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Travel and Tropical Medicine

Edited by
Harunor Rashid and Ameneh Khatami

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About the Editors

Harunor Rashid is a Senior Research Officer at the Australian National Centre for Immunisation Research and Surveillance (NCIRS), and a Clinical Associate Professor, Discipline of Child and Adolescent Health, Faculty of Medicine and Health, the University of Sydney, Australia. He is a fellow of the Faculty of Travel Medicine, the Royal College of Physicians and Surgeons (RCPS), Glasgow, UK, and a councillor of the Australasian College of Tropical Medicine. Harunor is particularly interested in the epidemiology of vaccine-preventable infections among travellers, and actively contributes to the International Society of Travel Medicine (ISTM).

Ameneh Khatami is a senior lecturer in Child and Adolescent Health for The University of Sydney, based at The Children's Hospital at Westmead where she also works as a paediatric infectious diseases physician. Her current research focus is on novel therapeutic options for difficult to treat infections and optimisation of antimicrobial therapy for children with cystic fibrosis. Ameneh is the paediatric representative for the Australasian Society for Infectious Diseases Clinical Research Network with a goal to increase involvement of children in clinical trials, particularly early phase studies. Her previous areas of work include clinical vaccine trials in paediatrics with the University of Oxford and she maintains an ongoing interest in vaccine and public health research.

Preface to "Travel and Tropical Medicine"

International travel has become more accessible and is no longer enjoyed only by the privileged few, in part due to lower costs and more efficient transport modes. This has resulted in large segments of the global population being able to travel far and wide, in search of better economic opportunities, for leisure, to advance knowledge, and for fulfillment of their spiritual needs. Many people are also forced to travel because of conflict or natural disasters. It is estimated that 1.5 billion people took foreign trips every year before the COVID-19 pandemic, and that mass religious gatherings such as Kumbh Mela (India), Arbaeen (Iraq), and Hajj (Saudi Arabia) attracted about 50 million, 25 million and 3 million visitors respectively. Regardless of purpose, travel is often associated with detrimental health effects: communicable, psychological, and environmental hazards being well-known threats of travel. On the other hand, increased international trade and travel regulations have created new research opportunities in the area of preventive medicine.

This book contains a suite of published original articles, reviews and case reports/case series that provide an overview of common health issues and emerging travel-related diseases. The prevention and control of travel-related diseases in tropical and resource-poor settings are discussed, as are the travel-related health consequences for attendees of mass gatherings. This collection of articles is authored by experts from academia, hospital practice, laboratory medicine and social science.

The book is aimed at health professionals in travel medicine, tropical medicine, infectious diseases, migrant health, and immunisation and infection control. We thank the authors, reviewers and the editorial team for making this book possible.

Harunor Rashid, Ameneh Khatami
Editors

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Editorial

Special Issue: Travel and Tropical Medicine

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Historically, travel is known to be associated with an amplified risk of acquisition and transmission of infectious diseases, including pandemics. In his travelogue, "Rihla", Moroccan explorer Ibn Battutah record that his team contracted a febrile illness, most likely malaria, while in Kuzestan (Iran). Battutah keenly observed that "visitors to these countries in the hot season generally suffer from fever, as happens also in Damascus and other cities which have abundant waters and fruits". He narrowly escaped the mediaeval black death of 1348 in Syria on his journey to Mecca for the Hajj pilgrimage [1]. However, compared to mainstream specialties of medicine, there is paucity of research in the field of travel medicine.

In this Special Issue, we present a suite of publications on various aspects of travel medicine ranging from refugee and immigrant health to mass gathering medicine. Highlights of this Special Issue include a case report of melioidosis in the United States in a Filipino immigrant [2] and another report of five cases of histoplasmosis among film crew members who acquired the illness in Guatemala and presented in Australia [3]; although rare, both these infections are important in travel medicine and practitioners should be aware of these exotic infections during pre-travel advice sessions. Nicknamed a "great mimicker", for imitating other chronic infections, melioidosis should be considered in travellers returning from the Asia-Pacific. Histoplasmosis is a possibility in travellers with histories of exposure to bird or bat droppings, especially if immunocompromised.

Antimicrobial resistance (AMR) is a serious concern for travellers; two publications explored two different aspects of AMR: an original study showed fewer than 5% of health care workers (HCWs) who knew about a standard clinical guideline on antibiotic prescription (e.g., NICE-CG69, Centor Criteria) practised it correctly for Hajj pilgrims [4] and, in a systematic review, Fouz and colleagues show AMR genes can be detected in wastewaters from mass gatherings [5]. Both these publications highlight the importance of considering AMR among travellers and their external environments.

Two original articles focussed on the health of Rohingyas, among the most persecuted minorities in the world. One article assessed health literacy and health status of Rohingya refugees before their exodus to Bangladesh in 2017, showing that the majority (70%) of the 192 deaths that occurred in 1634 families in the year before their migration to Bangladesh occurred in men and 44% were claimed to be due to homicide [6]. A focussed survey involving 670 infants aged < 2 months showed that about 15% of children had watery or purulent discharge from their eyes [7]; although the study design did not include establishment of microbiological diagnosis, given the high likelihood of sexually

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transmitted infections among Rohingya refugees [8], some of these eye symptoms could be due to gonococcal ophthalmia neonatorum and, thus, deserve public health attention.

Two other review articles looked at rather unique topics in travel medicine: a systematic review on the understanding of immigrant populations' knowledge and attitude on the human papillomavirus (HPV) vaccine showed immigrants often lacked sufficient knowledge of HPV infections and some had negative attitudes towards vaccination [9] and another study described pathophysiology of disproportionate thrombotic tendency of COVID-19 and its implications for travellers, along with guidance based on the severity of COVID-19 and coagulopathy [10].

One original study focussed on Hajj pilgrims' understanding and practice of hand hygiene [11] and another of meningococcal vaccination [12]. A cross-sectional study conducted during the 2019 Hajj identified that most pilgrims knew hand hygiene could prevent respiratory and gastrointestinal infections, but many pilgrims did not know about precise hand washing methods. A follow up study conducted during the 2020 Hajj amidst the COVID-19 pandemic showed no improvement and pilgrims' mean hand hygiene knowledge score remained essentially unchanged (mean score 6.7 ($\pm$1.9) vs 6.4 ($\pm$1.35) of total 12) [11,13]. Another cross-sectional study conducted in 2017 and 2018 showed that 13.4% of Hajj pilgrims certainly missed meningococcal vaccination and another 4.8% were unsure about their vaccination status, which is a great concern [12].

Since 2000, when the quadrivalent meningococcal vaccination was made a Hajj visa-requirement, the uptake among overseas pilgrims ranged from 93% to 100% [12,14,15], but the vaccination rate among domestic pilgrims was lower ($\leq$82%) [11,12,16,17]. A recent study with detailed breakdown on vaccination history has shown that, even among overseas pilgrims, the actual vaccine uptake is only 77%. At least 11% of vaccination certificates are fake and another 12.6% are dubious; in 0.5%, an incorrect vaccine (e.g., bivalent vaccine) is recorded, making the certificate non-valid [18]. Suboptimal vaccination rates have also been reported among HCWs at Hajj [19,20]. These findings forecast a serious problem that could arise following the implementation of "vaccination passports" to allow passengers to avoid official border restrictions and quarantine as part of the global COVID-19 control strategy [21]. Non-vaccination and use of false vaccination certificates to cross international borders would jeopardise global efforts to curb the pandemic. Stringent measures supervised by international public health observers are needed to ensure the safety of world health.

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Case Report

Persistent *Burkholderia pseudomallei* Bacteremia in A Filipino Immigrant to the United States: A Case Report

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Abstract: Melioidosis is rare in the United States and endemic to Southeast Asia and Australia. Treatment includes an initial intensive phase of intravenous ceftazidime or meropenem monotherapy depending on severity. The following report describes a case of persistent bacteremia with ceftazidime failure and prolonged meropenem therapy on a ceftazidime-susceptible strain of *Burkholderia pseudomallei*.

Keywords: *Burkholderia pseudomallei*; melioidosis; ceftazidime; resistance; persistent bacteremia

1. Introduction

Burkholderia pseudomallei is a Gram-negative, aerobic bacillus found naturally in the water and soil of many countries in Southeast Asia, the Pacific Islands, and northern Australia [1]. Transmission of this bacterium occurs through inoculation, ingestion, or inhalation. Historically, in the 19th and 20th centuries, this bacterium had been used as a bioterrorism weapon due to its translocation in water leading to infection during monsoon season in areas where it is endemic [2,3]. Infection with *B. pseudomallei*, commonly known as melioidosis, presents as pneumonia in nearly 50% of patients, but may also cause bone, skin/soft tissue, or central nervous system infections. In many cases, the infection progresses to bacteremia that can rapidly become fatal [1,3]. Risk factors for melioidosis include travel to, or residence in, endemic areas, immunosuppression, smoking, chronic lung disease, diabetes mellitus, chronic liver disease, and renal insufficiency [4]. Melioidosis may present as an acute, chronic, or latent infection, and inappropriate treatment or shortened duration of therapy may lead to relapse or re-infection [4]. Relapse rates vary based on patient-specific risk factors, but have ranged from 5%–10%, and the mortality rates following *B. pseudomallei* infection are as high as 34.8%, despite appropriate ceftazidime or meropenem intensive therapy [5]. Although rare in the United States (US), the number of reported cases to the Centers for Disease Control and Prevention continues to grow, with most patients reporting recent travel outside of the US. Due to this rarity of melioidosis in the US, there are no established US treatment guidelines and available treatment information consists of international studies and guidelines.

Current treatment recommendations in Australia include an intensive phase of intravenous (IV) antibiotics followed by a prolonged oral eradication phase, which minimizes the risk of relapse [6,7]. These recommendations include a minimum of 10–14 days of IV ceftazidime or meropenem followed by a three- to six-month eradication phase using oral sulfamethoxazole/trimethoprim, doxycycline, or amoxicillin/clavulanate depending on the source of infection and other patient-specific factors [8].

Several studies have established ceftazidime monotherapy as the first-line intensive therapy agent. An open randomized trial compared 120 mg/kg/day ceftazidime to conventional therapy, which consisted of the combination of chloramphenicol, doxycycline, and sulfamethoxazole/trimethoprim, and found that ceftazidime reduced mortality from 74% to 37%, comparatively [9]. Ceftazidime monotherapy has also been compared to ceftazidime plus sulfamethoxazole/trimethoprim and was found to have similar rates of both mortality and recurrences [10]. Observational studies have shown that meropenem therapy may be clinically preferable over ceftazidime due to the concerns of potential resistance through penicillin binding protein 3 gene deletions in *B. pseudomallei*, lack of growth of resistant strains on agar plates, and early relapse of bacteremia associated with ceftazidime [11]. Meropenem is currently preferred over ceftazidime in cases of neuromelioidosis, persistent bacteremia, and critically ill patients [12,13]. Currently there is no strong evidence comparing carbapenems and ceftazidime, but there is a randomized, blinded trial comparing ceftazidime to meropenem underway in Thailand [14].

Persistent bacteremia with *B. pseudomallei* correlates to increased risk of death from melioidosis. Therefore, blood cultures should be performed weekly. Repeating cultures from other sites have shown no benefit for prognostic value [15]. Furthermore, when growing *B. pseudomallei,* it is recommended to utilize Ashdown agar in preference to blood agar when available due to potential organism misidentification as *Pseudomonas* or *Burkholderia* spp., which could delay appropriate treatment [15].

2. Patient Case

The patient's written informed consent and Southern Illinois University Edwardsville IRB approval were obtained for this case report. A supplemental table (Table A1) has been provided in Appendix A, which outlines antibiotic therapy and culture reports throughout the patient's hospital admission.

Our patient is an 81-year-old, 53 kg, 160 cm tall female from the Philippines who presented to our emergency department in southwestern Illinois with shortness of breath. Her past medical history is significant for asthma, hypertension, dyslipidemia, cerebral aneurysm, and arthritis. She reported moving to the US in 1979 and had recently returned from a one-month long visit to Quezon City, an urban area in the Philippines, five days prior to admission. Risk factors for melioidosis in this patient included: Travel to an endemic country and asthma (controlled at baseline). The patient had no sick contacts during or after her visit to the Philippines. There were no adverse weather events and the patient had no exposure to rural or agricultural areas during her stay.

The patient expressed having increased shortness of breath while ambulating or performing any exertional activities, along with decreased appetite, left-sided chest pain, weakness, fever, cough, and wheezing. Computed tomography (CT) of the chest demonstrated left upper lobe pneumonia, small bilateral pleural effusions, and bibasilar atelectasis. Chest x-ray showed patchy left basilar opacity suggestive of an infiltrate. Blood cultures and a respiratory culture were obtained in the emergency department prior to initiation of ceftriaxone and azithromycin for empiric treatment of suspected community-acquired pneumonia. On admission, her white blood cell (WBC) count was 12,900 cells/μL, temperature was 38.6 °C, and the patient was admitted with an initial diagnosis of pneumonia with acute respiratory distress.

On day three of admission, WBC count continued to trend up to 20,900 cells/μL while on ceftriaxone and azithromycin. The blood cultures drawn on day one of admission reported growth of *Burkholderia* species in one out of two bottles, and the respiratory culture resulted with no growth as the final result. Based on a creatinine clearance of 48 mL/min, ceftazidime 2 grams IV was initiated at a decreased dosing frequency of every 12 h, instead of every eight h, on day four of admission.

While on ceftazidime, the blood cultures drawn on day four reported the same growth of *Burkholderia* species. The patient's WBC count continued to increase, peaking at 33,300 cells/μL, before trending down to 16,200 cells/μL by day eight of admission (day five of ceftazidime therapy).

On day nine of admission, the patient's WBC count increased to 18,600 from 16,200 cells/μL, and CT with contrast of the chest was performed and demonstrated progressive complete consolidation of the superior lingular segment of the left upper lobe and new internal cavitation, which was concerning for necrotizing pneumonia. A two-dimensional (2D) echocardiogram did not demonstrate any vegetation at this time. Given the worsening CT findings coupled with persistent bacteremia, therapy was changed to meropenem 1 g IV every eight h.

On day 13 of admission, the regional laboratory reported the results of the culture drawn on day one of admission. Identification and susceptibility were the following: *B. pseudomallei*, susceptible to chloramphenicol, ceftazidime, meropenem, and sulfamethoxazole/trimethoprim. The Centers for Disease Control and Prevention were notified of the results from the regional public health laboratory. A CT of the abdomen and pelvis was performed and did not demonstrate any fluid collection or abscesses. Later that same day, temperature increased to 37.2 °C. Due to identification and susceptibility testing, and decrease in patient's creatinine clearance, repeat blood cultures were obtained and the dose of meropenem was increased to 2 grams every 12 h, and sulfamethoxazole/trimethoprim (320 mg trimethoprim component) twice daily was added to the therapy regimen. Incidentally, the blood cultures drawn on day 13 were drawn before starting sulfamethoxazole/trimethoprim and returned no growth after a total of 10 days of IV therapy (five days of ceftazidime and five days of meropenem).

Meropenem was continued for four days after the first negative blood culture was drawn (on day 13 of admission) for a total of 14 days IV intensive therapy. The patient was discharged home on oral sulfamethoxazole/trimethoprim (320 mg trimethoprim component) twice daily for a planned duration of three months.

Two weeks after hospital discharge, the patient was readmitted due to hyponatremia, hyperkalemia, and acute kidney injury. The sulfamethoxazole/trimethoprim dose was decreased during that admission. The previous dose was resumed on discharge as the admitting diagnoses resolved. Blood cultures repeated during this hospitalization were negative.

One month after the initial hospitalization, the patient was seen in clinic and remained free of symptoms of infection. No fevers or decrease in oxygen saturation was documented.

One month into treatment with sulfamethoxazole/trimethoprim, the patient developed acute kidney injury with a serum creatinine of 1.30 mg/dL (baseline of 0.64 mg/dL). Due to this adverse effect, therapy was switched to oral doxycycline 100 mg twice daily to complete the remaining course of maintenance therapy. Completion of the antibiotic regimen was confirmed through follow up with the patient in an outpatient infectious diseases clinic.

3. Discussion

Since this patient was not critically ill on hospital admission and had a pulmonary source of infection, ceftazidime therapy was not initiated until blood cultures returned positive for *Burkholderia* spp. Treatment was broadened to meropenem due to lack of clearance of bacteremia after approximately a week of ceftazidime therapy, in the setting of worsening of pneumonia with cavitation on CT. Sulfamethoxazole/trimethoprim was added in addition to meropenem due to a mild fever and one positive blood culture while on meropenem. We later found out that the blood cultures that were drawn immediately before starting sulfamethoxazole/trimethoprim were negative. Thus, microbiological cure was achieved after prolonged persistence of bacteremia on meropenem monotherapy.

Both ceftazidime and meropenem dosing were adjusted based on the patient's renal function, though guidelines make no recommendation regarding renal dose adjustment for either of these medications in the treatment of *B. pseudomallei* bacteremia. The recommended dosing interval for both drugs is every eight h. According to drug compendia, the recommended frequency is every 12 h, when considering this patient's creatinine clearance, due to drug elimination being slowed in renal impairment. With the dose adjustment, the patient did receive lower than studied doses of ceftazidime and meropenem [9,16].

The treatment of persistent bacteremia caused by *B. pseudomallei* after failed ceftazidime therapy is not well-studied or mentioned in the international treatment recommendations. While meropenem and ceftazidime have similar mortality rates according to observational studies, meropenem is typically reserved for septic shock and those patients deemed critically ill [12]. One study demonstrated emerging resistance to ceftazidime therapy not evident on susceptibility reports due to the poor growth of the bacteria on typical laboratory culture medium (ex. agar plates) [11]. Our facility's microbiology laboratory used blood agar and chocolate agar as culture media for this isolate, which typically yields identification of *Burkholderia* spp. or *Pseudomonas* spp. After the specimen was identified as *Burkholderia* spp., by our laboratory, the specimens were sent to a regional public health lab for identification and susceptibility testing. The culture media used by that laboratory was not disclosed to the authors, though Ashdown agar is recommended for culture media. [14] The susceptibility report that we were sent by a regional public health laboratory confirmed susceptibility to ceftazidime; however, the patient remained bacteremic after six days of ceftazidime, prompting the change to meropenem. Given the delay in identification of *B. pseudomallei*, and the lack of proper culture media for testing, meropenem therapy could have been initiated more expeditiously had the resources been in place to identify this organism earlier in the patient's hospital stay.

The risk of mortality from infection with *B. pseudomallei* is increased with persistent bacteremia; therefore, it is prudent to complete blood cultures once weekly. [15] In our case, blood cultures were drawn far more frequently than weekly, as the authors were not immediately aware that the pathogen was *B. pseudomallei*. Had identification occurred earlier in the patient's stay, the ordering of excessive blood cultures could have been avoided, which would have also decreased risk of exposure to laboratory personnel.

Based on our treatment, we are not able to determine whether or not sulfamethoxazole/trimethoprim should be used in combination with meropenem after ceftazidime failure. However, this case does support the use of meropenem monotherapy for the microbiological cure of persistent *B. pseudomallei* bacteremia in cases of clinical ceftazidime failure, despite ceftazidime susceptibility being shown *in vitro*. Further studies will be necessary to determine efficacy of meropenem vs. ceftazidime monotherapy in achieving microbiological cure in persistent bacteremia with *B. pseudomallei*. Furthermore, given the patient's travel history and the rarity of melioidosis in the United States, it would have been beneficial to have had appropriate identification and susceptibility testing earlier in the patient's hospitalization so that therapy could have been optimized sooner.

4. Conclusions

This is the first case report published on persistent bacteremia with *B. pseudomallei* in the United States. Despite *in vitro* testing confirming susceptibility for ceftazidime against *B. pseudomallei*, we report a case where a patient had persistent bacteremia with ceftazidime monotherapy, with persistent positive blood cultures on initial meropenem therapy. Prospective clinical trials are needed to determine the efficacy of ceftazidime compared to meropenem for microbiological cure.

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Appendix A

Table A1. Antibiotic therapy and culture results throughout admission.

Day of Admission [1]	Culture Results	Antibiotic Therapy
Day 1 (blood culture #1 collected)	–	–
Day 2	–	Ceftriaxone/Azithromycin
Day 3	#1 *B. pseudomallei* (1 out of 2) [2]	Ceftriaxone/Azithromycin
Day 4 (blood culture #2 collected)	–	Ceftazidime 2 grams Q12H
Day 5	–	Ceftazidime 2 grams Q12H
Day 6 (blood culture #3 collected)	#2 *B. pseudomallei* (2 out of 2) [2]	Ceftazidime 2 grams Q12H
Day 7	–	Ceftazidime 2 grams Q12H
Day 8	#3 *B. pseudomallei* (2 out of 2) [2]	Ceftazidime 2 grams Q12H
Day 9	–	Ceftazidime 2 grams Q12H/Meropenem 1 gram Q8H
Day 10 (blood culture #4 collected)	–	Meropenem 1 gram Q8H
Day 11	–	Meropenem 1 gram Q8H
Day 12	#4 *B. pseudomallei* (2 out of 2) [2]	Meropenem 1 gram Q8H
Day 13 (blood culture #5 collected)	-	Meropenem 2 grams Q12H/Sulfamethoxazole/Trimethoprim DS
Day 14	No growth Day 1	Meropenem 2 grams Q12H/Sulfamethoxazole/Trimethoprim DS
Day 15	No growth Day 2	Meropenem 2 grams Q12H Sulfamethoxazole/Trimethoprim DS
Day 16	No growth Day 3	Meropenem 2 grams Q12H/Sulfamethoxazole/Trimethoprim DS
Day 17	No growth Day 4	Meropenem 2 grams Q12H/Sulfamethoxazole/Trimethoprim DS
Day 18	No growth Day 5	Sulfamethoxazole/Trimethoprim DS

[1] Intensive IV antibiotic therapy does not include days of ineffective antibiotic treatment for *B. pseudomallei* (days 1–3). DS—double strength; 800 mg sulfamethoxazole/160 mg trimethoprim); Q—every, H—hours (Q12H—every 12 h). [2] Identification and susceptibility testing for these cultures did not result until day 13 of hospital admission.

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Case Report

Acute Pulmonary Histoplasmosis Outbreak in A Documentary Film Crew Travelling from Guatemala to Australia

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Abstract: *Histoplasma capsulatum* is an endemic mycosis with a widespread distribution, although it is infrequently reported in travellers. In April 2018, five television crew members developed an acute febrile illness after filming a documentary about vampire bats in Guatemala. Patients developed symptoms after travelling to Australia, where they presented for medical care.

Keywords: histoplasmosis; travel; outbreak; Guatemala

1. Case Report

From 24 March to 4 April 2018, 12 television crew members filmed a nature documentary about vampire bats in the Cueva de Juan Flores, Petén Department, Guatemala. All film crew members received general pre-travel assessment and counselling, including recommended pre-travel vaccinations, including influenza, hepatitis A, typhoid and rabies vaccination. Although crew members were encouraged to wear a mask as a general safety measure, they were periodically removed due to perceived discomfort in the humid conditions of the cave.

The television crew then travelled to Australia, where some members flew to Melbourne to begin filming another nature documentary, while the others remained in Sydney. Two patients presented to our health service (both camera operators), while the other three presented to local health services in Sydney, Australia (an audio engineer, television producer and host). The two patients who presented to our health service were both males, aged 33 (patient 1) and 54 (patient 2), with no significant past medical history. A written informed consent for publication was obtained from both patients. Their symptoms presented over the course of 24 h, including severe fatigue, neck and shoulder pain, headache, fevers, chills and cough. Symptom onset in both patients was 8 days after completion of filming.

On examination, both patients were febrile between 39–41 °C and haemodynamically stable. Oxygen saturation was $\geq$ 97% in both patients. Physical examination of both patients was otherwise unrevealing, with a clear chest on auscultation and no lymphadenopathy or organomegaly. Full blood examination was within normal limits for both patients, with frequent atypical and reactive lymphocytes on blood film. Both patients had normal renal and hepatic function with moderately elevated C-reactive protein. Both patients also received a chest x-ray, which was unremarkable in patient 1. The chest x-ray of patient 2 showed mild patchy reticular markings, but was otherwise normal. Additional investigations for influenza, malaria, typhoid, leptospirosis, rickettsia, arboviruses and atypical pneumonia were negative.

Given the exposure of both patients to caves in Central America, histoplasmosis serology was performed via immunodiffusion (Westmead Hospital, Sydney, Australia) and a urinary antigen (by EIA) was sent to Indianapolis, Indiana (MiraVista Diagnostics). Patient 2 provided a sputum sample, which was sent to Westmead Hospital, Sydney for prolonged incubation fungal culture.

Both patients were monitored for 72 h, by which point symptoms had significantly improved without antifungal therapy, and treatment was supportive, in concordance with IDSA guidelines [1]. Subsequent correspondence with both patients confirmed recovery by 4 weeks. Clinical information regarding the three unwell film crew members who travelled to Sydney was unavailable to our unit.

Histoplasmosis serology was initially negative by immunodiffusion in both patients (Westmead Hospital, Sydney, Australia). Histoplasma urinary antigen (MiraVista, Indianapolis, IN, USA) was detected in both patients, with a turn-around time of 13 days. The diagnosis was subsequently confirmed serologically in patient 1, who returned to his primary healthcare physician in the United States one week after discharge, where complement fixation demonstrated a 1:16 titre to yeast-phase antibody, and immunodiffusion detected a positive M band (Quest Diagnostics, West Hills, CA, USA). In patient 2, the diagnosis was confirmed in sputum, which was culture-positive after 4 weeks.

2. Discussion

The key challenge for clinicians reviewing travellers from regions endemic for *H. capsulatum* is the non-specific presentation of patients with acute pulmonary histoplasmosis (APH), which may mimic a number of bacterial and viral infections seen in returned travellers. A unique form of epidemiological evidence available to clinicians in this case included video footage viewed by the treating team, which confirmed hundreds of bats flying overhead in a swarming fashion, probably disturbed by the human activity and exposure to artificial light. Bat guano was also recorded on film to be falling directly onto our patients within the caving system (Figure 1, recorded by patient 1).

Figure 1. Image of bats filmed by patient 1 (particulate matter seen falling from above in foreground).

An increasing number of clustered cases in travellers have been reported in the literature, largely due to a rise in international travel and increasing rates of ecotourism (Table 1) [2–8]. The generally high attack rate is indicative of the large inoculum of infection, as exposure to caves and bat guano remains a key feature in the majority of previous reports. Diagnostic testing and treatment in these published reports also varies widely. Despite endemnicity in Australia, the unavailability of histoplasmosis urinary antigen continues to impair rapid diagnostic testing, particularly in acute cases, prior to seroconversion and culture positivity.

Table 1. Outbreaks of acute pulmonary histoplasmosis in travellers from South and Central America.

Location	Year	Population/Activity	Attack Rate, %	Diagnosis	Treatment (%, Primary Indication)
Ecuador (2)	1999	US high school students cave exploring	11/17 (65%)	Urine Ag 0/2 (0%) Serology: 4/7 (57%)	3/17 (18%, prolonged symptoms)
Nicaragua (3)	2001	US "adventure travellers" cave exploring	12/14 (85%)	Urine Ag 7/12 (58%) Serology 14/14 (100%)	9/12 (75%, symptom severity)
Belize (4)	2002	Canadian high school students cave exploring	14/15 (93%)	Urine Ag 5/7 (71%) Serology: 3/15 (20%)	1/15 (7%, prolonged symptoms)
Guatemala, El Salvador (5)	2004	Norwegian tourists cave exploring	16/19 (84%)	Serology: 8/14 (57%)	3/16 (19%, not reported)
El Salvador (6)	2008	US missionaries renovating a church	20/33 (61%)	Antigen (serum/urine) 7/20 (35%)	Not reported
Ecuador (7)	2012	Polish tourists (organised tour) cave exploring	4/4 (100%)	Serology: 4/4 (100%)	2/4 (50%, prolonged symptoms)
Brazil (8)	2013	Scientists, researching histoplasmosis in caves	4/8 (50%)	Serology 0/4 (0%) Sputum cytology, culture 4/4 (100%)	2/4 (50%, prolonged symptoms)

Our cases demonstrate the utility and feasibility of performing urinary antigen testing, which is reportedly the most sensitive test to diagnose APH [9]. Although APH is cross-reactive with other endemic mycoses, this is not a consideration in cases acquired in Australia, further supporting previous calls for its introduction in a national reference laboratory [10]. When used alone to diagnose APH, urinary antigen is limited by its poor overall performance [11]. However, a more recently developed EIA (MiraVista Diagnostics) measures both immunoglobulin G (IgG) and IgM, reporting a sensitivity of 96.3% for APH, when combining both antigen and antibody assays [11]. As demonstrated in the case of patient 1, convalescent serology should be repeated after several weeks, if negative during initial testing.

The severity of illness in our patients was mild, requiring a short period of hospitalisation for observation, diagnostic work-up and supportive management. It is difficult to quantify the inoculum of exposure, as masks were intermittently removed during filming. Patients were educated on the need for compliance with personal-protective equipment, and also counselled regarding the risk of reactivation in the event of immunosuppression. Both of our patients travelled overseas soon after their hospitalisation. These cases continue to highlight the dynamic interaction between adventure tourism and human curiosity, trans-continental travel and the evolving human-animal-ecosystem interface.

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Article

Pilot Survey of Knowledge, Attitudes and Perceptions of Hajj Deployed Health Care Workers on Antibiotics and Antibiotic Prescriptions for Upper Respiratory Tract Infections: Results from Two Hajj Seasons

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Abstract: Antimicrobial resistance (AMR) is a global public health issue. Upper respiratory tract infections (URTIs) are common illnesses during Hajj, for which antibiotics are often inappropriately prescribed. Hajj healthcare workers' (HCW) knowledge, attitudes and perceptions (KAP) about AMR and antibiotic use for URTIs are not known. We conducted a survey among HCWs during Hajj to explore their KAP regarding antibiotic use for URTIs in pilgrims. Electronic or paper-based surveys were distributed to HCWs during the Hajj in 2016 and 2017. A total of 85 respondents aged 25 to 63 (median 40) years completed the surveys. Most participants were male (78.8%) and were physicians by profession (95.3%). Around 85% and 19% of respondents claimed to have heard about AMR and antimicrobial stewardship programs, respectively, among whom most had obtained their knowledge during their qualification. Implementation of URTI treatment guidelines was very low. In conclusion, HCWs at Hajj have significant knowledge gaps regarding AMR, often do not use standard clinical criteria to diagnose URTIs and display a tendency to prescribe antibiotics for URTIs.

Keywords: Saudi Arabia; Hajj; mass gathering; survey; health care workers; knowledge; attitudes; perceptions; antimicrobial resistance; antimicrobial stewardship; upper respiratory tract infection; guideline

1. Introduction

Antimicrobial resistance (AMR) is a growing global concern affecting human health. The World Health Organization (WHO) has addressed AMR on several levels: surveillance [1], action plans [2] and guidelines including antimicrobial uses other than for medical purposes [3]. With the increasing risk of AMR, it is predicted that it will be the main underlying reason for lives lost by the year 2050, with a predicted toll of 10 million people per year [4]. Tackling AMR requires a multi-disciplinary effort, including reducing unnecessary antimicrobial prescriptions by healthcare workers (HCWs) [5]. HCWs also play key roles in minimizing antimicrobial misuse through interventions such as promoting vaccination and infection control measures [5]. Therefore, assessing HCWs' knowledge, attitudes and perceptions (KAP) on AMR is important to formulate an action plan to tackle drug resistance. This is crucially important in settings like travel, mass migration and during mass gatherings such as the Hajj pilgrimage in Makkah, Saudi Arabia.

Hajj is an annual religious mass gathering where over 2 million pilgrims assemble for around one week within a confined area measuring around 40 km^2, where the risk of transmission of infectious diseases is amplified [6]. Hajj has already been associated with an increased risk of airborne, foodborne and zoonotic infections [7]. Recent studies have demonstrated that pilgrims are at high potential risk of acquiring and transmitting AMR enteric bacteria [8,9], including multidrug resistant *Acinetobacter* spp., carbapenemase-producing *Escherichia coli* [10] and extended-spectrum cephalosporin- and colistin-resistant non-typhoidal *Salmonella* [11]. Although there are studies documenting AMR acquisition during Hajj, there are little available data on the knowledge, attitudes and perceptions of HCWs regarding antimicrobial use for the treatment of upper respiratory tract infections (URTIs) for pilgrims.

URTI is the most common medical complication among Hajj pilgrims [12–15], and studies showed that the majority of URTIs are treated with antibacterial agents (henceforth referred to as antibiotics without specification) [16], even though up to 95% at onset of symptoms, during Hajj, are known to be viral [17]. A recent study revealed that around 93% of pilgrims develop respiratory symptoms, of which 78% of tested samples were positive for at least one pathogen [18]. The most commonly found bacterial causes for respiratory infections were *Haemophilus influenzae* and *Staphylococcus aureus*, and viral causes were human rhinoviruses [19–21]. Since 1978, a law has been passed in Saudi Arabia prohibiting pharmacists from dispensing any drug, including antibiotics, without a prescription issued from a licensed physician, unless excluded by the Ministery of Health as "over-the-counter" medications [22]. In Saudi Arabia, a physician is recognised as anyone with a university medical degree, that is approved by the Saudi Commission For Health Specialties to practice medicine in Saudi Arabia [22]. However, enforcing this legislation is still a struggle [23]. In 2011, around 40% of requests to pharmacists in Saudi Arabia were for antibiotics without a physician's prescription; 98% of these requests were obliged [24]. Moreover, in Hajj contexts, it has previously been noted that 78% of antibiotics used by Australian Hajj pilgrims were dispensed without a physician's prescription [25]. These proportions are expected to improve further with the Saudi government enforcement of the drug dispencing law. However, to our knowledge, there are no antimicrobial stewardship programs (ASPs) that are specific to Hajj; moreover, with the exception of the 2009 influenza A(H1N1) pandemic, there are no readily available local guidelines for treating URTIs in the Hajj setting.

In this survey, we attempted to assess the KAP of Hajj deployed HCWs regarding antibiotics, AMR, URTIs and their understanding of evidence-based medicine (EBM) practices that are specific to treating URTIs; such as the use of Centor criteria and the National Institute for Health and Care Excellence clinical guideline 69 (NICE-CG69) [26,27]. The latter of which, as of September 2019, is under review and is expected to be updated [28].

2. Methods

2.1. Setting and Study Questionnaire

The study was conducted over two Hajj seasons (2016 and 2017). On both occasions, the Hajj Research Team were recruited via online portals and networking. The team members were senior university students or graduates who were residents of Makkah city at the time of Hajj. Transportation was provided to designated catchment areas for conducting the survey, such as Mina, Aziziya and Holy Mosque areas in Greater Makkah. The research team visited HCWs, primarily physicians, in their place of work during the peak period of the Hajj ritual week and invited them to take part in the survey after providing relevant study information. The anonymous survey was initially conducted online in 2016; however, due to a low response rate, a paper-based questionnaire was used in 2017. Both questionnaires are provided in the Supplementary Materials (Document S1). Completion of the survey was taken as implied consent to participate in the research, and the study protocol and related documents were approved by the King Abdullah Medical City Institutional Review Board (IRB number: 16-293).

2.2. Participants

For the 2016 Hajj season, recruitment of HCWs was done by 39 research team members (18 female). Following verbal consent of HCWs, an online link to the survey was sent to potential participants. Links and email invitations were generated and sent through the RedCap® (Vanderbilt University, Nashville, TN, USA) system. Responses could only be submitted when complete; they were stored as incomplete if not submitted or not answered.

For the 2017 Hajj season, recruitment of HCWs was done by five research team members (only one female). A paper-based survey was distributed among HCWs after they had consented to participate. The responses were subsequently entered by the first author (HB) into RedCap®.

Inclusion criteria were that the respondent should be an HCW, work during Hajj, work in a facility that may serve pilgrims and have the authority to prescribe or dispense antibiotics.

2.3. Data Analysis:

Raw data were extracted from RedCap® and cleaned through Excel 2016 (Microsoft Office 2016, Microsoft Corporation, Redmond, Washington, DC, USA). The data were exported to SPSS®22 (SPSS® Inc., IBM® Corporation, Armonk, New York, NY, USA.) for analysis. Missing data were excluded from the analysis for each field, with the denominator representing all valid responses for each question.

3. Results

Figure 1 outlines the recruitment of HCWs and response rates to the questionnaires in each year of the study. Sixty-seven out of the included 85 respondents (78.8%) were male. Other demographic and Hajj deployment related information are provided in Table 1.

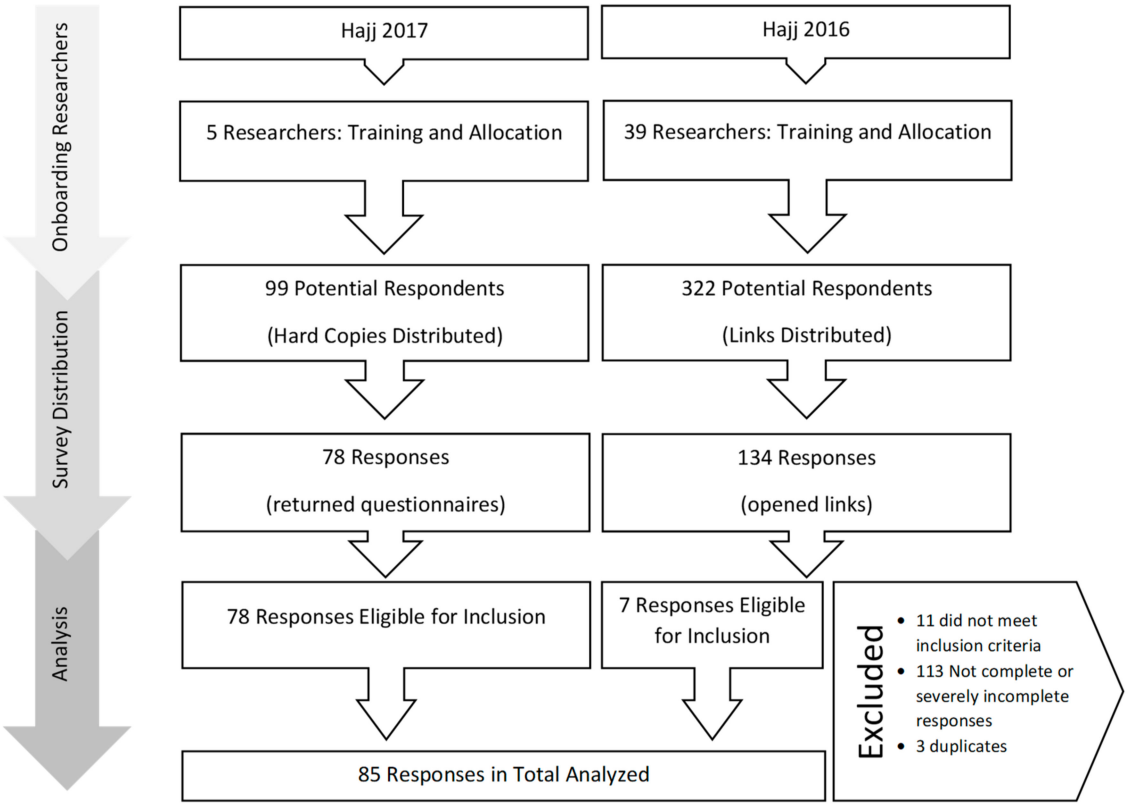

Figure 1. Flowchart for recruitment and analyzed responses.

Table 1. Demographics and responses to Hajj deployment related questions.

Query	Valid Response	Count; n (%)	
Gender	Male:Female	4.2:1	
Age	Years; Median (Range)	40	(25–63)
Nationality	Saudi Arabia	27	(33.7)
	Egypt	24	(30.0)
	Sudan	18	(22.5)
	Pakistan	9	(11.3)
	Other [1]	2	(2.5)
	Missing	5	
Occupation	Physician	81	(95.3)
	Pharmacist	3	(3.6)
	Nurse	1	(1.2)
Qualification Level	Bachelor degree (Physicians)	33	(39.2)
	Bachelor degree (Other)	3	(3.6)
	Specialist	36	(42.9)
	Consultant	11	(13.1)
	Diploma [2]	1	(1.2)
	Missing	1	
Stationing during Hajj	Hospitals	69	(94.5)
	Primary Healthcare Centers	3	(4.1)
	Community Pharmacy	1	(1.4)
	Unspecified or missing	12	

Table 1. *Cont.*

Query	Valid Response	Count; n (%)	
Number of previous Hajj seasons of deployment	First time	29	(35.4)
	1–3 times	22	(26.8)
	4–9 times	23	(28.0)
	≥10 times	8	(9.8)
	Missing	3	
Hajj working days [3]	≤15 days	70	(89.7)
	≥20 days	8	(10.3)
	Unspecified or missing	7	
Hajj working hours (daily shift)	8 h shift	17	(22.4)
	12 h shift	59	(77.6)
	Unspecified or missing	9	
Number of patients seen daily at Hajj	≤25 patients/day	22	(29.3)
	≥26 patients/day	53	(70.7)
	Unspecified or missing	10	

[1] India (1) and Syria (1); [2] Pharmacist; [3] There were no responses documented for 16–19 days.

Most (73/80, 91.3%) HCWs felt that their working conditions were "crowded" with respect to patient visits during Hajj. Among respondents stating they saw more than 25 patients/day, at least one response was up to 200 patients/day. Interestingly, regardless of HCWs' perception of crowding in the workplace, 68.6% (48/70) of HCWs stated that their decision to prescribe antibiotics was not affected by the patient load on their services. Only 20.0% (14/70) would prescribe fewer antibiotics during Hajj than in non-Hajj contexts.

3.1. KAP Regarding Antibiotics and AMR

The proportion of Hajj deployed HCWs who were aware of reports of resistance to antibiotics ranged from 84.7% (61/72) for penicillin to 22.0% (13/59) for colistin. Interestingly, four HCWs were not aware that colistin (two HCWs), isoniazid (three HCWs), pyrazinamide (two HCWs) and ethambutol (one HCW) were antibiotics. An overview of HCWs' KAP regarding regulatory procedures for, and use of, antibiotics are found in Table 2.

Table 2. Knowledge and attitude of Hajj deployed healthcare workers regarding regulations and uses for antibiotics.

Query	Valid Responses	n (%) Out of (N); Missing		
Did you hear about antimicrobial resistance?	Yes—from any source	72	(90.0)	80; 5
	Yes—from academic studies	56	(80.0)	70; 2
	Maybe	7	(8.8)	80; 5
	No	1	(1.2)	80; 5
Did you hear about antimicrobial stewardship programs?	Yes—from any source	16	(20.0)	80; 5
	Yes—from academic studies (qualification at any time)	10	(66.7)	15; 1
	Academic qualification—Post-1996	9	(90.0)	10; 0
	Maybe	18	(22.5)	80; 5
	No	46	(57.5)	80; 5
Physician's recommendation should be required for dispensing antibiotics		76	(98.7)	77; 8
Antibiotics should only be dispensed with a prescription		70	(93.3)	75; 10
There should be compliance visitations to pharmacies by the governing body		65	(87.8)	74; 11
There should be evidence-based criteria for antibiotic prescriptions		72	(93.5)	77; 8

Table 2. *Cont.*

Query	Valid Responses	n (%) Out of (N); Missing		
Do antibiotics treat bacterial infections in Hajj?	Yes—during Hajj	72	(93.5)	77; 8
	Yes—also in non-Hajj contexts as a potential choice of treatment	69	(100.0)	69; 3
	Yes—in both contexts but as the main choice of treatment	39	(58.2)	67; 2
	Maybe	4	(5.2)	77; 8
	No	1	(1.3)	77; 8
Is there evidence that antibiotics treat bacterial infections in Hajj?	No evidence supporting antibiotic used for treatment	5	(6.7)	75; 10
	No evidence to refute antibiotics as treatment	22	(29.3)	
Do antibiotics treat viral infections in Hajj?	Yes—during Hajj	10	(12.5)	80; 5
	Yes—also in non-Hajj contexts as a potential choice of treatment	3	(50.0)	6; 4
	Yes—in both contexts but as the main choice of treatment	2	(66.7)	3; 0
	Maybe	13	(16.3)	80; 5
	No—not during Hajj	57	(71.2)	80; 5
	No—does not treat also in non-Hajj contexts	32	(74.4)	43; 14
	No—in both contexts, but would treat for any reason if "warranted"	8	(32.0)	25; 7
	Warranted—Secondary bacterial infections	5	(71.4)	7; 1
Is there evidence that antibiotics treat viral infections in Hajj?	No evidence supporting antibiotics used for treatment	31	(40.8)	76; 4
	No evidence to refute antibiotics as treatment	11	(14.5)	

3.2. KAP Regarding URTIs and Antibiotic Prescription for URTIs

The majority (71.8%, 61/74) of Hajj deployed HCWs believed there should be guidelines for prescribing antibiotics for URTIs during Hajj. Specific reasons included the need for Hajj health services to be evidence-based (51/61, 83.6%), to save time (40/61, 65.6%) and for greater standardization of health services (33/61, 54.1%). A summary of the KAP of HCWs deployed during Hajj regarding URTIs and related management is provided in Table 3.

Table 3. Knowledge and perceptions of Hajj deployed healthcare workers regarding upper respiratory tract infections (URTIs) and related treatment information.

Query	Valid Response	Count; n	(%)
Perception of the proportion of patients presenting with tonsillitis	More in Hajj context	15	(24.6)
	The same in Hajj and non-Hajj contexts	29	(47.5)
	More in non-Hajj context	21	(34.4)
	Missing	24	
Perception of the proportion of patients presenting with common cold	More in Hajj context	9	(13.4)
	The same in Hajj and non-Hajj contexts	33	(49.3)
	More in non-Hajj context	25	(37.3)
	Missing	18	
Perception of the proportion of patients presenting with sore throat	More in Hajj context	6	(9.1)
	The same in Hajj and non-Hajj contexts	40	(60.6)
	More in non-Hajj context	20	(30.3)
	Missing	19	

Table 3. *Cont.*

Query	Valid Response	Count; n	(%)
First choice of treatment for URTI	amoxicillin alone	26	(34.7)
	azithromycin alone	9	(12.0)
	amoxicillin with antibiotics other than azithromycin	22	(29.3)
	azithromycin with antibiotics other than amoxicillin	4	(5.3)
	antibiotics including amoxicillin and azithromycin	11	(14.7)
	antibiotics other than amoxicillin and azithromycin	3	(4.0)
	Missing	10	
Knowledge of existence of NICE-CG69 [1]	Yes	29	(40.8)
	No	23	(32.4)
	Not sure	19	(26.8)
	Missing	14	
Knowledge of existence of Centor criteria	Yes	22	(31.9)
	No	33	(47.8)
	Not sure	14	(20.3)
	Missing	16	

[1] National Institute for Health and Care Excellence clinical guideline 69.

Only 3.6% (1/28) of those who knew of NICE-CG69 practiced it correctly; a surprisingly lower proportion than among those who denied knowledge of its existence (20.0%, 3/15). 32/72 (44.4%) Hajj deployed HCWs would, as per the guideline, advise their patients that antibiotics were not required when not prescribing antibiotics for URTIs, compared with 20.8% (15/72) and 6.9% (5/72) of those providing immediate or delayed prescriptions, respectively. Only 5.0% (1/20) of those who knew of the Centor criteria provided responses to demonstrate correct use of such criteria, whereas 6.9% (2/29) of HCWs who claimed not to have heard of the Centor criteria were practicing the guideline correctly.

4. Discussion

This survey demonstrates a substantial gap in knowledge about antibiotics, AMR and antibiotic treatment protocols for URTIs among HCWs deployed during Hajj. The key results are the knowledge gaps identified with respect to bacterial resistance to specific antibiotics, ASP and pathogens treated by antibiotics. HCWs should know that antibiotics do not treat viruses; however, Hajj deployed HCWs' understanding that antibiotics do not treat viruses (36%) is only slightly higher than that of the Saudi general public (24%) [29]. This may not reflect a true lack of knowledge about antibiotics but could be due to lack of clarity about the questions since several conflicting results were identified that suggest some HCWs were confused with the use of English terms such as 'antimicrobial' and 'antibacterial'. As such, we would recommend future studies use questionnaires with Arabic translations when addressing HCWs in Hajj.

Although HCWs' knowledge regarding AMR is not lower than the perceived global clinician HCW average (90% vs. 69%) [30], most HCWs surveyed did not know about ASP, which are relatively new and were not prominent in the medical literature at the time of most respondents' graduation [31]. This highlights the importance of continuing medical education (CME), which is mandatory for physicians working under most jurisdictions, and suggests greater emphasis on education on AMR and ASP is required for HCWs in such settings [32]. Nevertheless, all of the valid responses from Hajj deployed HCWs with respect to their attitudes towards various methods of restricting antibiotics were positive.

Awareness among Hajj deployed HCWs regarding the existence of guidelines for prescription of antibiotics for URTIs is low, and their reported compliance with such guidelines is even lower. This is a commonly encountered problem in many settings. For instance, a study conducted in Boston, USA found that even though several guidelines were available on prescribing antibiotics for URTIs, up to 66% of physicians failed to adhere to those guidelines [33]. Among HCW surveyed in this study, there

was a tendency to prescribe amoxicillin and/or azithromycin as the first choice of antibiotics (96%). Data derived from the World Health Organization's Eastern Mediterranean Region also demonstrated high consumption of these two drugs, although the report does not show if there was any association between the consumption of amoxicillin and/or azithromycin and the treatment of URTI [34]. While our survey did not specifically ask about treatment of URTIs in the context of Hajj, the responses received suggest that the same practices would occur during the Hajj season. The limited available literature on this topic suggests low appropriateness of antibiotic prescriptions during Hajj. Only around 40% of French pilgrims who were prescribed antibiotics in one report had an appropriate indication according to French recommendations [35]. Similarly, another study found 98.6% of patients attending an ear, nose and throat clinic during Hajj were prescribed antibiotics, even though 44.6% were diagnosed with viral infections [16]. We suggest that recommendations for treating URTIs during Hajj should be devised and their implementation studied by local authorities, in response to requests from HCWs to have such guidelines readily available.

Some of the findings of this survey may be due to a lack of incorporation of EBM principles in the undergraduate medical curriculum. A study of medical students in Saudi Arabia found that 70% did not attend EBM workshops and only 24.4% would follow such evidence [36]. Moreover, negligible EBM training in CME programs and limited access to EBM resources might have further widened this knowledge gap [37]. Factors related to high patient loads may also affect implementation of knowledge into practice. Most of the Hajj deployed HCWs surveyed worked for 84 h a week, mostly while working 12 h shifts, during which most saw at least 182 patients per week. In comparison, family physicians in the USA work for about 51 h a week, for a working day of about 8.5 h, and see an average of 99 patients per week [38].

Limitations of this survey include missing information; potential language barriers and misunderstanding of terminology; a small sample size that is skewed to male respondents; limitations of the survey questions that did not fully address the effect of CME on HCW's KAP regarding AMR, use of antiviral agents or specific differences between Hajj and non-Hajj settings. Moreover, there were reports from participants that the survey was too long for Hajj contexts. As highlighted above, future research in this area should incorporate a larger and more diverse participant population, with surveys conducted in Arabic in addition to, or instead of, English. In addition, it would be useful to reconduct similar surveys over several years to assess for improvements in Hajj deployed HCWs' KAP regarding AMR, antibiotics and URTI treatment. Furthermore, additional insights regarding justifications for antibiotic use may be gained through semi-structured focus-group interviews involving physicians deployed during Hajj.

5. Conclusions

Hajj deployed HCW have low awareness about AMR, ASP and EBM principles. Some lack precise knowledge about antibiotics and their use. Most HCWs at Hajj do not use standard criteria to diagnose URTIs and they work long hours with high patient loads. Specific education on AMR, ASP and EBM implementation should start early in medical training, and continue through practicing years. Further studies are needed to address potential benefits that may be gained from Hajj-specific antibiotic guidelines and antimicrobial stewardship programs.

Supplementary Materials: The following are available online at: http://www.mdpi.com/2414-6366/5/1/18/s1, Document S1: Survey questionnaire.

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Tropical Medicine and Infectious Disease

MDPI

Review

The Contribution of Wastewater to the Transmission of Antimicrobial Resistance in the Environment: Implications of Mass Gathering Settings

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Abstract: Antimicrobial resistance (AMR) is the major issue posing a serious global health threat. Low- and middle-income countries are likely to be the most affected, both in terms of impact on public health and economic burden. Recent studies highlighted the role of resistance networks on the transmission of AMR organisms, with this network being driven by complex interactions between clinical (e.g., human health, animal husbandry and veterinary medicine) and other components, including environmental factors (e.g., persistence of AMR in wastewater). Many studies have highlighted the role of wastewater as a significant environmental reservoir of AMR as it represents an ideal environment for AMR bacteria (ARB) and antimicrobial resistant genes (ARGs) to persist. Although the treatment process can help in removing or reducing the ARB load, it has limited impact on ARGs. ARGs are not degradable; therefore, they can be spread among microbial communities in the environment through horizontal gene transfer, which is the main resistance mechanism in most Gram-negative bacteria. Here we analysed the recent literature to highlight the contribution of wastewater to the emergence, persistence and transmission of AMR under different settings, particularly those associated with mass gathering events (e.g., Hajj and Kumbh Mela).

Keywords: antimicrobial resistance (AMR); antimicrobial resistant bacteria (ARB); antimicrobial resistant genes (ARG); wastewater; Hajj and Kumbh Mela

1. Introduction

1.1. The Current Status of AMR as a Major Global Health Challenge

Antibiotics are one of the greatest tools of medicine. However, since the development of fluoroquinolones in early 1970, no new major groups of antibacterial drugs have been developed [1].

This paucity in development is accompanied by an increasing threat of antimicrobial resistant (AMR) organisms [1,2]. AMR is the major issue posing a threat to public health, with many reports warning of the significant risk of a post-antimicrobial era in which common infections can kill [1,3–5]. Recently, the World Health Organization (WHO) Global Antimicrobial Surveillance System (GLASS) reported increased levels of resistance in a number of serious and common bacterial infections in many regions of the world [6]. Currently, resistant infections result in 700,000 deaths every year, but the global resistance-associated mortality is estimated to top 10 million lives per year in 2050 [2]. The European Center for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention (CDC) have reported that AMR infections resulted in 25,000 and 23,000 deaths every year in high-income countries in Europe and the USA, respectively [7]. In low- and middle-income countries, AMR infections have been responsible for the deaths of 58,000 children and 38,000 adults in India and Thailand, respectively [7].

1.2. WHO AMR Priority Pathogens List

Recently, the WHO identified 12 bacterial species and their accompanying AMR profiles that pose the greatest threat to human health [8]. This list mainly includes Gram-negative bacteria and the most common etiologic agents associated with hospital- and/or community-acquired infections. These AMR bacteria have been divided into three categories: critical, high and medium priority, according to their impact on human health and the urgency for the development of new antimicrobial drugs to treat resistant infections. The critical category includes *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant) and various *Enterobacteriaceae* members, including *Klebsiella* spp., *Escherichia coli*, *Serratia* spp., and *Proteus* spp. (carbapenem-resistant and extended-spectrum ß-lactamase (ESBL)-producing), which are associated with severe and, often deadly, infections, including bloodstream infections and pneumonia. The high-priority category includes *Enterococcus faecium* (vancomycin-resistant); *Staphylococcus aureus* (methicillin-resistant, vancomycin-intermediate and resistant); *Helicobacter pylori* (clarithromycin-resistant); *Campylobacter* spp. (fluoroquinolone-resistant); *Salmonella* spp. (fluoroquinolone-resistant) and *Neisseria gonorrhoeae* (cephalosporin-resistant and fluoroquinolone-resistant), which are causative agents associated with more common infections, such as general infections, gastroenteritis and gonorrhoea. The medium-priority category includes *Streptococcus pneumoniae* (penicillin-non-susceptible), *Haemophilus influenzae* (ampicillin-resistant) and *Shigella* spp. (fluoroquinolone-resistant).

1.3. The Main Drivers of AMR Transmission

AMR is driven by complex interacting factors that could be described as a resistance network [9]. This network forms links between clinical factors (e.g., human health, animal husbandry and veterinary medicine) and other components, including human activities (e.g., travel [10,11], human displacement and over and misuse of antimicrobial drugs [12–14]) and environmental factors (e.g., persistence of antimicrobial drugs and AMR organisms in soil and water). For example, the variations in AMR patterns among different regions of the world have been associated with differing rates of consumption of, and exposure to, antimicrobial drugs [2]. This is alarming, with the data available on AMR transmission suggesting increasing consumption of antibiotics in humans during the past two decades, primarily in low- and middle-income countries [15]. The selective pressure associated with the exposure to antimicrobials in healthcare, agriculture and the environment enhances the development of new AMR variants and novel resistance mechanisms [16]. Other factors, including lack of access to clean water sanitation and healthcare service, poor personal hygiene, failure of AMR detection and treatment and poor vaccination coverage [17] in the community, have been shown to also contribute to the global transmission of AMR [18].

1.4. The Environmental Reservoir of AMR from Water and Sewage

Transmission of AMR can spread between people, animals and the environment via a number of different routes [19]. The environment acts as a bridge for different compartments, between animals to compost to soil to water to sediments to sewage [20]. While the environment acts as the reservoir, it also works simultaneously to mix mobile genetic elements (MGEs) that interact and diffuse into other parts or into human and animal hosts [19,21,22].

Many studies have highlighted the impact of the diverse nature of the reservoirs of AMR genes (ARGs) on promoting the emergence and transmission of AMR organisms [23]. AMR is ancient and ubiquitous in the environment, with many lines of evidence suggesting that transfer of ARGs occurs among different environments (e.g., from environmental to pathogenic bacteria) [24,25]. Although it has been well-established that the genetic transfer of ARGs is likely to occur between closely-related species, recent studies have suggested that this transfer can also occur among phylogenetically distant species and even among organisms belonging to distinct phyla [26], adding further challenges in the continuous evolution of new variants of AMR organisms. High concentrations of antibiotic residues, ARGs and AMR organisms have been reported from environmental samples recovered from hospital and urban and treated wastewaters and soils treated with animal manure [27–29].

Many studies have highlighted the role of sewage as a major environmental reservoir of AMR, as it represents an ideal environment for AMR microorganisms and ARGs to persist [30–32]. The situation of ARGs is more complex, because they are not degradable and can be spread among microbial communities in the environment through horizontal gene transfer, which is the main resistance mechanism in *Enterobacteriaceae* [33,34].

In this study, we aimed to systematically review the literature to identify the role of wastewater in promoting the transmission of AMR and to characterise the key factors implicated in the persistence of ARB and ARGs in this environmental component. We extended the analysis to characterise AMR transmission in environmental samples associated with key religious mass gathering events—Kumbh Mela and Hajj in India and Saudi Arabia, respectively.

2. Materials and Methods

2.1. Search Strategy

Searches were systematically carried out in four databases: Embase, Medline, PubMed and Web of Science Core Collection to obtain all articles that reported AMR in sewage samples. The key terms "antimicrobial resistance" OR "AMR" in combination with "sewage" were used to obtain the articles available between 2009–2019, with the search conducted on 21st June 2019. EndNote X7.5 (Thomson Reuters) was used for bibliography management. The duplicates were removed, and initial screening was performed by assessing titles, abstracts and keywords with an explicit focus on the use of molecular approaches, including whole genome sequencing and metagenomics, in detecting AMR. The search was extended to include special settings, such as mass gatherings at Hajj and Kumbh Mela.

2.2. Selection Criteria

Articles were included if they were written in English and included an observational study design where sewage samples were investigated molecularly for the detection of AMR. We excluded articles if they were written in languages other than English, reviews, opinion articles and editorials. Potential articles were evaluated on the inclusion criteria by retrieving the full text and were subsequently included in the analysis (Figure 1).

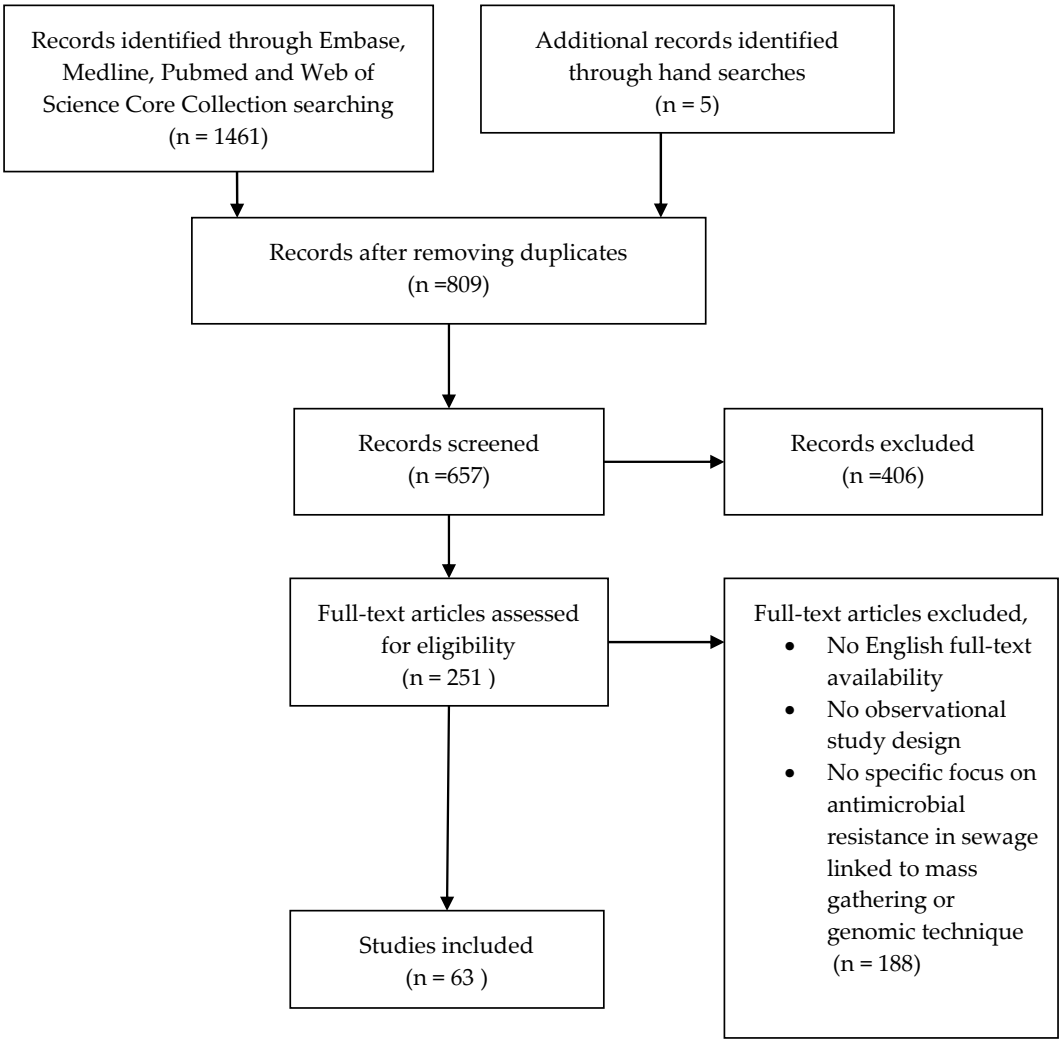

Figure 1. Flow-chart of literature search.

2.3. Data Analysis

1461 articles were obtained in the initial literature search and five articles found by hand searches, which included 809 duplicates. After removing the duplicates, the first screening removed non-English records and irrelevant abstracts, resulting in 251 remaining articles. Full-text was retrieved to screen the articles on the selection criteria, and a total of 63 papers were eligible for inclusion in the analysis (Figure 1). All papers were dissected to summarise the key information and findings, including year of publication, country study site, source and type of wastewater, abundance of ARGs and AMR microbial communities and methods used for AMR detection.

3. Results

3.1. Dissemination of Antimicrobial Resistance in Wastewater

From the 1466 articles that were identified, 63 studies conducted on wastewater samples between 2004 and 2018 (published in the period 2009–2019) were included in the data analysis. The analysed studies documenting the detection of ARBs and/or ARGs in different types of wastewaters are listed in Table 1. The source and type of wastewater samples investigated and the key findings highlighted by these studies are summarised (Table 1). Detailed information on location and time of sample collection, structure of ARBs populations and/or ARGs detected and the technology used in AMR characterisation are provided in Table S1.

Table 1. Overview of the studies included in this systematic review. AMR: antimicrobial resistant, ARG: antimicrobial resistant genes, ARB: antimicrobial resistant bacteria and MGE: mobile genetic elements.

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
1	**Nahar et al., 2019** [35]	Sewage and environmental water	Sewage, river, pond and swimming pool water	AMR bacteria of *E. coli* and *Salmonella* spp. were detected in all environmental samples.
2	**Qin et al., 2019** [36]	Sewage	Hospital sewage	Novel species (*Acinetobacter cumulans*) containing ARGs conferring resistance to carbapenems, cephalosporin or aminoglycoside were identified.
3	**Haberecht et al., 2019** [37]	Sewage and environmental waters	Sewage water, wastewater treatment plant (WWTP) (influent and effluent) and surface water (ambient water)	Increased abundance of ARB and multidrug resistant (MDR) strains were detected in influent compared to effluent wastewater. Extended-spectrum β-lactamases (ESBL)-producing *E. coli* strains have been identified in environmental surface water.
4	**Sekizuka et al., 2018** [38]	Sewage	WWTP effluent	Carbapenem-producing strain of *K. pneumonia* carrying $bla_{\text{KPC-2}}$ was detected. This novel resistant strain rarely detected in clinical settings in Japan.
5	**Cahill et al., 2019** [39]	Sewage and environmental wastewater	Hospital and municipal wastewater (pre- and post-hospital)	Higher rates of carbapenemase-producing *Enterobacteriaceae* (CPE) have been detected in hospital effluent.
6	**Niestepski et al., 2019** [40]	Sewage, environmental water and human faeces	Hospital wastewater, WWTP (influent and effluent) and human faeces	The highest drug-resistance levels were observed in the strains isolated from influent and effluent WWTP water. Bacteria of *Bacteroides fragilis* group (BFG) isolated from the WWTPs characterised by higher resistant profiles than those that have been recovered from human and rat faeces.
7	**Hendrieksen et al., 2019** [41]	Sewage	Domestic sewage	Clinically relevant ARGs associated with resistance to macrolides, tetracycline, aminoglycoside, beta-lactams and sulfonamides were identified. The abundance of ARGs were higher in samples collected from low-income compared to high-income countries.
8	**Khan et al., 2019** [42]	Sewage and environmental waters	Hospital wastewater, WWTP samples and downstream water	β-lactamase genes, including $bla_{\text{IMP-1}}$, $bla_{\text{IMP-2}}$ and $bla_{\text{OXA-23}}$, were detected only in hospital sewage, while $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M-8}}$ and $bla_{\text{SFC-1}}$, $bla_{\text{VIM-1}}$ and $bla_{\text{VIM-13}}$ were only detected in downstream river water but not in the WWTP.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
9	**Tokajian et al., 2018** [43]	Sewage and environmental waters	Refugee camp sewage water and rivers effluent	Higher rates of AMR *E. coli* isolates, including ESBL-producing strains, and those which showed resistance to different antimicrobial drugs, including aminoglycosides, fluoroquinolones and trimethoprim/sulfamethosazole, were detected in samples from refugee camps.
10	**Parnanen et al., 2019** [44]	Sewage	Influent and effluent WWTPs samples from different countries	Significantly higher rates of ARGs were identified in effluent samples from low-income compared to high-income countries.
11	**Bougnom et al., 2019** [45]	Sewage	Urban wastewater for agriculture (three canals with different settings)	Higher rates of ARG that confer resistance to 11 major antimicrobial drug groups, including aminoglycoside, tetracycline, beta-lactams and macrolides, were detected in urban wastewater. There was difference in the composition of ARGs associated with ESBL within city water from three canals that received water from different environments, including hospitals.
12	**Gouliouris et al., 2019** [46]	Sewage	Municipal wastewater (untreated and treated) and hospital sewage	Higher rates of vancomycin and ampicillin-resistant *E. faecium* closely related to hospital isolates have been detected in untreated wastewater plants receiving directly from hospital sewage.
13	**Iweriebor et al., 2015** [47]	Sewage	Municipal and hospital wastewater	Ninety-one percent and 100% of the *Enterococcus* spp. (*E. faecalis* and *E. durans*) isolated from the hospital wastewater and final effluent wastewater, respectively, were resistant to vancomycin and erythromycin.
14	**Guo et al., 2017** [48]	Sewage	Aerobic-activated sludge (AAS) and an aerobically digested sludge (ADS)	Although MGEs, including plasmids; transposons; integrons (e.g., *intI1*) and insertion sequences (e.g., *ISSsp4*, *ISMsa21* and *ISMba16*) were abundant in both the activated and digested sludge. However, distinct microbial populations were associated with the two sledge samples.
15	**Wang JL, et al., 2015** [49]	Sewage	Pharmaceutical WTP (all stages of processing)	The abundance of clinically relevant ARGs, including *sul1*, *sul2*, *tetO*, *tetT*, *tetW* and *tetM*, remained consistently higher throughout the processing stages and discharged into the environment.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
16	**Conte D et al., 2017** [50]	Sewage and environmental waters	Hospital effluent, sanitary effluent, different sites within WWTP and upstream and downstream river water	ESBL-producing *K. pneumonia* and *E. coli* isolates were higher in hospital effluent and WWTP and river samples, respectively. Quinolone-resistant isolates were identified in hospital effluent, sanitary effluent, outflow sewage and surface water samples. MDR bacteria were detected in the hospital effluent and river waters.
17	**Baumlisberger et al., 2015** [51]	Sewage	Up- and downstream wastewater from nursing home	No obvious difference in ARG and MGE abundances were detected between up- and downstream samples.
18	**Adefisoye et al., 2016** [52]	Sewage	Final effluents of WWTP	MDR *E coli* isolates associated with neonatal meningitis; intestinal (enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and enteroaggregative *E. coli* (EAEC)) and ex-intestinal (UPEC) were identified.
19	**Suzuki et al., 2015** [53]	Sewage and environmental waters	Effluents of WWTP and surface water	High levels of ARGs associated with resistance to sulfamethosazole and oxytetracycline were detected in environmental surface water.
20	**Froes AM et al., 2016** [54]	Sewage	Hospital's wastewater	Diverse ARGs of serine β-lactamases, including uncommon β lactamase genes $bla_{PER,}$ bla_{VEB} and $bla_{GES,}$ were detected in hospital's wastewater.
21	**Laht M et al., 2014** [55]	Sewage	WWTP	High levels of ARGs associated with resistance to tetracycline, sulfonamide and β-lactam were detected in all stages in WWTP wastewater. No difference in ARGs abundance was identified after the purification process.
22	**Walsh et al., 2011** [56]	Sewage and environmental water	Seepage water, public tap water and control samples: sewage effluent samples from Wales	bla_{NDM-1}-producing bacteria were isolated from 17% (12 out 171) and 4% (2 out 50) of seepage and tap water samples, respectively. The detected strains included 11 species in which bla_{NDM-1} had not previously been reported (e.g., *Shigella boydii* and *Vibrio cholerae*).
23	**Zhang T et al., 2011** [57]	Sewage	Activated sludge of WWTP	Novel plasmids carrying ARGs associated with tetracycline, aminoglycoside and β-lactam resistance were identified. ARGs associated with resistance to tetracycline, macrolide and MDR were highly enriched in the activated sludge.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
24	**Chagas TP et al., 2011** [58]	Sewage	Influent, clarifier tank effluent and chlorine tank effluent from hospital STP (sewage treatment plant)	Multiresistant and ESBL-producing bacteria (high resistant rates to amikacin, trimethoprim/sulphametoxazole and cefalothin) were identified in the chlorine contact tank effluent.
25	**Szczepanowski et al., 2009** [59]	Sewage	Activated sludge samples and final effluent of WWTP	Clinically relevant ARGs associated with resistance to several antimicrobial drugs, including aminoglycoside and β-lactam, were identified in activated sludge and effluent wastewater.
26	**Li D et al., 2010** [60]	Sewage and environmental waste water	Wastewater, river water-up (RWU) and -downstream (RWD) associated with oxytetracycline production WWTP	High concentrations of oxytetracycline were identified in wastewater and in river water downstream but not in upstream waters. MDR phenotypes isolates were identified in the WW and RWD and less frequent in RWU.
27	**Zhang H et al., 2019** [61]	Sewage	Samples from 18 WWTPs	Activated sludge was the main reservoir of ARGs associated with resistance to sulfonamide (*sul1* and *sul2*) and tetracycline (*tetW*, *tetX* and *tetQ*).
28	**Li, B et al., 2015** [62]	Sewage, environmental water and faecal samples	Samples from AAS and ADS and different environmental waters and faecal samples (human, chicken and pig)	High level of ARGs, including those associated with MDR, and resistance to bacitracin; tetracycline; β-lactam; macrolide, lincosamide and streptogramin (MLS); aminoglycoside; quinolone and sulphonamide were detected in all samples.
29	**Hembach et al., 2017** [63]	Sewage	Influent and effluent water from seven WWTPs	*mcr-1* (associated with resistance to colistin) was detected in influent samples of all seven WWTPs and some of effluent waters (i.e., it was not eliminated during wastewater treatment reaching the aquatic environment). AMR strains of *A. baumannii*, *E. coli* and *K. pneumonia* were detected in both influent and effluents samples.
30	**Igbinosa IH et al., 2012** [64]	Sewage	WWTP	AMR *Aeromonas* spp. isolates resistant to penicillin, oxacillin, ampicillin and vancomycin were identified. Class A pse1 β-lactamase, class 1 integron and the bla_{TEM} gene were detected in 20.8%, 20.8% and 8.3% of the identified isolates, respectively.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
31	**Igbinosa EO et al., 2011** [65]	Sewage	Final effluents WWTP	*Vibrio* spp. strains (including *V. parahaemolyticus*, *V. fluvialis* and *V. vulnificus*) resistant to erythromycin, chloramphenicol, nitrofurantoin, cefuroxime and cephalothin were detected. SXT antibiotic resistance gene cluster (*floR*, *strB*, *sul2*, *dfrA18* and *dfrA1*) were also identified in % of these strains.
32	**Johnning A, et. Al 2015** [66]	Sewage and environmental water	Up and downstream WWTP and from industrially polluted sites and sediment samples from a pristine lake	Mutations in chromosomal genes *gyrA* and *parC*, associated with resistance to fluoroquinolone, were detected in *E. coli* communities. High abundance of mutations was correlated with the concentration of fluoroquinolones in investigated samples (i.e., samples polluted with high concentrations of fluoroquinolone).
33	**Sahlström L et al., 2009** [67]	Sewage and clinical samples	WWTP and isolates from humans and chickens	Vancomycin-resistant isolates of *Enterococcus* spp., including *E. faecium*, *E. hirae* and *E. durans*, were detected.
34	**Araújo C et al., 2010** [68]	Sewage	Sludge and sewage of urban and poultry slaughter house WWTP	Vancomycin-resistant isolates of *Enterococcus* spp., including *E. faecium*, *E. gallinarum* and *E. casseliflavus*, which were also resistant to varied groups of antimicrobial drugs (kanamycin, tetracycline, erythromycin, ciprofloxacin, ampicillin, streptomycin and gentamicin), were detected.
35	**Soge O et al., 2009** [69]	Sewage and environmental water	Water, soil and sewage	The majority of *Clostridium perfringens* strains recovered from water samples were found to carry more than one ARG encoding resistance to tetracycline and erythromycin.
36	**Zhang X et al., 2009** [70]	Sewage	WWTP	Enterobacteriaceae strains carrying class 1 integrons and ARGs associated with resistance to trimethoprim (*dfr17*) and streptomycin (*aadA5*) were detected.
37	**Odjadjare EO et al., 2010** [71]	Sewage	WWTP final effluent, discharge point and upstream and downstream of the discharge point	Most of the *Listeria* spp. isolates recovered from final effluents were MDR strains.
38	**Okoh AI et al., 2010** [72]	Sewage	WWTP final effluents	MDR *Vibrio* spp. strains that showed resistance to varied antimicrobial drugs (including sulfamethoxazole, trimethoprim, cotrimoxazole, chloramphenicol, streptomycin, ampicillin, tetracycline, nalidixic acid and gentamicin) were detected.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
39	**Yang H et al., 2010** [73]	Sewage and environmental waters	Soil, water and faecal samples	Higher copies of tetracycline ARGs and higher abundance of tetracycline-resistant bacteria were identified in farm (cattle), compared to nonfarm, wastewater samples.
40	**Garcia-Armisen T et al., 2011** [74]	Environmental water	Sewage-contaminated rivers	Most of the ARB detected in the Zenne river, downstream of Brussels, were MDR strains. The abundance of AMR communities (heterotrophic and faecal bacteria) was not correlated with the level of contamination of river water with sewage.
41	**Colomer-Lluch M et al., 2011** [75]	Sewage and environmental water	Urban sewage and river water	β-lactamase genes (bla_{TEM} and $bla_{CTX\text{-}M9}$) and one encoding penicillin-binding protein (*mecA*) were detected in the DNA phages recovered from all the samples.
42	**Fuentefria DB et al., 2011** [76]	Sewage and environmental water	Hospital wastewater and superficial water	Genetically distinct populations of AMR *Pseudomonas aeruginosa* were detected in these different environments (hospital wastewater and superficial water that received this wastewater discharge).
43	**Gaze WH et al., 2011** [77]	Sewage	Industrial waste, sewage sludge and pig slurry	Higher prevalence of class 1 integrons was detected in bacteria recovered from sewage sludge and pig slurry (exposed to antibiotic residues and detergents) compared to agricultural soils to which these waste products are amended. It has been estimated that ~10^{19} bacteria carrying class 1 integrons enter the United Kingdom environment by disposal of sewage sludge each year.
44	**Ma L et al., 2011** [78]	Sewage	WWTP	The abundance of bacteria (*E. coli*, *Klebsiella* spp. and *Aeromonas veronii*) carrying class I integronase gene *intI1* were higher in effluent compared to influent wastewater. *intI1* was detected in 20.4%, 30.9% and 38.9% of bacteria recovered from influent, activated sludge and effluent wastewater, respectively. This study suggested a role of activated sludge (characterized by high biomass and biodiversity) in developing AMR through the dissemination of integrons.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
45	**Mokracka J et al., 2011** [79]	Sewage	WWTP	Quinolone- and fluoroquinolone-resistant strains constituted 56% and 50.4% of recovered integron-bearing *E. coli* strains (including diarrheagenic and extraintestinal strains carrying virulence traits), respectively. Virulent extraintestinal strains constituted ~50% of all isolates and were detected in samples recovered from all wastewater treatment stages, including final effluent.
46	**Amaya E et al., 2012** [80]	Sewage and environmental waste water	Hospital wastewater and well waters	High levels of MDR *E. coli* isolates were recovered from samples collected from both hospital wastewaters and environmental well water. *E. coli* strains harbouring $bla_{\text{CTX-M1}}$ and $bla_{\text{CTX-M9}}$ were predominated in samples collected from wells and hospital wastewater, respectively.
47	**Mokracka J et al., 2012** [81]	Sewage	Municipal WWTP	MDR *Enterobacteriaceae* strains carrying class 1 and class 2 integrons (12.1%; 221 out of 1832) were identified in different stages of a municipal wastewater treatment plant (61.5%, 12.7% and 25.8% of ARB were originated from raw sewage, aeration tank and final effluent, respectively). The abundance of ARGs and MDR bacteria, particularly the level of ARG diversity and B-lactamase-producers, were higher in final effluent samples.
48	**Splindler A et al., 2012** [82]	Sewage	Untreated hospital effluents	Half of *Pseudomonas* spp. isolates recovered from untreated hospital effluent wastewater were MDR strains, while 41.9% (52 out of 124) of the isolates were found to carry *intlI*.
49	**Gundogdu, A. et al., 2012** [83]	Sewage	Untreated hospital wastewaters and WWTP	High level of ESBL-producing *E. coli* isolates were detected in untreated hospital wastewaters (bla_{SHV}), with distinct genotypes ($bla_{\text{CTX-M}}$) associated with the samples recovered from WWTP.
50	**Zarfel, G et al., 2013** [84]	Sewage and clinical samples	Sewage and human urinary tract infection samples	ESBL-producing bacteria carrying $bla_{\text{CTX-M}}$ were predominated in both sewage sludge ($bla_{\text{CTX-M-15}}$) and UTI ($bla_{\text{CTX-M-1}}$) samples. The study suggested the occurrence of a genetic exchange between the ESBL-resistant *E. coli* populations from human infections and those present in sewage sludge.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
51	**Colomer-Lluch M et al., 2013** [85]	Sewage and environmental water	Sewage and river water samples	Quinolone-resistant *E. coli* strains of clinically relevant ST69 and ST131 (carrying virulence traits) predominated in samples recovered from urban wastewater and both river and wastewaters, respectively. Similar virulence and macro-restriction profiles were identified in environmental and human isolates of ST131.
52	**Sadowy E et al., 2014** [86]	Sewage and environmental water	Wastewater, riverine estuary and anthropogenically impacted marine catchment basin	AMR isolates of *Enterococcus* spp., especially fluoroquinolone- and aminoglycoside-resistant *E. faecium* that shared virulence determinants and ST similar to nosocomial high-risk enterococcal clonal complexes (HiRECC), were detected.
53	**Gao P et al., 2015** [87]	Sewage	WWTP	Positive correlations were observed between the occurrence of heavy metals (e.g., zinc and lead and *ereB*, *mefA*&*E* and *ermB*) and antibacterial residues (e.g., triclosan with *ereA*, *ereB*, *mefA*&*E* and *ermB*) in urban wastewaters and the abundance of erythromycin-resistant genes.
54	**Nishiyama M et al., 2015** [88]	Sewage and environmental water	Sewage and urban river water samples	vanC-type vancomycin-resistant *E. faecium* and *E. faecalis*, which are the major types of enterococci in humans, were detected in both sewage and urban river water samples.
55	**Zhang S et al., 2015** [89]	Sewage	WWTP	Gram-negative and -positive isolates dominated WWTP influent and effluent samples, respectively. The frequency of detection of tetracycline-, sulphonamide-, streptomycin- and β-lactam-resistance genes (except *sulA* and bla_{CTX-M}) were higher in ARB from influent compared to effluent samples. The abundances of ARGs in activated sludge were higher in aerobic compartments than in anoxic ones.
56	**Simo Tchuinte PL et al.,** [90]	Sewage	Hospital effluent and sludge	Novel class 3 integrons with oxacillinase gene cassette, including aminoglycoside and β-lactam-resistant genes (bla_{OXA-10}, $bla_{OXA-368}$ or bla_{OXA-2}), were identified in *Acinetobacter johnsonii*, *Aeromonas allosaccharophila* and *Citrobacter freundii*, which were recovered from hospital effluent samples.

Table 1. *Cont.*

No.	References	Source of Samples	Types of Investigated Samples	Key Findings
57	**Young S et al., 2016** [91]	Sewage and environment water	Water and sediment samples from sewage spill site	Nosocomial pathogen; vancomycin-resistant *E. faecium* (harbouring *vanA* associated with a high resistance level) were isolated from water and sediment for up to 3 days after a sewage spill. *vanA* gene were found to persist for an additional week within these environments. Culturable levels of enterococci in water exceeded recreational water guidelines for 2 weeks following the spill, declining about five orders of magnitude in sediments and two orders of magnitude in the water column over 6 weeks.
58	**Lee J et al., 2017** [92]	Sewage	Food waste-recycling wastewater (FRW), manure and sewage sludge	The abundance of ARGs was greatest in manure, followed by sewage sludge and FRW. However, different patterns in the diversity and mechanisms of ARGs were identified. ARG associated with β-lactam resistance were higher in the FRW, and sulfonamides-resistant genes are higher in sludge. Total ARGs is associated with class 1 integron only in manure and sludge.
59	**An XL et al., 2018** [93]	Sewage	Influent, activated sludge and effluents of urban WWTP	High concentration of class 1 integron gene cassette (including trimethoprim, aminoglycoside and beta-lactam resistance genes) were identified in activated sludge.
60	**Haller L et al., 2018** [94]	Sewage	Hospital effluents	MDR bacteria belonging to *Enterobacteriaceae* and other species, including ESBL- and carbapenemase-producers, were identified.
61	**Galler H et al.,2018** [95]	Sewage	Activated sludge	Clinically relevant ARBs, including ESBL-*Enterobacteriaceae*, MRSA and vancomycin-resistant *Enterococcus* spp., were detected. ARG associated with resistance to β-lactam, vancomycin (*vanA*) and methicilin (*mecA*) were identified.
62	**Quach-Cu J et al., 2018** [96]	Sewage	Raw wastewater, activated sludge and secondary and tertiary WWTP effluent	The abundance of bla_{SHV}, bla_{TEM} and *sul1* were higher in raw wastewater than other samples.
63	**Yousfi K et al., 2019** [97]	Sewage	Hospital effluents	*Enterobacteriaceae* isolates (including *E. coli* and *K. pneumoniae*) and non-*Enterobacteriaceae* Gram-negative bacterial isolates (including *A. baumannii* and *A. hydrophila*) showed high levels of resistance to β-lactam and non-β-lactam-antibiotics, and most of them are multidrug-resistant. This study is the first study that found genes encoding carbapenemases, including bla_{OXA-23} and bla_{OXA-48}, like in *A. baumannii*, *K. oxytoca* and *S. xiamenensis* in Algerian hospital effluents.

These studies highlight the role of aquatic ecosystems, particularly wastewater, as a key reservoir of AMR bacteria and ARGs in the environment. High levels of both ARBs and/or ARGs were detected in samples collected from different types of wastewater, including municipal sewage [39,46,47,54,81] and influent and effluent samples from wastewater treatment plants (WWTPs) [37,42,44,55,63,64, 70–72,78,79]. Similarly, high levels of AMR were identified in industrial [66,77,92] and agricultural wastewater and samples recovered from pharmaceutical treatment plants [49,66]. High levels of clinically relevant ARBs and/or ARGs were identified in influent and effluent samples from hospital wastewater [36,39,40,42,50,54,58,76,80,82,83,90,94,97].

A number of studies have also demonstrated elevated levels of AMR detected in samples that have been collected from downstream water [36,44]; the surface water of rivers [35,37,53,75,86,88] and tap water [56]. Few studies have detected ARBs and clinically relevant ARGs in environmental samples that have been exposed to/contaminated with sewage [62,91].

3.2. ARB Populations Associated with Wastewaters

The majority of the studies have used integrated molecular and phenotypic approaches to characterise the resistance profiles and virulence contents associated with AMR bacteria. Many studies (n = 47) have used advanced molecular approaches, including polymerase chain reaction (PCR) followed by Sanger sequencing and/or quantitative PCR, to characterise the AMR genotypes. Recent studies (n = 16) have used whole-genome sequencing and metagenomic analyses to comprehensively detect the microbial and AMR determinants in wastewater samples. The latter aimed to assess the abundance and distribution of microbes and associated AMR agents (mobile genetic elements (MGE), including plasmids, transposons, integrons and insertion sequences) and to identify the factors that determine the persistence of AMR bacteria and ARGs in wastewater.

Phenotypic characterisation demonstrated that *Enterobacteriaceae* members, including *Escherichia coli*, *Klebsiella* spp., *Shigella* spp., *Salmonella* spp., *Vibrio* spp., *Acinetobacter* spp. and *Enterococcus* spp., were among the most common AMR bacteria identified in the wastewater samples investigated in the analysed studies (Table 1). Additionally, high levels of MDR bacteria and ARGs conferring resistance to varied classes of antimicrobial drugs, including beta-lactams, carbapenems, tetracyclines, aminoglycosides, fluoroquinolones, sulphonamides, macrolides, vancomycin and erythromycin, were documented in the analysed articles (Table 1).

3.3. Selective Pressure within Wastewater Environments Promote the Emergence of Novel Variants of ARGs and ARBs

Generally, high levels of ARB, including MDR strains and diverse ARGs, have been detected in influent wastewater (untreated) collected from various sources, particularly low-income settings [41,44]; hospitals [36,39,40,42,46,47] and pharmaceutical waste [49,60]. However, many studies demonstrated that effluent samples collected from urban, hospital and pharmaceutical-treated wastewater still contain elevated levels of diverse ARGs, ARB and antimicrobial drugs [49,58,60,63]. For instance, a recent study demonstrated that the abundance of ARGs was significantly higher in effluent wastewater samples collected from low-income compared to high-income countries [41,44]. High rates of ARGs have been identified in pharmaceutical wastewater treatment plants, with the rate of those associated with clinically important antimicrobial drugs (e.g., *sul1*, *sul2* and *tet*) being found to remain high throughout the different stages of the treatment process and, therefore, were subsequently discharged into the environment [49].

NDM-1 producing strains, including *V. cholerae*, *Shigella boydii* and *Aeromonas caviae*, which had not been previously reported to carry $bla_{\text{NDM-1}}$, have been isolated for the first time from drinking water (4%; 2 out of 50) and seepage samples (17%; 12 out of 171) from New Delhi [56]. This is in addition to the previously reported NDM-1-producing species, including *E. coli* and *K. pneumoniae* [56]. The carriages of $bla_{\text{NDM-1}}$-bearing plasmids by enterobacteria, aeromonads and *V. cholerae* have been shown to be stable, transmissible and exhibit the typical resistance pattern of NDM-1 [56]. Although the majority of strains

have previously carried $bla_{\text{NDM-1}}$ on plasmids, $bla_{\text{NDM-1}}$-bearing chromosomes have been first identified in environmental isolates of *Aeromonas caviae* and *V. cholerae* [56]. Another study has documented the isolation of novel species of *Acinetobacter cumulans* from hospital wastewater [36]. These strains have been found to contain ARGs associated with resistance to clinically important drugs, including carbapenems, cephalosporines and aminoglycoside [36]. Additionally, a carbapenemase-producing *K. pneumoniae* strain carrying $bla_{\text{KPC-2}}$, which is rarely detected in clinical settings, has been identified in WWTP effluent wastewater in Japan [38].

Additionally, many studies have detected high levels of integrons [77–79,81], including novel classes associated with oxacillinase gene cassette ($bla_{\text{OXA-109}}$, $bla_{\text{OXA-368}}$ and $bla_{\text{OXA-2}}$) in varied bacterial species recovered from wastewater samples [90]. Higher prevalence of class 1 integrons was detected in bacteria recovered from sewage sludge and pig slurry (environments that contain high concentrations of antibiotic residues and detergents) compared to agricultural soils to which these waste products are amended [77]. It has been estimated that ~10^{19} bacteria carrying class 1 integrons enter the United Kingdom's environment by the disposal of sewage sludge each year [77]. In another study, the investigated β–lactamase genes (bla_{TEM} and $bla_{\text{CTX-M9}}$) and *mecA* encoding for penicillin-binding protein were detected in all DNA phages that have been recovered from urban sewage and river water samples [75].

Collectively, the analysed studies demonstrate the potential role of wastewater (particularly untreated-, hospital- and pharmaceutical wastewaters) as an environmental reservoir that assists in the emergence and dissemination of novel variants of AMR bacteria. This is mainly promoted through the coexistence of diverse species of bacteria and high levels of ARGs in these environments, which increases the probability of the transfer of ARGs carried on mobile elements among closely related species. The untreated wastewaters also contain high levels of antimicrobial drugs, which pose an important selective pressure for the emergence and dissemination of AMR bacteria [98]. Recently, positive correlations were observed between the occurrence of heavy metals (e.g., zinc and lead and *ereB*, *mefA*&*E* and *ermB*) and antibacterial residues (e.g., triclosan with *ereA*, *ereB*, *mefA*&*E* and *ermB*) in urban wastewaters and the presence of erythromycin resistant genes [87]. However, the dynamic of the selective pressure and the emergence of novel variants of ARBs remain poorly documented and understood.

3.4. Hospital Wastewater and the Dissemination of Clinically Relevant ARGs and ARBs Populations

Recent studies have reported the detection of elevated levels of clinically important AMR bacteria and ARGs in hospital effluent wastewater and environmental water sources that receive untreated hospital waste [39,46,47,50,58]. Clinically important AMR bacteria, including MDR (e.g., carbapenemase-producing *Enterobacteriaceae*) and ESBL-producing bacteria (e.g., ESBL-producing *K. pneumoniae*) [39,50,58] and vancomycin and ampicillin-resistant *Enterococcus* spp., have been identified in hospital wastewater-associated samples [46,47].

3.5. Impact of Wastewater Treatment Processes on AMR Dissemination

Conventional and advanced WWTPs have employed different biological, physical and chemical process to clean wastewater from pollutants and contaminants so that they can be reused and/or returned back to the environment. The efficiency of removal of AMR bacteria from effluent wastewater (treated) varies according to the treatment procedure employed [99]. Therefore, it is not surprising that high levels of clinically important AMR bacteria, including MDR and ESBL-producing strains of *K. pneumoniae*, *Enterobacter cloacae* and *E. coli* [58]; MDR *Listeria* spp. [71] and MDR *Vibrio* spp. [72], have been detected in effluent samples. Another study demonstrated the detection of MDR *E. coli* strains associated with neonatal meningitis, intestinal and extraintestinal serotypes in final effluents of WWTPs [52]. High levels of resistance were identified in BFG bacteria isolated from WWTPs compared to those that have been recovered from human faeces investigated [40].

Importantly, there is growing evidence that wastewater treatment does not have a profound impact on eliminating the ARGs present in hospital wastewater, with no significant difference in ARG abundance between influent and effluent hospital wastewater samples [54]. Another study demonstrated the high abundance of ARGs and MGEs, including plasmids, transposons, integrons and insertion sequences among samples collected during different treatment processes using aerobic activation (aerobic-activated sludge (AAS)) or anaerobic digestion (anaerobically digested sludge (ADS)) [48]. However, a distinct microbial population has been identified in AAS compared to ADS samples, which suggests a role for the treatment process in promoting the dissemination of particular resistance patterns [48]. A number of recent studies have also demonstrated that novel ARG-bearing plasmids and ARGs that confer resistance to multiple clinically relevant antimicrobial drugs, including aminoglycoside and β-lactams, were highly enriched in activated sludge and effluent wastewater [57,59,61,89,92]. Additionally, activated sludge investigated in one study was found to contain varied ARBs, including ESBL-*Enterobacteriaceae*, MRSA and VRE, and several ARGs associated with resistance to β-lactam, vancomycin (*vanA*) and methicilin (*mecA*) [95].

Consistently, Gram-negative and -positive isolates dominated in WWTP influent and effluent samples, respectively, with the frequency of detection of tetracycline-, sulphonamide-, streptomycin- and β-lactam-resistance genes (except *sulA* and $bla_{\text{CTX-M}}$) being higher in ARB from influent compared to effluent samples [89]. The abundance of *intI1*-bearing bacteria (including *E. coli*, *Klebsiella* spp. and *Aeromonas veronii*) were higher in effluent compared to influent wastewater, with *intI1* being detected in 20.4%, 30.9% and 38.9% of bacteria recovered from influent, activated sludge and effluent wastewater, respectively [78]. In another study, MDR *Enterobacteriaceae* strains carrying class 1 and class 2 integrons (12.1%; 221 out of 1832) were identified in different stages of a municipal wastewater treatment plant (61.5%, 12.7% and 25.8% of ARB originated from raw sewage, aeration tank and final effluent, respectively) [81]. However, the abundance of ARGs and MDR bacteria, particularly the levels of ARG diversity and β-lactamase-producers, were higher in the final effluent samples [81].

Collectively, these studies demonstrated WWTPs as hotspots for the emergence of ARBs and highlighted the impact of the treatment technology employed and potential roles of specific stages of treatment processes, particularly those characterised by high biomass and biodiversity (e.g., activated sludge), in maintaining diverse ARGs and promoting particular populations of ARBs. Advanced treatment processes, including membrane filtration, ozonation and UV-irradiation, are highly efficient in reducing the abundance of AMR in effluent wastewater to levels observed in low-impacted surface water [99].

3.6. AMR Dissemination in Wastewater Associated with Mass Gathering Settings

Most of the studies that reported AMR in wastewater have been conducted within one or a few countries. The majority of the studies were conducted in Asia (n = 22), followed by Europe (n = 23), Africa (n = 10), South America (n = 9), North America (n = 7), Central America (n = 1) and Oceania (n = 2).

No studies have been conducted to investigate the transmission of AMR bacteria and ARGs in environmental samples associated with key religious mass gatherings (Kumbh Mela and Hajj) occurring in low-income settings. Kumbh Mela and Hajj are the largest and most diverse mass gathering events that have been associated with an increased risk of infectious disease emergence and transmission [100,101].

Kumbh Mela, the world's largest religious gathering that attracts millions of Hindu pilgrims, is celebrated at four riverbank pilgrimage sites, including Ganges-Yamuna-mythical Saraswati rivers confluence, Ganges, Godavari and Shipra [101]. The bathing of the pilgrims in these rivers is one of the key rituals, as they believe that it cleanses them of their sins. This raises serious public health issues with regards to the dissemination of waterborne diseases in a setting known to be endemic for cholera [102,103]. Recently a number of studies using metagenomic approaches have detected high levels of ARB, ARGs and antimicrobial residues in water and sediment samples collected from the Ganges

River [104]. In addition, ARGs related to different classes of clinically relevant antimicrobial drugs, including ß–lactams, aminoglycosides, fluoroquinolones, macrolides-lincosamide-streptogramins (MLS), rifampicin and sulphonamides, have been identified in samples collected from the confluence of the river Ganges with Yamuna [105].

Hajj has already been associated with an increased risk of airborne, foodborne and zoonotic infections [100]. Recent studies have demonstrated that pilgrims are at high potential risk of acquiring and transmitting AMR enteric bacteria, [106–109] including carbapenemase-producing *E. coli* [110] and extended-spectrum cephalosporin- and colistin-resistant non-typhoidal *Salmonella* [111], as well as MDR *Acinetobacter* spp. [110].

4. Discussion

The release of antimicrobial drugs, ARBs and ARGs originating from human and animal waste to the environment is a global problem that has serious implications on public health. Therefore, strengthening knowledge on the spread of AMR through surveillance and research was one of the key strategic objectives of the WHO global action plan that was launched in 2015 [112]. Here, we systematically analysed the recent literature to highlight the contribution of different types of wastewaters from various sources (e.g., low- and high-income countries and mass gathering settings) to the emergence, persistence and transmission of AMR in environments and their potential impacts on public health.

The analysed studies highlighted the role of wastewaters as major sources of antimicrobial agents, ARBs and ARGs in the environment. Particular types of wastewaters (e.g., untreated municipal-, hospital- and pharmaceutical wastewaters) have been characterised by high levels of clinically relevant ARBs and ARGs. These environments can provide an ideal platform allowing the transfer of ARGs among the bacterial populations either before or after being discharged into the environment. This is alarming considering that many clinically relevant bacterial species, including enterotoxigenic *E. coli* and typhoidal and non-typhoidal serotypes of *Salmonella,* have been shown to be able to persist in the environmental water for relatively long times [113–115].

Wastewater treatments have been shown to be effective in reducing the ARB loads in effluent samples. However, there is increasing evidence that effluent samples from wastewater treatment plants, wastewater discharges of pharmaceutical production facilities, hospitals and other healthcare facilities are hotspots (ideal platforms) for selective pressure processes that promote the emergence and dissemination of novel AMR mechanisms and new variants of ARBs and ARGs. However, it is noted that the positive selection process and the dynamics of the emergence of novel variants of ARGs and ARBs within WWTPs and their associated impacts on human health remain poorly documented and characterised. The WHO has highlighted the need for greater attention and action to develop quantitative microbial risk assessments and supporting guidance to address human health risks associated with environmental exposures to antimicrobial agents, their metabolites, ARBs and ARGs [32].

Interestingly, a recent pioneer study has proposed a culture-independent metagenomic analysis of untreated wastewater as an effective approach to track and predict the dissemination of AMR bacteria and ARGs globally [41]. The authors of this study have used a standardised metagenomic protocol to characterise the bacterial resistome content and to detect variations in the abundance and diversity of ARBs and ARGs in a global collection of untreated wastewater samples (collected from 79 sites in 60 countries). This study demonstrated that clinically relevant ARGs were more abundant in samples collected from low- and middle-income settings in Africa, Asia and South America, compared to those that have been collected from high-income settings in Europe, North America and Oceania. The variations in AMR gene abundance were found to strongly correlate with socioeconomic, health and environmental factors [41].

This approach can be applied in challenging settings (e.g., such as low-income countries and complicated mass gathering settings) to study the paradigm of AMR dynamics and epidemiology and

inform on the processes leading to the emergence and the dissemination of AMR infectious agents and, therefore, help in developing management strategies.

We conducted a research study that uses the opportunity presented each year by the Hajj pilgrimage and advanced shotgun-based metagenomic approaches to characterise the global population of enteric microorganisms circulating in environmental Hajj settings. This will provide an annual snapshot of the AMR bacteria and MGEs associated with each global locality and help in identifying the dynamics of emergence and dissemination of AMR in the environment.

Hajj is a unique mass gathering event that has been associated with an increased potential for the emergence and dissemination of AMR infections, raising major public health concerns within the host country and globally. The enormously diverse population of 3 million pilgrims, originating from 190 countries all over the world come together to perform the same activities within a relatively short period of time and over a limited area of land. Importantly, the pilgrims are required to stay in tents in Mina (a nonpopulated valley covering approximately 20 km^2 of land, of which only 4 km^2 can be occupied by pilgrims) for at least 3–5 days. The pilgrims are distributed in campaigns across Mina according to their geographical origin (i.e., country of origin). The wastewater is disposed of through septic tanks (onsite sewage facilities) that are associated with the pilgrims' campaign. We conducted the first study to use shotgun-based metagenomic analysis to characterise the abundance and distribution of microbial communities and resistance determinants in wastewater samples from septic tanks in Mina representing different campaigns (European, Middle East and North African (MENA) and East and Southeast Asian countries). The results indicated that high levels of ARGs, including ESBL and aminoglycoside markers, were detected in all sites tested. However, significant variations in the distribution of the bacterial species and the abundance of ARGs were identified.

Similarly, Kumbh Mela in India represents the world's largest periodic mass gathering event that involving bathing in small-specified rivers sites. Recent studies have highlighted the striking impact of mass bathing on river ecosystems, including the AMR microbial contents and dissemination of human infectious agents [105,116,117]. A recent study found a nearly 130-fold increase in bacterial load of human origin during the event. Moreover, metagenomic analyses demonstrated an increase in virulence and ARG loads during the MGEs [118].

Many studies have highlighted the roles of surface fresh and aquatic water, rural groundwater and sewage in the dissemination of AMR pathogens. The emergence of AMRs is part of a complicated ecological and evolutionary network, with the use of antimicrobial drugs anywhere within the system potentially selecting for resistance to that drug elsewhere in the network [23]. Gram-negative bacterial resistance, in particular, is promoted through horizontal gene transfer by the acquisition of mobile elements [119–121]. There is also increasing evidence that ARGs found in human microbial communities are likely to have been acquired from an environmental source [122,123]. The processing of human, farm and industrial waste together has a significant impact on the emergence of AMR to a wide range of the most clinically effective antibiotics [124,125]. In addition, even treated sewage samples discharged into rivers or lakes from treatment plants may contain significant concentrations of ARGs that enhance the development of AMR bacteria and raise major public health concerns [24,126–128].

Supplementary Materials: The following are available online at http://www.mdpi.com/2414-6366/5/1/33/s1, Table S1: Characterisation of studies detecting disseminated AMR genes in sewage.

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Article

A Rapid Assessment of Health Literacy and Health Status of Rohingya Refugees Living in Cox's Bazar, Bangladesh Following the August 2017 Exodus from Myanmar: A Cross-Sectional Study

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Abstract: Background: A survey was conducted among Rohingya refugees to assess their overall health literacy and health status. Methods: A questionnaire was developed to conduct face to face interviews among Rohingya refugees in Cox's Bazar, Bangladesh in November–December 2017. Families were selected using convenience sampling from four large refugee camps. Results: Primary respondents aged 10–90 (median 32) years, 56% male, representing 1634 families were interviewed and provided data of themselves and 6268 additional family members, 4163 (66.4%) of whom were children aged <18 years. Of all, only 736 (45%) primary respondents knew how to appropriately treat diarrhoea, 882 (54%) relied on unqualified village "doctors" for treatment, 547 (33.5%) reported a family member suffering injuries in the previous six months, with 8% (42/547) of injuries fatal. One hundred and ninety two (11.8%) primary respondents also reported deaths within their family in the preceding 12 months, with the majority (70% [134/192]) occurring in males, and 44% (85/192) of all deaths were claimed to be homicidal. Conclusion: This survey highlights overall poor health literacy, limited access to qualified health care, and a high rate of injuries and assaults among Rohingyas. However, these data come from an anecdotal survey that excluded some sensitive but important questions.

Keywords: Bangladesh; health literacy; health status; Myanmar; Rakhine; refugee; Rohingya

1. Introduction

The Rohingya people of Rakhine, Myanmar are considered one of the most persecuted populations in the world [1]. Rohingyas constitute about a third (now a quarter) of the population of Rakhine state (formerly known as Arakan), a western coastal state of Myanmar spreading over 36,760 square kilometres of land, with a population of about 3.2 million. Of the five districts of Rakhine, most Rohingyas are concentrated in Maungdaw. They are denied citizenship in Myanmar, which leads to negative discrimination, including denial of access to health and education. Rohingyas are also denied legal identities including birth certificates, and access to essential childhood vaccinations with 62% of Rohingya children under two receiving no parenteral vaccines [2]. Military crackdowns targeting Rohingyas have also occurred periodically, notably in 1978, 1991–1992, and most recently in 2017–2018, when approximately 700,000 Rohingyas crossed over the border to Bangladesh following escalating violence in Rakhine state, joining more than 200,000 Rohingya who were already in the country. Most of them now live in refugee camps in Cox's Bazar, a coastal district of Bangladesh [1,3–6]. From the very beginning, a number of United Nations (UN) agencies, including United Nations High Commissioner for Refugees, World Health Organization (WHO), International Organization for Migration, United Nations Children's Fund, and United Nations Population Fund; international humanitarian organisations including the International Federation of Red Cross and Red Crescent Societies, Médecins Sans Frontiers (MSF), CARE International, Save the Children Fund, and Orbis Eye Care; local non-government organisations including BRAC, Mukti, HOPE Foundation for Women and Children of Bangladesh, and Al-Markazul Islami are providing much needed humanitarian help. The Ministry of Health and Family Welfare, Bangladesh oversees and streamlines medical activities. There are medical clinics and dispensaries with facilities for minor surgeries within camps, and some over the counter drugs are available from shops and groceries around the camps accessible to both local residents and refugees. Patients needing secondary and tertiary care are transferred to local government medical college hospitals in Cox's Bazar or Chittagong. All treatment, medications and diagnostic tests are free for the refugees. Traditional healers and traditional birth attendants may be active in the camps, but they are not easily identified or recognised outside of the small communities in which they practice. Previous studies have noted high rates of malnutrition and low immunisation coverage among Rohingya refugees in Cox's Bazar who, thus, remain susceptible to infections including gastroenteritis, acute respiratory infections and acute jaundice syndromes [7–11]. Following the last mass migration, a large diphtheria outbreak and varicella and measles outbreaks have occurred, and cholera remains a constant threat [12–15]. Furthermore, Rohingya refugees suffer from a wide range of acute and chronic health conditions [7,14], including musculoskeletal and mental health problems that can be difficult to detect, assess and manage in this vulnerable population [16–18].

There has been some research to try to understand the magnitude of violence and fatalities occurring among Rohingyas [2,10,19]. A survey conducted among over 600 village leaders identified the primary reason for leaving Myanmar was violence in their village or in an adjacent village, perpetrated mostly by border police and the Myanmar military [19]. Another cluster of surveys led by MSF calculated the crude mortality rate (CMR) among those aged ≥50 years during the 2017 violence period was as high as 17.3 per 10,000 per day, an almost 15-fold higher CMR than in the same population before, and 9-fold higher CMR than after, the period of violence [10]. This is further corroborated by an in-depth interview of 22 survivors of a village called 'Chut Pyin', where an estimated 400 people with 99 children were killed in one day [20].

However, a comprehensive picture of the health status and health literacy (i.e., personal characteristics and social resources required for individuals and communities to access, understand and use information and services to make health decisions [21]) and other health care-related experiences among Rohingya refugees does not exist with respect to the most recent mass migration into Bangladesh. To this end, a rapid needs assessment survey was conducted among Rohingya refugees in Cox's Bazar in late 2017 as a first step to inform strategies to provide adequate health care, resource mobilisation and develop further action plans for this vulnerable population.

2. Materials and Methods

A brief proposal was prepared outlining key study steps and submitted to the Ministry of Health and Family Welfare, Bangladesh and received formal approval. In consultation with researchers experienced in refugee health, a questionnaire was devised using the WHO Europe's 'Toolkit for assessing health system capacity to manage large influxes of refugees, asylum-seekers and migrants' as a key reference. The questionnaire included questions on Rohingyas' demographics, health literacy about symptoms and prevention of common illnesses, access to health care, sanitation and immunisations, current illnesses, injuries in the preceding six months and fatalities and animal bites encountered in the past one year, as well as the presence of disabilities at the time of the survey. The questionnaire was written in the local dialect using vocabulary predominantly used by lay Rohingya people with little or no literacy.

The survey was conducted with the help of 19 trained interviewers in four refugee camps in Cox's Bazar from 25th November to 4th December 2017. Selection criteria for interviewers included an education level of at least 12th grade, ability to speak the local dialect ('Rohain' subdialect of Chittagonian Bangla language spoken in Cox's Bazar), experience in conducting public health surveys, and successful completion of a training workshop and post-workshop assessment. A three-day structured training workshop (22–24th November 2017) was arranged by experienced researchers who have previously conducted large health surveys in resource-poor settings to train potential interviewers on various aspects of data collection, including how to obtain consent, how and where to check for a Bacillus Calmette–Guérin (BCG) vaccination scar, how to record and store data, and maintain confidentiality. An important focus of this workshop was on the ethical conduct of research including key aspects of good clinical practice (GCP) and the International Council for Harmonisation (ICH) guidelines and the necessity to comply with those principles. Of the 21 attendees, 19 successfully completed the formal assessment which included conducting a mock survey. The field work and data collection were supervised by two medically qualified experienced researchers. A debrief session was conducted during a study closure meeting on 4th December.

Using a non-probability sampling method, consecutive houses from four refugee camps were surveyed starting from a corner of each camp which was chosen randomly. The camps where the survey was conducted are Balukhali Camp 01, Balukhali Camp 02, Moinergona Camp and Kutupalong Camp (Figure 1).

The interviewers approached the lead members (henceforth, called 'primary respondents') of the family and after explaining the survey aim and design, conducted a face to face interview to complete the questionnaire. Verbal agreement to participate in the survey and providing responses to interviewers' questions were considered implied consent to participate in the study. Participants' or their family members' identifiable information were not collected, but age was. For the purposes of the survey, a 'family' was defined as a group of people who sleep under the same roof and share meals from the same pot. All data were entered on a master Microsoft Excel spread sheet before importing to Statistical Package for Social Sciences (SPSS) software (IBM SPSS Statistics for Windows, version 25.0, Armonk, NY: IBM Corp). Categorical data were expressed as number and proportion, while continuous data were expressed as range with measures of central tendency.

No formal sample size calculation was attempted for this survey. The initial study proposal aimed to recruit 800–1200 refugee families, but as the number of refugees over the weeks escalated, the recruitment aim was increased to about 1500 families. Although no sample size calculation was done, the aim was to capture data from about 1% of the refugees who migrated to Bangladesh in late 2017 (n = 700,000), that is about 7000 individuals. It was estimated that there would be an average of five people in each family, requiring interviews of key informants from about 1500 families. This sample estimate was inflated by 10% to account for any incomplete data.

Figure 1. Map of Bangladesh showing Cox's Bazar and study camps in red dots (source: https://d-maps.com).

In 2017, following a large influx of Rohingyas into Bangladesh, the Directorate General of Health Services (DGHS), MHFW, Bangladesh approved immediate commencement of the study without prior ethics approval (Ref: DGHS/PHC/Rohingya/2017/163) as understanding the refugees' health status and risks was considered critically important for the refugees themselves and for the host

population. The study was conducted in compliance with the ICH and GCP guidelines. All key investigators were qualified clinical research professionals, including a WHO Monitor (M.R.R.), and the interviewers were assessed to ensure their understanding of ethical principles before being sent to the field. Verbal consent from each interviewee was obtained following detailed explanation of the survey methodology, including its purpose and its voluntary nature and explaining the participants' right to leave the interview at any time. The data were stored and managed confidentially and no one other than the investigators or their authorised personnel had access to the data. Children who provided data did so under supervision of their adult family members.

3. Results

3.1. Demographics

The demographics of primary respondents, their access to health care and their economic background are summarised in Tables 1 and 2, and the demographics of their family members are summarised in Table 3.

Table 1. Demographic characteristics of primary respondents among Rohingya refugees.

Particulars (N = 1634)	Overall n (%)	Male n (%)	Female n (%)	*p* Value
Number of participants	1634	913 (55.9)	721 (44.1)	
Age in years (median, mean ± SD)	10–90 (32, 36.3 ± 13.8)	10–90 (35, 37.7 ± 14.6)	10–80 (30, 34.4 ± 12.5)	<0.01
Children (aged < 18 years)	9 (0.6)	7 (0.8)	2 (0.3)	0.19
Occupation in Myanmar				
Farmer	423 (25.9)	393 (43.0)	30 (4.2)	<0.01
Homemaker	629 (38.5)	5 (0.5)	624 (86.5)	<0.01
Grocery businessman	211 (12.9)	199 (21.8)	12 (1.7)	<0.01
Sedentary workers	58 (3.5)	56 (6.1)	2 (0.3)	<0.01
Fisherman	32 (2)	32 (3.5)	0 (0)	<0.01
Student	32 (2)	24 (2.6)	8 (1.1)	0.03
Labourer	108 (6.6)	97 (10.6)	11 (1.5)	<0.01
Others	95 (5.8)	77 (8.4)	18 (2.5)	<0.01
Retired	32 (2.0)	25 (2.7)	7 (0.8)	0.01
Unemployed	14 (0.9)	5 (0.5)	9 (1.2)	0.13
Years of education received				
No education	389 (23.8)	241 (26.4)	148 (20.5)	0.01
1–5 years	834 (51.1)	403 (44.1)	431 (59.8)	<0.01
6–10 years	340 (20.7)	208 (22.8)	132 (18.3)	0.03
11–12 years	51 (3.2)	41 (4.5)	10 (1.4)	<0.01
>12 years	20 (1.3)	20 (2.2)	0 (0)	<0.01

SD = Standard deviation.

Table 2. Economic background of primary respondents among Rohingya refugees.

Particulars (N = 1634)	Overall n (%)
Owned lands in Rakhine	1278 (78.2)
Total arable land in acres, range (median; IQR)	0.4–144 (2; 0.8–4.0)
Own gold/jewelleries	1310 (80.2)
Total amount of gold in grams, range (median)	1–478 (23.3)
Family income per month in US$ before migration, range (median; IQR)	0–5200 (65; 65–195)
Have money deposited in a bank	12 (0.7)
Roof of your Myanmar house built with	
Leaves	909 (55.6)
Thatched	597 (36.5)
Corrugated iron sheets	111 (6.8)
Others	17 (1)

Table 2. *Cont.*

Particulars (N = 1634)	Overall n (%)
At home, where did you usually get your drinking water from?	
Tube well	1316 (80.5)
Pond	174 (10.6)
Deep well	105 (6.4)
Other sources	39 (2.4)
Have sanitary latrine for the family in Myanmar	1583 (96.9)
One latrine	1267 (77.5)
More than one latrine	316 (19.3)

IQR = Interquartile range.

Table 3. Demographic characteristics of family members among Rohingya refugees.

Particulars	Overall n (%)	Male n (%)	Female n (%)	*p* Value
Total number of participants	6268	2973 (47.4)	3295 (52.6)	
Age in years range (median, mean ± SD)	0.1–120 (12, 15.9 ± 14.6)	0.1–98 (11, 15.1 ± 14.7)	0.1–120 (13, 16.5 ± 14.4)	<0.01
Children (aged < 18 years)	4163 (66.4)	2114 (71.1)	2049 (62.2)	<0.01
Occupation in Myanmar				
Student	2217 (35.4)	1129 (38.0)	1088 (33.0)	<0.01
Homemaker	1111 (17.7)	19 (0.6)	1092 (33.1)	<0.01
Farmer	363 (5.8)	339 (11.4)	24 (0.7)	<0.01
Grocery businessman	198 (3.2)	189 (6.4)	9 (0.3)	<0.01
Labourer	150 (2.4)	145 (4.9)	5 (0.2)	<0.01
Others	334 (5.3)	182 (6.1)	152 (4.6)	0.01
Unemployed or too young to be employed	1832 (29.2)	940 (31.6)	892 (27.1)	<0.01
Retired	63 (1)	30 (1)	33 (1)	0.98
Years of education received				
No education	2064 (32.9)	1057 (35.6)	1007 (30.6)	<0.01
1–5 years	3322 (52.9)	1434 (48.2)	1888 (57.3)	<0.01
6–10 years	810 (13)	423 (14.2)	387 (11.7)	<0.01
11–12 years	51 (0.8)	39 (1.3)	12 (0.4)	<0.01
>12 years	21 (0.3)	20 (0.7)	1 (0.03)	<0.01
Ever received a vaccine	5255 (83.8)	2475 (83.2)	2780 (84.4)	0.23
BCG vaccination in children < 5 years (N = 1264)	764 (60.4)	381 (12.8)	383 (11.6)	0.15
Are they ill now? (Yes)	778 (12.4)	318 ()	460 (14)	<0.01

SD = Standard deviation.

A total of 1634 primary respondents were approached, and all agreed to participate in the survey and provided data on an additional 6268 family members. About 97% of primary respondents (1582/1634) hailed from Maungdaw township, the rest 3.2% (52/1634) were from other places including Buthidaung, Pauktaw, Rathedaung and Taungup. Most had no or limited literacy (75% of primary respondents and 86% of family members had, at most, five years of education). Some sort of employment was documented in 57% of primary respondents (927/1634), but only 18% of other family members (1108/6268). Excluding children aged <18 years (n = 4272) in the whole cohort, approximately 56.1% (2035/3630) of individuals for whom information was available were employed. The range of responses to income and ownership of land and gold varied widely, but median values were low. The median monthly income for primary respondents was US$65 per month.

3.2. Health Literacy and Health Status

Primary respondents' health awareness and access to health care are summarised in Table 4. Overall, there was poor understanding regarding treatment of common illnesses such as diarrhea. The majority (90%) of childbirths occurred at home, with only 4% occurring in the presence of a

trained health care worker. Data regarding injuries or animal bites suffered within the preceding six months, presence of ongoing disabilities and fatalities occurring in the last year among all family members are summarised in Table 5. Over a third (547/1634) of primary respondents reported injuries among themselves or their family members in the previous six months, and over one-eighth (192/1634) reported deaths among family members in the previous 12 months. At the time of the interview, 24.7% (403/1634) of primary respondents reported to have an illness, and only 62.1% (1015/1634) managed to have some sleep the previous night.

Table 4. Health literacy and access to health care among Rohingya refugees during their stay in Myanmar, as reported by primary respondents.

Questions	Number (%) (Total N = 1634)
How do you treat if someone at home suffers from diarrhoea?	
With oral rehydration salt	736 (45)
With medicine	247 (15.1)
Other	54 (3.3)
No response provided	597 (36.5)
Do you wash your hands with soap after the toilet? (Yes)	1092 (66.8)
Where do you go first when a family member is ill?	
Unqualified village doctor	882 (54)
Pharmacy/dispensary	449 (27.5)
Government hospital	274 (16.8)
Other	29 (1.7)
Any babies born in the family in the last one year? (Yes)	397 (24.3)
Did a pregnant woman in your family ever receive a vaccine? (Yes)	1102 (67.4)
Did a pregnant woman in the family ever receive antenatal care? (Yes)	562 (34.4)
Place of delivery of the last baby born to the family	
At home	1464 (89.6)
In hospital	63 (3.9)
Other places	107 (6.5)
Who delivered (or helped deliver) the last baby born in the family?	
A traditional birth attendant	1178 (72.1)
A relative	291 (17.8)
A nurse/mid-wife or doctor	71 (4.3)
Other	94 (5.8)

Table 5. Injuries, animal bites and deaths among Rohingya families as reported by primary respondents interviewed in Cox's Bazar.

Particulars	Number (%)
Any injury among family members in the last six months (N = 1634)? (Yes)	547 (33.5)
Injury type (N = 547)	
Assault	286 (52.3)
Accident	128 (23.4)
Occupational	51 (9.3)
Domestic task	41 (7.5)
Other	41 (7.5)
Assault caused by (N = 276)	
Stick	128 (46.4)
Bullet	65 (23.6)
Knife	16 (5.8)
Burn	4 (1.4)
Other	63 (22.8)

Table 5. *Cont.*

Particulars	Number (%)
Treatment received (N = 547)	
From pharmacy	211 (38.6)
From hospital	158 (28.9)
From primary care centre	47 (8.6)
No treatment received	108 (19.7)
Consequence of injuries (N = 547)	
Complete resolution	195 (35.6)
Ongoing complaint or disability	310 (56.7)
Death	42 (7.6)
Any snake bitesamong family members in the last six months (N = 1634)? (Yes)	48 (2.9)
Fatalities from snake bites (N = 48)	0 (0)
Any dog bitesamong family members in the last six months (N = 1634)? (Yes)	104 (6.3)
Fatalities from dog bites (N = 104)	5 (5%)
At least one death among family members in the last one year (N = 1634)	192 (11.8)
Two deaths in the family	25 (1.5)
Three deaths in the family	2 (0.1)
Gender of deceased (N = 192)	
Male	134 (69.8)
Female	54 (28.1)
Unspecified	4 (2.1)
Age of deceased in years, range (median)	0.1–113 (31)
Deceased aged≤50 years	116 (60.4)
When did the individual die? (N = 192)	
Within the preceding 4 months	114 (59.4)
4–12 months prior	78 (40.6)
Cause of death (N = 192)	
Homicide	85 (44.2)
Sudden unexpected death	34 (17.7)
Febrile illness	19 (9.9)
Paralytic illness	11 (5.7)
Accident	9 (4.7)
Maternal death	8 (4.2)
Coma	4 (2.1)
Other	18 (9.4)
Unknown	4 (2.1)

4. Discussion

Key findings of the survey include overall poor health literacy, limited access to health care, including primary and preventive care, as well as obstetric care, and a high rate of injuries and assaults. This survey was conducted in November–December 2017, three months after the start of the most recent violence in Rakhine state, Myanmar, giving an overall picture of the health status and health literacy of the Rohingya refugees that fled to Bangladesh and were temporarily settled in Cox's Bazar. The timing of the survey and the questions that were asked, generally assessing health parameters over the preceding 12 months, reflect the status of this population prior to their migration and provide important information regarding health needs for service providers in Bangladesh.

The demographic characteristics of the respondents in this survey demonstrate the basic existence with which most Rohingyas live, such as living in thatched or leave-roofed houses in the vast majority, with a median monthly income of US$ 65, which is just above the World Bank definition of absolute poverty set at US$ 1.90/day in 2015 [22]. This is in line with previous reports assessing the socioeconomic status of this marginalised population [2]. Although access to health care is multifactorial and complex, economic factors play a key role [23]. As such, over 80% of Rohingyas, while in Rakhine, predominantly relied on traditional village "doctors" or "pharmacists" for their medical care. Such traditional healers play an important role in the health and well-being of many marginalised and vulnerable populations, and ongoing constructive dialogue between traditional health providers and formally recognised

medical services is essential to ensure all health and mental health needs of communities in need are met [24]. Access to antenatal and obstetric care was also limited. Almost two-thirds of pregnant women did not receive any antenatal care and 90% relied on domiciliary care by unregulated or unqualified health care providers for their deliveries. Most of these findings confirm those published previously on this subject [2,10,19]; however, this study also uniquely identifies that Rohingyas have poor health literacy, with over half of primary respondents unable to answer questions on how to appropriately treat diarrhoea. This is in the context of a setting in which diarrhoea is endemic, and a leading cause of death [25].

However, there were other positive findings from the survey. Over 80% of families had access to a tube well (an iron pipe well meant for suctioning water from underground aquifers) for water, which has also been reported by other researchers [2], and 97% had at least one sanitary latrine for the family; although, hand washing with soap was suboptimal, with only two-thirds of primary respondents reporting use of soap and water to wash hands after going to the toilet. Despite this seemingly low rate of basic infection prevention, this is an improvement compared to a previous survey among community members in rural Myanmar conducted in the 1980s, which found that only 5% to 12% of people regularly used soap to wash their hands after visiting the toilet [26]. However, this should be considered against the real-life context that the vast majority of Rohingyas were struggling hard to make ends meet and were forced to choose between the purchase of soap and the most basic essentials such as food.

Around 84% of primary respondents reported ever receiving a vaccine, and a BCG scar was noted in 60% of children among all family members under five years of age. No further information regarding vaccinations received was sought and the reports were not corroborated by viewing vaccination certificates, which were unlikely to be available. This is because one of the aims of this survey was to crudely gauge the refugees' prior access to preventive medicine rather than establishing a full immunisation record. Unfortunately, this suggests that up to 16% of individuals may not have received any immunisations in the past, including those in the WHO Expanded Program on Immunisation schedule, leading to a significant risk of both individual and community vulnerability to disease outbreaks as have occurred with diphtheria, measles and varicella [12,13,15]. However, our results are more favourable than those of other recent surveys that demonstrated 43% of children under the age of four had not received any doses of an injectable vaccine in Myanmar [2], and that only 23% of Rohingya children under five years of age had received a measles vaccine [10]. Explanations for these differences are multifactorial and discussed below.

Another important finding from this survey is the relatively large proportion of Rohingyas who had suffered injuries within a six-month period. Among injuries reported, the largest proportion were those due to assault, including by stick (46.4%), bullet (23.6%) and knife (5.8%). This gives a crude estimate of the assault rate in this population, excluding homicides, as approximately 2417 per 100,000 persons within the previous year, significantly higher than the background rate of non-fatal assaults occurring in Myanmar of 8.7 cases per 100,000 persons in 2016 [27]. This survey did not ask for further information regarding how injuries occurred, and by whom assaults were perpetrated; however, in a separate survey conducted subsequent to ours, 64% of respondents reported violence against civilians occurring during the military campaigns in Rakhine by Myanmar security forces during August–September 2017 [19]. In addition, almost two-thirds of injuries reported in the current survey led to death or ongoing complaint or disability, reflecting the severity of the injuries.

Fatalities were common, with 192 deaths occurring among all family members over the preceding year, with the most common cause being homicide (44%), although illness and accidents were also responsible for many of those deaths. This high number of fatalities corroborate other estimates that suggest around 6700 Rohingyas died as a result of violence in the initial 31 days following the outbreak of unrest [10,28]. Similarly, Bhatia et al. record 10.7% of Rohingya families surveyed reported one death in the family, 2.5% reported two deaths, and 1.2% reported three deaths in the one year preceding the survey [2]. In the current survey, victims of fatalities were predominantly male (approximately

70%), consistent with the findings of another survey conducted by MSF [10]; however, in contrast to their results, we note more people aged 50 years or younger dying compared to those aged over 50 years. This difference could be explained by the temporal relation to the commencement of violence in Rakhine, wherein our study took place within three months of the outbreak of violence, while the MSF study began in February 2017, six months before the 2017 violence, although it continued until November 2017. Sadly, eight of the fatalities (4%) reported here were maternal deaths indicative of poor/non-access to adequate obstetric and perinatal care for Rohingyas [1].

In addition, 48 primary respondents reported a snake bite in a family member in the preceding year. This roughly translates to a snake bite incidence in this population of 0.6% compared to 0.12% incidence in central Myanmar; although generally, 1 in 15 snake bites in Myanmar are fatal [29], and no fatal snake bites were reported in this survey. Dog bites among families were reported by 104 primary respondents, with five (5%) of these resulting in death. This may be related to rabies infection, which is still a major public health concern in Asia, including Myanmar. The 2015 estimated rate of rabies mortality across Myanmar was 0.2 per 100,000 population, and the rate is claimed to be lower in Rakhine [30]. These five fatal dog bites among a cohort of 7900 people translates to a mortality rate of 63 per 100,000 persons. Although some proportion of these deaths may have been due to blood loss, organ damage, wound infection or other causes, rather than rabies, it is likely that the poor public health infrastructure in Rakhine results in an under-reporting and under-estimation of rabies deaths among Rohingyas.

Despite the generally young age of the population surveyed (median age of primary respondents was 32 years, and of their family members was 12 years with 71% [4477/6268] of family members aged ≤18 years), around 25% of primary respondents and 12% of their family members reported illness at the time of the survey, and just over 60% of primary respondents managed to get sleep in the preceding night. These questions again only broadly assess ongoing stress and mental health issues among the refugees and highlight the large un-met need to access health care, including mental health care. A cross-sectional study conducted among existing Rohingya refugees in Bangladesh before the 2017 exodus showed that 36% suffered from post-traumatic stress disorder (PTSD) and 89% suffered from depression [16]. Unsurprisingly, high rates of mental health problems were also common among children, with 52% of Rohingya children in Bangladesh having results in the abnormal range for emotional symptoms on the Strengths and Difficulties Questionnaire (SDQ), and 25% with results in the abnormal range for peer problems [31]. Rohingya refugees in Malaysia have similar high rates of comorbid mental health disorders, including 32% with PTSD, 9% with generalised anxiety disorders, and 12% with major depressive disorder [32].

Such high rates of illness and mental health problems in these refugees suggest ongoing vulnerability to disease that is much higher than would be expected by the population demographics. For example, recently, 38 COVID-19 cases with two fatalities have been reported among Rohingya refugees, including among residents of our study camps, which is a grave concern due to the crowded and difficult social circumstances within these camps [33]. Lockdown measures have been introduced following the detection of early cases, and surveillance continues. In addition to monitoring the direct health and mental health impact of the pandemic in these camps, assessing both the efficacy of lockdown measures, as well as negative effects on access to health care and other services will be crucial in the coming months.

The strength of this study is its large sample size, which was selected in a systematic manner from four large refugee camps, and which is probably representative of the population of Rohingya refugees currently displaced to Cox's Bazar in Bangladesh. The study was conducted within three months of the influx of refugees and the researchers had field experience in this context and were familiar with the local culture and language.

There are several limitations to the study. Firstly, the anecdotal nature of the survey, and the interviewers' inability to objectively corroborate statements (with the exception of BCG scars in children), means that both under- and over-estimations are highly likely and only general conclusions

can be drawn. Secondly, some primary respondents were children aged as young as 10 years, although children aged 10 to <18 years only accounted for 0.6% of all primary respondents. Thirdly, for cultural and political reasons, we did not ask for details regarding injuries and fatalities, in particular, the perpetrators of injuries and fatalities. Thus, although we postulate, based on the timing of the majority of deaths that occurred in the preceding four months, that a large proportion resulted from the violence occurring in Rakhine, we cannot confidently support or refute this. Finally, it is likely that despite efforts to ensure the surveys were conducted in a language and culture-sensitive manner, a degree of misunderstanding occurred. For example, the question regarding receipt of a vaccine may have been interpreted as having received a vaccine after arriving in Bangladesh, since several vaccination campaigns were rolled out to curb epidemics of diphtheria, measles, varicella and cholera [34]. Thus, it is possible that the routine immunisation rate in this population is higher than reported in this survey; although, based on the results of other similar surveys referred to earlier, it is unlikely that the under-estimation is large. Similarly, some data conflict with the results of other surveys. For example, in our survey, only 24% of primary respondents and 33% of their family members were reported as not having any education, while in another survey, 76% of Rohingya household members older than 15 years had no formal education, and 53% of Rohingya children aged younger than 15 years did not attend school [2]. This could be due to a failure of the survey in distinguishing formal and informal education, such as that provided by religious and village leaders. Finally, the data cannot be broadly generalised as they are drawn from only 1634 families representing just 1% of the Rohingya influx and were recruited using convenience sampling.

5. Conclusions

This survey provides a broad-strokes overview of Rohingya refugees' health status and health literacy and highlights overall poor health literacy and limited access to qualified health care in Myanmar. A high rate of injuries, accidents and assaults, as well as fatalities, have occurred in this population in the preceding 12 months, with the majority of deaths occurring in the preceding four months, coinciding with the outbreak of violence in Rakhine state in Myanmar that lead to the mass migration of Rohingyas into Bangladesh. Furthermore, despite the generally young age of the population surveyed, there appears to be a high rate of ongoing or persistent illness and disability, reflecting the multifactorial trauma and socioeconomic disadvantage experienced by these individuals. These findings make timely and multi-pronged health, educational and political interventions imperative to ensure the physical, mental, social and spiritual wellbeing of this vulnerable population.

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Article

Prevalence of Eye Problems among Young Infants of Rohingya Refugee Camps: Findings from a Cross-Sectional Survey

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Abstract: Early detection of pediatric eye problems can prevent future vision loss. This study was to estimate the prevalence of common eye problems among infants born in a resource-constrained emergency setting with a broader aim to prevent future vision loss or blindness among them through early detection and referral. We conducted a cross-sectional survey among 670 infants (0–59 days old) born in Rohingya refugee camps in Bangladesh between March and June of 2019. The most common eye problem found was watering from the eye and accumulation of discharge by which 14.8% of the children were suffering (95% CI: 12.2–17.7). More than 5% of the infants had visual inattention (95% CI: 3.5–7.0), and 4% had redness in their eyes (95% CI: 2.7–5.8). Only 1.9% of infants (95% CI: 1–3.3) had whitish or brown eyeballs, and 1.8% of children might have whitish pupillary reflex (95% CI: 0.9–3.1). None of the eye problems was associated with the gender of the infants. The prevalent eye problems demand eye care set up for the screening of eye problems in the camps with proper referral and availability of referral centres with higher service in the districts.

Keywords: pediatric eye problem; eye care; infant; Rohingya refugee

1. Introduction

A global initiative launched in 1999 named VISION 2020: The Right to Sight recognised blindness in children as a priority area of disease control [1]. In 2001, it was estimated that in low- and middle-income countries, more than half a million children with severe visual impairment and blindness had avoidable causes [2]. Later in 2010, a global estimate showed that around approximately 17.5 million children were at risk of developing low vision [3]. Childhood blindness can be caused by some common pediatric eye problems such as cataract, amblyopia, childhood tearing, and ptosis during infancy. The importance of prevention of childhood blindness is related to Disability Adjusted Life Years (DALY). Early detection of these is considered to be effective to avoid visual handicap worldwide.

In a recent survey, the prevalence of childhood blindness in rural Bangladesh was observed to be as 6.3 per 10,000 children [4], according to a national case series study, more than two-thirds of such cases could be avoided [5]. A qualitative study in Bangladesh identified barriers that influence eye care provision for children, which showed that barriers to early detection of symptoms, eye examination, and referral services could lead to permanent blindness in children which can be prevented by first empowering communities to recognise childhood cataract and take action [6]. A national campaign launched in 2004 found more than thirty-two thousand children as blind in Bangladesh, and highest number of those cases were found through key informant (KI) method which worked by providing

short training to local volunteers to detect cases and refer to the health centres [7]. Also, a validation study found that in low-income settings, KI was an effective and a low-cost method for identification of cases with disabilities and visual impairment, KI was highly sensitive (100%) and specific (69%) [8]. Therefore, similar approaches can be applied to identify cases of common pediatric eye problems in order to ensure their early detection and treatment.

The largest refugee camp in the world is now located in Bangladesh, with around 915,000 Rohingya population [9]. These enormous numbers of displaced people have limited access to healthcare with higher health risks [10]. Evidence suggests that the prevalence of vision impairments and blindness among refugees are common and often higher than the general population. A recent systematic review found that the prevalence of blindness in the refugee camps can range from 1.3% to 26.2% [11]. A study with Afghan refugees in Pakistan revealed that 2.1% of all refugees there were blind, and 6.9% were visually impaired [12]. Also, a study in Uganda concluded that in refugee settlement camp setting, the prevalence was much higher than outside [13]. These pieces of evidence demand urgent attention to look into this issue in the refugee camps in Bangladesh. Studies show that the major interventions, in most places, including refugee camps, to control childhood blindness are public health in nature (vitamin A supplementation and measles immunisation) [14]. However, the need for eye care interventions for refugees are unique, and in every stage of displacement, such interventions should be targeted [11].

Childhood blindness causes a significant economic burden on the family and community [15]. In an extremely resource-constrained setting as refugee camps, this disease burden poses additional pressure to the government of the country and donors working to improve the health of such displaced people. In Bangladesh, it is a common problem that, parents often do not recognise the eye problems of their children and seek eye care in time. This study aimed to identify common eye problems among young infants born in refugee camps by trained refugee volunteers. The broader aim was to set an evidence-based ground that would help assess the need for eye care facilities in the camps. We conducted a cross-sectional survey among Rohingya infants during March– June of 2019.

2. Materials and Methods

2.1. Population

The target population for this study was 0–59 days old infants born and raised in the refugee camps. In Bangladesh, the integrated management of childhood illness (IMCI) protocol implemented at Primary Health Care (PHC) setting has incorporated eye component for early detection and prevention of childhood blindness. The IMCI protocol is divided into two components: one for age group 0 to 59 days, and another for 2 months to 5 years; for each set, there are age-specific eye components. For early screening and eye care provision, we chose the 0 to 59 days age group. Also, the Non-Communicable Disease Control Program (NCDC) of Directorate General of Health Services (DGHS), Ministry of Health and Family Welfare (MOHFW), with the technical assistance of World Health Organization (WHO) developed a training manual for health care providers working at PHC setting [16].

Based on a study done in 2018, the birth rate inside the Rohingya refugee camp is 35.6 per 1000 population [17]. The population of Rohingyas, according to UNHCR data [10], is about 915,000 in the camps. Thus, the total number of infants born in a year would be about 33,000.

2.2. Sample Size and Sampling

We calculated the sample size for the prevalence survey with finite population correction. As the prevalence of eye problems among the population of interest was unknown, considering prevalence as 50%, the precision of 5%, population size as 33,000 (estimated based on crude birth rate [17]) and confidence level at 95%, the estimated sample size obtained was 380. Estimating the design effect as 1.75, we got the final sample size as 665.

For sampling, we used cluster sampling method. There are two refugee settlements located at two different sub-districts (locally called 'Upazila') of Cox's Bazar, a coastal district of Bangladesh. The settlement located at Ukhiya Upazila, named Kutupalong camp, is known as the largest refugee camp in the world, housing more than 630,000 Rohingya refugees. Therefore, we selected this settlement for the study.

The Kutupalong settlement is a cluster of 20 camps, and we decided to select eight camps randomly based on the coverage this project could allow and the fact that the movement of the refugees of a camp is restricted within their camp boundary. Each camp is divided into individual blocks, and eight camps consisted of a total of 44 blocks. We formed 22 clusters each consisting of two blocks. From each cluster, a frontline health worker (FHW), who is a volunteer from the refugee community, was recruited to be trained and collect data. The details of the randomisation process were as follows:

(1) To select the camps randomly, we put the 20 pieces of folded paper, each with different camp numbers (from 1 to 20) written on them, and
(2) requested one of the researchers in our team who was not involved in the study to choose eight folded pieces of paper after shaking the box each time. Hence, we selected eight camps. The characteristics of the camps are shown in Table 1:

Table 1. Characteristics of camps surveyed.

Selected Camp No.	Total Population *	Total No. of Women of Reproductive Age *	No. of FHWs Assigned
2	29,918	7221	2
4	32,115	7622	4
5	25,117	5939	2
10	32,963	7791	4
11	31,346	7249	2
13	41,735	9819	2
14	31,917	7301	2
15	49,443	11,542	4

* UNHCR Population Data - 31/03/19 [18].

The samples were selected by trained FHWs who took their adjacent household as the starting point and then surveyed every household clockwise in the camps to find infants matching the criteria. The inclusion criteria of our sample were as follows:

(a) The infant is 0 to 59 days old and home-based in the study area.
(b) Mother present as the primary caregiver and at least 18 years old.
(c) Mother agreed to give written consent and share information for the study.

The exclusion criteria for sampling were—

(a) Mother of an infant who does not want to give consent for the study,
(b) An infant with fever or other physical illness that may affect the examination by the FHW.

In the selected eight clusters, FHWs identified a total of 814 infants. Among them, the 22 trained FHWs interviewed 670 (82.3% response) mothers of the babies who gave consent to enrol their babies in the study and examined the babies. We surveyed from March 2019 to June 2019.

2.3. Survey Tools

The survey tools included the following forms: a screening form, a consent form and a questionnaire on eye problems. We prepared all forms in the Burmese language which the refugees use to read and

write. The questionnaire on eye problems included closed questions along with checkboxes for answers (Yes/No) and short instructions along with each question to check the eyes of the infants according to the guideline mentioned above (a sample questionnaire is given as supplementary material). The survey form included short instructions along with each question in order to ensure identical methods of assessment during the survey.

2.4. Training

All FHWs were trained using the training guideline and flashcards developed for PHC providers by NCDC in 2016 [16]. The manual and the materials are endorsed by the national eye care program and used for training workers to identify and refer to eye problems of the infants nationwide.

2.5. Ethical Approval

We obtained ethical approval of the study from Bangladesh Medical Research Council (BMRC) – Registration number 141 14 08 2018.

2.6. Data Collection, Processing, and Analysis

The FHWs collected data daily. Before data collection, they were instructed to ensure if the eyes were adequately cleaned and visible. The FHWs checked and reported the following symptoms or signs for the eyes of the young infants:

(1) If the eyeball looks whitish or brownish,
(2) If there were watering or tearing from the eye while the baby is not crying and if there were any accumulation of discharge,
(3) If there is any redness present on the sclera of the eyeball.
(4) If there is any visible sign of injury present in the eye,
(5) If there was any structural deformity of the eye present in the infant,
(6) If the mother reports any problem of normal vision for her child (whitish pupillary reflex on examination),
(7) If any visual inattention is present by asking mothers if the child looks at her face and smiles.
(8) For any symptom or sign found present, the workers verbally referred the mother to the nearby health facility.

The data forms were weekly collected from the FHWs by a field coordinator, quality checked and sent to the main office to be checked, entered, cleaned and validated. After all data entry and validation processes, we analysed the data using IBM SPSS Statistics 21. We conducted the univariate and bivariate analysis for this study. To find the association between variables, we used the chi-square test with risk ratio estimate.

3. Results

The age of the infants ranged from 1 day to 49 days (M = 36.4, SD = 7.5) with 51.2% identified as boys and 48.8% as girls. The distribution of the surveyed infants over the camps are shown in Table 2:

Table 2. Distribution of infants (0–59 days) among clusters.

Camp No.	Number of Infants (0–59 Days)	Percentage (%)	Boys (n)	Girls (n)
3	63	9.4	31	32
4	123	18.4	56	67
5	60	9.0	37	23
10	93	13.9	42	51
11	86	12.8	41	45
13	63	9.4	33	30
14	55	8.2	26	29
15	127	19.0	77	50
Total	**670**	**100**	**343**	**327**

The most common problem among the infants was watering from the eye (14.8%, 95% CI: 12.2–17.7). Visual inattention was reportedly found as the second most common problem in the infants reported by mothers (5.1%, 95% CI: 3.5–7.0). Also, the redness of the eye was prevalent in 4% of infants (95% CI: 2.7–5.8). An almost similar percentage of children were found to have whitish or brown eyeballs (1.9%, 95% CI: 1.0–3.3) and problem in normal vision (1.8%, 95% CI: 0.9–3.1). The health workers observed directly whitish or brown eyeballs in the infants, while mothers reported that they thought their children had a problem in normal vision. Very few children had been found with structural deformity (0.6%, 95% CI: 0.2–1.5). None of the children had any sign of injury in their eyes (95% CI: 0–0.5). Table 3 below shows details of the prevalence against each checked symptoms or signs:

Table 3. Prevalence of eye conditions among refugee infants (0–59 days) and proportions among boys and girls.

Indicators for Eye Problems	N	Prevalence (95% CI)	Boys, n (%)	Girls, n (%)
Watering from eye or accumulation of discharge	99	14.8 (12.2–17.7)	56 (16.3)	43 (13.1)
Visual inattention	34	5.1 (3.5–7.0)	21 (6.1)	13 (4)
Redness of eye present	27	4 (2.7–5.8)	15 (4.4)	12 (3.7)
Eyeball whitish or brown	13	1.9 (1.0–3.3)	9 (2.6)	4 (1.2)
Problem with normal vision (whitish pupillary reflex)	12	1.8 (0.9–3.1)	8 (2.3)	4 (1.2)
Structural deformity	4	0.6 (0.2–1.5)	1 (0.3)	3 (0.9)

We found no significant difference in the prevalence of any of the eye problems between boys and girls. Mother's education level or age had no association with any of the eye problems among the infants.

4. Discussion

The most common problem found in the infants in this study was watering from the eyes which have been found common in other studies as well [19–21] and can be caused by a variety of problems [22]. Also, in a population study where 20% children were found to have this abnormality, in almost all cases (95%) the onset of watering from the eye was during the first month of age [21]. Nasolacrimal duct (NLD) obstruction is considered to be the most common diagnosis for watering from eyes with discharge among infants [23]. Also, the canalisation of the NLD is a common occurrence during the first month of life [24]. According to a recent book published, 30% of the infants may have watering of the eye which can be easily cured. The study also showed that 96% of the cases are resolved spontaneously [21]. However, if the condition is left untreated, it may lead to prolonged nasolacrimal

duct impotency and the complications of secondary infection [19]. Therefore, a structured referral mechanism may need to be established in refugee setting for better examination of infants' eyes.

A variety of causes can cause visual inattention in infants [25], and ophthalmologists can confirm the diagnosis. In our study at the field level, the eye problems identified validates the need for further investigation of the infants by clinically trained care providers or ophthalmologists in the refugee camps. As blindness among children and adults is a common problem in most, if not all, refugee communities, inadequate eye care services and a scarcity of literature on eye problems lead to generating less stimulation and involvement of the donors and funders to take initiatives to prevent blindness of growing children [26]. Our project trained and enabled the community members to work within their communities and identify signs for eye problems in the refugee children. Similarly, to identify cases of blindness in children of the host country key informants method had been used, validated and succeeded in identifying blindness in children [5,8,27]. The present study suggests that this approach can be applied in the refugee context as well to identify and prevent cases of childhood blindness.

Studies in other refugee camps in a resource-constrained country such as Uganda [13], Pakistan [12] addressed the prevalence of eye impairments, eye diseases, and blindness in the refugees. The prevalence survey in Pakistan among Afghan refugees found that the leading cause of blindness was cataract and uncorrected refractive errors [12] which could easily be prevented if detected earlier at a younger age. To address the need for a vulnerable population, comprehensive vision screening, improved access to eye care centres and creating evidence-based guidelines are essential [28]. Our findings from this study reinforce the idea in the Rohingya refugee camps in Bangladesh as well.

5. Conclusions

The prevalent eye problems demand eye care set up for the screening of eye problems in the camps with proper referral and availability of referral centres with higher service in the subdistricts and districts. This study validates the need to revisit screening facilities for common eye problems inside camps and provide the community with options to avail eye care referral services provided at higher facilities within the districts.

6. Limitation

The study could not follow up the referred cases and ensure the required eye care of the screening positive infants with eye problems. The reason for the higher prevalence of watering from the eye was not investigated whether it is ophthalmia neonatorum or not and any preventive measures could not be formulated.

Supplementary Materials: The following are available online at http://www.mdpi.com/2414-6366/5/1/21/s1, Table S1: Eye Questionnaire for 0-59 days of Rohingya infants.

Author Contributions: A.E.H. conceived the study, provided administrative support in carrying out the study and drafted the manuscript. Z.A.A. was a co-investigator, carried out the experiment, performed the analytic calculation, interpreted the study findings, and wrote the first draft of the manuscript. K.I. coordinated the overall study, helped supervise the project and commented on several drafts of the manuscript. A.E.K. contributed to adapting the eye questionnaire from the Integrated Management of Childhood Illness (IMCI) protocol and drafting the manuscript. R.H. designed its protocol, supervised the study, helped secure funding and commented on several drafts of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Tropical Medicine and Infectious Disease

MDPI

Review

Knowledge, Attitudes and Perceptions of Immigrant Parents Towards Human Papillomavirus (HPV) Vaccination: A Systematic Review

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Abstract: Background: Our understanding about knowledge, attitudes and perceptions (KAP) of immigrants regarding human papillomavirus (HPV) vaccine is poor. We present the first systematic review on KAP of immigrant parents towards HPV vaccine offered to their children. Methods: Major bio-medical databases (Medline, Embase, Scopus and PsycINFO) were searched using a combination of keyword and database-specific terms. Following identification of studies, data were extracted, checked for accuracy, and synthesised. Quality of the studies was assessed using the Newcastle Ottawa Scale and the Joanna Briggs Institute Qualitative Assessment tool. Results: A total of 311 titles were screened against eligibility criteria; after excluding 292 titles/full texts, 19 studies were included. The included studies contained data on 2206 adults. Participants' knowledge was explored in 16 studies and ranged from none to limited knowledge. Attitudes about HPV vaccination were assessed in 13 studies and were mixed: four reported negative attitudes fearing it would encourage sexual activity; however, this attitude often changed once parents were given vaccine information. Perceptions were reported in 10 studies; most had misconceptions and concerns regarding HPV vaccination mostly influenced by cultural values. Conclusion: The knowledge of HPV-related diseases and its vaccine among immigrant parents in this study was generally low and often had negative attitude or perception. A well-designed HPV vaccine health educational program on safety and efficacy of HPV vaccination targeting immigrant parents is recommended.

Keywords: cervical cancer; human papillomavirus; HPV vaccine; knowledge; attitudes and perceptions

1. Introduction

Human papillomavirus (HPV) infection is a sexually transmitted disease and both women and men are rapidly exposed to it after the onset of sexual intercourse [1,2]. Oncogenic HPV can cause cervical, anogenital, head and neck cancers [3,4].

Cervical cancer is the fourth most common cancer found in women and the third most frequent cause of death with approximately 570,000 cases and 311,000 deaths in 2018 worldwide [5,6]. In developed countries nearly half of the cervical cancer cases are diagnosed in women aged less than 50 years old [6,7]. Rates of HPV infection vary greatly between geographic regions and population

groups. In developed countries, cervical cancer has been declining for many years largely due to the cervical cytology screening programme which is now being replaced by HPV screening. However, cervical cancer is increasing in developing countries where nationwide cervical cancer screening is currently unavailable. It is the second most common cancer in countries with a lower human development index ranking and is the most common cancer in about 28 countries [6,8]. The high-risk types, HPV 16 and HPV 18, cause 70% of all invasive cervical cancers and HPV types: 6, 11, 16, 18, 31, 33, 45, 52 and 58 together can cause 95% of cervical cancers.

HPV vaccination is the most effective method of preventing HPV infection [9]. The immunity gained via HPV vaccination is mainly responsible for the reduction in HPV infection and related cancers [10]. The main goal of this vaccination is to avoid persistent infections that may progress to an invasive carcinoma [10,11]. HPV vaccine is safe, well tolerated and has the potential to significantly reduce the incidence of HPV-associated precancerous lesions [12,13]. It can also effectively protect against certain HPV types that can lead to genital warts. This vaccine is most beneficial if delivered prior to the commencement of sexual activity [13,14]. During the last 12 years, over 80 countries have introduced national HPV vaccination programs [15]. The United States of America (USA), Australia, Canada and the United Kingdom (UK) were among the first countries to introduce HPV vaccine into their national immunization programs (Table 1). All countries programs target young adolescent girls, with some countries also having programs for adolescent males [16]. Specific target age groups differ as do catch-up vaccination recommendations. The majority of countries are delivering vaccine through school-based programs, health centres or primary care providers [15]. National HPV vaccination programs of two or three dose schedules have demonstrated a dramatic impact on population level HPV prevalence, persistent HPV infection, genital warts, and cervical intraepithelial neoplasia [17]. The coverage of HPV vaccine achieved by the national programs has been highly variable within the countries [13]. During the past ten years, since HPV vaccine was licensed, there has been an increase in immigrants from different cultures and languages travelling to the Western countries. Most of the immigrants originate from socio-economically underprivileged countries [17,18], and do not have a nationally funded HPV vaccination program (Table 1); therefore, it is reasonable to believe that most immigrants do not have a background knowledge about HPV vaccination.

Table 1. Human papillomavirus (HPV) vaccination programs in several countries that receive high numbers of immigrants from developing countries.

Countries	Year Vaccination Introduced	Vaccination Strategy	Recent Reported Coverage (Year of Data) *	Immigrant's Countries of Origin	HPV Vaccination in Countries of Origin
USA	2006	Primary care/health centre-based	48.6% (2017)	Mexico, China, Vietnam, South Korea, Portugal, Puerto Rico, Brazil, Argentina, Colombia, Peru, and other parts of South America; South Asia; Somalia, Ethiopia, Eritrea, and other African countries	Many countries of South America notably Mexico, Argentina, Brazil and Colombia have implemented national HPV vaccination, in the remaining countries it has not been implemented or still at preparatory stage
Denmark	2008	Primary care/health centre-based	40% (2018), but improving now	Poland, Syria, Turkey, Lebanon, Iraq, Palestine	In most of these countries there is no publicly funded national human papillomavirus vaccination programme

Table 1. *Cont.*

Countries	Year Vaccination Introduced	Vaccination Strategy	Recent Reported Coverage (Year of Data) *	Immigrant's Countries of Origin	HPV Vaccination in Countries of Origin
Netherlands	2010	Mixed: School-based and primary care-based	45.5% (2018)	European countries, Japan, USA, Australia, Indonesia, Turkey, Surinam, Morocco and Somalia	Except for the Western immigrants, most non-Western immigrants don't have a vaccination policy in their home countries.
UK	2008	School-based	83.8% (2017/18)	Indian subcontinent, Poland, China, Nigeria, Somalia, Central America, and many other countries of Asia, Africa and Europe	In large majority of these countries there is no publicly funded national human papillomavirus vaccination programme but started in some countries (e.g., Sri Lanka) in recent years
Sweden	2011	School-based	80% (2018)	Middle East, Africa, Asia, Eastern Europe	In large majority of these countries there is no publicly funded national human papillomavirus vaccination
Puerto Rico	2006	Primary care/health centre-based	49.9% (2014)	Mainly from Dominican Republic	In Dominican Republic school-based HPV vaccination was announced in 2016

* If not specified this coverage data is for adolescent girls.

Knowledge and understanding of HPV infection and HPV vaccine are important factors in decision-making about disseminating the vaccine [13]. Since the licensure of HPV vaccine in 2006, research regarding the uptake of HPV vaccine among ethnic minorities, immigrants and refugees, has been limited [18,19]. This is attributed to factors such as language barrier and cultural differences, legal issues, religion, education, lack of specialized migrant health services and lack of awareness among migrants of their rights [20]. To our knowledge, there is no systematic study on immigrant parents' knowledge, attitudes and perceptions (KAP) towards HPV vaccination. This study aims to address this research gap by systematically synthesising published data on immigrant parents' KAP towards HPV disease and vaccination offered to their children to inform future efforts to increase HPV vaccine coverage.

2. Materials and Methods

Literature searches were performed using OVID Medline (1946–April 2019), OVID Embase Classic (1947–April 2019), PsycINFO (1806–May 2019) and SCOPUS (1945–May 2019). The searches used a combination of data base-controlled vocabulary terms and text word terms. These included "Papillomavirus vaccines", "Human Papillomavirus vaccine", "knowledge, attitudes, perceptions", "emigrants", "immigrants", "population groups", "ethnic groups", "refugees", "mothers", "fathers" and "parents". Searches were conducted from 2007 to 2019. The final search was conducted on 1 May 2019. No language or date restrictions were applied. The OVID Medline search strategy used is available upon application to authors. We additionally searched the reference lists of review articles to identify original research articles describing knowledge, attitudes and perceptions of HPV vaccine among immigrant parents.

For inclusion in this review, papers needed to discuss knowledge or attitudes or perceptions of immigrant parents (defined as parents who have been permanently living in a foreign country along with their children) and/or primary immigrant caregivers towards HPV vaccine. Papers were excluded if they did not include the views of parents or only discussed other childhood vaccines. Perception was defined as how parents interpreted/perceived HPV vaccine in light of their life experiences, and attitude was defined as their reactions to those perceptions. After screening the titles, full texts were retrieved and reviewed, and data were extracted in an Excel sheet by the first author. The data collection form included the author, year, country of study, method, population, result of the study. Another author (HR) checked data abstraction and any discrepancy was resolved through discussion then data were synthesised. The quality of included studies was assessed by Newcastle Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp and by Joanna Briggs Institute (JBI) Critical Appraisal tools for use in JBI Systematic Reviews Checklist for Qualitative Research https://joannabriggs.org/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Qualitative_Research2017_0.pdf.

3. Results

In this systematic review, 311 titles from four databases were retrieved in total. There were 134 duplicates leaving 177 records to be screened. Of 177 titles, 121 were excluded for not meeting inclusion criteria. The full texts of the remaining 56 titles were assessed. Of these 36 studies were determined to be out of scope of this systematic review and excluded with reasons, the remaining 19 articles met the eligibility criteria of the systematic review as shown in the PRISMA flowchart (Figure 1). There were 12 qualitative studies and five quantitative studies and two mixed method studies.

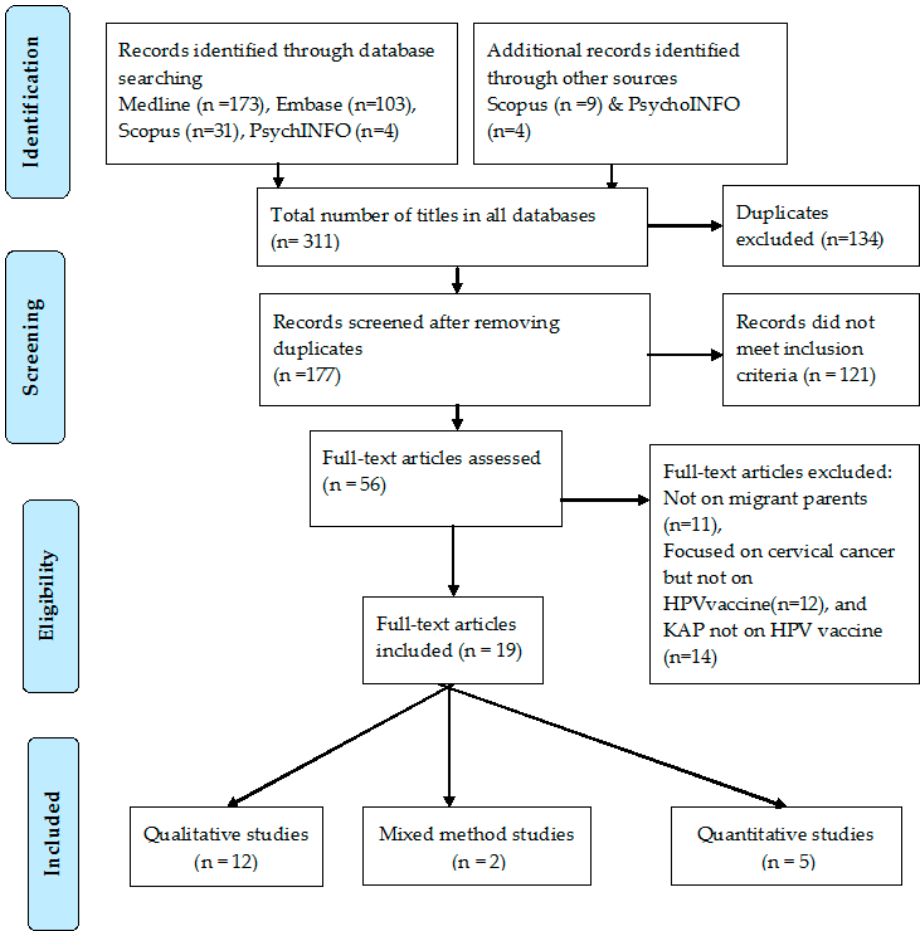

Figure 1. PRISMA flow diagram of the systematic review.

Total number of participants in all included studies was 2206 (M = 74, F = 1976 in addition to 156 parents with gender unclassified) with a male to female ratio of 1:27, where data were provided. Where age of interviewees was mentioned, the range varied from 18 to 66 years. Twelve studies were conducted in the USA, three in the UK, one in the Netherlands, one in Denmark, one in Sweden, and one in Puerto Rico. Six studies were conducted in community organizations including faith-based centres like churches and mosques [21–26], eight in health and social service agencies [27–34], two in schools and/or community groups [35,36], another two in social clubs [37,38], and one in a household [39].

Of the 19 studies, 16 reported on knowledge of the immigrant parents about HPV vaccine (Table 2), 13 reported their attitudes (Table 3) and 10 recorded perceptions (as defined by study author) towards HPV vaccine (Table 4). Four studies reported knowledge and attitudes [21,27,30,37] and one reported knowledge and perceptions [26], seven studies reported on all three outcomes (knowledge, attitude and perceptions) [22,23,29,35,36,38,39].

All included studies discussed the KAP of immigrant populations. If the study author(s) used the term "ethnic minority" to represent, we have similarly reported this term in the result tables.

For knowledge, the level of parents' knowledge about HPV disease and HPV vaccine ranged from no knowledge in 11 studies [21–24,26,27,29,33,35,37,39] to limited knowledge regarding HPV and HPV vaccine, as they heard about the vaccine but they did not know HPV vaccine's purpose, the eligibility requirements for the vaccine, and the vaccine's dosing/schedule requirements in three studies. Five studies revealed that some participants had not heard of HPV disease or HPV vaccine [27,33,35,39]. There were four studies that reported participants had no prior knowledge of HPV as a sexually transmitted disease or as a cause of cancer [25,30,32]. In four studies, participants described a lack of information and knowledge about the purpose of HPV vaccination, and HPV transmission [21,29,37]. Two studies found participants had limited knowledge regarding the relation between sexual transmission of HPV and cervical cancer [22,36] (Table 2).

In regards to attitudes towards HPV disease and HPV vaccine (Table 3), a number of non-vaccinating ethnic minority parents had negative attitudes to HPV vaccination thinking it would encourage unsafe sexual practices and promiscuity [22,30,35]. However, three studies showed that once parents were informed about the vaccine during the focus groups, they became keen to vaccinate their children [34,36,37]. Non-vaccinating and partially vaccinating parents from various ethnic backgrounds expressed concerns about potential side effects [35]; religious values and cultural norms also influenced vaccine decision-making [28,29], and a majority of participants (regardless of vaccination status) had a more positive attitude towards vaccination when they received information about HPV vaccine (Table 3).

Participants had misperceptions about HPV vaccine. The main reasons for declining HPV vaccine were their religious belief and culture; in particular, their belief that abstinence from sex before marriage would provide protection from disease [22,31,36]. Awareness of a health intervention is recognised as necessary but not sufficient condition for performing a health behaviour. As women become aware of HPV vaccine, they may have additional questions or concerns that may function as barriers to getting their daughters vaccinated [31] (Table 4).

Most studies were of generally good quality. When scored against the checklist used, ten qualitative studies received eight out of a possible 10 points, and one 10 of 10 [37]. Four of the eight quantitative observational studies scored eight of nine points, and the other scored seven of nine points (Table 5).

Table 2. Studies reporting knowledge of immigrants about HPV vaccine (16 articles).

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Countries of Origin	Population	Mean Age in Years (Range)	Gender (n)		Knowledge Results
						Male	Female	
Aragones et al., 2016 [21]	New York City, USA	Not reported	Colombia, Dominican Republic, Ecuador, Mexico	36 Latino immigrants	42 (25–65)	3	33	Most parents were either not informed or possessed inaccurate knowledge about HPV and HPV vaccine.
Forster et al., 2016 [35]	Southwest England, UK	1 March 2015–1 March 2016	Indian subcontinent, Caribbean, Africa	33 Minority ethnic	47 (36–62)	1	32	Thirteen immigrant parents had not heard about HPV vaccine.
Glenn et al., 2015 [27]	Los Angeles, USA	January 2009–January 2010	Latina, China, Korea, Africa and others	490 Minorities ethnic	44 (7.2)		490	One third of participants had never heard of HPV or HPV vaccine and had low knowledge. About 63% (n = 306) of respondents heard of HPV and another 61% (n = 294) heard of HPV vaccine.
Kepka et al., 2015 [24]	Salt Lake City, USA	Not reported	Mexico, Puerto Rico, Brazil, Argentina, Peru, and Portugal	118 Mexican immigrants	18–50 (±2.4)	18	97	Majority had no knowledge about HPV vaccine.
Mupandawana1 et al., 2016 [38]	North England, UK	Not reported	South Africa, Zimbabwe, Nigeria, Kenya, and Zambia	10 African immigrants	Not reported	5	5	Most participants had inaccurate knowledge about HPV vaccine.
Allen et al., 2012 [30]	Boston, USA	February–May 2008	Hispanic and African American	64 immigrants	Not reported	19	45	The majority of parents felt that they did not have adequate information about HPV or HPV vaccine to make an informed decision.
Salad et al., 2015 [29]	Netherlands	March–June 2013	Somalia	6 immigrants	(23–66)		6	Participants described a lack of information about HPV vaccine.
Salad et al., 2015 [29]	Netherlands	March–June 2013	Somalia	6 immigrants	(23–66)		6	Participants described a lack of information about HPV vaccine.

Table 2. *Cont.*

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Countries of Origin	Population	Mean Age in Years (Range)	Gender (n)		Knowledge Results
						Male	Female	
Luque et al., 2012 [26]	Georgia, USA	Not reported	Mexico and Honduras	12 Hispanic immigrants	(25–44)	7	5	Parents had little knowledge about HPV vaccine.
Bodson et al., 2016 [25]	Salt Lake City, Utah, USA	August 2013–October 2013	Mexico and others	108 Hispanic/Latino immigrants101 born out USA	(16– >50)	16	92	Participants born in Mexico or elsewhere (Spanish background) had lower factual knowledge than participants who were born in the United States. In total, 67.3% of participants had heard of HPV vaccine and 76.4% of HPV.
Marlow et al., 2009 [39]	UK	July–August 2008	Indian subcontinent, Caribbean, Africa, China	Ethnic minority	(16– >50)		601	Almost half of ethnic minority parents had not heard of the vaccine before being invited to vaccinate their daughters.
Greenfield, et al., 2015 [23]	Washington, USAWashington, USA	Not reported	Mexico, Somalia, Ethiopia and Eritrea	156 immigrants' parents	41		Not reported	Lack of knowledge about HPV vaccine was the main reason given by parents that their adolescents had not been vaccinated.
Zeraiq et al., 2105 [37]	Denmark	January 2011–January 2012	Lebanon, Iraq, Palestine	23 immigrants	Not reported		23	Ethnic minorities had lack of knowledge about HPV and HPV vaccine.
Grandahletal., 2012 [36]	Uppsala, Sweden	February–June 2011	Middle East, Africa, Asia, East ern Europe	50 immigrants	(18 [28]–60)		50	The participants had limited knowledge about HPV and cervical cancer and HPV vaccine. Lack of knowledge was the main reported barrier to vaccination.

Table 2. *Cont.*

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Countries of Origin	Population	Mean Age in Years (Range)	Gender (n)		Knowledge Results
						Male	Female	
Hopfer et al., 2017 [32]	CA, USA	July 21–August 20, 2016.	Latina and Vietnamese	48 immigrants	(18–26)		48	Lack of awareness about HPV was evident in women's stories, including confusing HPV with HIV, not knowing that HPV is a sexually transmitted infection. Vietnamese participants (96% (23/24)) were unable to elaborate on what HPV was, many were uncertain about its significance, 25% (2/8) unvaccinated Latina had never heard of HPV.
Stephens et al., 2014 [22]	Haiti, USA	October 2010–May 2011	Haiti	31 immigrants.	(18–22 yrs.)		31	Mothers had no knowledge about HPV (80.6% (25/31)), very knowledgeable (3.2% (1/31)), fairly knowledgeable (12.9% (4/31)), somewhat knowledgeable (3.2% (1/31)). Mothers had no knowledge about HPV vaccine (83.9% (26/31)), very knowledgeable, fairly knowledgeable (9.7% (3/31)), somewhat knowledgeable (6.4% (2/31)).
López, et al., 2016 [33]	San Juan, Puerto Rico	Not reported	Dominican Republic	60 immigrants	38.6 (±7.2 yrs.)	5	55	Parents had not heard about HPV (3.3% (2/60)) and yes heard (91.7% (55/60)). Parents had not heard about HPV vaccine for males (38.3% (23/60)), had heard (55% (33/60))

Table 3. Studies reporting attitudes of immigrants about HPV vaccine (13 articles).

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Population	Mean Rge in Years (Range)	Gender (n)		Attitudes Results
					Male	Female	
Aragones et al. 2016 [21]	NYC (New York City), USA	Not reported	36 immigrants	42 (25–65)	3	33	Parents were motivated to protect the health of their children and were keen to obtain more information regarding HPV and the vaccine.
Forster et al., 2017 [35]	Southwest England	1 March 2015–1 March 2016	33 Ethnic minorities	47 (25–65)	1	32	Ethnic minority mothers said HPV vaccine was unnecessary as they had been fine without it. Parents expressed a wide range of concerns about the vaccine. A number of non-vaccinating ethnic minority parents believed their daughters were not at risk of contracting HPV or developing cervical cancer.
Glenn et al., 2015 [27]	Los Angeles, USA	January 2009–January 2010	Ethnic minorities	44 (7.2)		490	Ethnic minorities had positive and negative attitudes towards HPV vaccine: 63% of participants expressed positive attitudes towards immunization against HPV disease is a good thing. Participants with negative attitudes (54%): that Immunizations have more side effects than benefits.
Albrigh et al., 2017 [28]	Colorado, USA	July 2012–January 2013	41 Ethnic minorities	(18– >50)	3	38	The most common reported reasons for non-initiation and non-completion among English-speaking parents included a low perceived risk of HPV infection, vaccine safety concerns, and distrust of government and/or medicine. Spanish-speaking parents who had either not encouraged initiation of HPV vaccine series or had not explained the necessity of completing the series, cited concerns that vaccination would encourage sexual activity.
Mupandawana et al., 2016 [38]	North England, UK	Not reported	10 Ethnic minority	Not reported	5	5	Majority of participants said HPV vaccine was unacceptable, with fear of promiscuity, infertility and concerns about it being a new vaccine with unknown side effects. Religious values and cultural norms influenced vaccine decision-making with fathers acting as the ultimate decision-maker.

Table 3. *Cont.*

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Population	Mean Rge in Years (Range)	Gender (n) Male	Gender (n) Female	Attitudes Results
Allen et al., 2012 [30]	Boston, USA	February–May, 2008	64 Ethnic minority	Not reported	19	45	Participants distrust medical providers and pharmaceutical companies.
Salad et al., 2015 [29]	Netherland	March to June 2013	6 Immigrants	(23–66)		6	Most mothers have distrust towards the Dutch health care system and government and doubts about HPV vaccine age.
Marlow et al., 2009 [39]	UK	July to August 2008.	Ethnic minority	(16– >50)		601	Parents with strong religious or cultural views were less likely to accept HPV vaccine. Consistency with attitudes to HPV testing, which some minority women felt reflected non-traditional cultural or religious practices and were concerned it encouraged premature sex.
Greenfield et al. 2015 [23]	Washington, USA	Not reported	156 immigrants	41		156 gender not distinguished	All three ethnic groups expressed a desire to access vaccine information in their respective languages.
Zeraiq et al., 2015 [37]	Denmark	January 2011 to January 2012	23 Ethnic minority	Not reported		23	All participating mothers accepted the vaccine for their daughters to prevent cervical cancer.
Grandahl et al., 2012 [36]	Uppsala, Sweden	February to June 2011	50 immigrants	(18–60)		50	Participants' expressed that they accepted the vaccination for their daughters, as it was important for their future health. Some women considered girls in the target group were too young and it would be better to wait until they were a little older and had become women.

Table 3. *Cont.*

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Population	Mean Rge in Years (Range)	Gender (n) Male	Gender (n) Female	Attitudes Results
Perkins et al., 2010 [34]	Boston, Massachusetts, USA	June 2007 to February 2008.	72 Immigrants	Not reported	3	69	Attitudes differed dramatically by ethnicity; only 11% of Caucasian parents endorsed school HPV vaccine entry requirements, compared with 78% of African-American parents, 60% of Afro-Caribbean and African parents, and 90% of Latino parents. Most parents expressed favorable opinions toward HPV vaccine for their own daughters.
Stephens et al., 2014 [22]	Haiti, USA	October 2010–May 2011	31 immigrants	(18–22)		31	Immigrant mothers who had little knowledge about HPV or the vaccine, felt unsure about vaccination; their concern centered on conflict with cultural values and perceptions of risks associated with HPV vaccine.

Table 4. Studies reporting perceptions of immigrants about HPV vaccine (12 articles).

Author(s), Publication Year [Ref]	Country of Study	Year of Study	Population	Age	Gender (n)		Perception Results
					Male	Female	
Forster et al., 2016 [35]	southwest, England	1 March 2015–1 March 2016	33 Ethnic minority	47 (25–65)	1	32	Non-vaccinating ethnic minority parents reassured themselves of their decision by reporting that there are approaches other than vaccination to protect against HPV, such as abstinence from sex before marriage, which was related to religious beliefs.
Mupandawana1 et al., 2016 [38]	North England, UK	Not reported	10 Ethnic minority	Not reported	5	5	HPV vaccine was generally unacceptable within this African community, with culture and religion influencing risk perceptions toward the vaccine and playing important roles in vaccination decision making.
Salad et al., 2015 [29]	Netherland	March–June 2013.	6 Immigrants	(23–66)		6	Participants' belief that abstinence from sex before marriage protect from diseases.
Luque t al., 2012 [26]	Georgia, USA	Not reported	Hispanic immigrant	Not reported	7	5	Participants had misperceptions about HPV vaccine. They think that the vaccine is unnecessary if they are not having sex.
Albright et al., 2017 [28]	Colorado, USA	July 2012–January 2013	41 Ethnic minority	(18 to 50)	3	38	Spanish-speaking parents concerned that vaccinating against HPV would encourage sex. These parents expected their daughters to abstain from sex until marriage, and they did not want to give their daughters the message that sexual activity was permissible or give them a false protection.
Marlow et al., 2009 [39]	UK	July – August 2008.	950 Ethnic minority	(16– >50)		601	The main reason for declining HPV vaccine was religious belief. The importance of religion appears to come from a strong belief in sexual abstinence until marriage.
Greenfield et al. 2015 [23]	Washington, USA	Not reported	156 Immigrants	41		156 gender not stated	All three minority ethnicities had misperceptions about HPV vaccine or HPV disease. Most participants do not believe children are at risk and believe the vaccine could lead to early initiation of sexual activity.
Grandahl et al., 2012 [36]	Uppsala, Sweden	February - June 2011	50 immigrants	(18–60)		50	Cultural influences on perceptions about protection: participants believed a woman did not have sexual intercourse with a man before marriage.
Baldwin et al., 2012 [31]	Texas, USA	December 2008–May 2010	256 Ethnic minority	42.3		256	Non-White participants were significantly less likely to have talked with others and looked for information about HPV vaccine than White participants. Mothers' perceptions of vulnerability, severity, varied by race/ethnicity.
Stephens et al., 2014 [22]	Haiti, USA	October 2010–May 2011.	31 Immigrants	(18–22)		50	Most mothers were willing to have their daughters vaccinated against HPV if it would protect or improve their health. Some mothers did not support HPV vaccine for their daughters; the remaining mothers were unsure because of their lack of knowledge. For those mothers who were unsure; concerns centred on conflict with cultural values and their perceptions of the risks associated with the vaccine.

Table 5. Quality assessment of the included studies.

Author (Ref)	Score	Remarks
Qualitative Studies Assessed by Joanna Briggs Institute Critical Appraisal Checklist		
Aragones et al., 2016 [21]	Met 8 of 10 positive criteria	
Allen et al., 2012 [30]	Met 10of 10 positive criteria	
Zeraiq et al., 2105 [37]	Met 10 of 10 positive criteria	
Albright et al., 2017 [28]	Met 8 of 10 positive criteria	
Grandahl et al., 2012 [36]	Met 8 of 10 positive criteria	
Stephens et al., 2014 [22]	Met 8 of 10 positive criteria	
Forster et al., 2016 [35]	Met 8 of 10 positive criteria	Nil
Mupandawana et al., 2016 [38]	Met 8 of 10 positive criteria	
Salad et al., 2015 [29]	Met 7 of 10 positive criteria	
Luque et al., 2012 [26]	Met 8 of 10 positive criteria	
Perkins et al., 2010 [34]	Met 8 of 10 positive criteria	
Hopfer et al. 2017 [32]	Met 8 of 10 positive criteria	
Quantitative Studies Assessed by Newcastle Ottawa Scale		
Baldwin et al., 2012 [31]	Scored 7 of 9 stars	
Bodson et al., 2016 [25]	Scored 7 of 9 stars	It is a mixed method study
Glenn et al., 2015 [27]	Scored 7 of 9 stars	Nil
Greenfield et al., 2015 [23]	Scored7 of 9 stars	It is a mixed method study
Kepka et al., 2015 [24]	Scored 7 of 9 stars	
López, et al., 2016 [33]	Scored 8 of 9 stars	Nil
Marlow et al., 2009 [39]	Scored 8 of 9 stars	

4. Discussion

This systematic review identifies gaps in knowledge, attitudes, and perceptions about HPV infection and its vaccine among immigrant parents in western countries. Our analyses indicate that although HPV vaccine has been in use for over a decade, information about this vaccine, and HPV infection in general, and its relation to cancer in particular, does not appear to have been well disseminated to immigrant parents. Most participants in 12 included studies had no knowledge about HPV vaccine (Table 2), one third of participants in two studies reported receiving no information about HPV vaccine, [27,35]. All participants in one study have not even heard of the vaccine [29]. This systematic review showed participants had both negative and positive attitudes towards HPV vaccination, and most participants had misconceptions about HPV vaccination.

In concordance with our systematic review findings, semi-structured interviews conducted with non-parent immigrant participants also showed limited knowledge about HPV infection its vaccine. For example, a study conducted in a Western Canadian province, found participants had limited knowledge about HPV. Most women perceived their risk of HPV to be low but reported willingness to receive the vaccine when recommended by their doctors [19]. Similarly [35], in Italy, knowledge and attitude toward HPV infection and vaccination among non-parent immigrants and refugees was low [40]. In Sweden, adolescent school students were interviewed in relation to their beliefs and knowledge about HPV prevention: HPV vaccination was found to be associated with ethnicity and the mothers' education level; i.e., girls with a non-European background, including those of Arabic background, and with a less educated mother were less likely to have received the vaccine. Vaccinated girls perceived HPV infection as more severe, had more insight into women's susceptibility to the infection, perceived more benefits of the vaccine as protection against cervical cancer and had a higher intention to engage in HPV-preventive behaviour [41].

Furthermore, another systematic review that explored knowledge and attitudes of Iranian people towards HPV vaccination found that the overall knowledge and awareness about HPV vaccination was low; however, their attitude toward HPV vaccination was positive and strong [42]. This corroborates the findings from three studies included in our systematic review that showed positive attitude towards HPV vaccines once parents were informed about it during focus groups. [34,36]. This could possibly

explain why the negative attitude to HPV vaccination found in most of the studies included in our systematic review was stemmed from poor knowledge/misconceptions and may change after providing the right information.

Unlike the immigrants, mainstream populations of USA had better knowledge and more positive attitudes toward HPV vaccine. A quantitative study conducted in Southern California compared knowledge and acceptability between US-born African Americans and African immigrants, and between US-born Latinas and Latina immigrants. African and South American immigrants were less likely to know where they can get/refer for HPV vaccine and less likely to have heard about HPV vaccine than South Americans and US-born Africans [43]. Similarly, a study in Denmark found that refugee girls, mainly from Muslim countries, had significantly lower HPV immunization uptake compared to Danish born girls, indicating that refugee girls may face challenges to access and use of immunization services [44].

A study in 2018 indicated that the increase in refusal and hesitancy of Muslim parents to accept childhood vaccination was identified as one of the contributing factors in the increase of vaccine-preventable diseases cases in several countries such as Afghanistan, Malaysia and Pakistan. News disseminated via some social media outlets claiming that the vaccine has been designed to weaken Muslims, reinforced the suspicion and mistrust of vaccines by parents [45]. A qualitative study of the views of young non-parent Somali men and women in the USA demonstrated that the participants had limited knowledge about the vaccination and had suspicions concerning the effectiveness or value of immunization, with most participants stating that the Somali community was mostly Muslim and did not engage in sexual activity before marriage [46]. A cross-sectional study included in our systematic review conducted to evaluate awareness of women from major UK ethnic minority groups (Indian, Pakistani, Bangladeshi, Caribbean, African and Chinese women) toward HPV vaccination identified that those from non-Christian religions were less accepting of the vaccine (17–34%). The study concluded that some cultural barriers could be addressed by tailored information provided to ethnic minority groups [47].

Attitudes toward HPV vaccine are important in HPV vaccine uptake. Our systematic review revealed certain attitude-related barriers to vaccine acceptability for adolescents, particularly vaccine hesitancy among some mothers. A qualitative study reported that Latin American immigrant mothers of adolescent daughters expressed more hesitancy regarding adolescent vaccines compared to childhood vaccines expressed an increased sense of belief in their ability to determine what is best for their children [48]. In contrast to the negative attitudes of immigrant parents as found in most of the included studies in our systematic review, most mainstream non-immigrant women had positive attitudes about receiving an HPV vaccine and high intention to receive the vaccine both for themselves and their daughters [49]. Variables associated with intention to vaccinate included knowledge, personal beliefs, confidence that others would approve of vaccination, and having a higher number of sexual partners [49]. However, negative or variable attitudes of parents to vaccinate their children have been reported in a systematic review involving Turkish population [50]. The systematic review showed that between 14.4% and 68.0% of Turkish parents were willing to have their daughters vaccinated with HPV vaccine and between 11.0% and 62.0% parents were willing to have their sons vaccinated [50], suggesting a negative attitude may not be just a phenomenon of immigrants, many non-immigrants in their own countries too may have negative attitudes towards HPV vaccination. However, since this attitude appeared amenable to change in our systematic review, innovative simple interventions may improve attitudes to HPV vaccination. For instance, a higher vaccination rate was achieved at three clinics in Texas, USA among children and adolescents through the involvement of patient navigators. The patient navigators met the parents of unvaccinated or incompletely vaccinated children while they waited for their children's health providers in private clinic rooms to confirm the need for additional HPV vaccine doses. Parents of children who needed ≥1 dose were offered personal counselling and given handouts in English or Spanish on HPV vaccine. Following such counselling about 67% parents

got their children vaccinated either immediately or at a follow-up visit soon thereafter, indicating that providing counselling in a clinic setting can improve vaccination acceptance [51].

To our knowledge this is the first systematically conducted review of HPV vaccination knowledge, attitudes and perceptions among immigrants. Most included studies were of acceptable quality. We failed to identify research regarding knowledge, attitudes and perceptions of immigrant parents towards HPV vaccine in developing countries. Some papers did not clearly distinguish between attitudes and perceptions as outcomes. However, these studies suggest that tailored educational programs to improve KAP on HPV vaccine among immigrant parents may be a valuable intervention for HPV vaccination uptake.

5. Conclusions

Parental knowledge and attitudes towards HPV vaccine have been examined in many recent studies and lower uptake of HPV vaccine among immigrants, refugees and ethnic minorities has been documented. Our results support the pressing need to develop an intervention aimed to improve HPV vaccination uptake in these populations. More research is needed in the design and evaluation of tailored educational resources for ethnic minority groups, particularly in the framework of the vaccination programme.

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Tropical Medicine and Infectious Disease

MDPI

Review

The COVID-19 Pandemic: Disproportionate Thrombotic Tendency and Management Recommendations

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Abstract: COVID-19 is an infectious disease caused by the SARS COV-2 virus. Patients with COVID-19 are susceptible to thrombosis due to excessive inflammation, platelet activation, endothelial dysfunction, and circulatory stasis, resulting in an increased risk of death due to associated coagulopathies. In addition, many patients receiving antithrombotic therapy for pre-existing thrombotic diseases can develop COVID-19, which can further complicate dose adjustment, choice and laboratory monitoring of antithrombotic treatment. This review summarizes the laboratory findings, the prohemostatic state, incidence of thromboembolic events and some potential therapeutic interventions of COVID-19 associated coagulopathy. We explore the roles of biomarkers of thrombosis and inflammation according to the severity of COVID-19. While therapeutic anticoagulation has been used empirically in some patients with severe COVID-19 but without thrombosis, it may be preferable to provide supportive care based on evidence-based randomized clinical trials. The likely lifting of travel restrictions will accelerate the spread of COVID-19, increasing morbidity and mortality across nations. Many individuals will continue to receive anticoagulation therapy regardless of their location, requiring on-going treatment with low-molecular weight heparin, vitamin K antagonist or direct-acting anticoagulants.

Keywords: anticoagulant; antiplatelet; antithrombotic therapy; COVID-19; SARS-CoV-2; thrombosis; disseminated intravascular coagulation

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1. Background

Travel has greatly accelerated the global spread of COVID-19 and has so far affected over 107 million people with more than 2.3 million deaths (as of 10 February 2021) [1]. SARS-CoV-2, the cause of the COVID-19 pandemic, replicates in the upper respiratory tract to enable active viral shedding with minimal symptoms [2]. Survival of the virus for 24 to 72 h on different types of surfaces further facilitates fomite transmission [3], allowing the virus to be readily transmissible in travel settings. The early symptoms of COVID-19, such as fever, fatigue, headache, cough, shortness of breath, diarrhea and myalgia, are similar to those in other viral infections [4]. The virus binds to the angiotensin converting enzyme 2 (ACE2) receptor, which is expressed at higher levels in males compared to females, and also in Asians compared to white Caucasians or Africans [5]. The clinical course of the disease can be divided into three phases: the viremic phase, the acute or pneumonic phase and the severe or recovery phase [6]. Much like other virulent zoonotic coronavirus infections such

as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome corona virus (MERS-CoV), COVID-19 can potentially lead to systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS), multi-organ dysfunction and shock [7]. Though severe COVID-19 and its complications are common in the elderly and individuals with comorbidities such as diabetes and cardiovascular diseases, younger and healthy persons are not always spared and can also develop severe and complicated disease [8]. Increases in lactate dehydrogenase, C-reactive protein (CRP), D-dimer, ferritin and interleukin-6 (IL-6) are common laboratory findings in patients with COVID-19 [4,9]. Plasma IL-6 levels can correlate with disease severity and pro-coagulant states [9].

2. Possible Pathophysiology of Coagulopathy

There is a complex interplay between pro-inflammatory cytokine/chemokine release, increased endothelial dysfunction/damage and potential sepsis-induced coagulopathy during the acute phase of the disease, which in severe cases can increase the risk of thrombosis (Figure 1). Increased pro-thrombotic characteristics of COVID-19 likely results from (a) severe and prolonged hypoxemia that stimulates thrombosis, (b) cytokine storms in critically ill patients, and (c) a presumed role of local pulmonary thrombotic phenomena. It is presumed that prothrombotic pulmonary endothelial dysfunction leads to severe acute inflammation (through release of complement and cytokines) and blood coagulation activation with vascular microthrombosis that triggers further coagulopathy, leading to disseminated intravascular coagulation (DIC) [10].

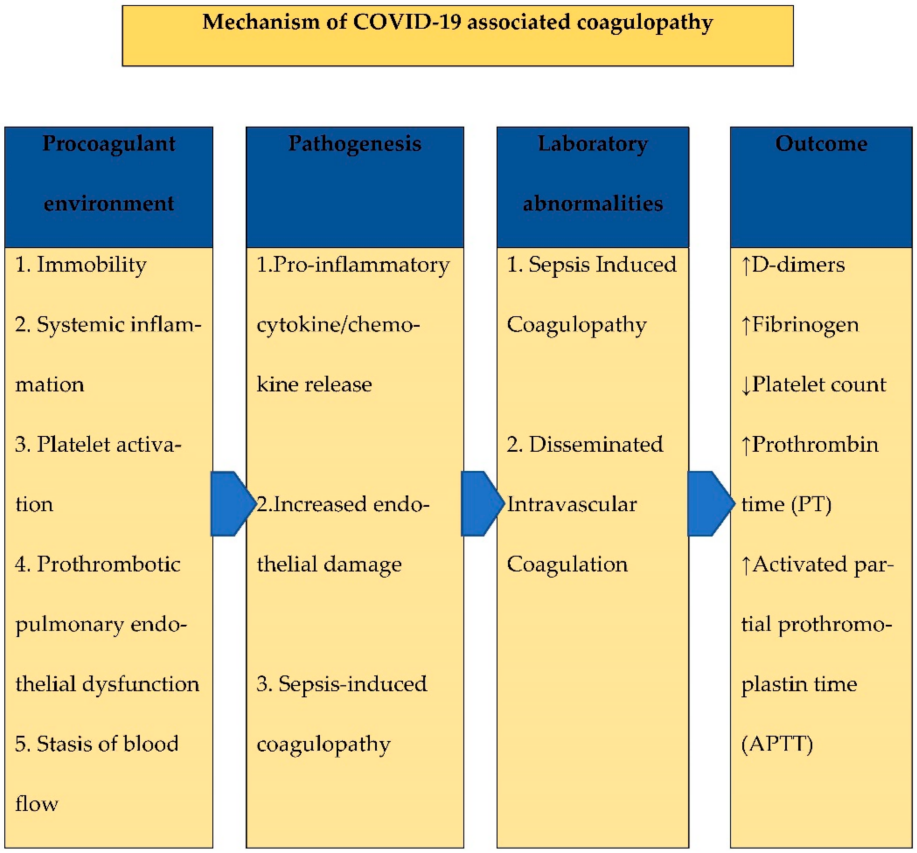

Figure 1. Mechanisms of COVID-19 associated coagulopathy.

Post-mortem histological similarities suggest that Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV), the cause of a previous endemic between 2002 and 2003, also causes ARDS with visible localized pulmonary hemorrhage, pulmonary oedema, desquamation with hyaline membrane formation and interstitial mononuclear inflammatory infiltrates.

Localized pulmonary arteriolar thrombosis observed with SARS has not yet been described in autopsy reports of patients with COVID-19 [11]. Pulmonary vasculature thrombosis is likely to result from severe hypoxia, which is a powerful stimulant of coagulation.

3. Biomarkers of Hemostasis

Thrombocytopenia and increased D-dimer levels are consistently associated with an increased need for mechanical ventilation, admission to the intensive care unit (ICU) or death [12,13]. The severity of COVID-19 is frequently associated with prolongations of prothrombin time (PT), international normalized ratio (INR) and thrombin time (TT), and a trend of increases in activated partial thromboplastin time (aPTT) [14–16]. Retrospective analysis of hospitalized patients with COVID-19 indicates high levels in D-dimers and fibrin degradation products, prolonged PTs and aPTT in non-survivors compared to survivors. It is estimated that 71% of patients succumbing to the complications of COVID-19 met the International Society on Thrombosis and Haemostasis (ISTH) criteria for DIC, compared to just 0.6% for survivors [17,18].

4. Potential Role of Complement Inhibition in COVID-19

Thrombotic microangiopathy (TMA) can occur in many different clinical conditions, including pathogenic complement activation. The complement system mediates the innate immune response that promotes inflammation, defends against bacterial infections, and often neutralizes infectious viruses [19]. Two murine studies investigated complement activation in coronavirus infections to determine whether activation of the system could be protective or pathogenic. In a murine model lacking C3 and unable to activate the common complement pathway, SARS-CoV infection severity was decreased with less respiratory dysfunction and lower cytokine levels despite equal viral loads [20], suggesting that a significant portion of SARS-mediated disease is likely immune mediated. There were increased concentrations of C5a and C5b-9 in sera and lung tissues in a mouse model of MERS-CoV infection [21]. Blocking C5a with an antibody alleviated lung and spleen damage, with decreased cytokine response and viral replication. Evidence is emerging that the complement system is overactivated in SARS-COV-2 as noted in previous coronavirus infections and this may play a central role in thrombosis and unbalanced immune response [22].

Excessive complement activation occurs in humans in a number of pathological settings, leading to diffuse TMA and end-organ dysfunction, e.g., atypical hemolytic-uremic syndrome (aHUS), a rare disorder of uncontrolled complement activation characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. TMA in aHUS results in renal dysfunction and, in rare cases, cardiac dysfunction. Importantly, aHUS is treatable with eculizumab, a C5 complement inhibitor. Early treatment with eculizumab can reverse both renal and cardiac dysfunction [23]. Although the use of complement inhibitors is limited to rare diseases, it should also be actively investigated in the treatment of COVID-19.

5. Role for Antivirals and Immunomodulatory Agents to Reduce the Development of Immunothrombosis

There are several potential control points in the pathophysiological cascade COVID-19, starting from the initial infection to later development of ARDS where targeted therapeutic interventions could reduce the severity of disease. There is a role for dexamethasone in the treatment of ARDS in moderate to severe COVID-19 infection; dexamethasone reduces mortality and has become the standard of care in addition to using anti-viral and immunomodulatory therapies. Excessive systemic inflammation in patients with severe COVID-19 is likely to deplete levels of Vit C, Vit D and Zn in many individuals. Several human and animal studies highlight the potential efficacy of supplementation with a combination of Zn, intravenous Vit C and oral Vit D. Inhibition of IL6 by tocilizumab shows beneficial effects in several clinical trials and could reduce microthrombosis. Moreover, when used at appropriate doses, these treatments generally have an exceptionally good

safety record. Aspirin (acetylsalicylic acid), the macrolide antibiotic azithromycin, oral or intravenous administration of NAC (N-acetylcysteine) has a role in inhibiting NF-κB and reducing the activation of the coagulation cascade in severe cases of COVID-19 [24].

6. D-Dimer in COVID-19 and Coagulations Disturbances

Patient health can deteriorate rapidly in severe cases of COVID-19, leading to ARDS, septic shock, metabolic acidosis and coagulopathy including DIC. Levels of D-dimers, which originate from the breakdown of cross-linked fibrin and are related to activation of coagulation and fibrinolysis, are often markedly elevated in severe COVID-19 patients (Table 1) [25,26]. A retrospective cohort study of 191 patients reported that D-dimer levels greater than 1.0 μg/mL were associated with increased mortality (p = 0.0033) in patients with COVID-19 [8]. Levels of 2.0 μg/mL or more on admission were reported as the optimum cut-off for predicting in-hospital mortality for COVID-19 [27]. Nearly 90% of inpatients with pneumonia have increased coagulation activity as marked by elevated D-dimer levels. The levels of D-dimers on admission can be used to triage patients into critical care [8,14]. Increased D-dimer levels are associated with worse outcomes even though many patients may not have full blown DIC and have near normal levels of PT, aPTT and TT.

Table 1. Levels of D-Dimers in Patients with COVID-19.

Study and References	Levels in Non-Severe Patients (Confidence Interval	Levels in Severe Patients (Confidence Interval)	Significance Level (p Value)	Comments
Huang et al. (2020) [15]	0.5 mg/L	2.4 mg/L	p = 0.0042	ICU patients had significantly higher levels of D-dimer than non-ICU patients
Tang et al. (2020) [17]	0.61 (0.35−1.29)	2.12 (0.77−5.27)	p < 0.001	Overall mortality was 11.5%, the non-survivors revealed significantly higher D-dimer levels
Zhou et al. (2020) [25]	0.6 (0.3−1)	5.2 (1.5−21.1)	p < 0.0001	D-dimer levels > 1 μg/mL can help with early identification of patients with poor prognosis
Zhang et al. (2020) [27]	0.41 mg/L (0.15–0.69)	4.76 mg/L (2.99–11.9)	p < 0.001	D-dimer levels > 2.0 μg/mL on admission can predict in-hospital mortality in patients with COVID-19 and could be a therapeutic marker
Guan et al. (2020) [28]	43.2% with >0.5 mg/L	59.6% with >0.5 mg/L	N/A	D-dimer levels higher in those requiring ICU admission and invasive ventilation; statistical analysis not performed
Tu et al. (2020) [29]	Median 0.66 g/mL	Median 3.306 g/mL	p < 0.001	D-dimer levels were significantly higher in non-survivors

7. COVID-19, Elevated Troponin and Thrombotic Disease

Increased troponin levels in patients with COVID-19 are associated with poor outcomes [30], but the differential diagnosis for elevated troponin levels in patients with COVID-19 is broad [31] and ranges from nonspecific myocardial injury, impaired renal function (leading to troponin accumulation), myocarditis, pulmonary embolism (PE) and types 1 and 2 myocardial infarction (MI) [32,33]. Similarly, elevated natriuretic peptide levels is nonspecific [32] and consideration for thrombotic events such as PE should always be guided by clinical findings. Mortality rates are higher in patients with underlying cardiovascular disease due to COVID-19 infection [34]. Levels of high-sensitivity cardiac troponin I (hs-TnI) are useful in monitoring disease progression and mortality [35].

A retrospective study of hs-TnI levels and death in patients with COVID-19 (based on SARS-CoV-2 RNA detection) reported a univariable odds ratio of 80.1 (95% CI 10.3–620.4, $p < 0.0001$), which was higher compared to other biomarkers such as D-dimers and lymphocyte counts [8]. Another study of 416 hospitalized patients reported that hs-TnI was elevated in 20% of COVID-19 patients on presentation [36]. These patients were more likely to require invasive (22% vs. 4%, $p < 0.001$) or non-invasive (46% vs. 4%, $p < 0.001$) ventilation, develop complications such as ARDS (59% vs. 15%, $p < 0.001$) or acute kidney injury (9% vs. 0%, $p < 0.001$). Clinicians should remain alert that increased levels of hs-TnI can also be related to non-ischemic causes of myocardial injury and thereby avoid inappropriate use of other resources [37].

8. Venous Thromboembolism

Venous thromboembolism (VTE) is common in patients with COVID-19, although the prevalence remains unknown. A recent scoping review reported the incidence of VTE to be 20%, with a risk of stroke of 3%; both VTE and risk of stroke are increased in severely ill patients [38]. ARDS in patients with COVID-19 can cause hypoxic pulmonary vasoconstriction, pulmonary hypertension, and right ventricular failure; further injury from severe PE can be irreversible. Risk of VTE can be screened by levels of D-dimer and fibrinogen; a retrospective study suggests that D-dimer concentrations greater than 1.0 µg/mL predicted the risk of VTE [39]. Patients with one or more predisposing factors for VTE (such as being older, elevated CRP, increased D-dimers, high fibrinogen levels, tachypnea, fever, critical illness, infectious etiology and immobility) are at greater risk of such events during hospitalization and require close monitoring.

9. Management of VTE in Patients with COVID-19

Therapeutic anticoagulation is the mainstay of VTE management in patients either with or without COVID-19 [40–42]. Prescribing an anticoagulant agent should take into consideration underlying comorbidities; bleeding risk and the treatment choices can change during hospital stay or at discharge. Parenteral anticoagulation, for example with unfractionated heparin (UFH), is preferable in some inpatients with VTE as it can be temporarily withheld or reversed as no significant interactions have been reported with investigational COVID-19 therapies. However, using UFH has some disadvantages such as the variable times to achieve therapeutically activated partial thromboplastin time ratios and increased risks of infection to health care workers during frequent blood draws. Using low-molecular-weight heparin (LMWH) may be preferred in patients who are unlikely to need further procedures. Advantages of oral anticoagulation with direct oral anticoagulant therapy (DOACs) includes minimal monitoring, improved discharge planning and outpatient management, while potential disadvantages include clinical deterioration and an inability to access reversal agents in a timely manner. Use of DOACs or LMWH is preferable in patients who are ready for discharge as it can minimize contact with health care personnel during INR monitoring. Catheter driven reperfusion and thrombolysis therapy is often recommended for management of patients with an unstable and large PE, but many patients with COVID-19 can have absolute or relative contraindications (such as coagulopathy, thrombocytopenia, a recent invasive procedure, pericarditis, age > 75 years) to thrombolysis [43,44].

10. Outpatient Management with Mild COVID-19

Patients with mild symptoms of COVID-19 should stay at home and the routine use of thromboprophylaxis is not recommended; they should be assessed for potential risks of VTE or bleeding and should continue anticoagulant treatment for other indications. Such patients should be counselled on the transition to DOAC after considering the risks of bleeding, potential drug interactions, affordability and availability of drugs, and recent INR status. There may be limitations to monitoring INR at home or at nearby laboratories due to the risk of exposure to SARS-CoV-2. Patients not suitable for treatment with DOAC

should use LMWH as a reasonable alternative. Patients with a stable INR and who did not require changes in dosage within the last six months can safely continue warfarin therapy [45].

11. Management of Hospitalized Patients with Moderate or Severe COVID-19 without DIC

Hospitalized patients with moderate to severe COVID-19 should be assessed for risks of VTE and DIC. Routine screening for VTE (e.g., with bilateral lower extremity ultrasound) in hospitalized patients with COVID-19 with elevated D-dimer levels (>1500 ng/mL) is not currently recommended. Signs of active bleeding should be monitored if DIC is suspected or confirmed. Every patient with moderate to severe symptoms should be offered thromboprophylaxis if not strictly contraindicated (for example, with severe thrombocytopenia, grossly deranged coagulation profiles or active bleeding). The choice of drugs, the dose and duration of treatment should follow national guidelines. Laboratory data monitoring, especially of D-dimers, should be checked every 2−3 days. Intermediate or therapeutic doses of thromboprophylaxis should be used at the discretion of the treating physician based on the risk of bleeding. A study of 92 ICU patients indicates a 21% overall rate of hemorrhagic events, of which nearly half (48%) received anticoagulation treatment [38]. Parenteral LMWH is the preferred choice for thromboprophylaxis due to its advantages related to dosing schedule and monitoring compared to intravenous heparin. Compliance is an important consideration in anticoagulant therapy; LMWH is administered mostly as a single daily dose, which improves compliance and thus outcomes. Drug interactions between antiviral treatments and DOACs, and the difficulty in maintaining stable INRs in patients prescribed vitamin K antagonists, means that LMWHs or UFH are preferable alternative treatments, either with or without mechanical prophylaxis.

12. Hospitalized Patients with Moderate or Severe COVID-19 and with Suspected or Confirmed DIC

Prophylactic anticoagulation should be administered to patients with moderate or severe COVID-19 diagnosed with DIC but without significant bleeding. There are currently insufficient data to consider routine therapeutic or intermediate-dose parenteral anticoagulation with UFH or LMWH in hospitalized patients with COVID-19 with suspected or confirmed DIC but with no overt bleeding. It is reasonable to consider the indications for anticoagulation therapy during dose adjustment or discontinuation in patients with moderate or severe COVID-19 already receiving chronic anticoagulation treatment and who develop suspected or confirmed DIC without overt bleeding. A common recommendation in such conditions is to reduce the dose of anticoagulant if the thrombotic risk is not excessive [45,46]. Patients with moderate or severe COVID-19 and receiving dual antiplatelet therapy (e.g., percutaneous coronary intervention within the past three months or recent myocardial infarction) should be assessed on an individual basis and serial platelet counts should be considered when making decisions on dose adjustments or discontinuation of treatment. In general, it is advisable to continue dual antiplatelet therapy if the platelet count is >50,000, reduce to single antiplatelet therapy if the platelet count is between 25,000 and 50,000, and discontinue antiplatelet therapy if the platelet count is below 25,000. These guidelines should be reviewed according to the risk of bleeding vs risk of thrombosis [17].

Risk assessment of VTE is reasonable when using pharmacological prophylaxis for up to 45 days post discharge. Pharmacological prophylaxis should be considered if there is an elevated risk for thrombotic events without a high bleeding risk. Patients should be counselled on the importance of ambulation and physical activity at home [45].

13. Patients with COVID-19 Presenting with Acute Coronary Syndrome (ACS)

Decisions regarding percutaneous coronary intervention or fibrinolytic therapy should be taken after assessing the severity of ST-elevation myocardial infarction (STEMI) and potential COVID-19 in patients and transmission risk to clinicians and healthcare providers [47].

14. Extended (Post-Discharge) VTE Prophylaxis

Post discharge extended thromboprophylaxis is recommended with LMWH or DOACs; even though these therapies reduce the risk of VTE, there remains the risk of bleeding events, including major bleeding [48–54]. Although there is little data specifically related to COVID-19, an individualized approach should be used after balancing the risks of hemorrhage and thrombosis, followed by extended prophylaxis (for up to 45 days) for patients at increased risk of VTE (e.g., reduced mobility, comorbidities such as active cancer, and elevated D-dimer levels more than twice higher than normal) but who are at a low risk of bleeding [50,55]. There is no clear guidance on thromboprophylaxis in patients quarantined with mild COVID-19 but having significant comorbidities, or for those without COVID-19 but who are less active because of quarantine measures. Such patients should be counselled about the importance of remaining physically active at home. Until more high-quality data are available, pharmacological prophylaxis should be reserved for patients with the highest risk, including those with limited mobility and a history of prior VTE or active malignancy.

15. Role for Empiric Therapeutic Anticoagulation without a Diagnosis of VTE

In view of the hemostatic derangements discussed above and from observations of other viral illnesses, some clinicians prefer the use of intermediate- or full-dose parenteral anticoagulation (rather than prophylactic dosing) for routine care of patients with COVID-19 based on the hypothesis that it could prevent microvascular thrombosis [56,57]. However, data to support this premise are primarily based on a subgroup analysis (n = 97) from a single retrospective study having limited control for potential confounders [17]. Another single-center study with 81 patients suggested that D-dimer levels greater than 1500 ng/mL have a sensitivity of 85.0% and specificity of 88.5% for detecting VTE events [58]. Many physicians prefer prophylactic anticoagulation treatment, while others consider the short-term use of intermediate or therapeutic doses as a reasonable approach. While physicians currently use a variety of prophylactic, intermediate, or therapeutic doses of anticoagulants in patients, the optimal dosing in patients with severe COVID-19 remains unknown.

16. Managing the Risk of Hospital-Associated VTE

Hospital-associated venous thromboembolism (HA-VTE) includes VTE presentation while hospitalized, and for up to 90 days post-discharge. Patients infected with COVID-19 are at increased risk of HA-VTE, especially if they become immobilized during critical care. It is unclear if hospitalized patients with COVID-19 are at increased risk for VTE compared to other patients with chest infections and elevated D-dimer values. Elevated D-dimer levels can also be used in a scoring system to identify those at increased risk of VTE [50,59]. Patients with severe COVID-19 are immobile, leading to an acute inflammatory state with a hypercoagulable state. There is also the possibility of endothelial cell activation/damage due to binding of the virus to ACE2 receptors [60].

17. COVID-19 and Interventional Therapies for VTE

The management of PE requires a multidisciplinary team [40,61–63]. It is important to note that there are limited data demonstrating lower mortality rates due to the routine use of advanced VTE therapies [64]. Therefore, the use of catheter-directed therapies during the current outbreak should be reserved for the most critical cases.

18. Additional Considerations

A lack of data makes it difficult to recommend transfusion thresholds in patients with COVID-19 that differ from those recommended for other critically ill patients. The prophylactic transfusion of platelets, use of fresh frozen plasma, fibrinogen, and prothrombin complex concentrate may be considered if invasive procedures are planned [18]. Lastly, patients requiring targeted temperature management often have prolongations of both PT and

aPTT without evidence of bleeding diathesis [65]. Therefore, correction of coagulopathy in unselected patients without overt bleeding is not advisable.

19. Management of Bleeding That Occurs in COVID-19

Clinically overt bleeding is uncommon in patients with COVID-19. Bleeding in COVID-19-associated DIC requires support with blood products and should be managed as per local guidelines [66]. The guidelines for blood product transfusion are as follows: (a) maintain platelet count >50 $\times$ 10^9/L in DIC patients with active bleeding or >20 $\times$ 10^9/L in those with a high risk of bleeding or requiring invasive procedures, (b) fresh frozen plasma (15 to 25 mL/kg) in patients with active bleeding with either prolonged PT or aPTT ratios (>1.5 times normal) or decreased fibrinogen (<1.5 g/L), (c) fibrinogen concentrate, or cryoprecipitate in patients with persisting severe hypofibrinogenemia (<1.5 g/L), and (d) prothrombin complex concentrate if fresh frozen plasma transfusion is not possible. Tranexamic acid is not recommended for routine use in COVID-19-associated DIC.

20. Management of Patients with Thromboembolic Disease without COVID-19

The main management goals for patients with pre-existing or new onset thrombotic disease but without COVID-19 is to provide adequate antithrombotic protection, while minimizing physical contact between patients and healthcare workers. Outpatient management or early discharge for acute VTE is recommended whenever possible [57], and it is reasonable to plan early discharge after medication stabilization for low-risk ACS or PCI for high-risk ACS [67,68].

In general, pharmacotherapy in patients without COVID-19 but with thrombotic disease should be provided according to usual management plans. There is little evidence that antiplatelet agents or anticoagulants increase vulnerability to infection with COVID-19, or of developing severe COVID-19. Patients education on (a) self-monitoring of symptoms is important, and (b) visits to the emergency department for minor bleeding is discouraged.

Patients receiving vitamin K antagonist who need frequent INR checks face logistical challenges due to the lockdowns, with an increased risk of exposure to SARS-CoV-2 in public places. It is useful to consider alternatives such as extended INR testing intervals if previous INR values were stable [69]. Other alternatives include home-based INR checks (provided this is promptly enabled), drive-through INR testing, or switching to a DOAC or LMWHs when clinically appropriate [45].

Whenever switching of anticoagulant is planned, care should be taken to ensure the patients of affordability and accessibility of the agent. DOACs should be used with caution in the elderly (greater risk of bleeding, especially gastrointestinal bleeding) and those with acute kidney injury/renal impairment. Contraindications to treatment with DOACs include patients with mechanical heart valves, valvular atrial fibrillation (AF), antiphospholipid syndrome (APLS), concomitant use of drugs that inhibit cytochrome P450-family 3-subfamily A, and P-glycoprotein and patients who are pregnant or breastfeeding. Patient education on appropriate dietary habits while receiving vitamin K antagonists is also important. The use of LMWHs should be considered in cases where DOACs are not available or not approved by insurance providers.

21. International Travel and COVID-19

Multiple studies reported that environmental and physiological changes occur during routine commercial flights that could lead to mild hypoxia and gas expansion, and which can exacerbate chronic medical conditions or even induce acute in-flight medical events. Long-haul flights increase the risk of VET several fold. COVID-19 has at least two effects relevant to air travel. Unlike normal pneumonia, in which patients experience cough, chest discomfort and significant breathing difficulties, patients with COVID-19 pneumonia initially may not always experience such symptoms, causing a condition termed "silent" or "happy" hypoxia. This may be aggravated by the hypobaric cruising cabin altitude pressure. Silent hypoxia occurs because the virus only causes the air sacs to collapse to reduce oxygen

levels without affecting the removal of carbon dioxide. It is important to detect hypoxia in these patients before they begin to experience dyspnea so that an early intervention can prevent the lungs from deteriorating further. Secondly, COVID-19 is associated with coagulopathy and endothelial damage resulting in VTE. As many economies return to normal, commercial aircrafts will resume operations whilst implementing preventative strategies. Pre- and on-board pulse oximetry screening can be used for early detection of silent hypoxia in unwell passengers boarding aircrafts. This is a simple procedure and can avoid on-flight emergencies. Identified risk factors may be accentuated by the procoagulant effects of undiagnosed COVID-19 that can increase the risk of VTE's. The "new normal" pre- and post-travel consultation will need to take all these considerations into account. Travelers with known risk factors (e.g., obese, male, pregnant, smoking history, previous/family history of VTE) should consider appropriate thromboprophylaxis [70].

22. Public Health Considerations Related to Care for Thrombotic Disease

Governments have enacted mandatory home quarantine for all non-essential personnel in areas most affected by COVID-19. There are several issues to consider related to thrombotic disease at a community level:

1. Many patients become sedentary given the recommendations to remain at home, and are thus at increased risk for VTE [71–73]. This is particularly the case for the elderly and high-risk patients [74].
2. As daily routines continue to be disrupted, many will experience dietary changes (especially in daily intake of green vegetables, which are the major sources of vitamin K in Western diets) that can affect treatment with vitamin K antagonists. As the quarantine measures become even more restrictive, changes in physical activity, diet and vitamin K intake are likely to impact INR values further.
3. The COVID-19 pandemic has devastated the economy of many countries, with the United Nations estimating that COVID-19 could cost the world economy more than $1 trillion in 2020 [75,76]. This will negatively impact the ability of many patients to receive treatment for thrombotic diseases. Socioeconomic disadvantages are linked to higher rates of VTE and adverse outcomes [77,78].

23. Conclusions

More information and data are needed to better define thromboembolic disease due to de novo COVID-19 and differentiate it from pre-existing thrombotic disease to guide optimal management strategies. A large international registry is currently accruing data from COVID-19 patients with VTE [66,67] and another adjudicated prospective registry is incorporating COVID-19 outcomes with cardiovascular risks (CORONA-VTE registry; BWH Thrombosis Research Group). A multicenter, multinational ACS registry has also been initiated, in addition to the new American Heart Association registry for cardiovascular care and outcomes in these patients. Special attention should also be given to patients with pre-existing thromboembolic diseases and with limited access to care due constraints on access to the health care system. The guidance provided in this review for thrombotic disease and antithrombotic therapy during the COVID-19 pandemic (summarized in Table 2) should supplement rather than substitute for clinical decision making. Nuances in conversations between patients and practitioners should be considered when making appropriate patient-centered decisions. Thrombotic diseases may be existing factors or incident complications in patients with COVID-19. Mindful prescribing of preventive and therapeutic doses of antithrombotic agents will mitigate the potentially lethal thrombotic and hemorrhagic events in these high-risk patients. Collaboration between funding agencies, professional societies, patients, clinicians and investigators is needed to address current knowledge gaps on coagulopathies inpatients with COVID-19 patients. Lastly, as governments ease lockdowns, international travel will impact on how we manage and advise patients who remain at risk of, or who are recovering from, COVID-19.

Table 2. Summary of management guidelines for thrombotic disease in patients with COVID-19.

Status of Patient	**Management Recommendations**
Mild COVID-19	1. Remain ambulatory at home 2. Continue thromboprophylaxis for other indications at usual doses 3. Switch to DOAC if available and affordable
Moderate to severe COVID-19 without DIC	1. D-dimer and platelet count checked every 2–3 days 2. Prophylactic dose thromboprophylaxis if not strictly contraindicated 3. Routine screening for VTE (despite elevated D-dimer levels (>1500 ng/mL) is not currently recommended.
Moderate to severe COVID-19 with DIC	1. Prophylactic dose thromboprophylaxis if not bleeding 2. Patients already on chronic anticoagulation, dose is reduced after careful consideration of indication 3. Patients on dual antiplatelet therapy should have dose adjustment based on serial platelet count
Bleeding in COVID 19	Uncommon but managed as per local guideline
Thromboembolic disease without COVID-19	1. Antithrombotic medication as per guideline. 2. self-monitoring of symptoms is important 3. visits to the emergency department for minor bleeding is discouraged 4. Extended INR checks for patients receiving vit K antagonists 5. LMWH or DOACs can be used if accessible and affordable

DOAC: Direct oral anticoagulants; DIC: Disseminated intravascular coagulation; VTE: Venous thromboembolism; INR: International normalized ratio; LMWH: Low-molecular weight heparin.

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Hand Hygiene Knowledge and Practices among Domestic Hajj Pilgrims: Implications for Future Mass Gatherings Amidst COVID-19

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Abstract: This study examined Hajj pilgrims' knowledge and reported practice of hand hygiene. In Hajj 2019, a cross-sectional survey was undertaken in Mina, Makkah, Saudi Arabia, of domestic Saudi pilgrims aged ≥18 years by using a self-administered Arabic questionnaire that captured data on pilgrims' socio-demographics, hand hygiene knowledge, and reported practices of hand cleaning following certain actions. A total of 348 respondents aged 18 to 63 (median 32) years completed the survey, of whom 200 (57.5%) were female. The mean (±standard deviation (SD)) hand hygiene knowledge score was 6.7 (±SD 1.9). Two hundred and seventy one (77.9%) and 286 (82.2%) of respondents correctly identified that hand hygiene can prevent respiratory and gastrointestinal infections respectively, but 146 (42%) were not aware that it prevents hand-foot-mouth disease. Eighty-eight (25.3%) respondents erroneously reported that hand hygiene prevents HIV. Washing hands with water and soap was the most preferred method practiced before a meal (67.5% (235/348)), after a meal (80.2% (279/348)), after toilet action (81.6% (284/348)), when hands were visibly

soiled (86.2% (300/348)), and after waste disposal (61.5% (214/348)). Hajj pilgrims demonstrated a good knowledge and practice of hand hygiene, but there are gaps that are vital to control outbreaks such as COVID-19.

Keywords: COVID-19; Hajj; hand hygiene; infection prevention and control; infectious disease; mass gathering

1. Introduction

COVID-19 pandemic has affecting about 38 million individuals with over one million deaths around the world (as of 13 October 2020) [1]. The disease is believed to transmit from human to human primarily via respiratory droplets, direct contact and indirect contact. Most mass gatherings (MGs), therefore, have been cancelled during this pandemic. MGs are defined as the concentration of people at a specific location for a specific purpose over a set period of time and which has the potential to strain the planning and response resources of the country or community [2]. MGs are known to accelerate the progression of a pandemic. A MG of two million people in Mexico, the Iztapalapa Passion Play that took place in 5–11 April 2009 is believed to have accelerated the spread of the last pandemic caused by influenza A(H1N1)pdm09 [3]. Each year, Hajj pilgrimage to Makkah, in Saudi Arabia, attracts two to three million people from around the world making it one of the largest annual MGs. Respiratory tract infections are the leading illnesses during Hajj affecting 40–90% of pilgrims [4]. Such religious and other MGs pose a significant risk for the spread of infectious diseases, as currently the case with the global pandemic of COVID-19. Saudi Arabia has reported about 338 confirmed cases with about 5000 fatalities [1], and despite banning of Umrah (minor pilgrimage to holy cities in Saudi Arabia) and subsequently downscaling the Hajj of 2020, there have been clusters of COVID-19 in Makkah and Medina, the two important pilgrimage cities [5].

MG-related confirmed cases of COVID-19 have been reported in South East Asian countries like Malaysia [6], and South Korea [7]. In the Middle East, the virus spread from a religious MG in Qom, a Shi'ite holy city (120 km south of the capital Tehran), Iran [8]. Intense crowding during Hajj is highly likely to amplify the risk of transmission of COVID-19. Approximately a third of Hajj attendees are elderly or have pre-existing health conditions, rendering them highly vulnerable to severe form of disease and fatality from infection [9].

In the absence of a definitive preventive measure, non-pharmaceutical measures such as social distancing, isolation, quarantine, and hand hygiene remain the mainstay of infection prevention and control against respiratory infections including COVID-19 [10].

The Saudi Arabian Ministry of Health (MoH) recommends an array of preventive measures annually to minimize the risk of transmission of respiratory infections during Hajj season, including hand washing, respiratory hygiene, and vaccinations [9]. Despite this advice, research demonstrates that the uptake of preventive measures varies among pilgrims [11].

Hand hygiene was found to be the most favored infection preventive measure for Hajj attendees [11]. It is significantly associated with a reduction in self-reported respiratory infections including influenza-like illnesses (ILI) [12], frequency of sputum production, myalgia [13], and fever [14]. Use of an alcohol-based hand sanitizer significantly reduces *Streptococcus pneumoniae* detection in respiratory samples [15]. There is a paucity of focused research that has examined knowledge and practice of hand hygiene among domestic Hajj attendees. This study examined hand hygiene knowledge and reported behaviors among Saudi Hajj pilgrims in 2019 and attempts to inform policy for the other MGs amidst COVID-19.

2. Materials and Methods

A cross-sectional study using a paper-based anonymous survey was conducted among Saudi pilgrims aged ≥18 years who attended Hajj in 2019 in Mina tent city, Greater Makkah, Saudi Arabia, a place where pilgrims spend at least four nights during Hajj. A convenient sampling strategy was used to recruit participants. The data collectors went to Mina and randomly approached the nearest two domestic tour groups (Hamlahs) who agreed to cooperate with the study. From these selected Hamlahs the sample was drawn by randomly asking pilgrims to participate in the study.

This paper-based self-administered anonymous survey was conducted in Arabic. Initially, a survey questionnaire in English was drafted using some exemplary questions used in published surveys [16,17]. Two public health researchers and one epidemiologist translated the questionnaire to Arabic independently. Subsequently, two professional translators performed backward translation separately (who have not seen the original survey or material). The survey underwent pilot testing via a focus group of eight respondents to discuss and answer the survey to ensure readability, personal interpretation of individual question and solving any problems in answering the questions. Following revision, the final version was prepared by the authors that addressed grammatical discrepancies.

The questionnaire consisted of three parts. Part 1 collected non-identifying participant sociodemographic data. Part 2 collected data about the participant's knowledge of hand hygiene using true/false questions. Additionally, there were questions on common myths and fallacies of hand hygiene reported in the literature, such as the misconceptions that hands should be held under water while lathering with soap and the adequate time used for hands rubbing before rinsing. This was assessed using a Likert scale that uses scoring from 0 to 12, with a higher score indicating considerable knowledge level on hand hygiene and a lower score indicating insufficient knowledge. Part 3 comprised of items related to self-reported hand washing behavior. Respondents were asked whether they washed their hands, and by what methods, in different situations during their Hajj journey. These included hand washing before and after eating, after using toilet, after caring a sick person, when hands are visibly dirty, after disposal of a garbage bag, after sneezing or coughing, and after handshaking.

Seven senior medical students (four males and three females) were selected as volunteers and trained as data collectors. The volunteers approached and explained the study purpose and methodology to domestic Saudi Hajj pilgrims residing in camps in Mina on 11th and 12th of August 2019. Pilgrims who agreed to participate were then given the questionnaires to complete, and respondents' queries, if any, were answered. This being an anonymous survey, no signed consent was obtained, and respondents' completion of the survey was considered as their implied consent. Ethics approval was obtained from King Abdullah Medical City, Makkah, Saudi Arabia (IRB ref 19-558).

To ensure correct and uniform entry of data, the collected data from hard copy questionnaires were entered into an electronic form using the Google Forms software (Google LLC, Mountain View, CA, USA) (https://forms.gle/syQt5ouVzJ5usr318). Subsequently, all the data were exported to a master Excel spreadsheet (Microsoft Office 356, version 2002, Redmond, WA, USA) for cleaning and coding before importing to Statistical Package for Social Sciences (SPSS) software (IBM SPSS Statistics for Windows, version 25.0, IBM Corp, Armonk, NY, USA). Descriptive statistics for socio-demographic characteristics, hand hygiene knowledge level, and hand hygiene practices of the respondents were reported. Where appropriate the difference between categorical variables was examined using the chi-squared test, and independent t-test was used to compare the gender differences in the knowledge score on hand hygiene. Binary logistic regression, using the backward Wald method, controlling for factors, such as age, gender, chronic medical conditions, educational level, employment status and the number of times respondents attended Hajj, was used to investigate variables related to hand hygiene knowledge and practices. Previous researchers found that at least 60% of respondents were practicing hand hygiene at Hajj; considering an error margin of 5% to be acceptable for this anonymous survey, a sample of 370 respondents was considered sufficient for this study.

3. Results

Volunteers approached and invited 380 pilgrims to participate in the study, and 348 (91.6%) agreed. The median age of respondents was 32 (range 18–63) years, 200 (57.5%) were female. Of all 348 respondents, 208 (59.8%) had a bachelor's degree or above, 108 (51.7%) were employed, and 43 (12.4%) reported having at least one chronic disease. Just over three-quarters (270, 77.6%) of the respondents reported attending Hajj that year for the first time, with the rest (78, 22.4%) reporting attending Hajj previously at least once (Table 1).

Table 1. Demographic characteristics of respondents (N = 348).

Variables	n (%)
Age, years	
Median (range)	32 (18–63)
Gender	
Male: Female	148:200 (42.5:57.5)
Education	
None	11 (3.2)
Primary/elementary school certificate	22 (6.3)
High school certificate	73 (21)
Diploma	34 (9.8)
University degree	182 (52.4)
Higher degree	26 (7.5)
Employment stats	
Unemployed	126 (36.3)
Student	42 (12)
Employed	180 (51.7)
Self-employed	19 (5.5)
Full time worker	148 (42.5)
Casual/part-time worker	13 (3.7)
Number of times attending Hajj	
Once	270 (77.6)
More than one	78 (22.4)

Of the 348 respondents, there were 230 (66.3%) who reported being aware of the annual Saudi MoH health recommendations for Hajj travelers issued, 262 (75.5%) who reported seeking some form of health advice before Hajj journey, 286 (82.2%) reported receiving the compulsory meningococcal vaccine, and 288 (82.8%) reported receiving other recommended vaccines (Table 2).

With respect to knowledge about hand hygiene, the mean (±standard deviation (SD)) of total scores (0 to 12) was 6.7 (±1.9). Less than half (155, 44.5%) of the respondents had a low knowledge score (defined as total score of ≤6), over half (175, 50.3%) had a medium score (defined as total score of between 7 and 9), and the rest (18, 5.2%) had a high score (defined as total score of ≥10). In multivariate logistic regression analysis, there was no significant association between the level of hand hygiene knowledge and possible factors, including gender, age, having chronic diseases, number of Hajj times, education, and employment status (all p values > 0.05). Nonetheless, both gender and employment status showed a near statistical significance (odds ratio (OR) = 1.73, 95% CI = 0.98–3.07, p = 0.06 and OR = 1.76, 95% CI = 0.97–3.16, p = 0.06, respectively) with females and employed respondents having higher knowledge level compared to males and unemployed individuals. The results of the respondents' level of hand hygiene are detailed in Table 3.

Table 2. Awareness of Saudi Arabian Ministry of Health (MoH) recommendations, pre-Hajj health advice, and vaccination status (N = 348).

Variables	n (%)
Awareness of MoH recommendations	
No	117 (33.6)
Yes	231 (66.4)
Sought pre-Hajj health advice	
No	85 (24.4)
Yes	263 (75.6)
Sources of Hajj health advice	
Doctors	48 (11.1)
Special Hajj websites (e.g., MoH)	81 (18.8)
Tour groups	80 (18.5)
Family and fiends	130 (30.1)
General websites	93 (21.5)
Vaccination status	
Mandatory meningococcal vaccine	286 (82.2)
Recommended influenza vaccine	280 (80.5)

Table 3. Hand hygiene knowledge level among respondents (N = 348).

Questions	Answered n (%)
Which of the following diseases can be transmitted by poor hand hygiene?	
Diarrhoeal diseases	
True §	286 (82.2)
False	62 (17.8)
Flu, cough (upper respiratory infection or ILI)	
True §	271 (77.9)
False	77 (22.1)
Hand-foot-mouth disease	
True §	202 (58)
False	146 (42)
HIV/AIDS	
True	88 (25.3)
False §	260 (74.7)
Skin infections	
True §	195 (56)
False	153 (44)

Table 3. *Cont.*

Questions	Answered n (%)
Eye infections	
True §	258 (74.1)
False	90 (25.9)
Diabetes	
True	16 (4.6)
False §	332 (95.4)
Are the following statements correct?	
Always keeping your hands clean may lower our body defence mechanism	
True	181 (52)
False §	167 (48)
Hands should be held under water while lathering with soap	
True	120 (34.5)
False §	228 (65.5)
An alcohol-based hand sanitiser that contains 40% alcohol is sufficient for hands disinfectant	
True	119 (34.2)
False §	229 (65.8)
Rubbing my hands until soap forms a lather for 10 s before rinsing is enough for hand disinfection	
True	202 (58)
False §	146 (42)
Temperature of water makes no difference in terms of the cleansing effect of hand cleaning	
True §	147 (42.2)
False	201 (57.8)
Mean (±SD) of Total Scores (0 to 12)	6.7 (±1.92)

§ Correct answer.

Table 4 summarizes the results of whether pilgrims practiced hand hygiene or not and by what methods in different situations during Hajj. Except for cleaning hands following handshakes, which was reported by just over half (177, 51.9%) respondents, an overwhelming majority of respondents reported cleaning their hands following other tasks. Hand washing using water and soap was the most commonly reported hand hygiene method among pilgrims across all situations. A small number (58, 16.7%) of the respondents reported barriers to using hand hygiene during Hajj season, notably unavailability of soap and hand rub (60.5% (31/58)), limited access to washrooms (23.3% (18/58)) and intense crowding (16.3% (14/58)).

Table 4. Respondents' hand hygiene practices and methods used in different situations during Hajj (N = 348).

Types of Hand Hygiene	Before Meal n (%)	After Meal n (%)	After Toilet Action n (%)	After Touch a Sick Person n (%)	When Hands Visibly Dirty n (%)	After Waste Disposal n (%)	After Sneeze/Cough n (%)	After Handshakes n (%)
Water only	60 (17.2)	29 (8.3)	33 (9.5)	33 (9.5)	12 (3.4)	44 (12.6)	60 (17.2)	30 (8.6)
Water and soap	235 (67.5)	279 (80.2)	284 (81.6)	138 (39.7)	300 (86.2)	214 (61.5)	89 (25.6)	68 (19.5)
Alcohol hand rubs	22 (6.3)	19 (5.5)	23 (6.6)	111 (31.9)	31 (8.9)	44 (12.6)	68 (19.5)	63 (18.1)
Water wipes	16 (4.6)	14 (4.0)	5 (1.4)	14 (4.0)	3 (0.9)	12 (3.4)	51 (14.7)	16 (4.6)
Did not practice hand hygiene	15 (4.3)	7 (2.0)	3 (0.9)	52 (14.9)	2 (0.6)	34 (9.8)	80 (23.0)	171 (49.1)

4. Discussion

This study explored hand hygiene knowledge and reported practices of domestic Saudi Hajj pilgrims. There is a paucity of research that has examined hand hygiene knowledge among Hajj pilgrims. In this study the pilgrims had a moderate knowledge of hand hygiene (6.7 ± 1.9). Most respondents had an accurate knowledge of the protective role of hand hygiene against common infectious diseases such as respiratory and gastrointestinal infections, but some were not aware of its role against some less common but nonetheless important infections. Concerningly, about 40% of respondents were not aware that hand hygiene can prevent hand-foot-mouth disease for which hand hygiene is strictly advised, and a quarter of respondents mistakenly thought hand hygiene prevented HIV infection. Pilgrims' knowledge also varied for responses pertaining to questions relating to common myths surrounding hand hygiene and knowledge on correct hand hygiene procedure. For instance, half of the respondents reported that persistent hand washing lowers immunity, half thought that temperature of the water makes a difference in terms of the cleansing effect of hand washing, a third did not know that hands should not be placed under water while lathering, about 58% erroneously thought rubbing hands just for 10 s is enough to ensure disinfection while it should be 20 s, and about a third thought 40% alcohol is sufficient for disinfecting hands whereas the correct answer should be minimum 60% alcohol [10].

A study conducted among Malaysian pilgrims during the 2018 Hajj showed 87.1% pilgrims knew that washing one's hands with hand sanitizers can protect one from flu-like illness, which compares with only 77.9% pilgrims in our study knowing the role of hand hygiene against flu/cough/ILI [18]. The lower proportion in our study could be explained by the fact that foreign pilgrims are generally better informed and better prepared for Hajj travel since health authorities in their countries of origin are mandated to ensure health advice to pilgrims on communicable diseases prompting months of preparation including attendance at pre-travel health seminar (usually more than once) before embarking on Hajj journey [9]. It is also encouraging to see that more domestic pilgrims sought pre-Hajj advice in the present study compared to the previous year (three-quarters versus half) [19]. In this study, there was no significant difference in knowledge level by gender or age as was found in other studies involving Saudi healthcare workers and trainees [20]. It is unsurprising that some pilgrims lacked knowledge in some more specialist themes like alcohol concentration needs to be in disinfectants, duration of time required for hand rubbing, prohibition of placing hands under water while lathering and insignificance of water temperature, yet the pilgrims seem to have similar or even better knowledge in some domains compared to community dwellers in another developed country in Asia [17].

Hand washing with soap and water was the most common type of hand cleaning reportedly used by the study respondents in almost all the situations followed by alcohol hand rubbing. The results of this study are consistent with, and corroborate, the findings of other studies, including a systematic review, which found among Hajj pilgrims hand washing with soap was more popular than hand gel or sanitizers [18,21]. In contrast, a study explored domestic pilgrims' uptake of health preventive measures during the peak Hajj days found the proportion of respondents washing their hands with soap and cleaning hands with hand sanitizers was same (65%); however, pilgrims who were concerned about food poisoning were more likely to clean their hands with hand sanitizers (adjusted OR 2.5, 95% CI 1.1–5.4) indicating pilgrims' degree of concern may dictate the mode of hand hygiene [19].

The results of the present study also showed a relatively poor hand hygiene behavior after touching a patient with only 39.7% washing hands with soap–water and 31.9% using alcoholic hand rub, after sneezing and coughing (respectively 25.6% and 19.5%) and following handshakes (respectively 19.5% and 18.1%). Similarly, low compliance with hand hygiene following these actions in previous studies, for instance 15% Australian pilgrims washed hands after touching a patient while at Hajj, despite the fact that 86% made an intention before Hajj to wash hands after touching an ill person, indicating there were practical issues that barred them [22], e.g., unavailability of soap and hand rub, limited access to washrooms and intense crowding reported as barriers to hand hygiene in this study. Another study conducted among members of public across Gulf countries showed that only 39% individuals washed their hands with soap after handshakes which may indicate that in a non-epidemic setting most people

would not wash hands following handshake [23]. Regardless of what products are being used by pilgrims, hand hygiene in general has been proven to be an effective measure against respiratory infections at Hajj [12–14] and is practiced by pilgrims from different nationalities [18,19].

The patchy knowledge gap and non-compliance on certain occasions warrant improvement. This is indicated by the findings from this and a previous study that showed pilgrims with a university-level education had a higher hand hygiene compliance compared to those with a lower education, and several other studies reporting lack of awareness as an important hindrance to hand hygiene, meaning education may improve the hand hygiene uptake [24]. Through a pre- and post-intervention survey conducted during the Hajj 2011, Turkestani and colleagues showed that direct health education to pilgrims is effective in improving hand hygiene compliance rate from 79.1% to 95.5% [25]. During a pandemic era, such as the current COVID-19 outburst, intensive pre-travel health education perhaps through a certification program following a short course on hygiene may be made compulsory for all pilgrims. Tour operators may conduct the course and should have, as a pre-requisite of running Hajj tours, more advanced knowledge of hygiene, health, and safety. This can be buttressed by direct health education and a random quick knowledge test at the points of entries supplemented by multi- lingual health messages on billboard with graphical illustration on how to perform hand hygiene, map a direction of hand hygiene facilities and resources. Effective pandemic health messaging during the Hajj 2009 was associated with higher compliance with protective measures and with shorter duration of respiratory illnesses [12]. Furthermore, giving advice by Islamic scholars about the importance of alcohol- based hand rubs use and reinforce how this practice does not harm pilgrims could potentially eliminate taboos surrounding the use of alcohol-based hygienic products and in turn enhance compliance [26].

Although a small number of respondents reported some barriers in practicing hand hygiene during Hajj such as unavailability of soap and hand rub, limited access to washrooms, and intense crowding, solutions, for example, providing supplementary hand hygiene products to pilgrims, could potentially increase the uptake. However, in Hajj setting, such improvements are not always feasible; therefore, pilgrims are encouraged to carry their own personal hygiene products.

Considering the continuance of the COVID-19 pandemic and its risks of spreading in mass gatherings, and the increase in average infections globally, the Saudi Government decided to downscale Hajj for this year 2020 allowing only a thousand local and resident foreigners perform the pilgrimage [27].

This study had some limitations. Firstly, the survey was conducted only among domestic Saudi pilgrims who unlike international pilgrims bypass many vicissitudes of travel hence these findings may not be generalizable. Secondly, the reported barriers were not qualitatively gauzed, and finally, being anecdotal in nature some data (e.g., vaccination history) could be at risk of recall bias. These limitations can be addressed by a mixed-method study involving an extended sample from multiple nationalities with different health, education and cultural backgrounds.

5. Conclusions

There is a paucity of research that has examined infection prevention and control measures in MGs. Hand hygiene was generally acceptable among domestic Saudi pilgrims but there were variable knowledge gaps in some aspects that may be improved by intensive health education and awareness-raising strategies. Hajj pilgrims demonstrated a good knowledge and reported practice of hand hygiene, although there were some gaps in their key areas that are vital to containing and mitigating outbreaks, particularly in the context of MGs and the current global COVID-19 pandemic.

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Article

Meningococcal Vaccine for Hajj Pilgrims: Compliance, Predictors, and Barriers

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Abstract: Background: Major intercontinental outbreaks of invasive meningococcal disease associated with the Hajj occurred in 1987, 2000, and 2001. Mandatory meningococcal vaccination for all pilgrims against serogroups A and C and, subsequently, A, C, W, and Y controlled the epidemics. Overseas pilgrims show excellent adherence to the policy; however, vaccine uptake among domestic pilgrims is suboptimal. This survey aimed to evaluate meningococcal vaccine uptake among Hajj pilgrims and to identify key factors affecting this. Methods: An anonymous cross-sectional survey was conducted among pilgrims in Greater Makkah during the Hajj in 2017–2018. Data on socio-demographic characteristics, vaccination status, cost of vaccination, and reasons behind non-receipt of the vaccine were collected. Results: A total of 509 respondents aged 13 to 82 (median 33.8) years participated in the survey: 86% male, 85% domestic pilgrims. Only 389/476 (81.7%) confirmed their meningococcal vaccination status; 64 individuals (13.4%), all domestic pilgrims, did not receive the vaccine, and 23 (4.8%) were unsure. Among overseas pilgrims, 93.5% certainly received the vaccine (6.5% were unsure) compared to 80.9% of domestic pilgrims ($p < 0.01$). Being employed and having a tertiary qualification were significant predictors of vaccination adherence (odds ratio (OR) = 2.2, 95% confidence interval (CI) = 1.3–3.8, $p < 0.01$; and OR = 1.7, CI = 1–2.5, $p < 0.05$, respectively). Those who obtained pre-Hajj health advice were more than three times as likely to be vaccinated than those who did not (OR = 3.3, CI = 1.9–5.9, $p < 0.001$). Lack of awareness (63.2%, 36/57) and lack of time (15.8%, 9/57) were the most common reasons reported for non-receipt of vaccine. Conclusion: Many domestic pilgrims missed the compulsory meningococcal vaccine; in this regard, lack of awareness is a key barrier. Being an overseas pilgrim (or living at a distance from Makkah), receipt of pre-Hajj health advice, and employment were predictors of greater compliance with the vaccination policy. Opportunities remain to reduce the policy–practice gap among domestic pilgrims.

Keywords: Hajj; meningococcal disease; vaccine uptake; pre-travel health advice

1. Introduction

Hajj is a large annual mass gathering that attracts more than two million Muslims from around the world to congregate within confined areas in Makkah, Saudi Arabia. A highly crowded and congested environment during Hajj amplifies risks associated with mass gatherings, including transmission of respiratory organisms, notably *Neisseria meningitidis* [1,2].

Neisseria meningitidis is associated with a substantially high rate of carriage (up to 86%) in crowded and closed populations, which resulted in large intercontinental outbreaks of invasive meningococcal disease during Hajj [3]. Following the Hajj in 1987, an intercontinental Hajj-related outbreak of meningococcal serogroup A (MenA) disease led to approximately 2000 cases [4], and its subsequent introduction into the African meningitis belt affected around 70,000 people [5]. Furthermore, in 2000 to 2001, a large outbreak of meningococcal disease resulted in at least 47 deaths, including 11 deaths in the United Kingdom, and affected no fewer than 2400 people in several countries throughout Asia, Africa, Europe, and North America. Serogroup W (MenW; a serogroup that was not previously known to cause large epidemics) sequence type 11 was responsible for over half of those cases [4,6].

Mandatory bivalent (serogroups A and C) meningococcal vaccination for all pilgrims from 1987 brought the disease under control during the Hajj for more than a decade [6,7]. Switching the vaccination policy to the quadrivalent (serogroups A, C, W, and Y) meningococcal (MenACWY) polysaccharide vaccine in 2002, coupled with chemoprophylaxis at the port of entry for pilgrims arriving from the African meningitis belt, again brought the subsequent epidemics under control [8]. Since then, no further Hajj-related meningococcal outbreaks occurred [6]. The mandatory vaccination policy also applies to residents of Hajj zones and to personnel who serve pilgrims during the Hajj, including healthcare workers (HCWs) (Table 1) [1,9].

Table 1. Current preventive measures mandated by the Saudi Arabian government to control meningococcal disease during Hajj.

Measure	When	Age Criteria	Target Group
MenACWY polysaccharide vaccine	Within last 3 years, but ≥ 10 days before arrival	Any individual > 2 years	a. Visitors to Saudi Arabia for Umra *, Hajj, or seasonal work ** b. Residents of Saudi Arabia as follows: - Residents of Makkah and Madina at the time of Hajj - Residents of all other provinces undertaking the Hajj ** - Hajj workers ***
MenACWY conjugate vaccine	Within last 5 years, but ≥ 10 days before arrival		
Chemoprophylaxis (ciprofloxacin, 1 tablet, 500 mg)	Upon arrival (at port of entry)	Any individual > 2 years (excluding pregnant women)	Visitors from African meningitis belt countries for Umra *, Hajj, or seasonal work **

MenACWY; quadrivalent meningococcal serogroup A, C, Y, and W. * A minor pilgrimage to Makkah outside of the Hajj season. ** Requirements for Hajj and Umra entry visa, and for Hajj permit for domestic pilgrims. *** Including individuals working at points of entry or in direct contact with pilgrims.

Monitoring the annual number of Hajj visas and mandating the vaccine as a prerequisite for the visa application both limited the numbers of overseas pilgrims and improved vaccination rates. For instance, reports on vaccine uptake among overseas pilgrims since 2006 showed a compliance of no less than 96% and reaching up to 100% [10–14]; however, concerns remain among this group of pilgrims, including the receipt of inappropriate vaccines and, due to the limited access to vaccines (including cost), the use of fraudulent vaccination certificates [11,15,16].

Since 2003, Saudi citizens and other expatriate residents in Saudi Arabia who intend to perform Hajj must apply for a Hajj permit with a MenACWY vaccine receipt stipulated as a requirement. Despite this, unauthorized domestic pilgrims often sometimes enter Hajj sites without a permit and

without formally registering with an official Hajj tour group. Additionally, despite being enforced and freely offered, the vaccine coverage was found to be very low (64%) in 2006 in the only published work reporting vaccine uptake among domestic pilgrims [12]. The rate was also unsatisfactory among domestic HCWs (ranging from 51.7% to 84.7%) in several studies conducted between 2009 and 2018 [17–20]. In recent years, the enforcement of the Hajj permit requirement by rigorous procedures at points of entry into Makkah reduced the number and proportion of domestic pilgrims (from 1.4 million (45%) in 2012 to 600,000 (26%) in 2018) [21]. However, there is no recent study assessing the uptake of meningococcal vaccines among these pilgrims. To this end, a survey was undertaken to evaluate the coverage of MenACWY vaccines among Hajj pilgrims and to identify the key predictors and barriers affecting their uptake, particularly among domestic pilgrims, which was not assessed in previous studies.

2. Materials and Methods

An anonymous cross-sectional survey was distributed among domestic pilgrims present in Mina, a tent city, and a main Hajj site on the outskirts of Makkah, and among overseas pilgrims who were staying in Aziziyah (before moving to tents in Mina), a borough of Makkah, adjacent to Mina, during the Hajj seasons of 2017 and 2018.

2.1. Participant Recruitment

Overseas and official domestic pilgrims were eligible to participate; all other non-pilgrims were excluded. In order to recruit a representative sample of both domestic and international pilgrims, the research team approached domestic pilgrims in their camps in Mina, and overseas pilgrims living in hotels/serviced apartments in Aziziyah. The research team (composed of research doctors and trained volunteer allied health or medical students) randomly approached tour operators to access their tent camps or housing and to invite pilgrims to the study. The research team, after obtaining permission from the tour group leaders, explained the study to their pilgrims, answered any queries they had, and invited them to participate. Participation depended primarily on the cooperation of the tour group leader, and then the pilgrim's willingness to participate.

No identifiable personal data were collected, and respondents' completion of the survey was considered implied consent. This study was reviewed and approved by the Institutional Review Board of King Saud University College of Medicine, Riyadh, Saudi Arabia (E-17-2534).

2.2. Survey Design

The survey was designed and reviewed by experts in the field of Hajj and vaccine-preventable diseases. The questionnaire collected data on socio-demographic characteristics (such as age, gender, educational level, and employment status), as well as uptake of meningococcal vaccines as a preparation for Hajj and reasons behind non-receipt of the vaccine in such cases. It also evaluated if this was the participant's first time to the Hajj, whether the vaccine was freely offered, and the receipt of pre-Hajj health advice. The survey was primarily in English, with Arabic translations available for those who preferred to complete the survey in Arabic. Survey responses were collected using a printed or web-based form securely hosted in Wufoo^{TM} (SurveyMonkey Inc., San Mateo, CA, USA). Written responses were entered into the web-based form, and all data were subsequently exported to a Microsoft Excel^{TM} (Microsoft Corp., Redmond, WA, USA) spreadsheet for analysis.

2.3. Statistical Methods

The proportion of participants responding to each question was reported. To measure the association between predictors and vaccine uptake, odds ratios (OR) with 95% confidence intervals (95% CI) based on the risk estimate statistics were calculated. Pearson's chi-squared test was used to compare categorical variables and determine associations and correlations. For questions evaluating sources of pre-Hajj health advice and reasons for non-receipt of the vaccine, one sample nonparametric

test (Jeffreys interval) was used to report the proportion of participants providing each response and the 95% CI for the point estimate.

All those who declared previous receipt of the vaccine, regardless of the year of vaccination, were considered as vaccinated; further analysis was done to determine the adherence to the vaccine policy time window. Participants who were unsure about their vaccination history were excluded from the analysis in the OR calculation. A p-value ≤ 0.05 was considered statistically significant. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS™) for Windows™ v.25.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Participant Characteristics

In total, 513 pilgrims agreed to participate in the study, of whom 509 completed the survey; the remaining four submitted blank forms and, hence, were excluded from the denominator. Only 444 respondents declared their age, ranging from 13 to 82 years (mean 36, SD ±12.6). Males comprised 86% of the sample, and local pilgrims accounted for 85%. Table 2 summarizes the demographic characteristics of the surveyed participants.

3.2. Meningococcal Vaccine Uptake

Of the 476 participants who declared their vaccination status, only 389 (81.7%) confirmed receipt of a meningococcal vaccine; 64 (13.4%), all domestic pilgrims, did not receive the vaccine, and 23 (4.8%) were unsure about their vaccination status. Almost all (93.5% (58/62)) overseas pilgrims declared receipt of the vaccine, although four (6.5%) were unsure, compared with 80.9% (321/397) of domestic pilgrims who received the vaccine ($p < 0.01$), 61/397 (15.3%) who did not, and 15/397 (3.8%) who were unsure (Table 3). Employed participants were twice as likely to be vaccinated as those who were not employed, and those who received pre-Hajj health advice from any source, and those with a tertiary qualification had a higher vaccination uptake rate. Among domestic pilgrims, those from Makkah province were almost three times more likely to miss out on the vaccine compared to those from other provinces.

Table 2. Demographic characteristics of the domestic and overseas pilgrims.

Characteristics	All *n/N* * (%)	Domestic Pilgrims *n/N* * (%)	Overseas Pilgrims *n/N* * (%)	*p*-Value
Number of participants	509**	416/489 (85.1)	73/489 (14.9)	
Age in years				
Mean (SD)	36 (± 12.6)	34.7 (± 12)	42 (± 13.4)	< 0.001 ***
Range (Median)	13–82 (33.8)	13–82 (32.6)	21–69 (38.9)	
Gender				
Male:female	6:1	7.5:1	3:1	0.03 ***
Country of residence				
Saudi Arabia	398/499 (79.8)	398/412 (96.6)	0	
Pakistan	33/499 (6.6)	0	30/73 (41.1)	
South Africa	28/499 (5.6)	0	28/73 (38.4)	
Egypt	11/499 (2.2)	7/412 (1.7) ¶	0	
Malawi	6/499 (1.2)	0	6/73 (8.2)	
Zambia	5/499 (1)	0	5/73 (6.8)	
Other	18/499 (3.6)	7/412 (1.7) ¶	4/73 (5.5)	
Highest qualification				
No formal education	4/499 (0.8)	2/411 (0.5)	1/70 (1.4)	
School certificate ‡	43/499 (8.6)	34/411 (8.2)	4/70 (5.7)	
High school certificate §	124/499 (24.8)	100/411 (24.3)	21/70 (30)	
Diploma	58/499 (11.6)	39/411 (9.5)	12/70 (17.1)	
University undergraduate degree	215/499 (43.1)	184/411 (44.8)	30/70 (42.9)	
University postgraduate degree	55/499 (11)	52/411 (12.7)	2/70 (2.9)	
Employed				
No	168/501 (33.5)	134/412 (32.5)	25/69 (36.2)	0.61
Yes	333/501 (66.5)	278/412 (67.5)	44/69 (63.8)	
Hajj attendance				
First time	285/500 (57)	212/409 (51.8)	60/71 (84.5)	< 0.001 ***
> 1 time previously	215/500 (43)	197/409 (48.2)	11/71 (15.5)	

SD—standard deviation. * The total number of respondents with complete information for each individual variable. ** Twenty participants with unknown allocation status (overseas or domestic). *** Statistically significant. ¶ Holders of any visa other than a Hajj visa were officially treated as domestic pilgrims. ‡ Year 10 equivalent; § year 12 equivalent.

Table 3. Meningococcal vaccine uptake.

Characteristics	Overall Meningococcal Vaccine Uptake			Meningococcal Vaccine Uptake among Domestic Pilgrims		
	n/N * (%)	OR ** (95% CI)	*p*	*n/N* * (%)	OR ** (95% CI)	*p*
All						
All participants ¶	389/476 (81.7)			321/397 (80.9)		
Gender						
Male	322/380 (84.7)	1.0 (ref)		270/326 (82.8)	1.0 (ref)	
Female	53/58 (91.4)	1.9 (0.7–4.98)	0.18	39/43 (90.7)	2 (0.7–5.9)	0.19
Origin						
Domestic	321/382 (84)	1.0 (ref)				
Overseas	58/58 (100)	n.a	<0.001 §			
Province of residence						
Makkah Province				59/80 (73.8)	1.0 (ref)	
Other				207/235 (88.1)	2.6 (1.4–5)	< 0.01 §
Hajj attendance						
First time	208/246 (84.6)	1.0 (ref)		158/193 (81.9)	1.0 (ref)	
≥ 1 time previously	174/199 (87.4)	1.3 (0.7–2.2)	0.39	158/183 (86.3)	1.4 (0.8–2.4)	0.24
Tertiary qualification						
No	122/150 (81.3)	1.0 (ref)		99/125 (79.2)	1.0 (ref)	
Yes	262/297 (88.2)	1.7 (1.0–2.5)	0.048 §	220/254 (86.6)	1.7 (0.97–2.98)	0.06
Employed						
No	113/144 (78.5)	1.0 (ref)		92/121 (76)	1.0 (ref)	
Yes	269/302 (89.1)	2.2 (1.3–3.8)	0.03§	226/258 (87.6)	2.2 (1.3–3.9)	< 0.01 §
Received pre-Hajj health advice						
No	64/89 (71.9)	1.0 (ref)		54/78 (69.2)	1.0 (ref)	
Yes	322/360 (89.4)	3.3 (1.9–5.9)	< 0.001 §	264/300 (88)	3.3 (1.8–5.9)	< 0.001 §

OR, odds ratio; 95% CI, 95% confidence interval; *p*, *p*-value; ref, reference value; n.a, not available. * Total number of respondents with vaccination status (excluding "unsure" respondents) and complete information for each individual variable. ** For OR calculation, responses with "unsure" for vaccination status were excluded. ¶ Includes all participants with vaccination status. § Statistically significant.

3.3. Participant Adherence to Vaccination Policy

Overall, among the 389 vaccinated individuals, 329 (84.6%) received the vaccine within the last three years, 12 (3.1%) received it over three years prior to Hajj attendance, and 48 (11.9%) did not declare the year of vaccination. Thus, 20.5% (70/341) of domestic pilgrims failed to confirm their adherence to the complete vaccination policy (either did not receive the vaccine at all, received it over three years prior, or were unsure about their vaccination status). This translates to an almost seven-fold increased risk of non-compliance with the vaccine policy compared to overseas pilgrims (OR = 6.8, 95% CI = 1.6–28.8), $p < 0.01$). Lack of awareness that the vaccine is a mandatory requirement (63.2%, 36/57) was the main reason given for not receiving the vaccine (Figure 1).

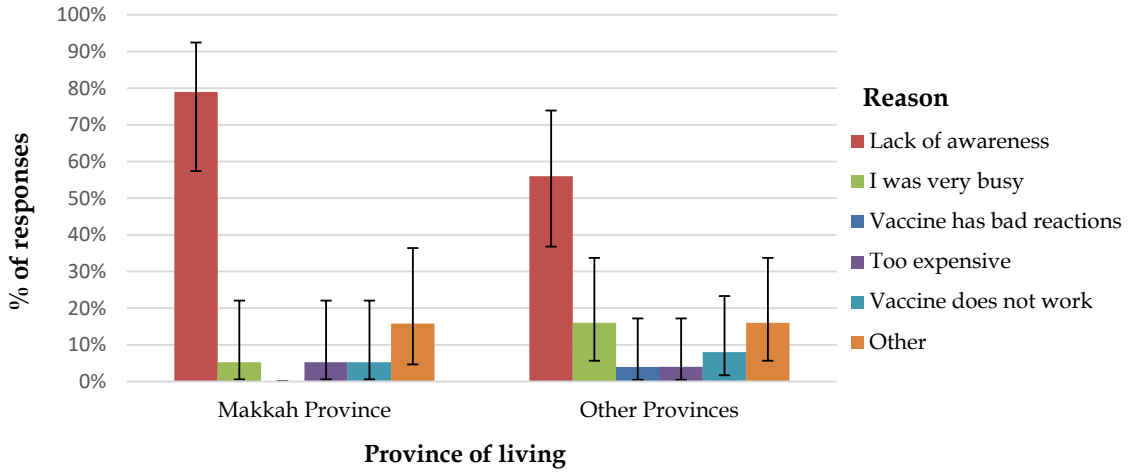

Figure 1. Reasons for non-receipt of meningococcal vaccine among unvaccinated domestic pilgrims: proportion of participants providing each reason with the 95% confidence interval for the point estimate.

3.4. Vaccination Venues

Domestic pilgrims were mainly vaccinated at primary health care centers (79.3%), while overseas pilgrims mostly visited hospitals or travel clinics (70.3%).

3.5. Cost of Vaccination

Overall, 55 (15.1%) participants paid for the vaccine. Overseas pilgrims, women, and those who attended Hajj for the first time were significantly more likely to pay for the vaccine than domestic pilgrims, men, or those who attended Hajj previously (Table 4).

3.6. Receipt of Pre-Hajj Advice

Only 19.4% (98/504) of participants received pre-Hajj health advice from one or more "professional" sources, including general practitioners or a specialized travel clinic; 61% (309/504) received advice from "non-professional" sources, and 19% (97/504) did not receive any advice (Figure 2). Notably, overseas pilgrims (64%) were more likely to receive advice from professional sources than domestic pilgrims (16%; OR = 9.6, 95% CI = 5.3–17.3, $p < 0.001$).

Table 4. Covering the cost of vaccination.

Characteristics		Proportion of Participants Who Paid for the Vaccine		
		n/*N* * (%)	OR (95% CI)	*p*
All				
All participants ¶		55/364 (15.1)		
Gender				
Male		38/302 (12.6)	1.0 (ref)	
Female		16/49 (32.7)	3.4 (1.7–6.7)	< 0.001 §
Origin				
Domestic		30/307 (9.8)	1.0 (ref)	
Overseas		23/48 (47.9)	8.5 (4.3–16.7)	< 0.001 §
	Pakistan	17/17 (100)		
	South Africa	3/20 (15)		
	Other	9/27 (33.3)		
Hajj attendance				
≥ 1 time previously		13/165 (7.9)	1.0 (ref)	
First time		41/193 (21.2)	3.2 (1.6–6.1)	< 0.001 §
Tertiary qualification				
Yes		31/246 (12.6)	1.0 (ref)	
No		22/112 (19.6)	1.7 (0.9–3.1)	0.09
Employed				
Yes		32/246 (13)	1.0 (ref)	
No		21/108 (19.4)	1.6 (0.9–3)	0.12

OR, odds ratio; 95% CI, 95% confidence interval; *p*, *p*-value; ref, reference value. * Total number of respondents with known source of payment and complete information for each individual variable. ¶ Includes all participants with known source of payment. § Statistically significant.

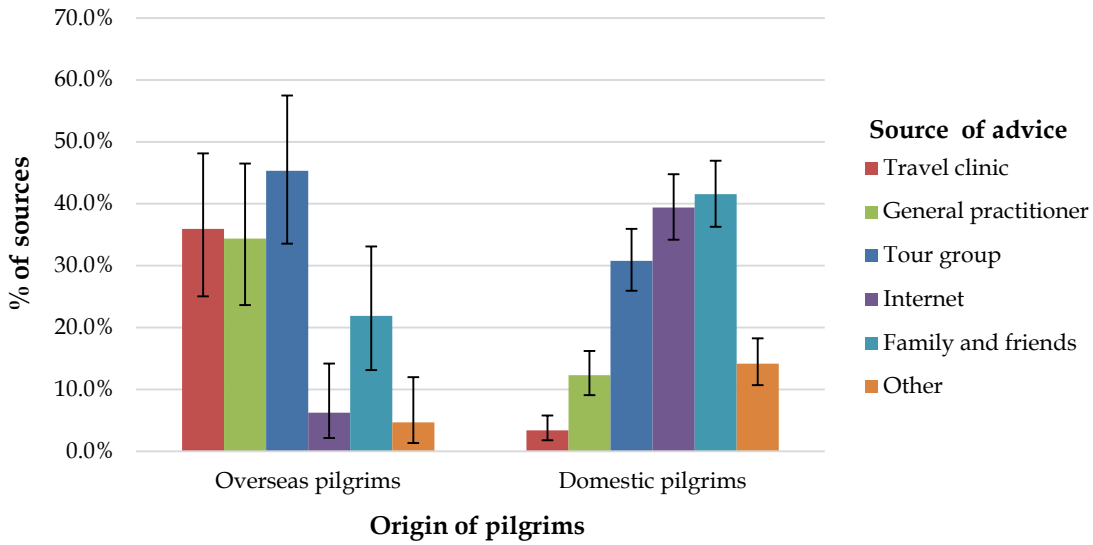

Figure 2. Sources of pre-Hajj health advice among participants who received such advice: proportion of participants providing each response with the 95% confidence interval for the point estimate.

4. Discussion

The key finding of this study is that around one-sixth of domestic Hajj pilgrims failed to receive the compulsory MenACWY vaccine in recent years. Meningococcal vaccination is a visa prerequisite for international pilgrims; thus, a high coverage among overseas pilgrims was expected and demonstrated (93.5%). In this regard, the findings of this study are consistent with previous reports. Compliance among overseas pilgrims ranged from 96% to 98% between 2006 and 2010 [13], and two recent studies conducted at King Abdul Aziz International Airport, among 796 and 5235 arriving overseas pilgrims in 2013 and 2014, revealed uptake rates of 98.2% and 100%, respectively [10,11]. However, assessing

compliance to other measures of the vaccination policy among overseas pilgrims, such as type and timing of vaccination, is recommended [11,16].

Nevertheless, it is concerning that, despite regulatory efforts, vaccine uptake among local pilgrims who form nearly one-third of total attendees at Hajj each year is unacceptably low. Although the vaccine uptake identified in this survey (85%) is higher than that reported by El Bashir et al. during the Hajj in 2006 (64% among domestic pilgrims who attended the National Guard Clinics in Makkah [12]), it appears that the official regulation that mandates meningococcal vaccination as a prerequisite for a Hajj permit for locals is less effective than that applied to international pilgrims, and a significant number of domestic pilgrims are able to avoid vaccination. Ensuring no Hajj permit is granted unless a valid certificate is provided may improve the situation; however, it is possible that there are more prevailing factors involved, including education of the general population, as well as HCWs.

Several studies demonstrated suboptimal meningococcal vaccine coverage among local HCWs, which, at best, did not exceed 85% among highly vulnerable hospital emergency room HCWs in Madina in 2015 [19]. A similar rate (82.4%) was also reported among HCWs working in Mina and Arafat, principal Hajj zones in Makkah, in 2003 [17]. Other studies found uptake rates as low as 67.1% and 76.1% among HCWs serving pilgrims in 2009 and 2018, respectively [18,20].

Longer distance of travel appears to act as a motivator for overseas pilgrims to better prepare for Hajj and to seek and follow health advice. This was also noted even among domestic participants in this survey. Similarly, in a previous vaccine uptake survey among domestic pilgrims, fewer pilgrims (50%) from Hajj zones (Makkah and Jeddah) were shown to be vaccinated against MenACWY than pilgrims from other regions in Saudi Arabia (71%) [12]. Moreover, pilgrims from Makkah city were found to have lower vaccination coverage against seasonal influenza than pilgrims from the rest of the country (adjusted OR = 0.52, 95% CI = 0.37–0.72, $p < 0.001$) [22].

An important finding of this survey is that receiving pre-travel health advice, regardless of the source, substantially increased compliance with the vaccination policy. The majority of overseas pilgrims received "professional" pre-Hajj health advice, while locals tended to rely on "social" sources. However, receiving any pre-travel health advice, being employed, and having a tertiary qualification were each individually associated with greater compliance with the vaccination policy. Previous reports on uptake of other recommended vaccines at Hajj also indicated that receiving pre-travel health advice was a considerable motivator for receiving vaccinations against other diseases [14,23]. Furthermore, in a large survey among residents of Gulf Cooperation Council countries, doctors' advice was the leading motivator for receipt of influenza vaccine [24]. Worksite immunization was shown to be effective in facilitating influenza vaccine uptake in Saudi Arabia [25]. Similarly, some employed participants of this survey indicated receiving the meningococcal vaccine at or through their workplace. This may explain the higher vaccine uptake among employed participants compared with those who identified themselves as unemployed. Additionally, more educated pilgrims were more likely to receive the meningococcal vaccine than those with lower educational attainment. A cross-sectional study of Australian Hajj pilgrims also demonstrated that having a university education was associated with a higher likelihood of receiving recommended Hajj vaccines (OR = 3.4, 95% CI = 1.7–6.7, $p = 0.01$) [14]. Previous reports also described a higher rate of vaccine uptake in women preparing to be pilgrims [20,26,27]. The association observed in this survey was in the same direction, but the difference with men was not statistically significant, which may be due to the low proportion of women who participated in the survey.

Unvaccinated domestic pilgrims named several barriers to vaccination; lack of awareness that the vaccine is compulsory was the most commonly cited reason, followed by lack of time. Lack of awareness as a barrier to vaccination is consistent with pervious findings on meningococcal vaccine uptake during Hajj among local HCWs [19,20]. In fact, lack of knowledge was also highlighted in previous studies reporting uptake of other Hajj recommended vaccines such as influenza vaccine, for both Saudi [22] and international pilgrims (during the influenza A (H1N1) pandemic) [28]. Lack of awareness was also the main reason reported by Australian pilgrims in 2014 for not receiving

Hajj recommended vaccines [14]. Lack of time was also found to be a barrier to vaccination among emergency room HCWs in Madina [19] and was shown to be a more significant barrier to influenza vaccination among domestic male pilgrims compared to female pilgrims [22].

Surprisingly, a substantial minority of domestic pilgrims also reported having to pay for their vaccine, which in principle should be provided freely in major public primary healthcare facilities across the country. Unfortunately, the wording of the pre-defined questionnaire had limited ability to identify this as a barrier among domestic pilgrims.

New, highly immunogenic, conjugate vaccines are replacing the older polysaccharide vaccines in many developed countries, and they are increasingly being recommended for Hajj pilgrims. Conjugate vaccines are more effective in controlling the carriage of meningococci [29,30] but are considerably more expensive. Meningococcal serogroups that are not covered by the current quadrivalent vaccine were frequently isolated from throat swabs collected from pilgrims, namely, serogroups B and, less frequently, X [31–33]. A recent systematic review concluded that serogroup B dominated the carriage acquisition among Hajj pilgrims [32], and most carriers received the polysaccharide vaccine, which is not expected to reduce the carriage acquisition of serogroups contained in the vaccine [34,35]. The opportunity to prevent future outbreaks depends on an ongoing review of the current mandatory vaccination policy in view of these and future developments.

Promisingly, most of the participants received some pre-Hajj health advice, but the fact that 84% of vaccinated domestic pilgrims (who certainly had a pre-Hajj contact with health professionals) stated non-receipt of advice from a "professional" source deserves careful attention. This provides an important reminder to local health authorities to take advantage of the national Hajj immunization program as an opportunity for providing face-to-face pre-Hajj health education.

The strength of this survey is that it provides a snapshot regarding the current situation with uptake of the compulsory meningococcal vaccine among mainly domestic Hajj pilgrims, and, for the first time, it provides insight into some of the barriers to vaccination. However, since pilgrims are often too busy to complete forms, the small sample size and the submission of incomplete responses are key limitations of this survey. Additionally, the small number of unvaccinated participants limits the ability to draw reliable conclusions regarding the true role of specific barriers. Furthermore, the data are self-reported, and we had no way of validating vaccination histories; moreover, the questionnaire did not differentiate between conjugate and polysaccharide meningococcal vaccines. Finally, we considered all those who stated previous receipt of the vaccine as vaccinated; however, since some respondents did not state the year of vaccination, the true uptake rates may be lower than reported here. The inability to include "unauthorized" domestic pilgrims also adds to the potential overestimation of the true vaccine uptake rate among domestic pilgrims.

In conclusion, this survey demonstrates that many domestic pilgrims miss the compulsory meningococcal vaccine prior to attending Hajj. Overseas pilgrims appear to have good uptake of the vaccine, as expected from the mandatory vaccination for visa policy. Receipt of pre-travel health advice, regardless of the source, is a key motivator for vaccine uptake, and lack of awareness about the vaccination policy is an important barrier. Improving vaccine uptake likely requires system-wide strategies, such as reducing financial barriers and increasing the availability of vaccination centers, as well as greater education of the public, particularly targeting those who are intending to perform Hajj, regarding Hajj-related health risks and prevention strategies. Strategies to improve the ability of local HCW to proactively provide preventive pre-Hajj health advice are also needed. Additionally, the success of the mandatory vaccination policy that is applied to international pilgrims should be modeled to improve compliance with the domestic policy through more rigorous checks and measures. Ongoing evaluation of such strategies is required to monitor the true uptake of vaccines and other health-promoting behaviors among domestic (and international) pilgrims, so that appropriate public health responses can be made to evolving situations.

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and A.A.; visualization, A.-M.B.; writing—original draft, A.-M.B., F.A., W.F., A.A., and A.K.; writing—review and editing, G.R.B., T.A., H.A., M.A.B., A.K., and H.R.

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