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High Prevalence of CTX-M-Type β-Lactamases among Clinical Isolates of *Enterobacteriaceae* in Bamako, Mali[▽]

In 2004, Weil et al. reported the presence of CTX-M-15 in *Salmonella enterica* (10), and recently Ruppé et al. reported the carriage of CTX-M-15-producing isolates in children in a remote village in Senegal (8). However, since the report of SHV-type enzymes in Nigeria (1), no survey has been performed on extended-spectrum β-lactamase (ESBL) producers in West African hospitals (4, 7). Between May 2004 and April 2006, in Hôpital du Point G, Bamako, Mali, of 1,193 nonrepetitive isolates of *Enterobacteriaceae* identified by the API 20E system (bioMérieux, Marcy l'Étoile, France), 256 produced an ESBL detected by the double-disk synergy test (9) (156/747 *Escherichia coli*, 82/217 *Klebsiella pneumoniae*, 15/81 *Enterobacter cloacae*, 2/12 *Morganella morganii*, and 1/36 *Proteus mirabilis* isolates). Enzymes were identified for 109 *E. coli* and 63 *K. pneumoniae* isolates randomly selected every 3 months and for all *E. cloacae*, *M. morganii*, and *P. mirabilis* isolates. These strains were isolated from patients hospitalized in the medical (n = 127 [66.3%]) and surgical (n = 27 [14.0%]) wards and intensive care units (n = 2 [1.2%]) and emergency patients (n = 4 [2.3%]) and outpatients (n = 30 [15.7%]) and from urine (n = 142 [74.7%]), blood (n = 12 [6.3%]), pus (n = 20 [10.4%]), and other sites (n = 16 [8.3%]) (stool, catheter, and pleural and peritoneal fluid). Antimicrobial susceptibility was determined by disk diffusion according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr/) (Table 1). Isoelectric focusing evidenced only one pI corresponding to a TEM-type enzyme, pI 5.4, in 121/190 isolates. Since this pI was considered as TEM-1 in most cases, we did not screen other TEM-type ESBLs by PCR. All 190 isolates were positive for bla_{SHV}- or $bla_{CTX\text{-}M}$-related genes, as assessed by PCR using previously described specific primers (2). After direct sequencing of PCR products (2), CTX-M-15 (n = 157), CTX-M-14 (n = 17), SHV-12 (n = 17), and SHV-27 (n = 1) were identified. Two isolates produced two ESBLs, CTX-M-15 and SHV-12 or SHV-27 (Table 1). Except in three isolates of *E. coli*, all SHV-type enzymes were produced by *E. cloacae* or *K. pneumoniae*, confirming the role of *E. coli* CTX-M-15 in the increase in ESBL incidence. To determine whether the ESBL frequency was due to the spread of some predominant strains, randomly amplified polymorphic DNA (RAPD) analysis was performed (2). A great diversity of RAPD types were observed (Table 1), and no isolate was the same as *E. coli* O25:H4-ST 131 (3, 5). In *E. cloacae* species, all SHV-12-producing isolates had similar RAPD patterns, in contrast to CTX-M-15-producing isolates, which were all different.

This study confirms that the worldwide diffusion of the CTX-M-15 enzyme has also reached West Africa. This was probably due to plasmid spread, since *E. coli* isolates, unlike SHV-12-producing *E. cloacae* isolates, were not genetically related (6). The spread of resistance in this area was more likely due to plasmids carrying antibiotic resistance genes than to bacterial transmission between patients. Maintenance of these plasmids was probably favored by antibiotic pressure. These findings emphasize the need for a policy of careful antibiotic use worldwide.

TABLE 1. Frequency of resistance to non-β-lactam antibiotics of ESBL-producing *Enterobacteriaceae* isolates

Species	No. of isolates	ESBL	% of isolates with resistance to[a]:									No. of RAPD types
			AMK	TOB	GEN	TET	CHL	NAL	NOR	CIP	TMP	
E. cloacae	6	CTX-M-15	0	100	100	100	100	100	100	100	100	6
	9	SHV-12	78	89	12	89	89	100	89	89	89	1
E. coli	11	CTX-M-14	0	100	100	82	82	100	100	100	100	9
	95	CTX-M-15	17	97	83	88	73	98	98	98	95	83
	1	CTX-M-15 + SHV-27	0	100	100	0	100	0	100	100	100	ND[b]
	1	CTX-M-15 + SHV-12	0	100	100	100	100	100	100	100	100	ND
	1	SHV-12	100	100	0	100	100	100	100	0	100	ND
K. pneumoniae	3	CTX-M-14	0	67	67	67	67	67	33	33	100	3
	54	CTX-M-15	20	96	89	84	40	93	96	95	100	34
	6	SHV-12	50	83	33	50	83	83	67	67	100	5
M. morganii	2	CTX-M-14	100	100	100	100	100	100	100	100	100	ND
P. mirabilis	1	CTX-M-14	0	0	0	100	100	100	100	100	100	ND

[a] AMK, amikacin; TOB, tobramycin; GEN, gentamicin; TET, tetracycline; CHL, chloramphenicol; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; TMP, trimethoprim.
[b] ND, not determined.

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