgM antibodies were detected less frequently than IgG antibodies ($p < 0.001$). The study provides information on the quality of different serological tests and indicates that most of the participants need to improve the sensitivity of their assays, in particular to detect IgM antibodies more reliably and be able to detect acute infections adequately.

Keywords: Chikungunya fever, emerging viral infection, external quality assurance, serology, virus diagnostic

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