Acquisition of Extended-Spectrum β -Lactamases by *Escherichia coli* and *Klebsiella pneumoniae* in Gut Microbiota of Pilgrims during the Hajj Pilgrimage of 2013

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We reported the acquisition of extended-spectrum- β -lactamase (ESBL)-producing bacteria in rectal samples of 129 pilgrims during the 2013 Hajj (pilgrimage to Makkah). When returning from the Hajj, there was a significant increase in the number of pilgrims carrying *E. coli* resistant to ceftriaxone ($P = 0.008$). The CTX-M gene was detected in rectal samples, with the detection rate increasing from 10.08% to 32.56% of samples after the Hajj ($P < 0.001$).

The Hajj (pilgrimage to Makkah) is well recognized as a source for transmission of infectious diseases (1–4). Pilgrims can be carriers of infectious diseases when returning home. Several reports have shown the dissemination of resistant genes and multidrug-resistant bacteria (MDR) in travelers (5, 6), including extended-spectrum- β -lactamase (ESBL)-producing bacteria in travelers from the Netherlands (7, 8), Spain (9), and Japan (10). However, studies of the acquisition of antibiotic resistance (AR) genes in pilgrims during the Hajj are few. We aim to investigate the change and acquisition of MDR bacteria in pilgrims returning from the 2013 Hajj with specific focus on *Escherichia coli* and *Klebsiella pneumoniae*.

Rectal swabs were collected from a cohort of 129 pilgrims traveling to Saudi Arabia with the same travel agency. Samples were collected 10 days before travel (22 September 2013) and 1 day before returning (23 October 2013) as described by Gautret et al. (11). One hundred microliters from each swab was inoculated into Trypticase soy broth (Becton, Dickinson and Company, Sparks, MD, USA). After 24 h of incubation, samples were plated onto Hektoen enteric agar (Oxoid Deutschland GmbH, Wesel, Germany). MacConkey agar with 2 mg/liter cefotaxime and Cepacia agar (bioMérieux, Marcy-l'Étoile, France) were used to isolate ESBL-producing bacteria and colistin-resistant bacteria. Species were identified by matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry (Microflex; Bruker Daltonic, Bremen, Germany). *E. coli* and *K. pneumoniae* isolates were tested against six antibiotics (colistin, ticarcillin-clavulanic acid, piperacillin-tazobactam, ceftriaxone, gentamicin, and imipenem) by the disk diffusion method for an overview of antibiotic-resistant bacteria in pilgrims and were interpreted using EUCAST guidelines (<http://www.eucast.org>).

Total DNA was extracted from *E. coli*, *K. pneumoniae*, and rectal samples using an EZ1 BioRobot machine (Qiagen S.A., Courtaboeuf, France) according to the manufacturer's instructions. ESBL-encoding genes (CTX-M, TEM, and SHV) were detected by PCR and sequencing and were then analyzed with the ARG-ANNOT (12) and NCBI databases. Primers used in this study have previously been described elsewhere (13–17). Multilocus sequence typing (MLST) was performed as described in the database (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/>, <http://bigsdweb.pasteur.fr/klebsiella/>). A phylogenetic tree was constructed

FIG 1 Percentages of pilgrims harboring resistant *E. coli* (a) and *K. pneumoniae* (b) before Hajj (light gray) and after return from Hajj (dark gray). CST, colistin; CRO, ceftriaxone; TIM, ticarcillin-clavulanic acid; GEN, gentamicin; TZP, piperacillin-tazobactam; IPM, imipenem. Significant changes before Hajj and after return from Hajj are indicated with an asterisk (*).

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TABLE 1 Antibiotic phenotypes and molecular characterization of 23 *E. coli* isolates and 5 *K. pneumoniae* isolates collected from Hajj pilgrims in 2013

Strain no.	Sample ^a	Species	ESBL genes			ST	Clonal complex	Antibiotic ^b					
			CTX-M	TEM	SHV			CST	CRO	TIM	GEN	TZP	IPM
31B	Before	<i>E. coli</i>	CTX-M-1	TEM-1		ST10	ST10 Cplx	0	1	1	0	0	0
48B	Before	<i>E. coli</i>	CTX-M-15	TEM-1D		ST361		0	1	1	1	0	0
47B	Before	<i>E. coli</i>	CTX-M-15	TEM-1D		ST361		0	1	1	1	0	0
47R	Return	<i>E. coli</i>	CTX-M-15	TEM-1		ST12	ST12 Cplx	0	1	1	0	0	0
68B	Before	<i>E. coli</i>	CTX-M-14	TEM-1		ST38	ST38 Cplx	0	1	1	0	0	0
68R	Return	<i>E. coli</i>	CTX-M-15	TEM-1		ST1312		0	1	0	0	0	0
76B	Before	<i>E. coli</i>	CTX-M-1	TEM-1		ST12	ST12 Cplx	0	1	1	0	0	0
76R	Return	<i>E. coli</i>	CTX-M-14	TEM-1		ST117		0	1	1	1	0	0
46R	Return	<i>E. coli</i>	CTX-M-15	TEM-1		ST3728		0	1	1	0	0	0
85R	Return	<i>E. coli</i>	CTX-M-15	TEM-1		ST656	ST10 Cplx	0	1	1	1	0	0
118R	Return	<i>E. coli</i>	CTX-M-15			ST195	ST10 Cplx	0	1	0	0	0	0
69R	Return	<i>E. coli</i>	CTX-M-15			ST226	ST226 Cplx	0	1	0	0	0	0
24R	Return	<i>E. coli</i>	CTX-M-15			ST552 ^c		0	1	1	0	0	0
111R	Return	<i>E. coli</i>	CTX-M-15			ST648		0	1	0	0	0	0
34R	Return	<i>E. coli</i>	CTX-M-2			ST457		0	1	1	1	0	0
87R	Return	<i>E. coli</i>	CTX-M-2			ST117		0	1	1	1	0	0
93R	Return	<i>E. coli</i>	CTX-M-97	TEM-1		ST46	ST46 Cplx	0	1	1	0	0	0
121R	Return	<i>E. coli</i>	CTX-M-8	TEM-1		ST448	ST448 Cplx	0	1	1	0	0	0
33R	Return	<i>E. coli</i>	CTX-M-8	TEM-1		ST58	ST155 Cplx	0	1	1	0	0	0
100R	Return	<i>E. coli</i>	CTX-M-8			ST602	ST446 Cplx	0	1	0	0	0	0
57R	Return	<i>E. coli</i>	CTX-M-14	TEM-1		ST69	ST69 Cplx	0	1	1	0	0	0
27R	Return	<i>E. coli</i>	CTX-M-27			ST131	ST131 Cplx	0	1	1	0	0	0
8R	Return	<i>E. coli</i>		TEM-1		ST224		0	1	1	1	0	0
8R1	Return	<i>K. pneumoniae</i>	CTX-M-15	TEM-1	SHV-67	ST273		0	1	0	0	0	0
8R2	Return	<i>K. pneumoniae</i>	CTX-M-15	TEM-1	SHV-106	ST15		0	1	0	0	0	0
62R	Return	<i>K. pneumoniae</i>	CTX-M-15	TEM-1	SHV-110	ST873		0	1	1	0	0	0
66R1	Return	<i>K. pneumoniae</i>	CTX-M-15	TEM-183		ST1548		0	1	1	1	0	0
66R2	Return	<i>K. pneumoniae</i>	CTX-M-14		SHV-161	ST179 ^c		0	1	1	1	0	0

^a Before, before Hajj; return, after Hajj.^b AST results. CST, colistin; TIM, ticarcillin-clavulanic acid; CRO, ceftriaxone; GEN, gentamicin; TZP, piperacillin-tazobactam; IPM, imipenem; 0, susceptible; 1, resistant.^c New ST type.

by concatenated MLST sequences using MEGA5 with minimum evolution analysis (18). McNemar's or Fisher's exact test, as appropriate, was used to calculate the change in the number of pilgrims harboring AR bacteria and ESBL genes present in the samples.

A total of 129 pilgrims were screened (77 women [59.7%] and 52 men [40.3%]); the mean age was 61.7 years (34 to 85 years), 81 pilgrims (62.8%) used antibiotics, and 30 pilgrims (23.3%) had diarrhea during Hajj (11). Before traveling, 18 of 129 pilgrims (14%) were colonized by *E. coli* that was resistant to any of the six antibiotics, increasing to 36 of 129 pilgrims (28%) upon return ($P = 0.009$). There was a significant increase in the number of pilgrims harboring *E. coli* resistant to ceftriaxone (3.9% versus 14.0%; $P = 0.008$) and ticarcillin-clavulanic acid (12.4% versus 22.5%; $P = 0.048$) (Fig. 1a). Five versus 13 (before versus return) ceftriaxone-resistant *E. coli* isolates were coresistant to ticarcillin-clavulanic acid (Table 1). The proportions of pilgrims carrying ceftriaxone-resistant and gentamicin-resistant *K. pneumoniae* increased to 2.3% and 3.1%, respectively. Colistin-resistant *E. coli* and *K. pneumoniae* isolates were found before and on return with an MIC range of 4 to 24 mg/liter (Fig. 1). One of 3 colistin-resistant *E. coli* isolates, 2 of 5 colistin-resistant *K. pneumoniae* isolates (before), and 2 of 4 colistin-resistant *K. pneumoniae* isolates (return) were resistant to ticarcillin-clavulanic acid. None of the isolates were resistant to piperacillin-tazobactam and imipenem.

In 23 ceftriaxone-resistant *E. coli* isolates, ESBL genes were identified (5 before versus 18 on return). CTX-M variants in return isolates were CTX-M-8, CTX-M-2, CTX-M-97, and CTX-M-27. One of the ceftriaxone-resistant *E. coli* isolates was TEM-1 (Table 1). The MLST tree of *E. coli* and *K. pneumoniae* is shown in Fig. 2. Nineteen sequence types (STs) were identified which showed the common ST361 in two pilgrims before travel (no. 47B and 48B). However, there was no relationship between them. Of 5 ceftriaxone-resistant *K. pneumoniae* isolates, there were four isolates with CTX-M-15 and one with CTX-M-14. The different ST isolates harboring dissimilar ESBL genes were found in pilgrim no. 66 (ST1548 and ST1798).

The acquisition of ESBL genes was determined in rectal samples. CTX-M genes were identified in 10.08% (13/129) of before-Hajj samples and in 32.56% (42/129) of return samples ($P < 0.001$). TEM and SHV were detected at high levels before departure and upon return (TEM, 78.29% versus 82.95%, $P = 0.3074$; SHV, 63.6% versus 72.9%, $P = 0.065$). Similarly, rates of ESBL *E. coli* and *K. pneumoniae* isolates in Saudi Arabia between 2009 and 2010 were high at approximately 30% (19). A previous report showed a high acquisition rate of the CTX-M gene in Netherlands travelers, rising from 9% to 33.6% after travel (8). A total of 17 CTX-M variants were found in the rectal samples of 48 pilgrims (Fig. 3). Of these, seven variants were present in before-Hajj and return samples: CTX-M-1, CTX-M-15, CTX-M-2, CTX-M-14, CTX-M-8, CTX-M-5, and CTX-M-77. Among those

FIG 2 Phylogeny of concatenated MLST sequences of *E. coli* (a) and *K. pneumoniae* (b) strains isolated before Hajj and after return from Hajj. Minimum evolution analysis with 1,000 bootstraps was used for analysis. Branch label represents *E. coli* (EC) or *K. pneumoniae* (KP), followed by the pilgrim's number and source of sample (B, before Hajj; R, after Hajj). Sample contained two different bacterial strains indicated by the number after source of sample. STs were indicated for each strain.

returning from Hajj, nine specific CTX-M variants were identified in pilgrims: CTX-M-3, CTX-M-20, CTX-M-27, CTX-M-28, CTX-M-44, CTX-M-56, CTX-M-78, CTX-M-115, and CTX-M-152. Different STs and variants of CTX-M confirmed the diversity of bacterial clones harboring ESBL genes that were acquired during Hajj. Our previous work reported the acquisition of cephalosporin- and colistin-resistant *Salmonella enterica* (20). Thus, pilgrims may acquire other ESBL-producing bacteria (21), which may transfer these genes to the gut bacteria. CTX-M variants present in our study have been described worldwide in humans, animals, and the environment (21). This may be due to contaminated food or water (22), such as raw vegetables (23) and meat (24), as reported in Saudi Arabia.

In conclusion, our findings show that pilgrims returning from the Hajj harbored significantly more ESBL-producing bacteria than before travel. However, antibiotics consumption was reported as the risk factor for the acquisition of ESBL-producing bacteria during travel (6) and our French pilgrims had a high prevalence of diarrhea, including the detection of extensively used antibiotics (11, 25, 26). The source and mode of transmission of these MDR bacteria remain unknown and may involve the environment, food, water consumption, hygiene, and human community contact (25). Monitoring of ESBLs from samples may be an important means of controlling endemic transmission in travelers. Additionally, there is a need for efficient infection control practices, including a global sur-

FIG 3 Diversity of CTX-M genes detected in rectal swab samples for each pilgrim in samples taken before and after return from Hajj. The rectangle represents the different types of CTX-M genes. The circle represents the number of pilgrims who are CTX-M positive. The blue dotted line represents CTX-M-positive results for each pilgrim before Hajj (B). The red line represents CTX-M-positive results for each pilgrim after return from Hajj (R).

veillance system to monitor not only pilgrims but also travelers in general while traveling.

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