Clonal Structure, Extended-Spectrum β -Lactamases, and Acquired AmpC-Type Cephalosporinases of *Escherichia coli* Populations Colonizing Patients in Rehabilitation Centers in Four Countries

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The prospective project MOSAR was conducted in five rehabilitation units: the Berck Maritime Hôpital (Berck, France), Fondazione Santa Lucia (Rome, Italy), Guttmann Institute (GI; Barcelona, Spain), and Loewenstein Hospital and Tel-Aviv Sourasky Medical Center (TA) (Tel-Aviv, Israel). Patients were screened for carriage of *Enterobacteriaceae* resistant to expanded-spectrum cephalosporins (ESCs) from admission until discharge. The aim of this study was to characterize the clonal structure, extended-spectrum β -lactamases (ESBLs), and acquired AmpC-like cephalosporinases in the *Escherichia coli* populations collected. A total of 376 isolates were randomly selected. The overall number of sequence types (STs) was 76, including 7 STs that grouped at least 10 isolates from at least three centers each, namely, STs 10, 38, 69, 131, 405, 410, and 648. These clones comprised 65.2% of all isolates, and ST131 alone comprised 41.2%. Of 54 STs observed only in one center, some STs played a locally significant role, like ST156 and ST393 in GI or ST372 and ST398 in TA. Among 16 new STs, five arose from evolution within the ST10 and ST131 clonal complexes. ESBLs and AmpCs accounted for 94.7% and 5.6% of the ESC-hydrolyzing β -lactamases, respectively, being dominated by the CTX-M-like enzymes (79.9%), followed by the SHV (13.5%) and CMY-2 (5.3%) types. CTX-M-15 was the most prevalent β -lactamase overall (40.6%); other ubiquitous enzymes were CTX-M-14 and CMY-2. Almost none of the common clones correlated strictly with one β -lactamase; although 58.7% of ST131 isolates produced CTX-M-15, the clone also expressed nine other enzymes. A number of clone variants with specific pulsed-field gel electrophoresis and ESBL types were spread in some locales, potentially representing newly emerging *E. coli* epidemic strains.

Escherichia coli is one of the key human pathogens, responsible for a variety of infections in both the hospital and the community. The most important is urinary tract infection and also bacteremia, intra-abdominal infections, nosocomial pneumonia, neonatal meningitis, and others (1). Treatment of these infections has become more and more problematic with time due to the increasing resistance of *E. coli* to antimicrobials, including the expanded-spectrum cephalosporins (ESCs). The main mechanism of resistance to ESCs in *Enterobacteriaceae* is the production of several types of β -lactamases, such as extended-spectrum β -lactamases (ESBLs) or AmpC-like cephalosporinases, and *E. coli* is one of the major producers of these (2, 3). The frequency of ESBL and acquired AmpC producers in nosocomial *E. coli* populations has been constantly growing since the 1980s and 1990s, and since the early 2000s, such strains have been increasingly observed in the community as well (2–4). Two main factors have been responsible for this increase, namely, the dissemination of plasmids carrying the ESBL and AmpC genes and the clonal spread of producer strains (5, 6).

The recent introduction of multilocus sequence typing (MLST) schemes (7) has accelerated the accumulation of knowledge on the clonality of *E. coli* populations worldwide. Numerous reports have described a number of clones, often forming clonal complexes, that have disseminated in all continents. Some of them, usually belonging to the more virulent phylogroups B2 and D (8), have been associated with the rapid spread of resistance,

such as ESBL production (6, 9). The main example has been the uropathogenic sequence type 131 (ST131), identified in 2008 as a global producer of the CTX-M-15 β -lactamase, which currently predominates among ESBLs (10–14).

The European Union-funded project MOSAR was a prospective study addressing various aspects of the spread of resistance in intensive care, surgical, and rehabilitation wards across Europe and Israel, as well as specific actions aimed at combating that (www.mosar-sic.org). One of its objectives was to reveal the clonal structure of ESC-resistant *Enterobacteriaceae* colonizing patients in rehabilitation units (RUs) in four densely populated areas. Here we show the results of the molecular analysis of *E. coli* strains identified during this project.

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MATERIALS AND METHODS

Study design and clinical isolates. Five RUs located in the north of Paris, France (Berck Maritime Hôpital [BM], Berck, France), Rome, Italy (Fondazione Santa Lucia [FS]), Barcelona, Spain (Guttmann Institute [GI]), and Tel-Aviv, Israel (Loewenstein Hospital [LH] and Tel-Aviv Souraski Medical Center [TA]), participated in the study upon the approval of their ethics committees. The units differ in types of patients and size (BM, 80 beds; FS, 106 beds; GI, 38 beds; LH, 45 beds; TA, 50 beds). From October 2008 to February 2011, rectal swab specimens for culture were collected from all patients at admission, 2 weeks later, and then once monthly and at discharge. Rectal swabs were plated onto Brilliance ESBL agar (Oxoid, Basingstoke, United Kingdom); *Enterobacteriaceae* colonies were identified using the manufacturer's instructions. One colony of each morphotype was frozen and shipped to the National Medicines Institute, Warsaw, Poland, for definite analysis. Species identification was done with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). The phenotypic detection of ESBL and AmpC expression was carried out using the ESBL double-disk synergy test with disks containing cefotaxime, ceftazidime, cefepime, and amoxicillin with clavulanate on Mueller-Hinton agar (Oxoid) nonsupplemented and supplemented with 250 µg/ml cloxacillin (15). *E. coli* isolates suspected of the high-level AmpC production (augmentation of inhibition zones upon supplementation with cloxacillin) were tested by PCR for acquired AmpC types (16).

Of the total of 2,389 ESC-resistant *E. coli* isolates identified in the RUs during the project, 376 patient-unique isolates with an ESBL and/or acquired AmpC, collected in 2008 and 2009, were randomly selected for the molecular analysis according to their study numbers (BM, $n = 31$; FS, $n = 108$; GI, $n = 32$; LH, $n = 64$; and TA, $n = 141$). The aim was to select at least 30 isolates per hospital; differences in numbers between particular sites resulted from differences in the timing of the clinical trials at the sites, the availability of cultures at the start of this analysis, and the overall number of ESC-resistant isolates at each institution. The majority of isolates from TA were also used in a separate study on colonization risk factors and the population dynamics of ESBL-positive *E. coli*, and a part of the molecular data are presented elsewhere (17).

Typing. Pulsed-field gel electrophoresis (PFGE) was performed as described previously (18) with the use of the XbaI restriction enzyme (Fermentas, Vilnius, Lithuania). PFGE types and subtypes were discerned visually using the criteria of Tenover et al. (19). In order to construct dendrograms, electrophoretic patterns were compared by the BioNumerics Fingerprinting software (version 6.01; Applied Maths, Sint-Martens-Latem, Belgium), using the Dice coefficient, and clustering by the unweighted pair group method with arithmetic mean with a 1% tolerance in band position differences. MLST was carried out as described previously (7); the database available at <http://mlst.ucc.ie>, was used for assigning sequence types (STs) and clonal complexes. The clonal diversity indexes and confidence intervals (CIs) were calculated as described by Grundmann et al. (20). Classification of isolates into major *E. coli* phylogenetic groups was done by PCR (21).

β-Lactamase analysis. β-Lactamase profiling was done by isoelectric focusing as reported previously (22), using a model 111 mini-isoelectric focusing cell (Bio-Rad, Hercules, CA). Identification of the *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-9}, *bla*_{CTX-M-25}, *bla*_{SHV}, *bla*_{TEM}, *bla*_{CMY-2}, and *bla*_{DHA}-like genes was done by PCRs (23–26). Sequencing of the genes was performed as reported previously (23), using sets of consecutive primers specific for each gene type.

RESULTS AND DISCUSSION

Clonal structure of the *E. coli* populations. The 376 *E. coli* isolates were typed by PFGE, followed by MLST of representatives for each PFGE type; for larger types with subtypes, MLST was done on several isolates per type. Overall, 240 isolates were analyzed by MLST. A total of 76 clones (STs) were found (Tables 1 and 2; Fig. 1). The clonal diversity index for the entire collection was 81.9% (CI, 77.9 to 85.8%) and varied from 73.8% (CI, 64.3 to

83.2%) in FS, 80.9% (CI, 74.8 to 87.0%) in TA, 83.0% (CI, 69.4 to 96.2%) in BM, and 86.0% (CI, 79.6 to 92.4%) in LH to 91.3% (CI, 85.4 to 97.3%) in GI. This indicated that in general the *E. coli* populations were differentiated; however, the ongoing spread of specific strains likely played a notable role as well. Indeed, the detailed analysis (PFGE and β-lactamase profiling) revealed clusters of closely related isolates in each center, most significantly in FS and TA (discussed below).

Major clones. Twenty-two clones were observed in at least two centers in different countries ($n = 292$; 77.7%), while 10 STs were identified in three or more hospitals each (Table 1). Of these 10 clones, 7 comprised at least 10 isolates each, namely, STs 10, 38, 69, 131, 405, 410, and 648, accounting for 65.2% of the study isolates ($n = 245$). The situation in which a large collection of *E. coli* isolates is dominated by a small number of clones was often observed, but the composition of such groups differed according to the region and the type and source of isolates (29–32). The seven clones represent different phylogroups (8), namely, groups A (STs 10 and 410), B2 (ST131), and D (STs 38, 69, 405, and 648), and were reported in many studies performed worldwide on isolates from hospital and community patients (6, 29, 31–34). Their isolates varied in virulence inter- and intraclonally (7, 29, 33, 35, 36); recently, they have often been associated with diverse resistance traits, including ESBLs and other β-lactamases (6, 29, 31–35, 37–40).

(i) **ST131.** The clonal distribution was dominated by ST131, to which 41.2% of the isolates ($n = 155$) were classified. It prevailed in all of the centers, though at various rates: from 25.0% in GI, 32.8% in LH, 41.1% in TA, and 41.9% in BM to 50.9% in FS. A single isolate from FS represented a new ST, ST1827, being a single-locus variant (SLV) of ST131 and belonging to its clonal complex. ST131 has been of the highest concern since 2008, when it was identified to be the global CTX-M-15 producer (10, 12, 13). It has been less frequent among susceptible isolates (13, 30, 41), but the apparent ease with which it acquired resistance combined with its virulence has fuelled its pandemic spread (13). Except for a few studies (27, 40), ST131 has been the dominant clone in most studies on ESBL-producing *E. coli* (6, 13, 31, 35, 42); therefore, its high frequency here was not surprising. The lowest rate in GI is concordant with other reports from Spain and Barcelona (33, 43, 44); so are the higher rates in France (BM) and Italy (FS) (41, 45). The data for Israel are the first from this country and some of the first from the Middle East (12, 34), showing a remarkable prevalence of ST131 that is higher than that in Egypt (34).

(ii) **ST10.** ST10 was far less frequent ($n = 19$; 5.1%), but like ST131, it was identified in all of the hospitals. Moreover, eight other clones found sporadically in two sites or one site each (STs 48, 167, 617, 744, 1488, 1830, 1831, and 2519) belonged to the ST10 complex, grouping 34 isolates (9.0%) altogether. Four of these STs were new: ST1488 (from FS) and ST1830 (GI) were SLVs of ST10, while ST1831 (FS) and ST2519 (LH) were SLVs of the ST10-related ST744 and ST617, respectively. In all but one case, such SLV pairs occurred in the corresponding centers; therefore, the new STs might have evolved in these. The complex was especially remarkable in the Israeli center LH, where it comprised 17.2% of isolates ($n = 11$). The significant contribution of ST10 to the spread of resistance has been a very recent observation; previously it was reported in Spain (44), Italy (46), Egypt (34), and Canada (31).

Minor clones. The 54 STs identified only in one center each

TABLE 1 Clonal complexes, geographic distribution, PFGE types, and ESBL or AmpC types of *E. coli* clones identified in at least two centers

ST/clone ^a	Clonal complex	Phylogroup ^b	Center(s)	No. (%) of isolates	No. of PFGE types	ESBL or AmpC variant(s) (no. of isolates or center)
ST131	ST131	B2	BM	13	6	CTX-M-15 (8) and -14 (5)
			FS	55	19	CTX-M-15 (50), -14 (2), -1 (1), and -2 (1); SHV-5 (1)
			GI	8	5	CTX-M-15 (3), -14 (1), and -27 (1); SHV-12 (3)
			LH	21	13	CTX-M-15 (15) and -27 (5); CMY-4 (1)
			TA	58	7	CTX-M-27 (34), -15 (15), -14 (2), -39 (1), and -55 (1); CMY-4 (5)
				155 (41.2)		
ST10	ST10	A	BM	1	1	CTX-M-15
			FS	4	4	CTX-M-2 (2) and -1 (1); CMY-2 (1)
			GI	3	2	CTX-M-14
			LH	8	4	CTX-M-15 (4); SHV-12 (4)
			TA	3	3	CTX-M-14 (1); SHV-5 (1) and -12 (1)
				19 (5.1)		
ST354	ST354	D	BM	1	1	TEM-24
			FS	2	2	CTX-M-1 (1); CMY-2 (1)
			GI	1	1	CTX-M-14
			LH	1	NT ^c	CMY-2
			TA	2	2	CTX-M-2 (1) and -15 (1)
				7 (1.9)		
ST405	ST405	D	BM	1	1	CMY-2
			FS	4	3	CTX-M-15 (2), -1 (1), and -14 (1)
			LH	3	2	CTX-M-9 (1) and -15 (1); CMY-2 (1)
			TA	10	7	CTX-M-15 (5) and -9 (3); SHV-12 (1); CMY-2 (1)
				18 (4.9)		
ST38	ST38	D	FS	3	1	CTX-M-2
			GI	1	1	CTX-M-14
			LH	4	4	CTX-M-15 (3) and -9 (1)
			TA	8	7	CTX-M-15 (4), -14 (2), -9 (1), and -27 (1)
				16 (4.3)		
ST648	ST648	D	BM	2	2	CTX-M-1 (1) and -15 (1)
			FS	4	4	CTX-M-15 (3) and -14 (1)
			LH	1	1	CTX-M-15
			TA	6	2 + 1 NT	CTX-M-15 (5) and -14 (1)
				13 (3.5)		
ST410	ST23	A	FS	3	2	CTX-M-15 (2); SHV-12 (1)
			LH	8	3	SHV-12
			TA	3	2	SHV-12
				14 (3.7)		
ST69	ST69	D	FS	1	1	CTX-M-15
			LH	2	2	CTX-M-14
			TA	7	3	CTX-M-14 (5) and -15 (1); CMY-4 (1)
				10 (2.7)		
ST57	ST350	D	FS	1	1	CTX-M-1
			GI	3	2	CTX-M-14 (2); SHV-12 (2)
			LH	1	NT	CMY-2
				5 (1.3)		
ST155	ST155	B1	FS	1	1	CTX-M-1
			GI	1	1	SHV-2
			LH	1	1	CTX-M-14
				3 (0.8)		
STs observed in two centers						
ST224		B1	FS, LH	5 (1.3)	4	CTX-M-1 (FS); CTX-M-1 and -15; SHV-12 (LH)
ST167	ST10	A	FS, LH	4 (1.1)	3	CTX-M-15 (FS); CMY-42 (LH)
ST88	ST23	A	FS, TA	3 (0.8)	2	CTX-M-1 (FS); CTX-M-100 (TA)
ST216		A	GI, TA	3 (0.8)	3	CTX-M-3 (GI); SHV-12 (TA)
ST617	ST10	A	FS, LH	3 (0.8)	2 + 1 NT	CTX-M-15 (FS); CTX-M-27; SHV-12 (LH)
ST48	ST10	A	FS, TA	2 (0.5)	2	CTX-M-1 (FS); SHV-12 (TA)
ST59	ST59	D	FS, TA	2 (0.5)	2	CTX-M-1 (FS); CTX-M-14 (TA)
ST117		D	FS, GI	2 (0.5)	2	CTX-M-1 (FS); CTX-M-14 (GI)
ST744	ST10	A	BM, LH	2 (0.5)	2	CTX-M-15 (BM); CMY-2 (LH)
ST929		B2	BM, TA	2 (0.5)	2	CTX-M-15 (BM); CTX-M-55 (TA)
ST940		B1	BM, TA	2 (0.5)	2	CTX-M-15 (BM and TA)
ST1431		A	FS, TA	2 (0.5)	2	CTX-M-2 (FS and TA)
Total				292 (77.7)		

^a STs are ordered according to (i) the number of centers in which these were identified and (ii) their prevalence in the entire collection of isolates. STs observed in two centers are presented one per line, without splitting into the center-specific groups.

^b *E. coli* phylogroups were adopted from reference 6, 9, 27, or 28 (STs 10, 38, 57, 59, 69, 117, 131, 155, 354, 405, 410, and 648) or determined in this work (STs 48, 88, 167, 216, 224, 617, 744, 929, 940, and 1431).

^c NT, nontypeable by PFGE due to repeated DNA degradation.

TABLE 2 Clonal complexes, PFGE types, and ESBL or AmpC types of *E. coli* clones identified in a single center

Center	ST/clone (clonal complex) ^a	No. of isolates	No. of PFGE types	ESBL or AmpC variant(s) (no. of isolates)
BM	23 (23), 58 (155), 127, 154, 345, 349 (349), 1594, 1664, 2164, 2647	10	10	CTX-M-1 (3), -9 (1), and -15 (2); TEM-24 (2) and -52 (1); CMY-2 (1); DHA-1 (1)
FS	20 (20), 63, 73, 120, 302, 359, 728, 1249, 1488 (10), 1823 , 1827 (131), 1831 (10), 1944 , 2518 , 2648 , 2928	17	15 + 1 NT ^b	CTX-M-1 (12), -9 (1), and -15 (2); SHV-2a (1); CMY-2 (1)
GI	156 (156)	4	2 + 2 NT	CTX-M-14 (2); CMY-2 (2)
	393 (31)	3	NT	CTX-M-32
	40 (40), 226 (226), 602 (446), 607, 1830 (10), 2197	6	6	CTX-M-14 (2) and -15 (1); SHV-12 (3)
LH	316 (278), 361, 542, 1011, 2519 (10), 2520	8	7	CTX-M-2 (1), -14 (1), -15 (5), and -39 (1)
TA	372	13	2	CTX-M-15 (1); SHV-5 (12)
	398 (398)	9	1	CTX-M-39 (8); SHV-5 (1)
	62, 95 (95), 348 (156), 409, 449 (31), 469 (469), 641 (86), 746, 1196, 1596 , 1597 , 1598	14	11 + 1 NT	CTX-M-2 (3), -14 (1), and -15 (7); SHV-5 (1) and -12 (2)

^a The first number refers to the ST of a particular clone, and the following number in parentheses refers to the clonal complex if a clone is a member of such a complex. Numbers in bold refer to new STs identified in this work. The STs observed in a center are shown in one line together, except for the more prevalent STs (ST156 and ST393 in GI and ST372 and ST398 in TA), which are presented separately.

^b NT, nontypeable by PFGE due to repeated DNA degradation.

(Table 2) encompassed 22.6% of the isolates ($n = 84$), with larger fractions in GI (40.6%) and BM (32.3%). Several clones in this group were locally significant, e.g., ST156 and ST393 in GI (12.5% and 9.4%, respectively) or ST372 and ST398 in TA (9.2% and 6.4%, respectively). A notable finding was 16 new STs, identified in each site, including the 5 aforementioned members of the ST10 and ST131 clonal complexes. The minor clones were an important source of the interhospital diversity, making, e.g., the *E. coli* population in GI relatively more specific than the *E. coli* populations in the others. Their position within the global MLST database varied; whereas the new STs were likely local clones, most of the minor STs had been found in other countries, and a number of them have spread globally or belonged to pandemic complexes. It is unclear why some of them, like the ST31 complex, were so scarcely observed. The ST31 complex (clonal group O15:K52:H1) is often associated with resistance (33, 47, 48) and was represented here only by three ST393 isolates in GI and one ST449 isolate in TA. It is easier to understand why just single isolates of ST73 (in FS) and ST95 (TA) were identified. These highly pathogenic clones have frequently been recovered from clinical samples but rarely, if at all, with ESBLs (29, 34, 49–51), which may explain their absence in the analyses targeted on ESBL-positive *E. coli* isolates (27, 31, 32, 44).

MLST versus PFGE. All of the *E. coli* clones of wider occurrence and/or higher prevalence were split into multiple PFGE types in the entire population and often so in the local ones (Tables 1 and 2; see Fig. S1 in the supplemental material). ST131 isolates were diversified into 5 to 7 types in BM, GI, and TA, 13 types in LH, and 19 types in FS, with similarity rates between two patterns even falling below 60%. Some types formed bigger clusters in each hospital (>5% of isolates in a center), usually being ST131, e.g., PFGE types FSEcoJ in FS ($n = 22$; 20.4%) and TAEcoI in TA ($n = 34$; 24.1%). Among the other clones, larger clusters were observed for ST410 in LH (type LHEcoO, 9.4%), and ST372 and ST398 in TA (TAEcoQ, 8.5%, and TAEcoAS, 6.4%, respec-

tively). The STs found in more than one hospital usually had different PFGE types in each center; only the two Israeli sites (LH and TA) shared closely related strains, including several types of ST131 (e.g., TAEcoI) and some types of STs: 38, 69, 405, and 648. In all these cases, isolates of the same PFGE type produced the same β -lactamase in both institutions (see below). The PFGE heterogeneity of ST131 and efficient spread of specific types have been observed previously, and it seems that while in general the diversity reflects the long-term evolution of the clone, the prevalent PFGE types may represent emerging subclones with even enhanced epidemic potential (31, 42, 52). Recently, Johnson et al. (35) have confirmed the earlier hypothesis that the increased virulence in combination with resistance is likely responsible for the ecological success of such variants (4, 6, 13), although on a smaller scale, similar observations could be made in this study for other clones with multiple PFGE types, like ST10, ST69, or ST648, in particular centers.

ESBLs and acquired AmpCs. Overall, 94.7% of the *E. coli* isolates produced ESBLs ($n = 356$) and 5.6% produced acquired AmpCs ($n = 21$); one isolate had both an ESBL and AmpC. Considering that two isolates coexpressed two ESBLs, the total number of ESC-hydrolyzing β -lactamases was 379, including 358 ESBLs and 21 AmpCs (Table 3). The ESBLs belonged to the CTX-M ($n = 303$; 79.9%), SHV ($n = 51$; 13.5%), and TEM ($n = 4$; 1.1%) families, while the AmpCs were CMY-2-like enzymes ($n = 20$; 5.3%) and DHA-1 ($n = 1$; 0.3%). The prevalence of CTX-M enzymes was 66.7% in GI, 69.2% in LH, 77.3% in TA, 78.1% in BM, and 94.4% in FS. They represented four variant groups: CTX-M-1 (CTX-M-1, -3, -15, -32, and -55), CTX-M-2 (CTX-M-2), CTX-M-9 (CTX-M-9, -14, and -27), and CTX-M-25 (CTX-M-39 and -100). The CTX-M-1- and -9-like types were ubiquitous and prevalent (50.1% and 23.5%, respectively), while the CTX-M-25-like types were found only in Israel (7.1% in TA). SHVs (SHV-2, -2a, -5, and -12) were prevalent in GI (27.3%) and

FIG 1 MLST-based population structure of ESBL- and/or AmpC-producing *E. coli* isolates identified in the five rehabilitation centers (BM, FS, GI, LH, and TA) during the MOSAR study. The scheme was constructed using eBURST analysis. STs are symbolized by dots; the size of a dot corresponds to the number of isolates belonging to an ST. SLVs are linked by solid lines.

TA (17.7%), whereas TEMs (TEM-24 and -52) occurred only in BM (12.5%). The CMY-2-type AmpCs included three variants (CMY-2, -4, and -42). Only three β -lactamases, CTX-M-14, CTX-M-15, and CMY-2, were identified in each of the centers.

CTX-M-15 was produced by 154 isolates of 29 clones (40.6% of the enzymes) (Table 3). Its frequency varied from 12.1% in GI, 28.4% in TA, 46.2% in LH, and 46.9% in BM to 60.2% in FS. CTX-M-14 was present in 39 isolates of 16 clones (10.3%), being the main enzyme in GI (39.4%). CTX-M-27 and CTX-M-1 were observed in three centers each and were produced by 42 and 30 isolates, respectively (11.1% and 7.9% of all of the enzymes, respectively). CTX-M-27 was prevalent in TA (24.8%), but it was expressed only by three STs; in contrast, CTX-M-1, frequent in FS (23.1%), was produced by 26 clones. CTX-M-100 from a single isolate from TA was a new CTX-M-25-like variant and has separately been described in detail (53).

All these results reflected well the rapid dissemination of the CTX-M-type enzymes in the last decade, especially CTX-M-15 and -14 (11, 14). Local specificities, like the predominance of CTX-M-14 in GI, the spread of CTX-M-1 among *E. coli* clones in FS, and the presence of the CTX-M-25 types in LH and TA, had been observed in Spain, Italy, and Israel, respectively (28, 44, 54). The SHV enzymes have remained significant, but TEMs seemingly

tend to disappear under CTX-M pressure, as reported elsewhere (23). They were identified only in France, where TEMs have evolved and extensively disseminated in the past (55).

Clones versus β -lactamases. Of the widely identified clones (Table 1), ST131 alternatively produced 10 different β -lactamases, including 7 CTX-M variants of all groups, SHV-5, SHV-12, and CMY-4. The ST131 PFGE types were usually associated with one enzyme each, like the FSEcoJ with CTX-M-15 in FS or TAEcoI with CTX-M-27 in TA and LH. A few ST131 types expressed various β -lactamases, like TAEcoE in TA ($n = 17$; 12.1%), which produced CTX-M-14, CTX-M-15, CTX-M-55, or CMY-4, depending on the PFGE subtype. The most prevalent enzyme in ST131 was CTX-M-15 (58.7% of ST131 isolates), present in multiple PFGE types in each center; however, its frequency varied from 25.9% of ST131 isolates in TA (less than that of CTX-M-27) and 37.5% in GI (equal to that of SHV-12) to 90.9% in FS. The lack of a strict correlation between an ST and a β -lactamase was also observed for almost all other clones of wider distribution or higher prevalence, but isolates of a given PFGE type expressed the same enzyme. This was the case for the aforementioned larger PFGE clusters of ST410 in LH (SHV-12) or ST372 in TA (SHV-5 and CTX-M-39, respectively). These results follow those of a number of other studies which demonstrated that the

TABLE 3 Geographic and quantitative distribution of β -lactamase variants in the study isolates

β -Lactamase	CTX-M group	Center(s)	No. of isolates	% of enzymes	No. of clones
CTX-M-15	1	BM, FS, GI, LH, TA	154	40.6	29
CTX-M-27 ^a	9	GI, LH, TA	42	11.1	3
CTX-M-14 ^b	9	BM, FS, GI, LH, TA	39	10.3	16
CTX-M-1	1	BM, FS, LH	30	7.9	26
CTX-M-2	2	FS, LH, TA	13	3.4	8
CTX-M-39	25	TA	10	2.6	3
CTX-M-9	9	BM, FS, LH, TA	8	2.1	4
CTX-M-32	1	GI	3	0.8	1
CTX-M-55	1	TA	2	0.5	2
CTX-M-3	1	GI	1	0.3	1
CTX-M-100	25	TA	1	0.3	1
SHV-12		FS, GI, LH, TA	33	8.7	14
SHV-5		FS, TA	16	4.2	5
SHV-2		GI	1	0.3	1
SHV-2a		FS	1	0.3	1
TEM-24		BM	3	0.8	3
TEM-52		BM	1	0.3	1
CMY-2		BM, FS, GI, LH, TA	12	3.2	8
CMY-4		LH, TA	7	1.8	3
CMY-42		LH	1	0.3	1
DHA-1		BM	1	0.3	1
Total			379 ^c		

^a CTX-M-27 was encoded by the *bla*_{CTX-M-27a} ($n = 41$) and *bla*_{CTX-M-27b} ($n = 1$) genes.

^b CTX-M-14 was encoded by the *bla*_{CTX-M-14a} ($n = 36$) and *bla*_{CTX-M-14b} ($n = 3$) genes.

^c The total number is higher than 376 isolates because three isolates coproduced two different β -lactamases.

pandemic *E. coli* clones have basically been spreading by themselves, while the acquisition of locally prevalent plasmids with resistance genes stimulates their further dissemination (6, 13). The combination of ST131 traits with those provided by *bla*_{CTX-M-15} carrying plasmids has probably made such organisms better fit for further expansion (5, 10, 13, 35), and this association was observed in this work too. The remarkable exception was the Israeli center TA, where the spread of the PFGE type TAecoI resulted in the predominance of CTX-M-27 in ST131 (~59%), while the association was the strongest in FS in Rome, where the vast majority of ST131 isolates expressed CTX-M-15. These values are close to the extremes reported in earlier studies (13, 45).

Conclusions. This study, being one of the largest *E. coli* population analyses conducted so far, showed a complex view of the epidemiology, with a number of similarities and differences between the clinical sites. The similar elements were the global clones, mainly ST131, and the predominant ESBL types, CTX-M-15 and -14. On the other hand, the local populations differed remarkably from each other; ~50% of the STs identified in a center were usually not observed in the others. This diversity was apparent even in the two Tel-Aviv sites, which had only eight global clones in common. If one considers that only two clones (ST131 and ST393) in GI in Barcelona were found among ESBL producers from another hospital in this city in 2008 (43), the results show how different *E. coli* populations may exist in health care institutions of the same geographic area. The new data, especially those from Israel, suggest the possible emergence of several clonal variants that may appear to successfully spread in the future.

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