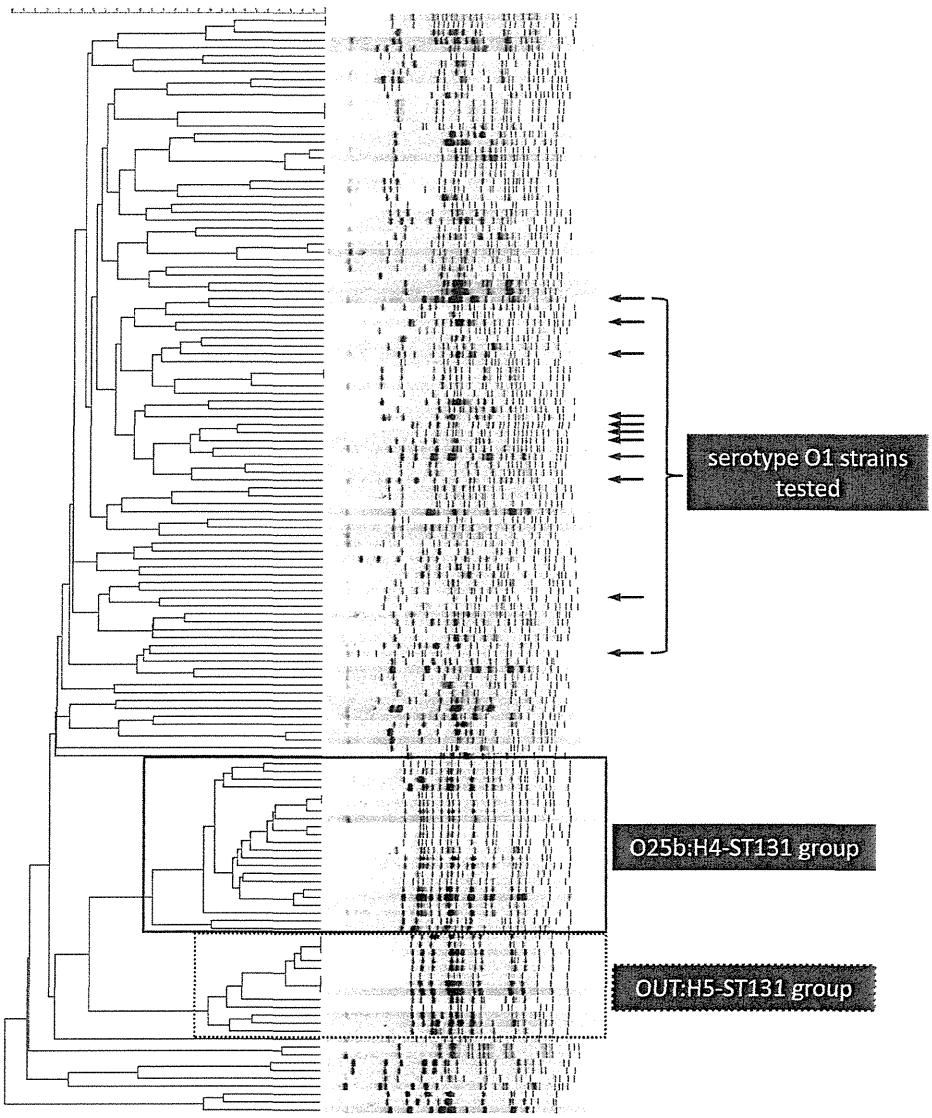

FIG 1 Dendrogram of PFGE types among the ESBL-producing *E. coli* isolates. The results of PFGE of XbaI-digested whole-genome DNA from 142 of the 145 ESBL-producing *E. coli* isolates recovered from 120 participants are shown. DNA from the remaining three ESBL-producing *E. coli* isolates (including one *E. coli* serogroup O15 isolate and two *E. coli* serogroup OUT isolates) became smeared during digestion with XbaI. The dendrogram was produced by the unweighted pair group method using arithmetic averages (UPGMA) algorithm based on the Dice similarity coefficient with a 1.0% band position tolerance. The O25b:H4-ST131 group and the OUT:H5-ST131 group are surrounded by squares with solid lines and dotted lines, respectively. Arrows, the rows for the serogroup O1 isolates tested.

bla_{CTX-M}-carrying plasmids to recipient cells was successful for 11 isolates, and the replicon types of the plasmids transferred were IncF (1 isolate), IncFIB (2 isolates), IncK (3 isolate), and IncI1 (1 isolate), as determined by PBRT. The Inc types of the plasmids from the remaining 4 isolates could not be determined. The $bla_{CTX-M-27}$ genes in the *E. coli* O25b:H4-ST131 isolates were carried by plasmids belonging to IncF or IncFIB.

Detection of multiple bla_{CTX-M}-harboring *E. coli* isolates with diverse serotypes. In 2 of the 13 long-term carriers (participants 49 and 60), $bla_{CTX-M-14}$- and $bla_{CTX-M-15}$-harboring *E. coli* isolates, respectively, were continuously recovered during the period of investigation, but the serotypes of the *E. coli* isolates were different in each participant (Table 4). In the case of participant 49, a $bla_{CTX-M-14}$-harboring *E. coli* O15:H6-ST354 isolate was repeatedly recovered for 7 months, and $bla_{CTX-M-14}$-harboring *E. coli* O125:H6 isolates belonging to ST354 were also recovered together with a $bla_{CTX-M-14}$-harboring *E. coli* O27:HUT-ST641 isolate in July 2010. Conjugal transfer of a $bla_{CTX-M-14}$-carrying plasmid to recipient cells was successful in the conjugation experiment, but the replicon types of the plasmids carrying the $bla_{CTX-M-14}$ gene could not be determined by PBRT. $bla_{CTX-M-15}$-harboring *E. coli* O169:H51 and OUT:HNM isolates, together with *E. coli* O1:H6-ST648, were recovered from the feces of participant 60, a long-term carrier, on different occasions. However, the replicon types of all 3 plasmids carrying the $bla_{CTX-M-15}$ gene were IncK, suggesting the probable conjugal transfer of the $bla_{CTX-M-15}$-carrying plasmids among genetically different commensal *E. coli* lineages in the bowel of participant 60.

TABLE 4 Characteristics of bla_{CTX-M}-harboring *E. coli* isolates repeatedly recovered from 13 long-term carriers and bla_{CTX-M}-harboring *E. coli* isolates simultaneously recovered from 2 participants

Participant[a]			Isolate		Donor			Transconjugant[b]			Tested month[c]															
											2010												//	2011		
			Serotype	MLST	ESBL type	Replicon type[b]	Resistant to[c]	ESBL type	Replicon type[b]	Resistant to[c]	1	2	3	4	5	6	7	8	9	10	11	12		3	~	12
29	F	52	O1:H6	ST648	CTX-M-14	ND	CIP, LVX, CTX, PIP	-	-	-		●	●	●												
60	F	60	O1:H6[d]	ST648	CTX-M-15	FIA, FIB, K, B/O	CIP, LVX, MIN, AZL, CAZ, CTX, PIP	CTX-M-15	K	AZL, CTX, PIP	×	×	×	●	●	●	●		●	●	●	●		●		
			O169:H51	ST297	CTX-M-15	K, B/O	MIN, AZL, CAZ, CTX, PIP	CTX-M-15	K	AZL, CTX, PIP							●									
			OUT:HNM	ST404	CTX-M-15	K, B/O	AZL, CAZ, CTX, PIP	CTX-M-15	K	AZL, CTX, PIP														●		
66	F	50	O1:H6	ST648	CTX-M-14	FIA, FIB	CIP, LVX, MIN, CTX, PIP	-	-	-	●	●	●	●	●	●	●		●		●	●		●		●
12	M	31	O25b:H4	ST131	CTX-M-27	FIA, FIB	CIP, LVX, MIN, AZL, CTX, PIP	-	-	-	●						●									
64	M	53	O25b:H4	ST131	CTX-M-27	FIA, FIB	CIP, LVX, MIN, CTX, PIP	CTX-M-27	FIB	CTX, PIP	●	●	●	●	×	×	●		×		×	×		×		×
69	F	58	O25b:H4	ST131	CTX-M-27	FIA, FIB	CIP, LVX, CTX, PIP	CTX-M-27	F	CTX, PIP	●	●	△	●	●	×		●	×		×	×		×		×
106	M	39	O25b:H4	ST131	CTX-M-27	FIA, FIB	CIP, LVX, MIN, CTX, PIP	CTX-M-27	FIB	CTX, PIP			×	●	●	●	×			×	●	×		×		
82	M	68	OUT:H5	ST131	CTX-M-14	FIA, FIB	GEN, CTX, PIP	-	-	-	●	●	●	●		●			×			×		×		×
88	F	45	OUT:H5	ST131	CTX-M-14	I1, FIA, FIB	CIP, LVX, CTX, PIP	CTX-M-14	I1	CTX, PIP	●	●	●	●	●○	●	●			●		×		×		×
49	F	38	O15:H6[d]	ST354	CTX-M-14	FIA	CIP, LVX, MIN, AZL, CTX, PIP	CTX-M-14	ND	CTX, PIP	●	●	●	●		●	●			×	×	×		×		×
			O125:H6	ST354	CTX-M-14	FIA	CIP, LVX, AZL, CTX, PIP	CTX-M-14	ND	CTX, PIP							●									
			O27:HUT	ST641	CTX-M-14	ND	CTX, PIP	CTX-M-14	ND	CTX, PIP							●									
87	M	52	O74:H6	ST38	CTX-M-14	ND	CIP, LVX, CHL, MIN, GEN, CTX, PIP	-	-	-		●				●										
26	F	42	O78:HUT	ST23	CTX-M-15	I1, FIB, P	CIP, LVX, MIN, AZL, CAZ, CTX, PIP	CTX-M-15	ND	AZL, CTX, PIP		●	●	●												
50	F	59	OUT:H4	ST 3407	CTX-M-15	FIB	AZL, CAZ, CTX, PIP	-	-	-	●	●	●	●	●	●	●	○	×	×	×	×		×		

[a] The data in the first three columns represent the personal identification number, gender (F, female; M, male), and age (in years), respectively.

[b] -, not transferred; ND, the replicon type was not determined by PBRT.

[c] ●, detection of bla_{CTX-M}-harboring *E. coli*; ×, no detection of bla_{CTX-M}-harboring *E. coli*, even though fecal screening was performed; △, prescription of antimicrobial treatment; ○, overseas travel.

[d] Isolates detected for a long period.

[e] CIP, ciprofloxacin; LVX, levofloxacin; CTX, cefotaxime; PIP, piperacillin; MIN, minocycline; AZL, aztreonam; CAZ, ceftazidime; GEN, gentamicin; CHL, chloramphenicol.

DISCUSSION

We first elucidated that bla_{CTX-M}-harboring *E. coli* isolates have colonized the intestinal tracts of healthy Japanese people, even in the absence of antimicrobial pressure, for from 3 months to up to 2 years. At one point in the study of ESBL producers from 2,230 participants, 70 (3.1%) participants were positive for ESBL producers. This proportion is comparable to that (6.4%) determined in other investigations involving 218 participants in Japan (24). On the other hand, 52 (15.6%) of the 333 participants whose feces were checked more than twice were found to be positive for ESBL producers. This proportion is far greater than that for the 2,230 participants evaluated once, but we assume that this value may well more accu-

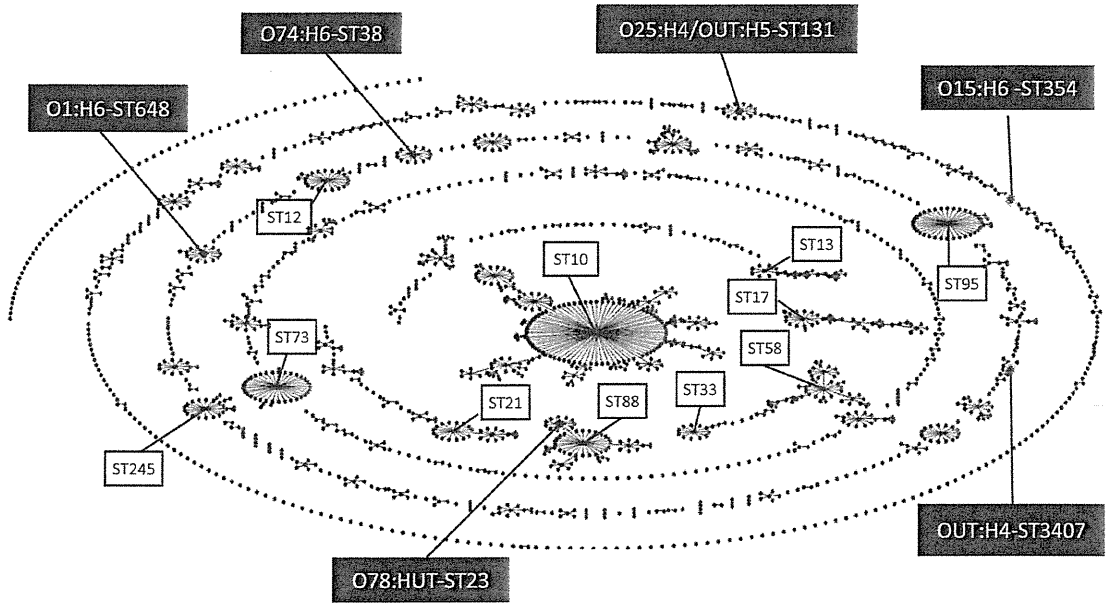

FIG 2 Result of eBURST analysis. A snapshot of the population created by eBURST analysis (http://eburst.mlst.net/) shows clusters of linked and unlinked sequence types in the *E. coli* MLST database (2,000 sequence types; http://mlst.ucc.ie/mlst/dbs/Ecoli). Sequence type labels were removed, but the serotypes and sequence types found for the ESBL-producing *E. coli* isolates repeatedly recovered from healthy people in the present study are shown.

rately reflect the state of fecal carriage of ESBL producers among healthy Japanese people. To our knowledge, our study, which involved more than 2,500 participants, is the first one to report the state of colonization by bla_{CTX-M}-harboring *E. coli* isolates in the intestinal tracts of people living in the community who have not apparently been exposed to antimicrobials.

There are some reports concerning the duration of fecal carriage of ESBL-producing *E. coli* isolates in humans who had been checked in clinical settings. An investigation enrolling 24 outpatients in Thailand demonstrated that the average period of carriage of ESBL producers in feces was 98 days and that 8 (33%) out of 24 patients carried ESBL-producing *E. coli* for 6 months (11). In a French study, 180 (40%) of 448 patients were persistent carriers of ESBL-producing *Enterobacteriaceae* for a median period of 6.6 months (25). After a nosocomial outbreak in southern Sweden, the long-term carriage of ESBL-producing *E. coli* (41 to 59 months) was observed in 5 (13%) of 39 patients (12). In a Netherlands study with 521 participants, 19 (16.8%) of 113 participants who acquired ESBL producers during foreign travel kept the ESBL producers for 6 months (26). A Swedish study also showed that 10 (24%) of 41 patients with traveler's diarrhea who carried ESBL-producing *E. coli* still carried the ESBL producers after 3 to 8 months (13). These findings are somewhat similar to our observation that 13 (25%) of the 52 carriers kept the ESBL producers for long periods. The present study may well be unprecedented, because more than 2,500 healthy participants were involved and the investigation was continued for more than 6 months with 10 carriers of ESBL producers and for up to 2 years in one case. Therefore, the results of our investigation suggest the actual state of the fecal carriage of ESBL producers among healthy people to whom no obvious antimicrobial was administered.

The affinity of particular *E. coli* serotypes to human intestines or the fitness of *E. coli* in human intestines would contribute to the long-term colonization of ESBL-producing *E. coli* in the intestinal tract. As for the serogroups of *E. coli* often recovered from human intestines, O1 (4.81%), both O1 (7.1%) and O25 (7.1%), and O25 (9.1%) were reportedly predominant in healthy subjects (27), patients suffering from sporadic diarrhea (28), and outpatients (29), respectively. These findings suggest that *E. coli* serogroups O1 and O25 are the ones that are most able to adapt to the human bowel. Serogroups O1 and O25 were predominant in the enteric flora of both American and Romanian people (30, 31), indicating that *E. coli* serogroups O1 and O25 generally accommodate themselves to the human intestines regardless of the human race. Among the people who participated in the present study, bla_{CTX-M}-harboring *E. coli* serogroup O1 or O25 was indeed continuously recovered from 7 of 13 carriers. Moreover, 1 case was found to carry $bla_{CTX-M-14}$-harboring *E. coli* O1:H6 for 2 years in the absence of antimicrobial treatment. These findings are consistent with those of several previous studies conducted in Japan and other countries. In the present study, the rates of detection of ESBL-producing *E. coli* serogroups O1 and O25 from the 120 people positive for ESBL producers were 7.6% (11/145) and 18.6% (27/145), respectively (Table 5). On the other hand, the rates of recovery of ESBL-producing *E. coli* isolates of serogroups O1 and O25 from patients who carried the ESBL producers for long periods were 23.1% (3/13) and 30.8% (4/13), respectively, and these values are much higher than those found for the 120 carriers of ESBL producers (Table 5). The acquisition of bla_{CTX-M} genes by *E. coli* serogroups O1 and O25 might well contribute to the long-term carriage of ESBL-producing *E. coli* in the intestines of healthy humans receiving no antimicrobial treatment. In addition, according to the results of PFGE analyses, it was suggested that the *E. coli* isolates belonging to serogroup O25 formed a clonal lineage, while the *E. coli* isolates belonging to serogroup O1 were genetically diverse (Fig. 1). It has been considered that bacterial strains harboring plasmids carrying

TABLE 5 Characteristics of the ESBL-producing *E. coli* isolates from the 120 overall carriers of ESBL producers and 13 long-term carriers of ESBL producers

Characteristic	No. (%) of isolates among:	
	145 isolates from 120 carriers	13 isolates from 13 long-term carriers
O serogroup		
O1	11 (7.5)	3 (23.1)
O25	27 (18.6)	4 (30.8)
O15	3 (2.1)	1 (7.7)
O74	5 (3.4)	1 (7.7)
O78	4 (2.8)	1 (7.7)
Other O serogroups	32 (22.2)	0 (0)
OUT	63 (43.4)	3 (23.1)
Molecular type of ESBL		
CTX-M-1 group	30 (20.7)	3 (23.1)
CTX-M-2 group	19 (13.1)	0 (0)
CTX-M-8 group	7 (4.8)	0 (0)
CTX-M-9 group	82 (56.6)	10 (76.9)
Non-CTX-M	7 (4.8)	0 (0)
Resistance to:		
Ceftazidime	25 (17.2)	3 (23.1)
Aztreonam	59 (40.7)	5 (38.5)
Gentamicin	34 (23.4)	2 (15.4)
Amikacin	1 (0.7)	0 (0)
Minocycline	96 (66.2)	8 (61.5)
Chloramphenicol	23 (15.9)	1 (7.7)
Fosfomycin	9 (6.2)	0 (0)
Ciprofloxacin	67 (46.2)	11 (84.6)
Levofloxacin	66 (45.5)	11 (84.6)

antimicrobial resistance genes tend to disappear sooner or later from human intestines and also that the plasmids carrying antimicrobial resistance genes are apt to be deleted from bacterial cells before long in the absence of antimicrobial treatment because the acquisition of antimicrobial resistance mechanisms usually impedes bacterial growth. However, ESBL-producing *E. coli* serogroups O1 and O25, which have a probable affinity for bla_{CTX-M}-bearing plasmids, could persistently inhabit the intestines of healthy people. This finding may suggest the possible acquisition of an additional ability for intraintestinal long-term colonization of *E. coli* isolates belonging to serogroups O1 and O25 that harbor IncF-group plasmids, which often carry various bla_{CTX-M} genes.

In the present study, we detected plasmids belonging to the IncF group, especially FIA and FIB, from 11 of 13 bla_{CTX-M}-harboring *E. coli* isolates repeatedly recovered, as reported previously (14). We also found that the $bla_{CTX-M-27}$ gene in *E. coli* O25b:H4-ST131 isolates was carried by plasmids belonging to the IncF or IncFIB group. The plasmid should have affinity for human-associated *E. coli* if the bla_{CTX-M}-carrying plasmid is kept for a long period in an *E. coli* isolate that colonizes human bowels even in the absence of antimicrobials. In fact, the plasmids bearing $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, or $bla_{CTX-M-27}$ often belong to the IncF type (32–34). These IncF-type plasmids were also frequently found in human-associated *E. coli* strains that can usually accept various types of plasmids carrying multiple drug resistance genes (35, 36).The IncF group of plasmids has frequently been detected in bla_{CTX-M} gene-harboring *E. coli* isolates repeatedly recovered from the feces of healthy people and could contribute to long-term carriage in the human bowel and to the widespread nature of bla_{CTX-M} genes among various Gram-negative bacilli belonging to the family *Enterobacteriaceae*.

The CTX-M-15-producing *E. coli* O25b:H4-ST131 isolates distributed worldwide are usually resistant to FQs. In the present study, FQ-resistant and bla_{CTX-M}-harboring *E. coli* isolates were continuously recovered from 11 (85%) of 13 long-term carriers, and FQ-resistant and $bla_{CTX-M-27}$-harboring *E. coli* O25b:H4-ST131 isolates were repeatedly isolated from 4 participants. The FQ resistance rates of ESBL-producing and ESBL-nonproducing *E. coli* isolates recovered from Japanese hospitals were about 63.3% and 30%, respectively (37, 38). Interestingly, the FQ resistance rate (85%) of the bla_{CTX-M}-harboring *E. coli* isolates colonizing Japanese food handlers for long periods found in the present study was considerably higher than the average FQ resistance rates of ESBL-producing and ESBL-nonproducing *E. coli* clinical isolates. This might predict the future endemicity of FQ-resistant *E. coli* O25b:H4-ST131 isolates harboring bla_{CTX-M} genes, especially $bla_{CTX-M-27}$, in Japan and surrounding Asian countries, as well as the global spread of FQ-resistant and CTX-M-15-producing *E. coli* O25b:H4-ST131 isolates.

In conclusion, our study elucidated that 70 (3.1%) of 2,230 healthy people were positive for ESBL producers when fecal specimens from these individuals were checked once and 52 (15.6%) of 333 people were positive for ESBL producers when fecal specimens were rechecked. This volunteer-based investigation could not systematically check all 2,563 participants through the investigation period, but 13 of the 52 carriers of ESBL producers who were checked more than twice (see Table S1 in the supplemental material) were found to carry ESBL producers for from more than 3 months to up to 2 years even in the absence of obvious antimicrobial treatment. Increasing human intestinal colonization of *E. coli* O25b:H4-ST131 and O1:H6-ST648 isolates that harbor bla_{CTX-M}-carrying plasmids belonging to the IncF, IncFIB, or IncK group would contribute to the augmented rates of long-term carriage of ESBL-producing *E. coli* isolates in the bowels of ordinary people receiving no antimicrobial treatment, as well as in patients admitted to hospital settings. FQ-resistant *E. coli* O25b:H4-ST131 isolates harboring $bla_{CTX-M-27}$ were repeatedly isolated from 4 of the 13 long-term carriers for several months. These observations might have implications for the choice of antimicrobial agents used for the treatment of community-acquired infections in the future, such as urinary tract infections and bacteremia caused by *E. coli*.

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