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Extended-spectrum β-lactamase–type SHV-12–producing Enterobacteriaceae causing septicemia in Tanzanian children: vectors for horizontal transfer of antimicrobial resistance

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Abstract

Septicemia caused by extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae was associated with high mortality in Tanzanian children. Conjugation experiments on the SHV-12–producing Enterobacteriaceae isolates showed that ESBL-encoding genes were transferred on large plasmids together with genes encoding resistance to aminoglycosides, resistance to ceftazidime, gentamicin, doxycycline, trimethoprim–sulfamethoxazole, and chloramphenicol.
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Extended-spectrum β-lactamases (ESBLs) are enzymes often encoded on conjugative plasmids. Determinants for resistance to non–β-lactam antimicrobial agents can reside on the same transmissible plasmids (Corkill et al., 2005; Naiemi et al., 2005; Schwaber et al., 2005). The SHV-12 type ESBL, 1st detected in Switzerland (Nuesch-Inderbinen et al., 1997), has now been reported from several parts of the world and, recently, also from Africa (Blomberg et al., 2005; Gangoue-Pieboji et al., 2005; Gray et al., 2006).

In the current study, we investigated the ESBL-encoding vectors in the SHV-12–positive Enterobacteriaceae isolates obtained as part of a prospective cohort study of pediatric septicemia at Muhimbili National Hospital in Dar-es-Salaam, Tanzania (Blomberg et al., 2007).

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Antimicrobial susceptibility testing was performed by the disk diffusion method (NCCLS, 1997) and MIC determination by Etest (AB Biodisk, Solna, Sweden). Enterobacteriaceae isolates were screened for ESBL production as recommended by the Clinical and Laboratory Standards Institute, and the ESBL phenotype was detected by ESBL Etests (AB Biodisk) as previously described (Blomberg et al., 2005). Polymerase chain reactions for detection of the bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$, *aac(6′)-I*, *aac(3)-II*, and *IntI1* genes were performed using genomic or plasmid DNA as template. Previously published primers and protocols were used (Rasheed et al., 1997; Dubois et al., 2002; Arpin et al., 2003; Pagani et al., 2003; Sundsfjord et al., 2004). Both strands of the amplicons were sequenced. Plasmid DNA was analyzed by both conventional gel electrophoresis and S1 nuclease-pulsed-field gel electrophoresis (S1-PFGE) (Barton et al., 1995). Conjugation experiments were performed by the filter mating method using the nalidixic acid (Nal)- and azide (Azi)-resistant strains *Escherichia coli* J53-1 Nal^r, *E. coli* J53-1 Nal^r Azi^r, and *E. coli* J53 Azi^r as recipients. Plasmid fingerprinting was done by *Eco*RI (Promega,

Table 1
Characteristics of the isolates and vectors for horizontal transfer of antimicrobial resistance

Organism	MIC (μg/mL)							Resistance genes	Cotransfer of phenotypic resistance [a]	Genetic relationship of plasmids [b]
	CTX	CAZ	GEN	DOX	SXT	CHL	CIP			
E. coli J53-1 (recipient)	0.064	0.125	0.125	2	1.28	8	0.125			
116 *E. cloacae*	4	32	256	>256	>640	>256	0.125	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		A
101 *K. pneumoniae*	4	32	128	>256	>640	>256	0.064	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*		
101Tc	1	8	32	64	1.28	>256	0.125	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*	GEN, DOX, SXT, CHL	
170 *E. cloacae*	32	256	>256	>256	>640	>256	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		B, profile I
133 *E. cloacae*	16	256	>256	128	>640	>256	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		B, profile I
171 *E. cloacae*	16	128	128	>256	>640	4	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		B, profile I
175 *Pantoea* spp.	8	32	256	>256	>640	>256	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		
175Tc	1	8	128	64	>640	>256	1	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$	GEN, DOX, SXT, CHL	A, profile II
176 *Pantoea* spp.	4	32	>256	128	>640	>256	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		
176Tc	1	8	32	128	>640	>256	0.50	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$	GEN, DOX, SXT, CHL	A, profile II
105 *K. pneumoniae*	4	>256	128	32	>640	>256	0.032	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*		
105Tc	2	>256	32	2	>640	>256	0.125	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*	CAZ, GEN, SXT, CHL	C, profile III
177 *E. cloacae*	32	64	>256	>256	>640	>256	>32	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		A
66 *S.* Newport	8	>256	256	64	>640	>256	0.25	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$		
66Tc	1	8	128	32	>640	>256	0.25	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$	GEN, DOX, SXT, CHL	A, profile II
107 *K. pneumoniae*	4	32	128	64	>640	>256	0.064	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*		
107Tc	4	16	64	2	>640	>256	0.125	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*	CAZ, GEN, SXT, CHL	C, profile III
109 *K. pneumoniae*	2	32	64	64	>640	>256	0.032	bla_{SHV}, $bla_{\text{TEM-1}}$, *aac(3)-II*		C, profile III
110 *K. pneumoniae*	4	32	64	4	>640	>256	0.032	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*		
110Tc	2	16	32	2	>640	>256	0.125	$bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, *dhfrA5*	CAZ, GEN, SXT, CHL	C, profile III

Tc = transconjugant; CTX = cefotaxime; CAZ = ceftazidime; GEN = gentamicin; DOX = doxycycline; SXT = trimethoprim–sulfamethoxazole; CHL = chloramphenicol; CIP = ciprofloxacin.

[a] Cotransfer of resistance (resistance or in vitro intermediate resistance for the cephalosporins) as determined by applied breakpoints.

[b] *Eco*RI profiles I, II, or III and hybridization with SHV probe to restriction fragment of the same size (A, B, or C). No designations are given if belonging to individual profiles or if positive signal to fragment of individual size was detected.

Madison, WI) digestion of plasmid DNA. Southern hybridization analysis of *Eco*RI-digested plasmid DNA with a $bla_{\text{SHV-12}}$ probe was performed with the kit AlkPhos Direct Labeling and Detection System (Amersham Biosciences, GE Healthcare, Buckinghamshire, UK).

The study included 13 Enterobacteriaceae isolates (Table 1). The isolates were almost uniformly resistant to gentamicin, doxycycline, trimethoprim–sulfamethoxazole, and chloramphenicol, and susceptible to ciprofloxacin and meropenem (Table 1). All isolates had both a $bla_{\text{TEM-1}}$ gene and 1 or more bla_{SHV} genes, but none had $bla_{\text{CTX-M}}$ genes. Twelve of the 13 isolates had the SHV-12 genotype. For the 5 *Klebsiella pneumoniae* isolates, specific bla_{SHV} alleles could only be identified by using plasmid DNA as template. The 5 *K. pneumoniae* isolates had the *aac(3)-II* gene, which encodes resistance to gentamicin, netilmicin, and tobramycin. Four of the *K. pneumoniae* isolates had a class 1 integron-type amplicon identified as the *dhfrA5* gene, which encodes type V dihydrofolate reductase conveying resistance to trimethoprim.

All isolates had at least 1 large plasmid detected by S1-PFGE. Conjugation experiments resulted in transconjugants for 7 of the donors (Table 1). The $bla_{\text{SHV-12}}$, $bla_{\text{TEM-1}}$, *aac(3)-II*, and *dhfrA5* genes, ESBL phenotype, and resistance to gentamicin, doxycycline, trimethoprim–sulfamethoxazole, and chloramphenicol were cotransferred by plasmids larger than 97 kb.

Fingerprinting of plasmid DNA by *Eco*RI digestion showed relatedness between plasmids (results not shown). Three of the *Enterobacter cloacae* isolates (133, 170 and

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