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LETTER TO THE EDITOR

Acquisition of *mcr-1* Plasmid-Mediated Colistin Resistance in *Escherichia coli* and *Klebsiella pneumoniae* during Hajj 2013 and 2014

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A plasmid-mediated transferable colistin resistance gene, *mcr-1*, was recently described in China (1) and was rapidly reported in several other countries (2). The spread of the *mcr-1* gene was not only reported in *Escherichia coli* but also associated with other *Enterobacteriaceae* species isolated from human clinical samples, farm animals, and travelers (2, 3). However, whether or not the *mcr-1* gene is acquired during the Hajj (the Muslim pilgrimage to Mecca) remains unknown.

We conducted two cohort studies of pilgrims traveling to Mecca in 2013 (22 September to 23 October) (4, 5) and in 2014 (19 September to 12 October) (6). A total of 440 rectal swab samples were collected from pilgrims (in 2013, 129 pilgrims [before and after their pilgrimage]; in 2014, 92 pilgrims before and 90 pilgrims after their pilgrimage) and were then screened for the presence of the *mcr-1* gene by real-time PCR and results confirmed by standard PCR and sequencing as described previously (7). All PCR-positive samples were then tested in an attempt to isolate *mcr-1*-resistant strains by culture on Cepacia agar (bioMérieux, Marcy-l'Étoile, France). Different colonies were tested by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF), antibiotic susceptibility testing (EUCAST), Etest (MIC susceptibility, ≤2 mg/liter), PCR, sequencing of the *mcr-1* and extended-spectrum-beta-lactamase (ESBL) genes (bla_{CTX-M}, bla_{TEM}, and bla_{SHV}) (5), and multilocus sequence typing (MLST) analysis (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/ and http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). All *mcr-1* and ESBL gene sequencing results were then analyzed with NCBI database.

The prevalences of *mcr-1*-positive isolates determined by PCR in rectal swabs of pilgrims were similar in 2013 and 2014, and the prevalence was significantly higher upon return (in 2013, 1.55% [2/129] before the pilgrimage versus 8.53% [11/129] after the pilgrimage [$P = 0.0104$]; in 2014, 1.02% [1/92] before the pilgrimage versus 9.18% [9/90] after the pilgrimage [$P = 0.0091$]). Ten *E. coli* isolates and 1 *K. pneumoniae* isolate from 23 pilgrims who were *mcr-1* positive by PCR were successfully identified by culture (Table 1). The sequence of the detected *mcr-1* gene showed 100% identity with the published sequence (1). Our colistin-resistant isolates were not resistant to all antibiotics (Table 1). MICs of colistin ranged from 3 to 4 mg/liter. Two unrelated pilgrims (no. 95 and 96) carried the common sequence type of *E. coli*, ST10, likely suggesting that the isolates from those two pilgrims represented the same clone. Also, two other unrelated pilgrims (no. 6 and 117) carried the same sequence type of *E. coli*, ST648. Conversely, two Moroccan pilgrims (no. 134 and 143) who formed a

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TABLE 1 Characteristics of pilgrims and *mcr-1*-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates during the Hajj in 2013 and 2014

Yr	Isolate no.	Sample collection time[a]	Species	Country origin of pilgrim	ST/CC	Gene	Colistin MIC (mg/liter)	Antimicrobial resistance pattern[b]
2013	44B	Before	*E. coli*	Algeria	ST93/CC168	bla_{TEM-1}	4	AMX-AMC-SXT
	1R	Return	*E. coli*	Algeria	ST453/CC86	bla_{SHV-1}	4	AMX-AMC-CRO-ATM
	6R	Return	*E. coli*	Algeria	ST648/CC648	bla_{TEM-1}	4	AMX-AMC-CRO-FOF
	85R	Return	*E. coli*	Algeria	ST656/CC10	$bla_{CTX-M-15}$, bla_{TEM-1}	4	AMX-AMC-FEP-CRO-ATM-SXT-GEN-NAL
	95R	Return	*E. coli*	Algeria	ST10/CC10	bla_{TEM-1}	4	AMX-AMC-SXT-GEN-NAL
	96R	Return	*E. coli*	Algeria	ST10/CC10	bla_{TEM-1}	4	AMX-AMC-SXT-GEN-CIP-NAL
	117R	Return	*E. coli*	Algeria	ST648/CC648	bla_{TEM-1}	4	AMX-AMC-CRO-FOF
	119R	Return	*K. pneumoniae*	Algeria	ST788[c]	bla_{TEM-1}	3	AMX-AMC-SXT
2014	1R4	Return	*E. coli*	Algeria	ST155/CC155	bla_{TEM-1}	4	AMX-AMC-SXT
	134R	Return	*E. coli*	Morocco	ST602/CC446	bla_{TEM-1}	3	AMX-AMC-SXT
	143R	Return	*E. coli*	Morocco	ST1300[c]	bla_{TEM-1}	3	AMX-SXT-GEN

[a] Before, before Hajj; return, after Hajj.
[b] AMX, amoxicillin; AMC, amoxicillin-clavulanate; ATM, aztreonam; FEP, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; FOF, fosfomycin; GEN, gentamicin; NAL, nalidixic acid; SXT, trimethoprim-sulfamethoxazole.
[c] CC not defined in the MLST database.

pair (wife/husband) carried different *E. coli* sequence types (ST602 and ST1300).

Overcrowded conditions, especially during the Hajj, are a major risk for dissemination of antibiotic resistance (AR) bacteria. Moreover, taking antibiotics during travel may play a major role in selecting AR bacteria (8). However, without colistin selection in pilgrims during the Hajj, *mcr-1*-resistant strains can disseminate among pilgrims, demonstrating a low transmission fitness cost. Additionally, some of the pilgrims who did not take any antibiotics during the Hajj were colonized by a *mcr-1* strain. In our study, we could not determine the sources and modes of transmission of this AR bacterium. The *mcr-1* gene has been reported to occur worldwide in many different sources, including foods, environments, animals, and humans (2, 9). Possible transmission of colistin-resistant bacteria between human and animals has been also reported (10, 11). Different types of sequence strains identified in pilgrims, including in the couple whose results indicated the acquisition of *mcr-1*, may come from multiple sources, including by direct and indirect transmission. Plasmids carrying the *mcr-1* gene may circulate in our cohorts and may spread when pilgrims return to their home countries. Our study results clearly demonstrate that *mcr-1* plasmid-mediated colistin resistance has already spread worldwide and that screening of stool samples from travelers is urgently needed.

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