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PCR investigation of infections in patients consulting at a healthcare centre over a four-year period during the Grand Magal of Touba

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ABSTRACT

Background: Respiratory and gastrointestinal symptoms and febrile illness are the most common complaints among ill pilgrims attending the Grand Magal of Touba (GMT) in Senegal.
Methods: Patients presenting with respiratory or gastrointestinal symptoms or febrile systemic illnesses were recruited between 2018 and 2021 at a healthcare centre close to Touba. Respiratory, gastrointestinal and blood samples were tested for potential pathogens using qPCR.
Results: 538 patients were included. 45.5% of these were female, with a median age of 17 years. Of the 326 samples collected from patients with a cough, 62.8% tested positive for at least one virus, including influenza viruses (33.1%). A high positivity rate of bacterial carriage was observed for *Haemophilus influenzae* (72.7%), *Streptococcus pneumoniae* (51.2%) and *Moraxella catarrhalis* (46.0%). Of the 95 samples collected from patients with diarrhoea, 71.3% were positive, with high rates of bacterial carriage, ranging from 4.2% for *Tropheryma whipplei* to 45.3% for Entero-pathogenic *Escherichia coli*. Of the 141 blood samples collected from patients with fever, 31.9% were positive including *Plasmodium falciparum* (21.3%), *Borrelia* sp. (5.7%) and dengue virus (5.0%).
Conclusion: This study provides insight into the aetiology of most common infections at the GMT on which to base therapeutic options.

1. Introduction

Every year in the holy city of Touba, in the Diourbel region of Senegal, a major event known as the Grand Magal of Touba (GMT) celebrates the departure into exile of Cheikh Ahmadou Bamba Mbacké. Between four and five million pilgrims participate in the GMT, making it the largest religious event in Senegal and in West Africa. Several activities take place during the event, but the most emblematic is the visit to the great mosque of Touba [1].

During the GMT, medical care is provided to pilgrims free of charge for five days. Most of the available local public medical infrastructures are involved in the medical preparation for, surveillance of and response to the event. At least 5,244 healthcare workers were mobilised during the GMT in 2018. The Ministry of Health set up 176 medical service delivery points, which included hospitals, health care centres and advanced medical posts [2].

Some syndromic surveillance data is available for previous years. During the 2015 GMT, of the 32,229 patients who consulted health services in Touba and the surrounding area during the five-day period of free medical coverage, the most common reasons for medical consultation were gastrointestinal symptoms (14%), respiratory and ear, nose and throat symptoms (10%), fevers of unknown origin (5%) and confirmed malaria (3%) [1]. In a similar study conducted among 20,850 patients during the 2016 GMT, the most prevalent symptoms were

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headaches (28.2%), gastrointestinal symptoms (22.0%), fever (17.2%), and respiratory symptoms (17.1%), and 2.4% had confirmed malaria [3].

With the exception of rapid malaria tests, no microbiological investigations were conducted, due to a lack of local laboratory resources. With the aim of closing this gap, in 2018 we conducted a preliminary PCR study among patients consulting at one health care centre in Mbacké, close to Touba, for three days during the GMT period [4]. We revealed that 42.3% of patients suffering from respiratory symptoms were positive for influenza A or B, and most patients (88.8%) carried bacterial pathogens including, notably, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*. In addition, 56.5% of patients suffering from diarrhoea were carrying bacterial pathogens including mostly *Escherichia coli* pathotypes. Finally, the most common pathogens identified from blood in patients with febrile systemic illness were dengue virus and *Plasmodium falciparum*. The survey was continued during the GMT between 2019 and 2021, revealing the absence of SARS-CoV-2 infections in patients with respiratory symptoms consulting at the Mbacké centre in 2020 and 2021, but a very high prevalence of influenza A (64.2%) in 2021 [5,6]. In this study, we describe the results of PCR pathogen screening in patients consulting at Mbacké centre with respiratory or gastrointestinal infection symptoms, or febrile systemic illness during the GMT between 2018 and 2021. In addition, we describe the implementation of a point-of-care laboratory (POC) in 2021.

2. Materials and methods

2.1. Data collection

The Mbacké healthcare centre (14°47′56″N, 15°54′36″W, 10 km from Touba) was selected because of its large number of patients, (1,115 patients during the 2015 GMT, unpublished data). It operated 24 h a day during the five-day period of free medical coverage of the GMT (26–28 October 2018, three-day pilot study, 15–19 October 2019, 4–8 October 2020, and 24–28 September 2021). The consultations were provided by medical doctors and nurses. The centre consists of an emergency room and a hospitalisation ward with a capacity of 28 beds. The inclusion criteria were to present with a cough, diarrhoea or febrile systemic illness as the main complaint. The medical team completed a demographic and clinical questionnaire and patients were offered appropriate sampling according to their symptoms. “Influenza-like illness” was defined as the combination of a cough, sore throat and fever [7]. “Diarrhoea” was defined by at least three liquid stools per day [8]. “Fever” was defined by an axillary temperature of at least 37.5C [9]. “Febrile systemic illness” was defined as documented fever at presentation without an identified source after clinical assessment. All patients fulfilling inclusion criteria and presenting from 8 a.m. to 17 p.m. were invited to participate.

2.2. Sample collection

Respiratory samples were collected from patients with a cough as their main complaint using commercial rigid cotton-tipped swab applicators (Medical Wire and Equipment, Wiltshire, UK), which were inserted in the anterior nares and the oro-pharynx and then placed in viral transport media (Sigma Virocult®). Rectal samples were collected from patients with diarrhoea as their main complaint using similar material. Blood was collected from patients with febrile systemic illness as their main complaint by venous puncture in an EDTA tube (BD vacutainer® K2E EDTA 18.0 mg, UK). In some patients for whom the main complaint was difficult to identify, respiratory and/or rectal and/or blood samples were concomitantly collected. Swabs and blood were kept at 4 °C before being transported to a laboratory in Dakar for storage at −80 °C and subsequently transferred to Marseille on dry ice for processing during the GMT 2018 to 2020 and processing in the months following sampling. In 2021, we implemented a POC laboratory to detect respiratory and blood pathogens in the health care centre in Mbacké, enabling results to be available on site within a few hours.

2.3. Identification of pathogens by PCR in Marseille

The EZ1 Advanced XL (Qiagen, Hilden, Germany) with the Virus MiniKit v2.0 (Qiagen) was used for DNA and RNA extraction according to the manufacturer’s recommendations. The respiratory pathogens tested by PCR were influenza A, influenza B, human rhinovirus, respiratory syncytial virus (RSV), human metapneumovirus, endemic coronaviruses (HKU1, 229E, OC43 and NL63), SARS-CoV2, human parainfluenza virus, adenovirus, *S. pneumoniae*, *Staphylococcus aureus*, *H. influenzae*, *Klebsiella pneumoniae*, *Bordetella pertussis* and *Mycoplasma pneumoniae* [10]. In addition, samples were investigated for *M. catarrhalis* DNA (gene copB) [11].

The gastrointestinal pathogens tested were hepatitis A virus, hepatitis E virus, adenovirus, astrovirus, rotavirus, norovirus, *Salmonella* sp, *Shigella*/enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), Entero-pathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), *Campylobacter jejuni*, *Tropheryma whipplei*, *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum/hominis* [10].

For blood samples, *P. falciparum*, *Coxiella burnetii*, *Borrelia* spp., *S. pneumoniae*, *Salmonella* spp., *S. aureus* and *Rickettsia* spp. [12] were amplified using the Light Cycler 480 Probes Master Kit (Roche Diagnostics France). Dengue virus (DENV) was detected by one-step simplex real-time quantitative RT-PCR amplification performed using Multiplex RNA Virus Master Kit (Roche Diagnostics, France) [13].

All quantitative real-time PCRs were performed using a C1000 Touch Thermal Cycle (Bio-Rad, Hercules, CA, USA). Negative controls (PCR mix) and positive controls (DNA from a bacterial strain or from the viral strain) were included in each run. A cycle threshold (CT) value of $\leq$35 was used to assess positive results of pathogen amplification. When two genes were tested for one pathogen, we considered the results of the pathogen to be positive if the CT value of the two genes was $\leq$35.

2.4. Identification of pathogens by PCR in Mbacké

In 2021, RNA was manually extracted from respiratory samples using the Quick-RNA™ MiniPrep kit (Zymmo research, USA) and DNA was manually extracted from blood samples using the E.Z.N.A® Tissue DNA kit (Omega, USA), according to the manufacturer’s recommendation. In this POC temporary laboratory, the respiratory pathogens tested for by PCR were influenza A and B, human rhinovirus, RSV and SARS-CoV2, and the blood pathogens tested were *P. falciparum*, *C. burnetii*, *Borrelia* spp., *S. pneumoniae*, *Salmonella* spp., *S. aureus* and *Rickettsia* spp. The amplification procedure was conducted as described above. The results from patients consulting in the morning were available that very afternoon, while those from patients consulting in the afternoon were available the next morning. All patients with a positive result were informed by telephone of their result.

2.5. Statistical analysis

STATA software version 14.2 (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 or Fisher’s exact tests, as appropriate. Small numbers with five cases or fewer were not included in the statistical analysis.

2.6. Ethics

Patients were invited to participate on a voluntary basis. Participants (or their parents when 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). It was performed in accordance

with good clinical practices recommended by the Declaration of Helsinki and its amendments.

3. Results

3.1. Study population

A 100% acceptation rate was achieved. The study included 538 patients distributed as follows: 88 were included in 2018, 215 in 2019,155 in 2020 and 80 in 2021. Of them, 245 (45.5%) were male, 290 were female (53.9%) and gender was not documented in three patients. Of the 529 patients whose age was documented, 197 were under the age of five (36.6%), 109 were aged between 5 and 15 years old (20.3%), 183 were aged between 16 and 45 years old (34.0%), and 40 were over the age of 45 (7.4%), with a median age of 17.1 (ranging from 0 to 91 years). A total of 295 (54.8%) patients came from other regions of Senegal or beyond to participate in the GMT and the remaining were local residents participating in the GMT.

A total of 377/538 patients reported at least one respiratory symptom with a cough the most frequent symptom (67.7% of 538 patients), followed by rhinitis (50.0%), and 6.9% reported an influenza-like illness. Some 239/538 patients reported gastrointestinal symptoms with diarrhoea being the most frequent (19.7%), followed by abdominal pain (19.3%) and vomiting (18.8%). A total of 264 patients (49.1%) reported fever, and 231 (42.9%) reported headaches and arthralgia (16.5) (Fig. 1). A total of 378/538 (70.3%) received antibiotic treatment and 25 (4.6%) received antimalarial drugs. Forty-three patients (8.0%) were hospitalised. Only one patient was transferred to a tertiary care hospital due to gastroenteritis with dehydration.

Based on clinician assessment, 196 patients had a cough as their main complaint, 52 had diarrhoea as their main complaint and 105 had a febrile systemic illness as their main complaint. In 185 patients, symptoms were not univocal (Supplementary Fig. 1).

3.2. Detection of pathogens in respiratory samples

Of the 326 samples collected, 97.2% tested positive for at least one pathogen, including 62.8% which tested positive for at least one virus, with influenza viruses (33.1%) and RSV (16.2%) being the most frequent. SARS-CoV2 was negative in all patients. A high positivity rate of bacterial carriage was observed (91.1%), notably for *H. influenzae* (72.7%), *S. pneumoniae* (51.2%) and *M. catarrhalis* (46.0%) (Table 1). We found that the positivity rates of some pathogens showed significant yearly variations. Influenza B virus was more frequently detected in 2018 and influenza A virus in 2021, while RSV was more frequently detected in 2019. *H. influenza* was more frequently detected in 2018 and 2019. The detection of *S. aureus* was lower in 2020. In addition, the detection of *S. pneumoniae* and *M. catarrhalis* tended to be higher in 2020.

Regarding the prevalence of respiratory pathogens by age group, we observed that influenza viruses were significantly more frequently detected in children aged between the ages of 5 and fifteen and in young adults (Table 2), while RSV was more frequently diagnosed among children under the age of five. Rhinovirus was more frequently detected among adults over the age of 45. *S. pneumoniae* and *M. catarrhalis* were more frequently detected in children under the age of 5 and *S. aureus* was more frequently detected in older adults.

3.3. Detection of pathogens in stool samples

Of the 95 samples collected, 71.3% were positive for at least one pathogen. Only 8.5% of patients tested positive for a virus, with adenovirus being the most frequent (6.3%). Higher rates of bacterial carriage were observed (63.1%) overall ranging from 4.2% for EHEC and *T. whipplei* to 45.3% for EAEC. Furthermore, 21.1% of patients tested positive for EPEC and 18.9% for *Shigella*/EIEC. Additionally, 14.7% patients tested positive for a parasite, with 9.5% for *G. lamblia* (Table 3). It was notable that EPEC detection was significantly higher in 2019 and 2020.

Regarding the prevalence of gastrointestinal pathogens by age group, we observed that EAEC was significantly more frequently detected in children under the age of five and *Shigella*/EIEC and *T. whipplei* was more frequently detected in older adults. Viral infections were almost exclusively observed in children under the age of five (Table 4).

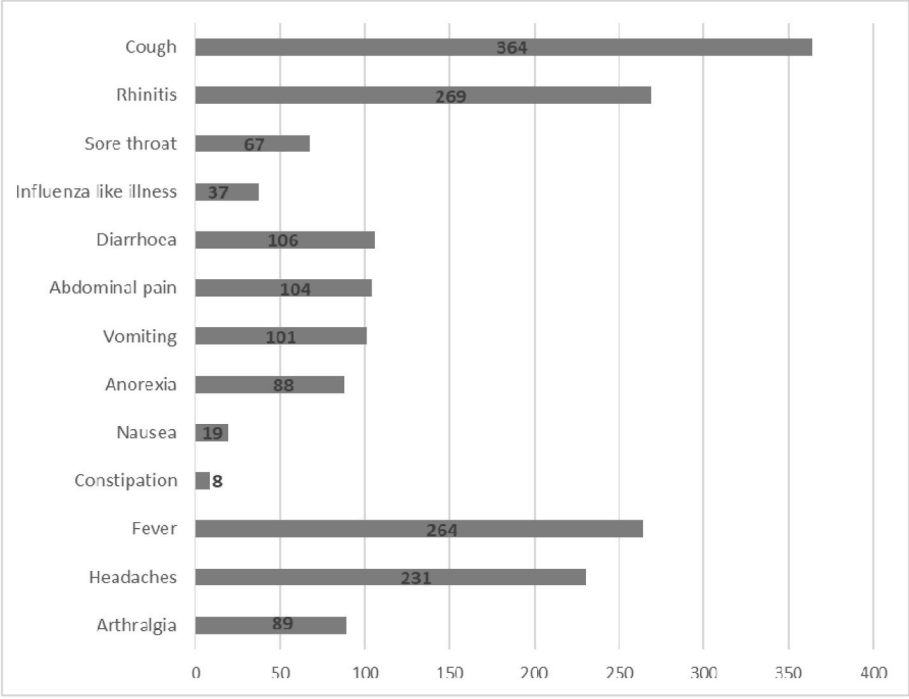

Fig. 1. Symptoms in 538 patients seen at the Mbacké healthcare centre over a four-year period at the Grand Magal of Touba.

Table 1
Prevalence of respiratory pathogens among 326 patients with respiratory samples available.

Pathogens	2018 N = 52	2019 N = 112	2020 N = 109	2021 N = 53	Total N = 326	P-value[a]
At least one pathogen	48 (92.3)	112 (100.0)	106 (97.2)	51 (96.2)	317 (97.2)	0.04
At least one virus	27 (51.9)	74 (66.1)	62 (56.9)	42 (79.2)	205 (62.8)	0.01
Influenza viruses	22 (42.3)	22 (19.6)	30 (27.5)	34 (64.2)	108 (33.1)	<0.0001
Influenza A virus	7 (13.5)	22 (19.6)	22 (20.2)	34 (64.2)	85 (26.1)	<0.0001
Influenza B virus	15 (28.8)	0 (0)	8 (7.3)	0 (0)	23 (7.0)	<0.0001
Respiratory syncytial virus	2 (3.8)	37 (33.0)	14 (12.8)	3 (5.7)	53 (16.2)	<0.0001
Human rhinovirus	3 (5.8)	18 (8.5)	6 (5.5)	4 (7.6)	27 (8.3)	0.03
Metapneumovirus	1 (1.9)	2 (0.9)	7 (6.4)	0 (0)	10 (3.1)	0.08
Adenovirus	0 (0)	5 (2.3)	3 (2.7)	0 (0)	8 (2.4)	0.20
Endemic coronaviruses	0 (0)	4 (1.9)	1 (0.9)	0 (0)	5 (1.5)	0.17
Human parainfluenza viruses	0 (0)	0 (0)	2 (1.8)	2 (3.8)	4 (1.2)	0.16
SARS-CoV2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
At least one bacteria	46 (88.5)	109 (97.3)	105 (96.3)	37 (69.8)	297 (91.1)	<0.0001
Haemophilus influenzae	33 (63.5)	91 (42.9)	89 (81.6)	24 (45.3)	237 (72.7)	<0.0001
Streptococcus pneumoniae	24 (46.1)	53 (25.0)	66 (60.5)	24 (45.3)	167 (51.2)	0.12
Moraxella catarrhalis	19 (36.5)	52 (24.5)	59 (54.1)	20 (37.7)	150 (46.0)	0.10
Staphylococcus aureus	15 (28.8)	39 (18.4)	8 (7.3)	9 (17.0)	71 (21.8)	<0.001
Klebsiella pneumoniae	2 (3.8)	1 (0.5)	0 (0)	0 (0)	3 (0.9)	0.09
Bordetella pertussis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Mycoplasma pneumoniae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Co-infection virus-bacteria	25 (48.1)	71 (63.4)	61 (56.0)	27 (50.9)	184 (56.4)	0.22

[a] Chi2 compares prevalence of pathogen between years.

3.4. Detection of pathogens in the blood

A total of 45/141 (31.9%) tested positive for at least one pathogen, including 21.3% who tested positive for *P. falciparum*, 5.7% for *Borrelia* sp. and 5.0% for dengue virus (Table 5). Out of 31 patients who tested positive for *P. falciparum,* nine (29%) were local residents, and out of eight patients infected with *Borrelia* sp, three (37.5%) were local residents. *P. falciparum* and *Borrelia* sp. were detected each year, while dengue virus was detected only at the 2018 GMT. *P. falciparum* was significantly more frequently detected in 2019.

Regarding the prevalence of blood pathogens by age group, no pathogen was detected in children under the age of five. The detection of *P. falciparum* tended to increase with age (Table 6).

4. Discussion

In this study, we were able to identify potential pathogens in 97.2% of respiratory samples obtained from patients with respiratory symptoms. Influenza viruses, *H. influenzae, S. pneumoniae and M. catarrhalis* were the most frequently detected among patients. In our study, conducted among pilgrims participating in the GMTs between 2017 and 2021, 55% of whom experienced respiratory symptoms, we observed that the acquisition of respiratory viruses overall was 18% and that the

Table 2
Prevalence of respiratory pathogens by age group (N = 321[a]).

Pathogens	<5 years N = 135	5–15 years N = 58	16–45 years N = 102	>45 years N = 26	P-value[b]
At least one pathogen	133 (98.5)	58 (100.0)	97 (95.10)	24 (92.3)	0.19
At least one virus	95 (70.4)	40 (69.0)	55 (53.9)	13 (50.0)	0.03
Influenza viruses	30 (22.2)	26 (44.8)	45 (44.1)	6 (23.1)	0.001
Respiratory syncytial virus	42 (31.1)	6 (10.3)	5 (4.9)	2 (7.7)	<0.0001
Human rhinovirus	16 (11.8)	6 (10.3)	4 (3.9)	5 (19.2)	0.05
Metapneumovirus	6 (4.4)	0 (0)	2 (2.0)	2 (7.7)	0.16
Adenovirus	7 (5.2)	1 (1.7)	0 (0)	0 (0)	0.06
Endemic coronaviruses	1 (0.7)	0 (0)	2 (2.0)	1 (3.8)	0.40
Human parainfluenza viruses	3 (2.2)	1 (1.7)	0 (0)	0 (0)	0.43
SARS-CoV2	0 (0)	0 (0)	0 (0)	0 (0)	NA
At least one bacteria	131 (91.4)	53 (91.4)	87 (85.3)	21 (80.8)	0.008
Haemophilus influenzae	106 (78.5)	45 (77.6)	67 (65.7)	15 (57.7)	0.06
Streptococcus pneumoniae	107 (79.3)	27 (46.5)	22 (46.5)	9 (34.6)	<0.0001
Moraxella catarrhalis	97 (71.8)	28 (48.3)	19 (18.6)	3 (11.5)	<0.0001
Staphylococcus aureus	23 (17.0)	16 (20.6)	21 (20.6)	10 (38.5)	0.05
Klebsiella pneumoniae	1 (0.7)	1 (1.7)	0 (0)	1 (3.8)	0.27
Bordetella pertussis	0 (0)	0 (0)	1 (1.2)	0 (0)	0.54
Mycoplasma pneumoniae	0 (0)	0 (0)	0 (0)	0 (0)	NA
Co-infection virus-bacteria	93 (68.9)	34 (58.6)	45 (44.1)	10 (38.5)	0.001

[a] Age was not documented in five patients.
[b] Chi^2 compares prevalence of pathogens between age groups.

acquisition of *H. influenzae, S. pneumoniae and M. Catarrhalis* ranged from 5% to 14% [14]. We also noticed that respiratory symptoms are independently associated with the acquisition of viruses and of *S. pneumoniae.* In a study conducted between August and December 2015 in 250 children and adults attending the Keur Socé healthcare centre in central rural Senegal, a prevalence of 50% of virus carriage was found in patients with acute respiratory infection symptoms, in line with our results in Mbacké [15]. The most prevalent viruses in the Tine study were rhinovirus (28%), influenza virus (16%) and endemic coronaviruses (12%). In another study conducted between 2009 and 2011 across Senegal with 232 patients over the age of 50 and suffering from ILI, 57% of samples tested positive for at least one virus, with the most prevalent viruses being influenza virus (29%) and rhinovirus (17%) [16]. Finally, influenza viruses were detected in 29% of 598 patients suffering from ILI in a one-year study conducted in Dielmo and Ndiop between December 2012 and August 2013 [17]. Similar to our results, high rates of *S. pneumoniae* were also found by Tine and colleagues in patients with acute respiratory infection symptoms, notably in children [15]. We observed significant differences in the rates of positivity of pathogens depending on the year, with influenza A virus being detected most frequently in 2018 and 2021, linked to epidemics of influenza A at the GMT [4,6]. Children under the age of five had the highest rate of RSV and *S. pneumoniae* positivity, as reported in several studies conducted in Senegal [18–20]. Respiratory infections have been associated with religious gatherings such as the Hajj in Mecca and linked to influenza virus infection [21,22]. In addition, Barasheed et al., identified rhinovirus as the most common cause of influenza-like illness among Hajj pilgrims in 2013 [23]. Finally, in a study conducted among French Hajj prilgrims with respiratory symptoms from 2014 to 2018, rhinovirus,

Table 3
Prevalence of gastrointestinal pathogens among 95 patients with rectal samples available.

Pathogens	2018 N = 23	2019 N = 54	2020 N = 15	2021 N = 3	Total N = 95	P-value[a]
At least one pathogen	14 (60.9)	41 (75.9)	11 (73.3)	1 (33.3)	67 (71.3)	0.40
At least one virus	2 (8.7)	5 (9.3)	1 (6.7)	0 (0)	8 (8.5)	0.95
Adenovirus	2 (8.7)	3 (5.6)	1 (6.7)	0 (0)	6 (6.3)	0.92
Rotavirus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Norovirus	0 (0)	2 (3.7)	0 (0)	0 (0)	2 (2.1)	0.49
Astrovirus	0 (0)	2 (3.7)	0 (0)	0 (0)	2 (2.1)	0.67
At least one bacteria	13 (59.1)	36 (66.7)	10 (66.7)	1 (33.3)	60 (63.1)	0.68
Enteroaggregative *Escherichia coli*	5 (21.7)	27 (50.0)	10 (66.7)	1 (33.3)	43 (45.3)	0.01
Shigella/ Enteroinvasive *Escherichia* coli	6 (26.1)	11 (20.4)	1 (6.7)	0 (0)	18 (18.9)	0.32
Enteropathogenic *Escherichia coli*	7 (30.4)	10 (18.5)	3 (20.0)	0 (0)	20 (21.1)	0.50
Enterohaemorrhagic *Escherichia coli*	0 (0)	4 (7.4)	0 (0)	0 (0)	4 (4.2)	0.23
Tropheryma whipplei	2 (8.7)	2 (3.7)	0 (0)	0 (0)	4 (4.2)	0.41
Campylobacter jejuni	0 (0)	4 (7.4)	1 (6.7)	0 (0)	5 (5.3)	0.41
Salmonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
At least one parasite	4 (17.4)	8 (14.8)	2 (13.3)	0 (0)	14 (14.7)	0.93
Giardia lamblia	2 (8.7)	5 (9.3)	2 (13.3)	0 (0)	9 (9.5)	0.88
Cryptosporidium sp.	1 (4.3)	3 (5.6)	0 (0)	0 (0)	4 (4.2)	0.65
Entamoeba histolytica	1 (4.3)	1 (1.8)	0 (0)	0 (0)	2 (2.1)	0.65

[a] Chi^2 compares prevalence of pathogens between years.

S. aureus and *H. influenzae* were the pathogens most commonly identified by PCR [24]. Despite intensive search we were not able to find relevant data about PCR investigation of patients consulting during religious MGs in Africa. The high rates of influenza virus (33%) and *S. pneumoniae* (51%) detection in ill GMT pilgrims consulting at the Mbacké centre has important public health consequences, since infections due to these two pathogens are vaccine-preventable and can be cured with oseltamivir and antibiotics, respectively. Finally SARS-CoV2 detection was negative, confirming that Senegal is one of the rare countries in Africa that has been able to contain the COVID-19 pandemic [25].

High rates of pathogens were detected among patients suffering from diarrhoea, mostly due to bacteria, with a large predominance of *E. coli,* notably EAEC. In the cohorts of pilgrims that we surveyed between 2017 and 2021, 13% of participants reported diarrhoea and 32% acquired bacterial rectal carriage with EAEC being the most frequent [14]. In this study, however, no association between gastro-intestinal microbes and diarrhoea was observed. This is likely because asymptomatic carriage of gastrointestinal pathogens is very frequent in Senegal, particularly when assessed using a very sensitive method such as PCR. This suggests that quantitative molecular methods should be used to better evaluate the aetiological role of potential gastrointestinal pathogens in this setting, as proposed by Liu et al. [26]. We found a very low rate of viral infections in our study, in contrast with a study conducted in Dakar in 2009–2010 [27]. This might be because our study was conducted during the rainy season, when viral infections appear to be less prevalent [27].

We found a pathogen in the blood of 32% of patients with febrile systemic illness, with a notable 21% prevalence of *P. falciparum*.

Table 4
Prevalence of gastrointestinal pathogens by age group (N = 92[a]).

Pathogens	<5 years N = 55	5–15 years N = 15	16–45 years N = 17	>45 years N = 5	P-value[b]
At least one pathogen	46 (83.6)	9 (60.0)	7 (41.2)	3 (60.0)	0.005
At least one virus	7 (12.7)	1 (6.7)	0 (0)	0 (0)	0.26
Adenovirus	6 (10.9)	0 (0)	0 (0)	0 (0)	0.15
Rotavirus	0 (0)	0 (0)	0 (0)	0 (0)	NA
Norovirus	1 (1.8)	1 (6.7)	0 (0)	0 (0)	0.42
Astrovirus	2 (3.6)	0 (0)	0 (0)	0 (0)	0.55
At least one bacteria	40 (72.7)	8 (53.3)	6 (35.3)	3 (60.0)	0.02
Enteroaggregative *Escherichia coli*	34 (61.8)	5 (33.3)	2 (11.7)	1 (20.0)	0.001
Shigella/Enteroinvasive *Escherichia coli*	11 (20.0)	3 (20.0)	11 (11.8)	2 (40.0)	0.001
Enteropathogenic *Escherichia coli*	12 (21.8)	5 (33.3)	2 (11.8)	0 (0)	0.50
Enterohaemorrhagic *Escherichia coli*	3 (5.4)	1 (6.7)	0 (0)	0 (0)	0.59
Tropheryma whipplei	0 (0)	2 (13.3)	1 (5.9)	1 (20.0)	0.04
Campylobacter jejuni	5 (9.1)	0 (0)	0 (0)	0 (0)	0.21
Salmonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	NA
At least one parasite	10 (18.2)	3 (20.0)	0 (0)	0 (0)	0.15
Giardia lamblia	6 (10.9)	2 (13.3)	0 (0)	0 (0)	0.32
Cryptosporidium sp.	4 (7.3)	0 (0)	0 (0)	0 (0)	0.29
Entamoeba hystolitica	0 (0)	1 (6.7)	1 (5.9)	0 (0)	0.17

[a] Age was not documented in three patients.
[b] Chi^2 compares prevalence of pathogens between age groups.

Table 5
Prevalence of blood pathogens in patients with blood samples available.

Pathogens	2018 N = 26	2019 N = 51	2020 N = 36	2021 N = 28	Total N = 141	P-value[a]
At least one pathogen	13 (50.0)	22 (43.1)	6 (16.7)	4 (14.3)	45 (31.9)	0.002
Plasmodium falciparum	5 (19.2)	18 (35.3)	4 (11.1)	3 (10.7)	30 (21.3)	0.02
Dengue virus	7 (26.9)	0 (0)	0 (0)	0 (0)	7 (5.0)	<0.0001
Borrelia sp.	1 (3.8)	4 (7.8)	2 (5.6)	1 (3.6)	8 (5.7)	0.83
Bartonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Tropheryma whipplei	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Coxiella burnetii	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Salmonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Streptococcus pneumoniae	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Staphylococcus aureus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Rickettsia sp.	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA

[a] Chi^2 compares prevalence of pathogens between years.

National surveillance data in 2019 showed that the district of Diourbel recorded 15,641/354,708 (4%) confirmed cases in Senegal, while three districts (Kédougou, Tamba and Kolda) accounted for 81% of cases [28]. Given, the incubation time of clinical infection with *P. falciparum*, cases observed during the GMT very likely resulted from infections acquired weeks previously. The majority of cases diagnosed at the Mbacké centre were in patients who had travelled to the Diourbel region for the purpose of participating in the GMT, in line with national monitoring data [28]. We found a 27% prevalence of dengue virus during the 2018 GMT, with no further cases in the following years. In 2018, dengue outbreaks occurred in Senegal [29,30]. The first cases were identified in the Fatick

Table 6
Prevalence of blood pathogens by age group (N = 138[a]).

Pathogens	<5 years N = 14	5–15 years N = 40	16–45 years N = 75	>45 years N = 9	P-value[b]
At least one pathogen	0 (0)	13 (32.5)	27 (36.0)	4 (44.4)	0.05
Plasmodium falciparum	0 (0)	7 (17.5)	19 (25.3)	3 (33.3)	0.13
Dengue virus	0 (0)	3 (7.5)	3 (4.0)	1 (11.1)	0.55
Borrelia sp.	0 (0)	3 (7.5)	5 (6.7)	0 (0)	0.63
Bartonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	NA
Tropheryma whipplei	0 (0)	0 (0)	0 (0)	0 (0)	NA
Coxiella burnetii	0 (0)	0 (0)	0 (0)	0 (0)	NA
Salmonella sp.	0 (0)	0 (0)	0 (0)	0 (0)	NA
Streptococcus pneumoniae	0 (0)	0 (0)	0 (0)	0 (0)	NA
Staphylococcus aureus	0 (0)	0 (0)	0 (0)	0 (0)	NA
Rickettsia sp.	0 (0)	0 (0)	0 (0)	0 (0)	NA

[a] Age was not documented in three patients.
[b] Chi^2 compares prevalence of pathogens between age groups.

region in early September, the second wave of cases occurred in the Diourbel region in late September and, finally, in the Saint Louis region [31]. The highest prevalence rates of dengue were in Touba, with 23 cases per 100,000 inhabitants, linked to a DEN3 outbreak [31]. Finally, we identified eight cases of *Borrelia* infection (6%). An 11.7% positivity rate of *Borrelia* was previously reported in febrile patients from the Niakhar district in Senegal tested by PCR in 2016 [32]. Few surveys have addressed the aetiology of non-malarial febrile illness in western Africa [33] and none has been conducted in the context of a mass gathering.

Because the results of PCR testing were available weeks after the survey was done, with the exception of a few pathogens in 2021, antibiotic treatments were empirically prescribed. A proportion of 69% of patients suffering from respiratory symptoms were prescribed antibiotics, as well as 84% of those with diarrhoea. Among patients with a negative malaria rapid test result, 62% were prescribed an antibiotic. These results are in line with previous observations at the GMT [3]. In 2021, we demonstrated that a POC laboratory can be functional in the setting of a healthcare centre receiving patients during the GMT period and could guide therapeutic options in the future.

Our study has some limitations. Patients presenting during evening and night duty hours were not included in this study. However, it is unlikely that these patients may have significantly differed from those seen during day-time. We have no information on the follow-up of the patients after they left the healthcare centre. We did not include healthy control patients. The numbers of patients included were limited and may not be representative of all patients consulting at healthcare centres during the GMT. PCR does not distinguish between live and dead pathogens. Nevertheless, we demonstrated that positivity rates of potential pathogens among patients suffering from respiratory or gastrointestinal symptoms, or febrile systemic illness were high. We were able to evidence the frequency of influenza viruses in patients suffering from respiratory symptoms, and that of both malaria and dengue in those with febrile systemic illnesses. This study helped to design POC laboratory methods that could be used in the context of the GMT for assessing the microbial aetiology of respiratory or gastrointestinal symptoms or febrile systemic illness upon which to base therapeutic management. Further investigation of the potential impact of the availability of a POC laboratory on antibiotic prescriptions is needed.

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CRediT authorship contribution statement

Ndiaw Goumballa: contributed to the experimental design, Writing – original draft, conducted the qPCR technique, administered questionnaires, followed patients and collected samples. **Masse Sambou:** contributed to the experimental design, conducted the qPCR technique. **Diouf Fatou Samba:** All authors contributed to ad approved. **Hubert Bassene:** contributed to the experimental design. **Marielle Bedotto:** All authors contributed to ad approved. **Adama Aidara:** contributed to the experimental design. **Mamadou Dieng:** All authors contributed to ad approved. **Van Thuan Hoang:** All authors contributed to ad approved. **Philippe Parola:** All authors contributed to ad approved. **Cheikh Sokhna:** contributed to the experimental design. **Philippe Gautret:** contributed to the experimental design, Writing – original draft, coordinated the work, All authors contributed to ad approved, the current version of the manuscript.

Declaration of competing interest

A conflicting interest exists when professional judgement concerning a primary interest (such as patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It may arise for the authors when they have financial interest that may influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2022.102515.

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