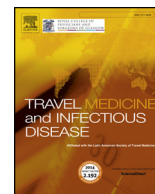




Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevier.com/locate/tmaid

Acquisition of enteric pathogens by pilgrims during the 2016 Hajj pilgrimage: A prospective cohort study

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ARTICLE INFO

Keywords:

Hajj
Mass gathering
Diarrhea
Escherichia coli
PCR

ABSTRACT

Background: Diarrhea can be frequent among Hajj pilgrims; however, data on its etiology are very limited.

Patients and methods: A prospective cohort study was conducted among Hajj pilgrims in 2016. Medical follow-up and systematic rectal swabbing were performed before leaving France and before leaving Saudi Arabia. Potential pathogens were identified using the BioFire FilmArray[®] Gastrointestinal multiplex qualitative PCR panel.

Results: 117 pilgrims were included and 13.7% experienced diarrhea during Hajj. Of the pre-Hajj samples, 32.5% were positive for at least one pathogen compared to 50% of post-Hajj samples ($p = 0.0033$). Diarrhea associated *Escherichia coli* strains, notably enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and Shiga-like toxin-producing *E. coli*, were acquired by 29.9%, 10.2%, and 6.5% pilgrims, respectively. Pilgrims with resolved diarrhea were significantly more likely to have post-Hajj EAEC positive samples, compared with those who did not suffer diarrhea (55.6% vs 16.5%). We found a lower prevalence of EPEC (22.5%) in pilgrims who declared washing their hands more frequently at the Hajj than usually as compared to others (40.0%).

Conclusion: The acquisition of diarrhea associated *E. coli* by Hajj pilgrims is of major concern given the high prevalence rate of third-generation cephalosporin-resistant *E. coli* in Saudi Arabia.

1. Introduction

Each year, 2–3 million Muslims from more than 180 countries gather in the Kingdom of Saudi Arabia (KSA) to perform the pilgrimage to Mecca, (the “Hajj”). The presence of a large number of pilgrims from many parts of the world in congested and crowded areas greatly increases the risk of spreading infectious diseases, particularly respiratory and gastro-intestinal diseases [1]. Available studies on the clinical prevalence of diarrhea among Hajj pilgrims indicate a mean prevalence of 2% with the highest prevalence (23%) reported among a group of French pilgrims in 2013. However, data on the etiology of diarrheal disease at the Hajj are very limited [2]. One study screened fecal samples from pilgrims with medically attended diarrheal illness from 40 countries during the 2011–2013 Hajj. Patient stools were screened for *Escherichia coli* pathotypes, *Campylobacter jejuni*, *Shigella* spp., *Salmonella* spp. *Yersinia enterocolitica* and *Vibrio cholerae* using a combination of previously established multiplex PCR assays. *Cryptosporidium* spp., *Giardia lamblia*, norovirus, rotavirus, adenovirus and astrovirus

were detected using commercial antigenic detection tests.

Bacteria were the main agents detected with a prevalence of *Salmonella* spp. of 11.4%, while diarrhea associated *E. coli* prevalence ranged between 1.3 and 8.8% according to pathotypes [3].

Recently studies on returning French pilgrims have shown that participation in the Hajj increases carriage rates of enteric pathogens including *Tropheryma whipplei* [4], multidrug-resistant nontyphoidal *Salmonella* [5], CTX-M type extended-spectrum β -lactamase producing *E. coli* and *Klebsiella pneumoniae* [6], colistin-resistant *E. coli* and *K. pneumoniae* and carbapenemase-producing *E. coli* [7,8]. Moreover, acquisition of CTX-M genes at the Hajj was associated with the occurrence of diarrhea and use of β -lactams [9]. Therefore, diarrhea at the Hajj is not only a concern for the pilgrims' health during their stay in Saudi Arabia but also a matter of public health with respect to potential dissemination of drug-resistant pathogens.

The aims of the present study were to evaluate the carriage of pathogens commonly associated with travelers' diarrhea among Hajj pilgrims with and without diarrheal symptoms during their stay in Saudi

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<https://doi.org/10.1016/j.tmaid.2018.05.017>

Received 16 February 2018; Received in revised form 28 May 2018; Accepted 30 May 2018
1477-8939/ © 2018 Published by Elsevier Ltd.

Arabia, and to better understand the dynamics of acquisition of enteric pathogens in this particular setting. We hypothesized that several factors may influence the outcome of gastrointestinal symptoms or pathogen carriage during the stay, including preventive measures, and use of antibiotics.

2. Materials and methods

2.1. Study population

The study was conducted among French Hajj pilgrims travelling together to Mecca, KSA, from August 27 to September 20, 2016, with one specialized travel agency in Marseille. Pilgrims stayed in the same hotels and tents all over their stay in Saudi Arabia with breakfast included. Pilgrims were free to eat in restaurants of their choice for lunch and diner while staying in Mecca (from August 27 to September 9) and in Medina (from September 15 to September 20). Pilgrims eat the same food during their stay in Mina, Arafat and Muzdalifah (from September 10 to September 14). Pilgrims older than 18 years were included on a voluntary basis, and participants were asked to sign a written consent form. Upon inclusion, the participants were questioned using a standardized pre-travel questionnaire, which included demographic data and chronic medical conditions. Health issues that occurred during the stay in the KSA were recorded by a medical doctor who travelled with the pilgrims. Diarrhea was defined by at least three loose or liquid stools per day. Each individual was classified in one of the three following categories: asymptomatic (those who did not experience any diarrheal symptoms during the entire stay in KSA), resolved diarrhea (those who experienced diarrhea during travel, but who recovered before return to France) and ongoing diarrhea (those with ongoing diarrheal symptoms once back in France). Nausea, vomiting, abdominal pain and constipation were also recorded. Overweight and obesity were defined by a body mass index of 25–30 and > 30, respectively. Compliance with preventive measures was investigated by questionnaires just before leaving KSA (higher or lower frequency of hand washing with soap during the stay in KSA, compared to usual frequency at home and use or not of hand disinfectant during the stay). Use of antibiotics in KSA was also recorded through questionnaires.

The protocol was approved by our Institutional Review Board (July 23, 2013; reference No. 2013-A00961-44). It was performed in accordance with the good clinical practices recommended by the Declaration of Helsinki and its amendments. All participants gave written informed consent.

2.2. Sample collection

The procedure included a rectal swab before departure and a rectal swab upon return. Rectal swabs were self-collected by each participant by using commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK), 10 days before departing from France (pre-Hajj specimens) and just 1 day before leaving the KSA (post-Hajj specimens). The swab was inserted past the anal sphincter (rotated for approximately 5 s) and replaced in its hermetic tube containing viral transport media (Sigma Virocult[®]). This standardized procedure was previously explained to the pilgrims by the investigators. Swabs were kept at room temperature before being transported to our laboratory in Marseille for storage in a –80 °C freezer within 48 h of collection.

2.3. Identification of gastro-intestinal pathogens

The analyses were carried out with a validated multiplex qualitative PCR method (FilmArray[®] GI Panel, bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions on a FilmArray 2.0 system. Hands-on time is less than 3 min and run time is approximately 1 h. The FilmArray[®] Gastrointestinal panel (BFGP, bioMérieux) included the

following bacterial, viral and parasitic targets: *Campylobacter* spp. (*C. jejuni*, *C. coli*, and *C. upsaliensis*), *Clostridium difficile* toxin A/B, enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC), Shigella/enteroinvasive *E. coli* (EIEC), *Plesiomonas shigelloides*, *Salmonella* spp., *Vibrio* spp. (*V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*), *Y. enterocolitica*, adenovirus F40/41, astrovirus, norovirus GI/GII, rotavirus A, sapovirus, *Cryptosporidium* spp., *Cyclospora cayotensis*, *Entamoeba histolytica* and *G. lamblia*.

2.4. Statistical analysis

Characteristics of the study population were summarized as frequencies and percentages for qualitative variables and as means, median and range for quantitative variables. Acquisition of pathogens was defined as the absence of a given pathogen in pre-Hajj stool samples and the presence of this pathogen in post-Hajj samples. The Student's *t*-test and Wilcoxon test, when appropriate, were applied to analyze the categorical variables. P values of 0.05 or less were considered significant. Statistical analyses were performed using SPSS (version 17.2).

3. Results

3.1. Demographics and gastro-intestinal symptoms

A total of 117 pilgrims were included upon departure from France (Table 1). The mean age was 61.0 years, and 46.2% were males. Most pilgrims were born in North Africa (89.8%) and had settled in France more than 20 years ago. Prevalence of chronic conditions was as follows: diabetes (31, 26.5%), hypertension (26, 22.2%), chronic respiratory disease (11, 9.4%), chronic cardiac disease (4, 3.4%), immunosuppressive condition (1, 0.9%), overweight (47, 40.2%) and obesity (38, 32.4%). A total of 75 (64.1%) pilgrims declared having used hand gel disinfectant during their stay and 51 (43.6%) declared having washed their hands more frequently than usually during their stay.

None of the pilgrims had digestive symptoms on leaving France; 16 (13.7%) experienced diarrhea in KSA and none of them took antibiotics for diarrhea; 31 (26.5%) pilgrims, however, took antibiotics during their stay, due to a respiratory tract infection. Overall, 16 pilgrims (13.7%) reported nausea, 6 (5.1%) vomiting, 9 (7.7%), abdominal pain and 13 (11.1%) constipation. Among 16 pilgrims with diarrhea, 5 reported nausea, 4 vomiting, 7 abdominal pain and 6 constipation. None were hospitalized.

Table 1

Demographic and baseline characteristics of the study participants (N = 117).

Characteristics	No. (%) unless otherwise specified
Mean age (IQR)	61.0 years (55–69 years)
Gender	
Male	54 (46.2)
Female	63 (53.8)
Birthplace	
Algeria	62 (53.0)
Tunisia	29 (24.8)
Morocco	14 (12.0)
Metropolitan France	7 (5.9)
Other ^a	5 (4.4)
Clinical symptoms during travel	
Diarrhea	16 (13.7)
Nausea	16 (13.7%)
Vomiting	6 (5.1%)
Abdominal pain	9 (7.7%)
Constipation	13 (11.1%)

^a Egypt (n = 1), Mali (n = 1), Senegal (n = 3).

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