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Travelers Can Import Colistin-Resistant *Enterobacteriaceae*, Including Those Possessing the Plasmid-Mediated *mcr-1* Gene

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Stool samples from 38 travelers returning from India were screened for extended-spectrum cephalosporin- and carbapenem-resistant *Enterobacteriaceae* implementing standard selective plates. Twenty-six (76.3%) people were colonized with CTX-M or DHA producers, but none of the strains was colistin resistant and/or *mcr-1* positive. Nevertheless, using overnight enrichment and CHROMagar Orientation plates supplemented with colistin, four people (10.5%) were found to be colonized with colistin-resistant *Escherichia coli*. One cephalosporin-susceptible sequence type 10 (ST10) strain carried a 4,211-bp IS*Apl1*-*mcr-1*-IS*Apl1* element in an IncHI2 plasmid backbone.

The emergence of colistin-resistant (COL-R-*Ent*) *Enterobacteriaceae* harboring the plasmid-mediated *mcr-1* gene has raised serious concerns (1, 2). These strains (especially *Escherichia coli*) have been isolated worldwide from humans (1, 3–10), food-producing animals (4, 6, 11–14), the food chain (7, 10, 15–17), and the environment (4, 15). However, most of these studies searched for *mcr-1* in previously stored extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-*Ent*) (8, 12, 13, 15, 17). This leads to a question about the actual prevalence of *mcr-1*-positive *Enterobacteriaceae* (*mcr-1-Ent*), especially because *mcr-1* can be carried by plasmids not coharboring extended-spectrum β-lactamase (ESBL), plasmid-mediated AmpC (pAmpCs), and/or carbapenemase genes (5, 7, 12, 14, 18). Moreover, although it is known that the prevalence of intestinal colonization with ESC-R-*Ent* in returning travelers is very high (19), data regarding the *mcr-1-Ent* are needed. Only Arcilla et al. have explored this phenomenon, indicating that 0.9% of the ESBL producers isolated from stools of Dutch travelers co-possessed *mcr-1* (8).

We analyzed the pre- and posttrip stool samples (both within 1 week) of 38 people living in Switzerland and traveling to India during January to August 2015. A questionnaire was also filled out at each sampling time indicating that in the 12 months before going to India, participants frequently visited other countries but never suffered diarrhea. On the other hand, after the journey to India (mean stay, 17.8 days), 39% of the travelers suffered from travelers' diarrhea and additional symptoms, although antibiotics were not taken (see Table S1 in the supplemental material).

To detect ESC-R-*Ent*, ~20 μg of fresh stools was enriched overnight in 10 ml LB broth containing a 10-μg disk of cefuroxime. Then, 100 μl was plated on BLSE, ChromID ESBL (bioMérieux), and Supercarba selective plates (20). After overnight incubation, colonies were identified using the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker). Microdilution GNX2F panels (Trek Diagnostics) were used to obtain the antibiotic MICs. The CT103XL microarray was used to detect *bla* genes. Multilocus sequence typing (MLST) for *E. coli* and *Klebsiella pneumoniae* (Warwick and Pasteur schemes, respectively) was used to determine the sequence type (ST); the *E. coli* phylogenetic group was also determined (21). The remaining stool samples were stored at −80°C without cryoprotectant.

Based on this initial screening, three (7.9%) people had stool samples positive for ESBL-producing *E. coli* before traveling, whereas 29 (76.3%) of them returned from India colonized with ESC-R-*Ent*, such as 26 CTX-M- and 3 DHA-producing *E. coli*. No carbapenemase producers were detected (Table 1). Overall, *E. coli* strains found in returning travelers were of non-hyperepidemic STs and were mainly of phylogenetic groups A1 ($n = 11$) and B1 ($n = 8$). Notably, all of these ESC-R-*Ent* had polymyxin MICs of ≤0.5 μg/ml and did not contain *mcr-1* as determined by PCR (1).

To improve our ability to detect possible Col-R-*Ent* (including those non-ESC-R), stored stools were enriched in 10 ml LB broth with two 10-μg disks of colistin. After overnight incubation, 100 μl was spread on four agar plates: MacConkey agar without or with colistin (4 μg/ml) and CHROMagar Orientation plus colistin (4 μg/ml) and vancomycin (8 μg/ml) without or with cefotaxime (2 μg/ml). MacConkey plates yielded numerous undistinguishable colonies of species naturally resistant to polymyxins (e.g., *Proteus* spp. and *Serratia marcescens*). In contrast, thanks to the typical color appearance of the different Gram-negative organisms, the CHROMagar Orientation plates allowed us to clearly identify Col-R *E. coli* (Col-R-*Ec*) colonies.

As depicted in Table 1 (rows highlighted in gray), only one

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TABLE 1 Extended-spectrum cephalosporin- and/or colistin-resistant *Enterobacteriaceae* detected in stool samples from 38 people before and after traveling to India during 2015[a]

			Result from stool samples before traveling								Result from stool samples after traveling							
								MIC, μg/ml[c]								MIC, μg/ml[c]		
ID	Age/sex	No. of days in India	Species detected (strain)	*bla* gene(s)[b]	ST	CC	Phy	COL	PolB	PCR$^+$ for *mcr-1*	Species detected (strain)	*bla* gene(s)[b]	ST	CC	Phy	COL	PolB	PCR$^+$ for *mcr-1*
3[d]	49/m	31	–								–							
8[g]	26/m	ND	–								*E. coli* (8R-*Ec*)	CTX-M-15-like	2451	131	$B2_3$	≤0.25	0.5	–
9	25/m	8	–								*E. coli* (9R-*Ec*)	CTX-M-15-like, TEMwt	34	10	A1	≤0.25	≤0.25	–
14	44/f	15	–								*E. coli* (14R-*Ec*)	CTX-M-15-like, TEMwt	10	10	A1	≤0.25	0.5	–
18	52/f	9	–								–							
19	53/f	20	–								–							
23[h]	67/f	14	–								*E. coli* (23R-*Ec*)	DHA	200	40	B1	≤0.25	0.5	–
											E. coli (26R-*Ec*-R-I)	CTX-M-15-like	3052	NA	D2	0.5	0.5	–
26[e]	50/f	14	*E. coli* (26-*Ec*)	-	69	69	D1	>4 (4)	>4	–	*K. pneumoniae* (26R-*Kp*)	CTX-M-15-like, TEMwt, SHVwt	48	NA	NA	≤0.25	0.5	–
29	31/m	15	*E. coli* (29-*Ec*)	CTX-M-gr.9, TEMwt	648	648	D2	≤0.25	≤0.25	–	*E. coli* (29R-*Ec*)	CTX-M-15-like	349	349	D2	≤0.25	0.5	–
31[f]	40/m	14	–								–							
33	40/f	32	–								*E. coli* (33R-*Ec*)	CTX-M-15-like, TEMwt	New[i]	NA	A0	≤0.25	≤0.25	–
34	36/f	13	–								*E. coli* (34R-*Ec*)	DHA	29	29	B1	≤0.25	≤0.25	–
37	43/f	27	–								*E. coli* (37R-*Ec*)	CTX-M-15-like, TEMwt	4388	NA	B1	≤0.25	≤0.25	–
											P. mirabilis (37R-*Pm*)	VEB	NA	NA	NA	NA	NA	–
41	68/f	15	–								*E. coli* (41R-*Ec*)	CTX-M-15-like, TEMwt, TEM-17-like	602	446	B1	≤0.25	0.5	–
43	37/f	17	–								*E. coli* (43R-*Ec*)	CTX-M-15-like	617	10	A1	≤0.25	0.5	–
45[f]	56/f	14	–								*E. coli* (45R-*Ec*)	CTX-M-gr.9	443	205	B1	≤0.25	≤0.25	–
50	33/m	30	*E. coli* (50-*Ec*)	CTX-M-15-like	131	131	$B2_3$	≤0.25	≤0.25	–	*E. coli* (50R-*Ec*)	CTX-M-15-like	155	155	B1	≤0.25	≤0.25	–
51	49/m	19	–								–							
52[g]	29/f	14	–								–							
55[d]	46/f	31	–								*E. coli* (55R-*Ec*)	CTX-M-gr.9	New[i]	NA	B1	≤0.25	≤0.25	–
56	42/m	9	–								*E. coli* (56R-*Ec*)	CTX-M-15-like	New[i]	NA	D1	≤0.25	≤0.25	–
59[e]	50/f	14	*E. coli* (59-*Ec*)	CTX-M-15-like, TEMwt	652	NA	A1	≤0.25	0.5	–	*E. coli* (59R-*Ec*)	CTX-M-15-like, TEMwt	3203	NA	A1	≤0.25	0.5	–
60	53/f	10	–								*E. coli* (60R-*Ec*)	CTX-M-15-like	48	10	A1	≤0.25	0.5	–
61	42/m	9	–								–							
63	33/f	15	–								–							
66	45/f	18	–								*E. coli* (66R-*Ec*)	CTX-M-gr.1, TEMwt	2076	NA	D1	≤0.25	≤0.25	–
68	ND/m	20	–								*E. coli* (68R-*Ec*)	CTX-M-15-like, TEMwt	410	23	A1	≤0.25	≤0.25	–
70	30/m	27	–								*E. coli* (70R-*Ec*)	CTX-M-15-like	4305	NA	A1	≤0.25	≤0.25	–
71	59/f	16	–								–							
73[h]	53/f	15	–								*E. coli* (73R-*Ec*)	CTX-M-15-like	4121	NA	A0	≤0.25	0.5	–
											E. coli (78R-*Ec*-MacI)	DHA	206	206	A0	≤0.25	0.5	–
78	57/f	20	–								*E. coli* (78R-*Ec*)	CTX-M-15-like	New[i]	NA	D2	>4 (12)	>4	–
80	26/f	19	–								*E. coli* (80R-*Ec*)	CTX-M-15-like	10	10	A1	≤0.25	0.5	–
83	34/f	23	–								*E. coli* (83R-*Ec*)	CTX-M-15-like	394	394	D1	≤0.25	0.5	–
											E. coli (86R-*Ec*-ChromI)	CTX-M-15-like	517	NA	A1	≤0.25	≤0.25	–
86	53/f	15	–								*E. coli* (86R-*Ec*)	DHA	3075	NA	B1	>4 (6)	>4	–

88	25/m	8	–	*E. coli* (88R-*Ec*-ChromI)	CTX-M-15-like, TEMwt	48	10	A1	≤0.25	≤0.25	–
				E. coli (88R-*Ec*)	CTX-M-15-like	New[i]	NA	D1	>4 (4)	>4	–
96	46/f	35	–	*E. coli* (96R-*Ec*)	CTX-M-15-like	1491	NA	A1	≤0.25	≤0.25	–
97	42/f	20	–	*E. coli* (97R-*Ec*)	CTX-M-15-like, TEMwt, TEM-11-like	2914	NA	D2	≤0.25	0.5	–
				E. coli (100R-*Ec*-Drig)	CTX-M-gr.1, TEMwt	155	155	B1	≤0.25	≤0.25	–
100	57/f	14	–	*E. coli* (100R-*Ec*)	TEMwt	ST10	10	A1	>4 (12)	>4	+

[a] Abbreviations used throughout: ID, identification code for the traveler; m, male; f, female; ND, not defined; ST, sequence type; CC, clonal complex; Phy, phylogenetic group; COL, colistin; PolB, polymyxin B; −, negative; +, positive; NA, not available or not applicable; wt, wild type (non-ESBL). Data highlighted in gray represent the colistin-resistant strains. All of these strains were detected with the CHROMagar Orientation plates containing colistin (4 μg/ml) plus vancomycin (8 μg/ml) with or without cefotaxime (2 μg/ml).
[b] Based on the CheckPoints CT103XL microarray results.
[c] MICs were obtained in microdilution with the GNX2F Trek panels; data in parentheses represent Etest results. For both methodologies, the MICs were obtained using colonies that grow on MacConkey agar plates without antibiotics.
[d] Relatives or couples traveling together.
[e] Relatives or couples traveling together.
[f] Relatives or couples traveling together.
[g] Friends traveling together.
[h] Friends traveling together.
[i] The new STs could not be assigned because whole-genome sequencing for the strains was not performed as requested by the curator of the *E. coli* MLST scheme (see Table S3 in the supplemental material).

person was colonized with a Col-R-*Ec* strain before traveling, whereas four (10.5%) returning travelers had stools positive for Col-R-*Ec* (of which three coproduced ESBLs or pAmpCs). Remarkably, two (26-*Ec* and 100R-*Ec*) out of the overall five Col-R-*Ec* strains were non-ESC-R (see Table S2 in the supplemental material) and were identified only with the CHROMagar Orientation plates containing colistin but not cefotaxime. Notably, Col-R *Klebsiella* spp., *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Salmonella* species strains were not found (2, 22). We cannot exclude that storing the stools at −80°C could influence the viability of Col-R strains.

Overall, one (2.6%) ST10/A1 *E. coli* strain (100R-*Ec*) was PCR positive for *mcr-1* (Table 1). This strain had a colistin MIC of 12 μg/ml by Etest and was coresistant to quinolones, trimethoprim-sulfamethoxazole, and tetracyclines (see Table S2 in the supplemental material). The strain carried HI2, FII, FIB, and X1 plasmid replicon types (PBRT kit; Diatheva). Experiments with transformation into chemically competent DH5α *E. coli* failed to obtain *mcr-1*-carrying cells (23). Conjugation experiments using *E. coli* recipient strain JF33 (rifampin resistant) performed at 25 and 37°C and with selection on MacConkey plates containing colistin (2 μg/ml) and rifampin (50 μg/ml) were also unsuccessful.

To complete the bacterial typing of the *mcr-1*-positive strain and to identify the resistome and mobilome, raw genome data (MiSeq; Illumina) were screened by ResFinder-2.1 and PlasmidFinder-1.3 (24, 25). 100R-*Ec* carried resistance genes for aminoglycosides [*aadA1*, *aadA2*, and *aph*(3′)-*Ia*], β-lactams (bla_{TEM-1}), polymyxins (*mcr-1*), quinolones (*qnrS1*), macrolides [*mph*(A)], phenicols (*cmlA1*), sulfonamides (*sul3*), tetracyclines [*tet*(*A*)], and trimethoprim (*dfrA12* and *dfrA14*).

By PlasmidFinder, the presence of IncF, IncX1, and IncHI2 plasmids was confirmed in the 100R-*Ec* strain. The *mcr-1* gene was identified at the end of a 26-kb contig (GenBank accession no. KX090925), flanked by two copies of IS*Apl1* in the opposite orientation forming a 4,211-bp IS*Apl1*-*mcr-1*-IS*Apl1* element. The 22,133-bp sequences preceding the IS*Apl1*-*mcr-1*-IS*Apl1* element shared >99% identity with DNA sequences of large IncHI2 plasmids, such as pKST313 from *Salmonella enterica* serotype Typhimurium (GenBank accession no. LN794248), pAPEC-O1-R from avian-pathogenic *E. coli* (GenBank accession no. DQ517526) (26, 27), and pSA26 carrying IS*Apl1*-*mcr-1* from an ST68 *E. coli* strain (GenBank accession no. KU743384). The *mcr-1* element was inserted into the *tra1* locus of the IncHI2 plasmid backbone immediately downstream of the conjugative protein gene *trhY*. The disruption of the *tra* region may explain the defective conjugation ability of this plasmid. Notably, the presence of *mcr-1* between duplicated IS*Apl1* insertion elements has so far only been observed in one human KPC-2-producing *E. coli* isolate in Germany and two IncHI2 plasmids isolated in the United Kingdom (4, 10).

Taking into account that 100R-*Ec* was of ST10/A1, and due to the high identity of the *mcr-1*-containing element with plasmids found in bacteria from animal origin, we speculate that this Col-R *E. coli* strain was acquired during the trip in India via the food chain or from environmental sources. Remarkably, the traveler was cocolonized with an ST155 CTX-M-producing *E. coli* strain (100R-*Ec*-Drig). A follow-up screening of the stool samples 3 and 6 months after returning from the trip indicated that the person was no longer colonized with both *E. coli* strains.

The remaining *mcr-1*-negative but Col-R *E. coli* strains (26-*Ec*, 78R-*Ec*, 86R-*Ec*, and 88R-*Ec*) underwent conjugation experi-

ments, but all failed to demonstrate a hypothetical plasmid-mediated mechanism of colistin resistance. We should note that such strains have been previously observed in humans and animals (8, 9, 28) and that they are Col-R, probably due to chromosomal mutations (10, 29). Notably, the stool samples from three of the travelers mentioned above were negative for Col-R *Ent* at the 3- and 6-month follow-up screenings. (ID-78 did not submit the stool samples for screening.) The risk factors determining the intestinal colonization with Col-R-*Ec* of people visiting India are unclear and should be investigated in the near future.

Our results indicate that the prevalence of intestinal *mcr-1-Ent* is probably underestimated because some isolates carrying only *mcr-1* (like 100R-*Ec*) could be routinely undetected when the screening is targeting the ESC-R and/or carbapenem-resistant strains (5, 7, 8, 12, 14, 18). It is possible that the IS*Apl1-mcr-1*-IS*Apl1* element present in 100R-*Ec* possesses the ability to transpose frequently, moving the *mcr-1* gene into the IncHI2 scaffold but also promoting its transposition in different plasmids. Multifocal plasmid types (IncP, IncX4, and IncI2) carrying *mcr-1*, but not *bla* genes conferring resistance to extended-spectrum cephalosporins (ESCs), have been previously reported, and some of them demonstrated a great potential for dissemination by conjugation among diverse Gram-negative species of the gut flora (1, 4, 18). Such colonizing bacteria may subsequently develop into difficult-to-treat infections, as observed for the ESBL producers acquired by people traveling from low- to high-prevalence countries (30–32).

The spread of non-ESC-R *mcr-1-Ent* could contribute to the silent expansion of this life-threatening resistance gene in both human and nonhuman settings. Specific and sensitive surveillance programs should be rapidly implemented to prevent unexpected outbreaks due to *mcr-1-Ent*.

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