

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 μ g of fresh stools was enriched overnight in 10 ml LB broth containing a 10- μ g disk of cefuroxime. Then, 100 μ l 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 GN2F 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 deter-

mine 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-hyperendemic 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 μ g/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- μ g disks of colistin. After overnight incubation, 100 μ l was spread on four agar plates: MacConkey agar without or with colistin (4 μ g/ml) and CHROMagar Orientation plus colistin (4 μ g/ml) and vancomycin (8 μ g/ml) without or with cefotaxime (2 μ g/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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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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