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Epidemiology of bacterial resistance at the Grand Magal of Touba in Senegal

Ihssane Ouaddane [a,b], Ndiaw Goumballa [a,b,c], Xuan Duong Tran [a,b], Coumba Diouf [a,b,c], Seydina M. Diene [a,b], Jean-Marc Rolain [a,b], Cheikh Sokhna [a,b,c], Philippe Gautret [a,b,*]

[a] Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France
[b] IHU-Méditerranée Infection, Marseille, France
[c] VITROME, Campus International IRD-UCAD de L'IRD, Dakar, Senegal

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ABSTRACT

Background: The Grand Magal of Touba (GMT) associates with risks of infection, but no study on the circulation of resistant bacteria has yet been conducted.
Materials and methods: qPCR was performed on rectal samples from GMT pilgrims between 2018 and 2021, before and after their participation in the gathering. Rectal samples from between 2018 and 2020 were also cultured on specific media, and antibiotic susceptibility testing was performed.
Results: Forty-one of the 296 (13.8%) pilgrims had at least one gastrointestinal symptom and 91/290 (31.4%) acquired pathogenic bacteria, mostly *Escherichia coli*. A total of 54.7% of pilgrims reported washing their hands more frequently than usual and 89.2% used soap. One hundred and five (36.2%) acquired at least one resistance gene, notably CTX-M A (21.0%), SHV (16.5%) and TEM (8.2%). The strains isolated by culture were mostly *E. coli*. These bacteria were found to be sensitive to carbapenems and resistant to amoxicillin and amoxicillin-clavulanic acid. The acquisition of enteroaggregative *E. coli* was independently associated with CTX-M A and TEM acquisition.
Conclusion: Pilgrims presented a risk for acquisition of CTX-M A after the GMT. Surveillance of the prevalence of resistant bacteria and the occurrence of associated clinical infections among pilgrims are necessary in the future.

1. Introduction

Mass gatherings are planned events where the number of people attending could result in significant difficulties in terms of planning, surveillance, and healthcare responses for the host country or community [1]. The Grand Magal of Touba (GMT) is a Muslim religious event celebrated in Senegal on the 18th of Safar, according to the Islamic calendar. It is estimated that between four and five million pilgrims attend the event each year [2]. Pilgrims come from all over Senegal but also from neighbouring countries and the diaspora.

There are risks of the transmission of infectious diseases during mass gatherings, such as the Hajj, in Saudi Arabia, where outbreaks of cholera, meningitis and respiratory infections have been described [2,3]. Ndiaw Goumballa et al. conducted a study between 2017 and 2021 on pathogens acquired by pilgrims at the GMT. Compared to gastrointestinal viruses (1.7%) and parasites (2.9%), the acquisition of gastrointestinal bacteria was higher (32.3%), with enteroaggregative *Escherichia coli* (18.9%) and enteropathogenic *E. coli* (10.5%) being the most frequent [4].

Bacterial resistance is now considered to be one of the most serious health problems in the world. This phenomenon has major consequences, with higher healthcare costs due to the need to use more expensive antibiotics, deaths due to untreatable infections, and the potential danger it poses for immunocompromised patients [5]. In a study conducted in 2010 at the Fann University Hospital in Dakar, the incidence of nosocomial infections with multidrug-resistant bacteria was five cases per 1 000 patient-days. The most frequently isolated germs were extended-spectrum beta-lactamase (ESBL)-producing bacteria (62%), *Pseudomonas aeruginosa* (13%), and coagulase negative staphylococci (12%) [6]. In contrast to the Hajj pilgrimage in Saudi Arabia, where a significant acquisition of resistant bacteria was observed [7], no study has yet been conducted on pilgrims at the GMT.

The first objective of this study was to detect the presence of antibiotic resistant bacteria by taking two complementary approaches,

* Corresponding author. VITROME, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13385, Marseille, Cedex 05, France.
E-mail address: philippe.gautret@ap-hm.fr (P. Gautret).

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namely the detection by qPCR of different resistance genes, and the isolation of resistant bacteria on specific culture media in pilgrims returning from the GMT between 2018 and 2021. The second objective was to establish the phenotypic profile of resistance to different antibiotics for each strain isolated by culture, by performing an antibiogram. Finally, we looked for possible correlations between the acquisition of resistance genes and different factors such as the demographic and medical characteristics of the participants, the preventive and therapeutic measures they followed, and their clinical symptoms.

2. Materials and Methods

2.1. Study population

The pilgrims involved in this study participated in the GMT between 2018 and 2021 and hailed from two villages in southern Senegal, Dielmo (13°43′ 22.07″ N, 16°24′40.09″ W) and Ndiop (13°41′08.01″N, 16°23′01.01″ W), in the Fatick region. All pilgrims were identified by the nurses in charge of the primary healthcare centres in both villages. The pilgrims were interviewed before their departure, using a standardised survey. The survey covered demographic data and chronic conditions. Gastrointestinal health problems occurring during and after the pilgrimage were recorded by a nurse who travelled to Touba with the group of pilgrims. The dates of the pilgrimage and hand hygiene measures were documented using standardised questionnaires, administered on return from Touba. Antibiotic consumption during the journey was also documented.

2.2. Sample collection

The procedure included the collection of stool samples between one and three days before departure from the villages (pre-Magal samples) and between three and six days after return (post-Magal samples). Participants provided stool samples in sterile containers supplied by the investigators. The investigators then collected small amounts of stool using swabs placed in a suitable transport medium (Sigma Virocult®). This standard procedure was explained to the pilgrims beforehand by the investigators. The swabs were kept at 4 °C before being transported to the laboratory in Dakar for storage in a −80 °C freezer within 48 h of collection, and then transferred to Marseille on dry ice for processing.

2.3. DNA extraction

DNA was obtained by semi-automated extraction. 200 μL of transport medium from each rectal swab was added to 350 μL of G2 lysis buffer (Qiagen) and glass powder in a tube and homogenised in a FastPrep BIO 101 machine (Qbiogene, Strasbourg, France) at maximum power for 40 s. Incubation took place at 100 °C for 10 min to allow complete lysis. The tubes were then centrifuged at 10 000 g for 1 min. Subsequently, 200 μL of supernatant was collected in additional tubes and enzymatically digested with 20 μL of proteinase K (20 mg/mL, Qiagen) and incubated overnight at 56 °C. The automated procedure, using the EZ1 Advanced XL (Qiagen, Hilden, Germany) with the DNA tissue kit (Qiagen), was performed according to the manufacturer's recommendations.

2.4. Identification of gastrointestinal bacteria

Gastrointestinal bacteria were detected by quantitative real-time PCR directly from stools, using the LightCycler® 480 Probes Master kit (Roche Diagnostics, France), according to the manufacturer's recommendations. Enteroaggregative *E. coli* (EAEC), Enterohaemorrhagic *E. coli* (EHEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC/Shigella), *Tropheryma whipplei*, and *Salmonella* spp were investigated before and after the GMT between 2018 and 2021 [8].

2.5. Identification of resistance genes

Resistance genes were identified directly from the stool samples by qPCR using primers and probes specific to the genes studied. Genes encoding β-lactamases (blaTEM, blaSHV, blaCTX-M-A, blaCTX-M-B), genes encoding carbapenemase (blaKPC, blaNDM, blaVIM, blaOXA-23, blaOXA-24, and blaOXA-48) and colistin resistance genes (MCR1 to 5 and MCR-8) were investigated (Supplementary Table 1). The qPCR amplifications were performed using the LightCycler® 480 Probes Master kit (Roche Diagnostics, France) according to the manufacturer's recommendations. The PCR cycling (39 cycles) was as follows: an initial denaturation at 50 °C for 4 min followed by 39 cycles, each of which included: denaturation at 95 °C for 5 min, hybridisation at 95 °C for 5 seconds and, finally, elongation at 60 °C for 30 s. Detection of resistance genes using qPCR was performed using a C1000 Touch™ thermal cycler (Bio-Rad, Hercules, CA, USA). A negative control (Reactif Mix) and a positive control (extracted DNA + Reactif Mix) were included in each cycle. A cycle threshold (CT) value ≤ 35 was selected for positive results.

2.6. Identification of resistant bacteria by culture

Culture was performed on all rectal samples collected before and after participation in the GMT. Four specific media were used to isolate the strains present in these samples (ESBL medium (Chromid Ref AEB525770), VRE (vancomycin-resistant enterococcus) medium (Chromid Ref 43 004), medium for MRSA (Methicillin-resistant *S. aureus*) (Chromid Ref 43 451), and CARBA/OXA medium (Carbapenemase Producing Enterobacteriaceae) (Chromid Ref 414 685)). Bacteria isolated by culture were identified by MALDI-TOF. Antibiotic susceptibility testing (antibiogram) was subsequently performed using the Kirby-Bauer disk diffusion method. The results were interpreted according to the EUCAST 2017 guidelines. ESBL isolates were tested against 18 antibiotics: amoxicillin, amoxicillin + clavulanic acid, amikacin, ciprofloxacin, ceftriaxone, doxycycline, ertapenem, cefepime, fosfomycin, gentamicin, imipenem, trimethoprim + sulfamethoxazole, piperacillin + tazobactam, aztreonam, ceftazidime, meropenem, ticarcillin + clavulanic acid, and ticarcillin.

2.7. Statistical analysis

STATA version 14.2 software (Copyright 2015 Stata Corp LP, College Station, Texas, USA http://www.stata.com) was used for statistical analysis. Differences in proportions were tested using Pearson's chi-square test or Fisher's exact test when appropriate. Associations between the adjustment variables and the main variable (resistance gene acquisition) were examined by univariate analysis. Results were presented as odds ratios (OR) with 95% confidence intervals (95%CI). Only variables with a prevalence ≥5.0% were considered for multivariate analysis. Variables with a P value < 0.2 in the univariate analysis were included in the multivariate analysis. Logistical regression was used to estimate adjusted odd ratios (aORs) for factors potentially related to resistance gene acquisition. A P value < 0.05 was considered significant.

2.8. Ethics

Pilgrims were invited to participate on a voluntary basis. Participants (or their parents when they were minors) were asked to sign a written consent form. The protocol was approved by the National Ethics Committee for Health Research in Senegal (SEN17/62) and was carried out in accordance with good clinical practices recommended by the Declaration of Helsinki and its amendments.

3. Results

3.1. Study population characteristics

A total of 296 pilgrims were included: 49 in 2018, 75 in 2019, 79 in 2020, and 93 in 2021. Of these, 45.6% were female. The median age was 25 years, with young adults aged between 16 and 45 accounting for 60.5% of the cohort. Very few participants reported having chronic conditions (Table 1).

3.2. Preventive measures

Some 54.7% of the pilgrims reported washing their hands more frequently than usual and 89.2% did so using hand soap. We observed that hand soap was significantly more frequently used in the 2020–2021 period than in 2018–2019 (Table 1).

3.3. Prevalence of clinical symptoms

Between 2018 and 2021, 19.3% of pilgrims reported at least one gastrointestinal symptom following participation in the GMT, and this proportion significantly decreased, to 9.9%, in 2021–2021 (Table 2). The most common symptoms were abdominal pain (6.8%), diarrhoea (4.7%), and constipation (3.4%), and 0.7% of pilgrims received antibiotics during their stay for any reason.

3.4. Prevalence and acquisition rate of gastrointestinal bacteria

A total of 295 pilgrims provided rectal swabs before the Magal and 291 after the Magal. 290 of these were matched. The prevalence of bacteria was high both before (48.5%) and after the Grand Magal (45.7%), and 31.4% of pilgrims acquired a bacterium, mostly EAEC (19.7%), EPEC (7.2%), and EHEC (6.9%) (Supplementary Table 2). The acquisition of bacteria was significantly lower during 2020–2021, notably for EPEC and EHEC (Table 2).

3.5. Prevalence and acquisition of resistance genes

The overall prevalence of resistance genes was relatively high before and after the pilgrimage (88.1% and 79.0%), and this was mostly due to TEM and SHV, and, to a lesser extent, to CTX-MA (Supplementary Table 3). One hundred and five pilgrims (36.2%) acquired at least one resistance gene with the highest rate (21.0%) for CTX-MA, followed by SHV (16.5%). OXA 24, OXA 48, VIM, NDM-1, KPC and colistin resistance genes were not identified in any samples (Table 3). The acquisition of TEM was significantly higher during 2020–2021.

3.6. Risk factors for the acquisition of resistance gene

Only CTX-M A, SHV and TEM were included in the analysis. In a univariate analysis (Table 4), the acquisition of TEM was almost four times higher in the 2020–2021 period than between 2018 and 2019. In addition, women and children under the age of 15 were more at risk of acquiring SHV. Finally, the acquisition of EAEC was associated with the risk of acquisition of CTX-M A and SHV. In a multivariate analysis (Table 4), being female remained associated with SHV acquisition, and EAEC acquisition with CTX-MA and SHV acquisition. In addition, being younger was associated with TEM acquisition and the acquisition of EPEC was associated with a lower risk of acquiring TEM.

3.7. Strains isolated in culture

Between 2018 and 2021, resistant strains isolated in culture samples were mostly ESBL (notably *E. coli*), with some cases of bacteria resistant to carbapenem and oxacillin (Supplementary Table 4).

3.8. Antibiograms

The results of the antibiograms showed that *E. coli* strains were resistant to amoxicillin and cephalosporins (Supplementary Figure). However, they were sensitive to carbapenems. *A. nosocomialis* strains were susceptible to aminoglycosides and susceptible-to-intermediate for cephalosporins. The only *A. baumannii* strain isolated was susceptible to imipenem, meropenem, gentamicin and ticarcillin, and resistant to amikacin, aztreonam, fosfomycin and trimethoprim + sulfamethoxazole. The two strains of *K. pneumoniae* were susceptible to carbapenems and aminoglycosides, and were resistant-to-intermediate for amoxicillin. The two strains of *E. cloacae* were susceptible to carbapenems, aminoglycosides and cephalosporins and resistant to trimethoprim + sulfamethoxazole.

4. Discussion

Our study population was characterised by the young age of the participants, and few elderly pilgrims. This result reflects the familial nature of the Grand Magal of Touba, in which children also participate. Chronic diseases are, therefore, rare in this population. Hand hygiene is a widespread practice in the studied population and the use of soap increased during the COVID-19 pandemic. Despite these measures, pilgrims experienced digestive symptoms suggestive of gastroenteritis

Table 1
Demographics, chronic diseases and preventive measures against gastrointestinal infections.

Variable N (%)	2018 N = 49	2019 N = 75	Subtotal 2018–2019 N = 124	2020 N = 79	2021 N = 93	Subtotal 2020–2021 N = 172	Total N = 296	*P* value[a]
Female	26 (53.1)	29 (38.7)	55 (44.3)	31 (39.2)	49 (52.7)	80 (46.5)	135 (45.6)	0.71
Male	23 (46.9)	46 (61.3)	69 (55.6)	48 (60.8)	44 (47.3)	92 (53.5)	161 (54.4)	
Median age (years)	31	28	29	23	21	22	25	0.0003
Age category (years)								
<16	11 (22.4)	18 (24.0)	29 (23.4)	17 (21.5)	32 (34.4)	49 (28.5)	78 (26.3)	0.03
16–45	27 (55.1)	44 (58.7)	71 (57.3)	54 (68.3)	54 (58.1)	108 (62.8)	179 (60.5)	
>45	11 (22.4)	13 (17.3)	24 (19.3)	8 (10.1)	7 (7.5)	15 (8.7)	39 (13.2)	
Chronic diseases								
High blood pressure	0 (0)	1 (1.3)	1 (0.8)	1 (1.3)	2 (2.1)	3 (1.7)	4 (1.3)	0.49
Cardiovascular disease	0 (0)	1 (1.3)	1 (0.8)	0 (0)	1 (1.1)	1 (0.6)	2 (0.7)	0.82
Other chronic disease[b]	3 (6.1)	0 (0)	3 (2.4)	2 (2.5)	1 (1.1)	3 (1.7)	6 (2.0)	0.68
Hand washing								
As per usual	20 (40.8)	41 (54.7)	61 (49.2)	29 (36.7)	44 (47.3)	73 (42.4)	134 (45.3)	0.25
More frequently than usual	29 (59.2)	34 (45.3)	63 (50.8)	50 (63.3)	49 (52.7)	99 (57.6)	162 (54.7)	
Use of hand soap	38 (77.5)	60 (80.0)	98 (79.0)	74 (93.7)	92 (98.9)	166 (96.5)	264 (89.2)	<0.0001

[a] Chi^2 compares the 2018–2019 subtotal to the 2020–2021 subtotal.
[b] Malignant tumour (1), chronic fatigue (1), motor disability (3), gastritis (1).

Table 2
Gastrointestinal symptoms, use of antibiotics and acquisition of gastrointestinal bacteria (PCR results).

Variable N (%)	2018 N = 49	2019 N = 75	Subtotal 2018–2019 N = 124	2020 N = 79	2021 N = 93	Subtotal 2020–2021 N = 172	Total N = 296	*P* value[a]
Clinical symptoms								
Gastrointestinal symptoms	5 (10.2)	19 (25.3)	24 (19.3)	5 (6.3)	12 (12.9)	17 (9.9)	41 (13.8)	0.02
Before the GMT	1 (2.0)	7 (9.3)	8 (6.4)	2 (2.5)	2 (2.1)	4 (2.3)	12 (4.0)	0.07
After the GMT	4 (8.2)	12 (16.0)	16 (12.9)	3 (3.8)	10 (10.7)	13 (7.6)	29 (9.8)	0.12
P value[b]	0.18	0.16	0.07	0.50	0.02	0.02	0.004	
Diarrhoea	3 (6.12)	5 (6.7)	8 (6.4)	0 (0)	6 (6.4)	6 (3.5)	14 (4.7)	0.24
Nausea	1 (2.0)	3 (4.0)	4 (3.2)	1 (1.3)	0 (0)	1 (0.6)	5 (1.7)	0.08
Vomiting	1 (2.0)	3 (4.0)	4 (3.2)	1 (1.8)	3 (3.2)	4 (2.3)	8 (2.7)	0.63
Abdominal pain	1 (2.0)	7 (9.3)	8 (6.4)	3 (3.8)	9 (9.7)	12 (7.0)	20 (6.8)	0.86
Constipation	2 (4.1)	4 (5.3)	6 (4.9)	3 (3.8)	1 (1.1)	4 (2.3)	10 (3.4)	0.24
Fever	1 (2.0)	4 (5.3)	5 (4.0)	2 (2.5)	0 (0)	2 (1.2)	7 (2.4)	0.11
Use of antibiotics	1 (2.0)	1 (1.3)	2 (1.6)	0 (0)	0 (0)	0 (0)	2 (0.7)	0.09
Acquisition of gastrointestinal bacteria								
At least one bacteria	28 (65.1)	19 (25.3)	47 (39.8)	19 (24.0)	25 (26.9)	44 (25.6)	91 (31.4)	0.01
EAEC	13 (30.2)	9 (12.0)	22 (18.6)	11 (13.9)	24 (25.8)	35 (20.3)	57 (19.7)	0.72
EHEC	7 (16.3)	9 (12.0)	16 (13.6)	2 (2.5)	2 (2.1)	4 (2.3)	20 (6.9)	<0.0001
EPEC	15 (34.9)	3 (4.0)	18 (15.2)	0 (0)	3 (3.2)	3 (1.7)	21 (7.2)	<0.0001
EIEC/Shigella	4 (9.3)	3 (4.0)	8 (6.8)	3 (3.8)	5 (5.4)	8 (4.6)	16 (5.5)	0.43
Tropheryma whipplei	1 (2.3)	0 (0)	1 (0.8)	3 (3.8)	0 (0)	3 (1.7)	4 (1.4)	0.52
Salmonella	2 (4.6)	0 (0)	2 (1.7)	0 (0)	0 (0)	0 (0)	2 (0.7)	0.08
Campylobacter jejuni	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA

[a] Chi^2 compares the 2018–2019 subtotal to the 2020–2021 subtotal.
[b] Fisher exact compares symptoms before versus after participation in the GMT. GMT: Grand Magal de Touba. EAEC: Enteroaggregative *Escherichia coli*, EHEC: Enterohaemorrhagic *E. coli*, EPEC: Enteropathogenic *E. coli*, EIEC: Enteroinvasive *E. coli*.

Table 3
Acquisition of resistance genes (PCR).

Variable N (%)	2018 N = 43	2019 N = 75	Subtotal 2018–2019 N = 118	2020 N = 79	2021 N = 93	Subtotal 2020–2021 N = 172	Total N = 290	*P* value[a]
ARG	27 (62.8)	13 (17.3)	40 (33.9)	20 (25.3)	45 (48.4)	65 (37.8)	105 (36.2)	0.50
CTX-MA	13 (29.6)	9 (12.0)	22 (18.6)	13 (16.5)	26 (28.0)	39 (22.7)	61 (21.0)	0.41
CTX-MB	8 (18.2)	0 (0)	8 (6.7)	4 (5.1)	0 (0)	4 (2.3)	12 (4.1)	0.06
SHV	16 (36.4)	4 (5.3)	20 (16.9)	7 (8.9)	21 (22.6)	28 (16.3)	48 (16.5)	0.88
TEM	2 (4.5)	2 (2.7)	4 (3.4)	3 (3.8)	17 (18.3)	20 (11.6)	24 (8.2)	0.01
OXA-23	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	1 (0.6)	1 (0.3)	0.41

[a] Chi^2 compares the 2018–2019 subtotal to the 2020–2021 subtotal. ARG: at least one resistance gene, CTX-M: cefotaximase Munich, SHV: Sulfydryl variable, TEM: Temoneria, OXA-23: Oxacillinase-23.

twice as frequently after attending the Magal than prior to their attendance. The prevalence of these symptoms, however, decreased during the COVID-19 pandemic, probably due to improved compliance with hand hygiene measures. Antibiotic use was very low. One in three pilgrims acquired a gastrointestinal pathogen after staying in Touba. These were mainly *E. coli*, particularly EAEC and EPEC, which likely resulted from food or water contamination. Although their symptoms were mild, they run the potential risk of transmission of gastrointestinal disease to family and friends upon return home. Nine in ten pilgrims were carriers of TEM, one in two were carriers of SHV, and one in five were carriers of CTM-X A before participating in the Magal. Genes encoding for colistin resistance were not found. Participation in the Magal resulted in the acquisition of new resistance genes in one in every three participants. These were mainly CTX-M A. The resistant bacteria isolated were mainly *E. coli* resistant to amoxicillin, to the combination amoxicillin + clavulanic acid and to cephalosporins, but were sensitive to carbapenems. We did not observe an increase in the prevalence of resistant genes over time before participation in the GMT during the years studied. This suggests that no significant transmission of resistant genes occurred in the village following the pilgrims' return, which likely results from a short carriage period after acquisition of resistant bacteria. This hypothesis should be challenged by sampling at six months following the return of positive pilgrims. Risk factor analysis shows an independent association between CTX-M A and EAEC acquisition.

We observed a very low prevalence of resistant *K. pneumoniae* isolates in our study. Another study conducted in 2015–2016 on 336 pregnant Senegalese women showed a prevalence of 40.2% *Klebsiella pneumoniae* gastrointestinal carriage with a low resistance rate to quinolone, other aminoglycosides and third-generation cephalosporins (about 3% each). ESBL *K. pneumoniae* were almost absent (0.7%) and multidrug resistance was about 11.7%, indicating that its spread in the community is still limited in Senegal, aligning with our study results [9]. In a systematic review [10] addressing the prevalence and incidence of carbapenem-resistant *K. pneumoniae* colonisation, mainly based on studies conducted in a healthcare setting, a high variability of the distribution was revealed in different geographic areas, with a low prevalence of 0.07% in Africa. We found no evidence of colistin-resistant bacteria in our study. Studies conducted on poultry products in Senegal revealed that 21% of salmonella isolates were resistant to quinolones and fluoroquinolones after being isolated from 300 chicken carcasses [11]. Another study conducted on 32 chicken farms in Senegal showed that 68% of isolates from environmental, faecal and drinking water samples, carcasses, and carcass washes, were multidrug-resistant, suggesting that chickens in Senegal could be a reservoir for humans antimicrobial resistance [12].

Our study has some limitations. The number of subjects included is relatively small. The study population living in rural areas of southern Senegal is not representative of all pilgrims attending the GMT. The

Table 4
Risk factors for acquiring resistance genes (univariate and multivariate analysis).

Variables	CTX-M A	SHV	TEM
Univariate analysis			
2018–2019	REF	REF	REF
2020–2021	1.28 [0.69–2.42] 0.41	0.95 [0.48–1.89] 0.88	3.75 [1.20–15.44] 0.01
Male	0.90 [0.49–1.65] 0.72	0.44 [0.21–0.68] 0.01	0.98 [0.39–2.52] 0.97
Age <15 years	REF	REF	REF
Age: 16–45 years	0.63 [0.32–1.24] 0.14	0.44 [0.22–0.91] 0.01	0.53 [0.20–1.46] 0.16
Frequent hand washing	0.63 [0.34–1.16] 0.11	1.2 [0.60–2.35] 0.59	1.16 [0.46–3.05] 0.72
Use of soap	0.70 [0.28–1.93] 0.42	0.77 [0.28–2.44] 0.59	NA
At least one gastrointestinal symptom after Magal	0.76 [0.21–2.16] 0.60	0.79 [0.19–2.45] 0.67	1.92 [0.44–6.40] 0.25
Acquisition of EHEC	0.64 [011–2.34] 0.50	1.75 [0.47–5.43] 0.30	0.56 [0.01–3.90] 0.58
Acquisition of EAEC	3.12 [1.56–6.15] 0.0003	3.51 [1.67–7.22] 0.0001	1.77 [0.58–4.80] 0.22
Acquisition of EPEC	1.99 [0.64–5.58] 0.15	2.16 [0.65–6.30] 0.12	0 [0.00–1.90] 0.15
Acquisition of EIEC/ Shigella	1.76 [0.46–5.80] 0.30	1.74 [0.39–6.08] 0.35	0 [0.00–2.56] 0.21
Multivariate analysis. Only significant results are shown.			
2018–2019	REF	REF	REF
2020–2021	–	–	–
Male gender	–	0.40 [0.21–0.78] 0.008	–
Age <15 years	REF	REF	REF
Age: 16–45 years	–	–	0.61 [0.40–0.93] 0.02
Frequent hand washing	–	–	–
Use of soap	–	–	–
Gastrointestinal symptom after Magal	–	–	–
Acquisition of EHEC	–	–	–
Acquisition of EAEC	3.12 [1.65–5.89] < 0.0001	3.74 [1.87–7.46] < 0.0001	–
Acquisition of EPEC	–	–	0.10 [0.02–0.35] < 0.0001
Acquisition of EIEC/ Shigella	–	–	–

EAEC: Enteroaggregative *Escherichia coli*, EHEC: Enterohaemorrhagic *E. coli*, EPEC: Enteropathogenic *E. coli*, EIEC: Enteroinvasive *E. coli*.

duration of the stay in Touba is generally short, which does not allow the acquisition of pathogens to be formally attributed to the participation to the GMT. We did not screen stool samples for colistin-resistant bacteria using a specific culture medium, although the PCR method usually has a sensitivity similar to that of culture [13].

5. Conclusion

Our results show a risk of acquisition of CTX-M A by pilgrims following their participation in the Grand Magal of Touba, while the prevalence of these resistance genes is relatively low in the villages of origin. This risk is not limited to a particular demographic category, and it is therefore not possible to identify a target population for enhanced preventive measures. The low use of antibiotics and the lack of correlation with hand hygiene measures provide no leverage for implementing preventive measures. Surveillance of the prevalence of carriage of resistant bacteria and the occurrence of clinical infections with these bacteria in pilgrims and their relatives could be of interest in the future.

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Credit author statement

CS, NG and PG contributed to the experimental design. IO, PG and wrote the manuscript. IO and NG conducted the qPCR technique. CD, IO and NG administered questionnaires, followed patients and collected samples. SD, JMR and CS critically reviewed the manuscript. PG and CS coordinated the work. All authors contributed to and approved the current version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2024.102709.

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