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Vaccine 36 (2018) 7415–7422

Contents lists available at ScienceDirect

Vaccine

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

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ARTICLE INFO

Article history:
Received 4 April 2018
Received in revised form 13 August 2018
Accepted 10 September 2018
Available online 17 September 2018

Keywords:
S. pneumoniae
Carriage
Pilgrim
Hajj
qPCR
Vaccine

ABSTRACT

Background: The Islamic Hajj pilgrimage is the largest annual mass gathering in the world. The overcrowding of people promotes the acquisition, spread and transmission of respiratory pathogens, including *Streptococcus pneumoniae*.
Methods: We conducted a methodological review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines. The objective was to summarize the available data regarding the prevalence of pneumococcal carriage among Hajj pilgrims and about carriage acquisition and circulation of *S. pneumoniae* among pilgrims before and after participating in the Hajj according to their vaccination status.
Results: Eight articles met eligibility criteria for pneumococcal carriage and impact of pneumococcal vaccination on carriage. Seven of them showed a significant increase in nasopharyngeal carriage of pneumococci following the pilgrimage, with acquisition rates ranging from 18 to 36%. Serotypes 3, 19F and 34 are the most common. A significant increase in antibiotic resistant strains was observed following participation in the Hajj. A lower prevalence was found in pilgrims treated with antibiotics, those who used a hand sanitizer, or those who washed their hands more frequently than usual. An increased carriage of pneumococcal serotypes included in pneumococcal vaccines (10-valent pneumococcal conjugate vaccine (PCV10), 13-valent pneumococcal conjugate vaccine (PCV13), 23-valent pneumococcal polysaccharide vaccine (PPV23)) was observed following participation in the Hajj. To date, no study has shown a significant reduction in pneumococcal carriage among pilgrims after vaccination with PPV23 or PCV. In fact, no significant difference was currently observed in the prevalence ratio of pneumococcal carriage between vaccinated and unvaccinated pilgrims.
Conclusion: The studies analyzed in this review showed an increased carriage of pneumococcus in post-Hajj pilgrims compared to pre-Hajj pilgrims, including vaccine serotypes. Further studies are needed to investigate the possible relationships between carriage, disease and vaccine in pilgrims.

1. Introduction

The Hajj is the largest annual mass gathering in the world. The Islamic pilgrimage to Mecca in Saudi Arabia attracts 2–3 million pilgrims from more than 180 countries [1]. The overcrowding of people promotes the acquisition, spread and transmission of pathogenic micro-organisms. Respiratory diseases are the most common infections during the pilgrimage. Community-acquired pneumonia (CAP) is a leading cause of hospitalization and the primary cause of critical illness among pilgrims [2–4]. Pneumonia occurs preferentially in the second week, between days 5–15 coinciding with the actual pilgrimage, when the pilgrim density is maximal [5]. A more recent study showed that most cases of CAP related to the Hajj, whom 71% required hospitalization, occur the week after the pilgrimage, just after the peak, corresponding to the incubation time after the acquisition of the bacteria before the onset of symptoms [3]. The etiology of respiratory tract infection is complex, and a wide variety of bacteria and viruses

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https://doi.org/10.1016/j.vaccine.2018.09.017
0264-410X/

are associated with respiratory disorders, with *Streptococcus pneumoniae* being one of the most common pathogens isolated from patients [5]. The prevalence of CAP attributable to *S. pneumoniae* among pilgrims was evaluated to 18% based on culture and urinary antigen detection test [3].

There are more than 90 different serotypes of *S. pneumoniae* [6], and their distribution varies by age, geographical area and clinical syndrome. Pneumococcal acquisition and subsequent nasopharyngeal carriage are considered the main source of pneumococcal horizontal transmission between persons, and a precursor for pneumococcal diseases. The natural route of pneumococcal infection is initiated by nasopharyngeal colonization with the homologous strain, and may progress to invasive disease especially if natural immunological barriers are crossed [7]. The nasopharyngeal colonization is an important key to the occurrence of pneumococcal disease including bacteriemic and non bacteriemic CAP, invasive pneumococcal infection (IPD) but also for its prevention [7]. Several host and environmental factors influence pneumococcal carriage including age, immune status, antibiotic consumption, pneumococcal vaccination, seasons or geographical area [7,8] Cremers et al. showed that nasopharyngeal microbiota could also influence pneumococcal acquisition [8].

Moreover, a significant number of asymptomatic carriers of *S. pneumoniae* was observed [7,9,10]. Children aged less than 5 years constitute the most significant reservoir of *S. pneumoniae*. The rate of pneumococcal carriage observed in young children can reach 60%, including after vaccine implementation [10], and coincides with the highest incidence of IPD among children [11]. *S. pneumoniae* carriage rate decreases to approximately 10–20% in adults, and paradoxically, despite the high prevalence of IPD and CAP in elderly people, a lower asymptomatic carriage rate (5–22%) is observed in this population [9,11,12]. Indeed, among elderly people, the risk of IPD is related to underlying medical and/or socio-demographic conditions and less clearly to the frequency of carriage [11]. The Hajj pilgrim population is characterized by a significant proportion of individuals aged > 65 years with chronic medical conditions, including diabetes mellitus, cardiovascular disorders, asthma, immune deficiency and chronic respiratory disease, placing them at frequent risk for IPD [13]. It is estimated that about 33% of Hajj pilgrims are at risk of pneumococcal disease due to age or presence of underlying comorbidities [14]. Furthermore, crowding conditions experienced by pilgrims favor the transmission of respiratory pathogens [15]. The acquisition of carriage of potentially pathogenic respiratory microbes is very frequent during the Hajj and is well demonstrated for viruses [16]. However, there are fewer studies addressing bacterial acquisition. Characterizing the carriage of pneumococcus and assessing the impact of vaccination in pilgrims can offer relevant information about their risk for IPD.

The objective of this review is to summarize available data about the prevalence of *S. pneumoniae* carriage among Hajj pilgrims, as well as data about carriage acquisition and circulation of *S. pneumoniae* among pilgrims before and after participating in the Hajj, according to their vaccination status.

2. Methods

2.1. Search strategy and selection criteria

The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (http://www.prismastatement.org). The following databases were searched, attempting to identify all relevant studies published from January 1980 to February 2018: Scopus (http://www.scopus.com/) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed). The latest search was conducted on February 28, 2018. The topic search terms used for searching the databases were as follows: #1: "hadj" OR "hajj" OR "pilgrimage"; #2: "*Streptococcus pneumoniae*" OR "Pneumococcus"; #3: "#1 AND #2". Only articles published in English were included, based on the common language shared by the authors. For inclusion the article needed to fulfill the following criteria: [i] it needed to be related to the Hajj pilgrimage; [ii] report on screening in asymptomatic or symptomatic participants; [iii] present bacteriological data and; [iv] report on *S. pneumoniae* carriage prevalence. Reporting on vaccination status was used as an optional criterion. We excluded case reports and studies conducted among ill pilgrims in the hospital and outpatient setting because our paper focuses only on *S. pneumoniae* carriage" The reference lists of reviews were screened to identify studies possibly missed by the search. Two researchers (S.E. and P.G.) independently performed the screening of the abstracts. Any discordant result was discussed in consensus meetings. After screening the abstracts, the full text of the articles was assessed for eligibility by the same two researchers and selected or rejected for inclusion in the systematic review.

2.2. Data collection process

The following data (if available) were extracted from each article: year of the study, study design, study population, type of sample taken, microbiological methods used, pathogen prevalence, pneumococcal vaccination rates and their effect on *S. pneumoniae* carriage prevalence.

2.3. Data synthesis and analysis

The study results were summarized to describe the main outcomes of interest (i.e., the prevalence of *S. pneumoniae* before and/or after participation in the Hajj).

To evaluate the effect of pneumococcal vaccine, the dichotomous data on the number of subjects with *S. pneumoniae* carriage post-Hajj in the vaccinated group and unvaccinated control group were extracted from each study with subsequent determination of the odd ratios (OR) and their 95% confidence intervals (CI). We combined data statistically using random effects (Mantel-Haenszel method) model due the differences in among the studies. Chi^2 and I^2 statistics were used to assess the heterogeneity among the included studies. Values of I^2 can be interpreted as low (25–50%), moderate (50–75%), and high (75% and greater) levels of heterogeneity. Meta-analyses were performed using Review Manager (RevMan 5.3, Cochrane Collaboration). Results were considered to be statistically significant with a p value of < 0.05.

3. Results

3.1. Study selection

A total of 55 articles were selected from the search, and no additional reference was found through a manual search. After screening of titles and abstracts, eight articles were selected for full-text assessment [17–21]. All eight articles were included in the qualitative synthesis of the systematic review (Fig. 1).

3.2. Prevalence and acquisition of S. pneumoniae carriage in pilgrims

To date, eight studies reported the prevalence of nasopharyngeal carriage of *S. pneumoniae* among Hajj pilgrims (Table 1 and Fig. 2). Most of the studies used specific qPCR for *S. pneumoniae* detection [19–21], two used only a bacterial culture approach [17,18] and one study used both qPCR and culture with a

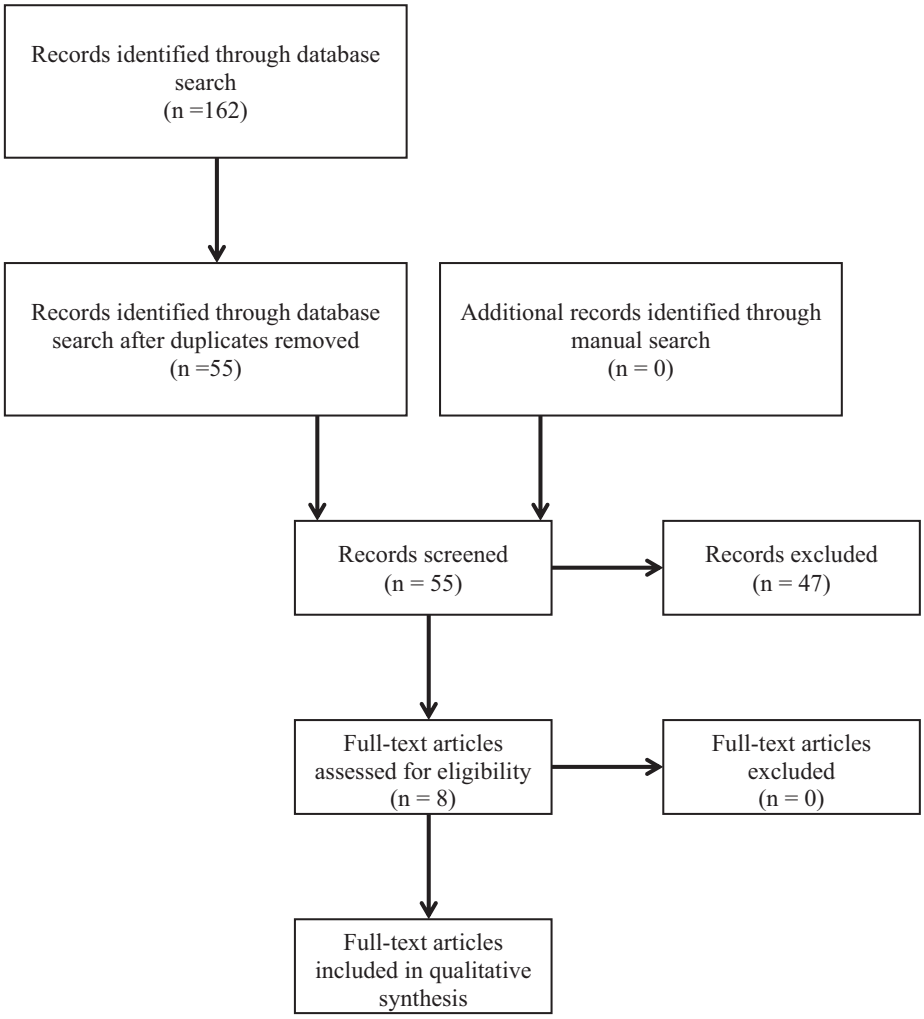

Fig. 1. Search strategy flow diagram.

comparative approach [22]. Two studies were elaborated by the same team that proposed prospective longitudinal studies in cohorts of French pilgrims to investigate the nasopharyngeal carriage of *S. pneumoniae* [19,20]. The nasopharyngeal samples were collected before the departure from France, during the pilgrimage only in symptomatic people presenting acute respiratory signs, and at the end of the pilgrimage for 2 consecutive years [19,20]. Benkouiten et al., reported the first work describing *S. pneumoniae* nasal carriage in 169 pilgrims during the Hajj season of 2012 [20]. They reported a significant increase in *S. pneumoniae* prevalence between pre-Hajj samples and post-Hajj samples (7.3% versus 19.5%, p = 0.0001). The prevalence of *S. pneumoniae* among the 70 symptomatic pilgrims additionally sampled during the pilgrimage was 7.1%. A lower prevalence was found in pilgrims treated with antibiotics compared to other pilgrims (17% versus 25.5%, respectively, p = 0.224) but this difference was not significant [20]. The subsequent study during the 2013 Hajj included 129 French pilgrims in whom a throat specimen was collected pre- and post-Hajj. The carriage of *S. pneumoniae* was estimated at 50% in pre-Hajj and 62% in post-Hajj specimens (p = 0.053) and was significantly higher than the previous years [19]. Nasal acquisition of *S. pneumoniae* was reported in 18.2% of pilgrims sampled in the 2012 Hajj, while 36.3% pharyngeal acquisition was demonstrated during the 2013 Hajj, suggesting important inter-individual transmission of the bacteria in an overcrowding context. The prevalence of the bacteria was lower in patients using hand sanitizer (55.2% versus 76.2%, p = 0.021) and in those performing frequent hand washing (54.7% versus 69.2%, p = 0.08) [19]. Among the 63 subjects with positive qPCR for pneumococcus before departure for the 2013 Hajj, 12 (19%) were negative at the end of the pilgrimage with 10 out of 12 having receive antibiotic treatment during their stay [19], supporting the data showing a lower prevalence of nasopharyngeal carriage after antibiotic therapy.

Memish et al. conducted a larger scale carriage study enrolling 3203 pilgrims originating from Asia and Africa during the 2011 and 2012 Hajj [17]. A nasopharyngeal sample was collected pre- and post-Hajj and culture techniques were used to identify the carriage organisms. They found a significant increase in carriage of *S. pneumoniae* (4.4% versus 7.5%, p = 0.0002), a significant increase in at least one antibiotic non-susceptible strain (2.5% versus 6.1%, p < 0.0001) and a significant increase in multiple antibiotic non-susceptible strains (0.6% versus 2.2%, p < 0.0001) between pre-Hajj and post-Hajj cohorts. Fifty-two serotypes were identified, with serotypes 3 (17%), 19F (5%) and 34 (5%) being the most common. The cross sectional design of the study based on non-paired cohorts did not allow calculation of acquisition rates. A paired cohort culture-based study including 1175 pilgrims from various origin was conducted during the 2013 Hajj and showed an increase in *S. pneumoniae* carriage from 1.8% pre-Hajj to 7.1% post-Hajj (p = 0.0016) [18]. Serotype 3 was also the most common (9.1%), followed by serotypes 6A (7.3%), 19F (6.4%), 23F (5.5%) and 34 (5.5%). Another carriage study was performed during the 2013 Hajj, when

Table 1
Study characteristics and prevalence of *Streptococcus pneumoniae* carriage in Hajj pilgrims.

Study	Hajj seasons	Study design	Subjects	Type of sample	Detection tools	Vaccination rate against invasive pneumococcal disease	Prevalence of *Streptococcus pneumoniae*	References
1	2011–2012	Non-paired cross-sectional cohort survey	1590 pre-Hajj and 1613 post-Hajj pilgrims from 18 countries in Africa and Asia	Nasopharyngeal swab	Culture	11.3% (type of vaccine not documented)	4.4% pre-Hajj/ 7.5% post-Hajj	[17]
2	2012	Paired prospective cohort survey	169 French pilgrims	Nasal swab (anterior nares)	qPCR	35.9% (polysaccharide vaccine)	7.3% pre-Hajj/ 19.5% post-Hajj	[20]
3	2013	Paired prospective cohort survey	129 French pilgrims	Oropharyngeal swab (throat)	qPCR	51.2% (polysaccharide vaccine)	50.0% pre-Hajj/ 62.0% post-Hajj	[19]
4	2013	Paired prospective cohort survey	692 pilgrims from 11 countries in Africa, Asia, Europe and US	Nasopharyngeal swab	qPCR	1.9% (type of vaccine not documented)	5.6% pre-Hajj/ 12.7% post-Hajj	[21]
5	2013	Non-paired cross-sectional cohort survey	514 pre-Hajj and 470 post-Hajj pilgrims from 13 countries in Africa, Asia, Europe and US	Nasal swab (anterior nares)	qPCR	0.2% (type of vaccine not documented)	4.7% pre-Hajj/ 13.6% post-Hajj	[21]
6.	2013	Paired prospective cohort survey	1175 pilgrims (multinational)	Nasopharyngeal swab	Culture	1.4% (conjugate vaccine PCV7)	1.8% pre-Hajj/ 7.1% post/Hajj	[18]
7.	2013	Cross-sectional cohort survey	551 post-Hajj pilgrims from Malaysia	Sputum and oropharyngeal swab	Culture	79.3% (type of vaccine not documented)	1.81% post-Hajj	[23]
8.	2014	Non-paired cross-sectional cohort survey	183 during-Hajj and 93 post Hajj pilgrims from Australia	Nasopharyngeal or oropharyngeal swab	During Hajj (qPCR) Post Hajj (Culture)	27.5% (conjugate vaccine PCV13)	14.2% during Hajj/1.1% post Hajj	[24]
9.	2016	Paired prospective cohort survey	807 Indian pilgrims	Oropharyngeal swab and nasal swab	qPCR and culture	0%	19% pre- Hajj/ 21.8% post Hajj 6.5% pre-Hajj/ 8.2% post Hajj	[22]

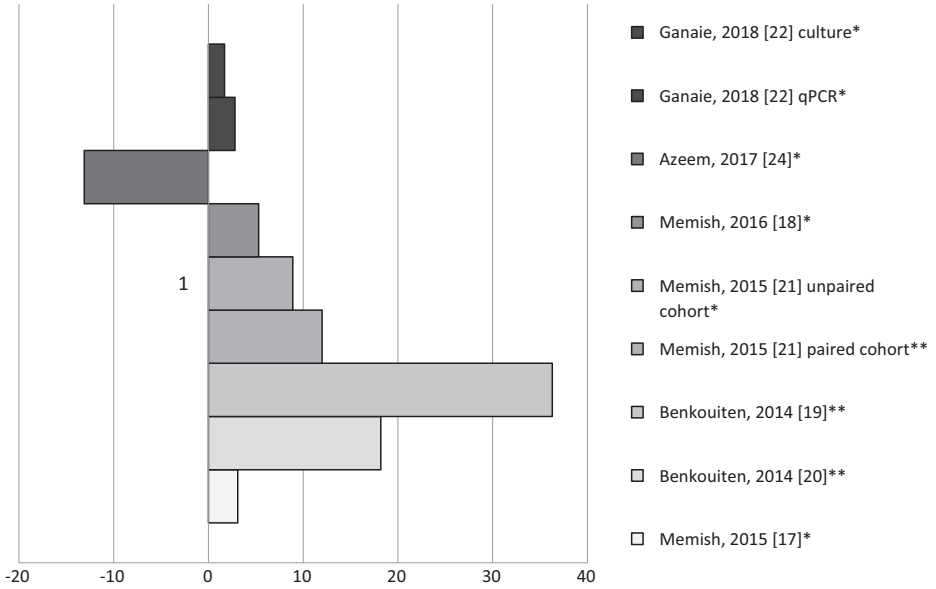

Fig. 2. Differences between prevalence of *Streptococcus pneumoniae* before and after the Hajj or acquisition rates. *Prevalence of *S. pneumoniae* carriage post-Hajj minus prevalence of *S. pneumoniae* carriage pre-Hajj, **acquisition rate (proportion of pilgrims positive for *S. pneumoniae* post Hajj and negative pre-Hajj).

pilgrims from 13 different countries in Asia, Africa and Europe and the United States were enrolled and tested by qPCR [21]. Two cohorts (one paired and one non-paired) were followed. The prevalence of *S. pneumoniae* increased significantly among pre-Hajj nasal specimens (5.6%) compared to post-Hajj nasal specimens (12.7%) ($p < 0.001$) in the paired cohort ($n = 632$). A significant difference was also observed in the non-paired cohort ($n = 514$ arriving and $n = 470$ departing pilgrims), 4.7% versus 13.6% ($p < 0.001$). A 1.81% pharyngeal carriage of *S. pneumoniae* was described in 551 Malaysian pilgrim returning from Hajj 2013 using culture; pre-Hajj data were not collected [23]. In 2014, pneumococcal carriage was evaluated to 14.2% using qPCR in pilgrims from Australia at the fifth days of the pilgrimage and decrease to 1.1% in pilgrims sampled two months after the pilgrimage [24]. However, only 30/246 pilgrims were paired in the 2 phases of their study and the diagnostic test used for the samples collected during and after

the Hajj differed. They used qPCR to test the sample collected at the fifth days and then culture to test the sample collected after the pilgrimage, respectively. More recently, nasopharyngeal carriage of *S. pneumoniae* was evaluated during the Hajj 2016 in 807 Indian pilgrims at 19% before the pilgrimage and 21.8% after the pilgrimage using qPCR (p = 0.048) and to 6.5% and 8.2% using culture (p = 0.064). Multidrug resistant pneumococcus carriage also increased from 11% in the pre-Hajj pilgrims to 32% in the post-Hajj pilgrims (p = 0.0002). Thirty-two different serotypes were identified, with serotypes 19F (20%), 9 V (7.2%) and 1 (6.2%) being the most common during the Hajj. Multiple serotypes were found in 12% of the pilgrims [22].

3.3. Effect of vaccination on pneumococcal carriage at the Hajj

3.3.1. Vaccine coverage against S. pneumoniae

Currently, two types of vaccines against *S. pneumoniae* are available, including the 23-valent pneumococcal polysaccharide vaccine (PPSV23) and the 10 and 13-valent pneumococcal conjugate vaccine PCV10 and PCV13. The first pneumococcal conjugate vaccine PCV7 was licensed in 2000 and replaced by PCV13 in 2010 [25]. Few studies report the proportion of vaccinated people among pilgrims. This prevalence is variable according to the country of origin of pilgrims, but is globally low, even in at-risk individuals (Table 2). The proportion of those vaccinated has tended to increase in recent years, but still remains very low in some countries [26]. In French pilgrims, 31.4% were vaccinated in 2009 versus 51.2% in 2013 [19], while in Australian pilgrims, 28.5% were vaccinated in 2011 versus 14.2% in 2013 [27]. The vaccination rate in multinational cohorts varied from 0.2% [21] to 11.3% [17]. The lower vaccination rate (0%) was reported in India during the Hajj 2016 [22] and the higher vaccination rate was reported in a Malaysian cohort during Hajj 2013 with 79.9% of vaccinated pilgrims [23].

3.3.2. S. pneumoniae carriage according to vaccination status

To date, only one study has shown a significant reduction in pneumococcal carriage after vaccination among pilgrims. In the multinational cohort of the 2011 and 2012 Hajj, the rate of post-Hajj pneumococcal carriage was higher in vaccinated pilgrims compared to unvaccinated pilgrims, although the difference was not statistically significant (10.0% versus 6.5%, respectively) [17]. Moreover, the prevalence ratio of pneumococcal carriage between pre-Hajj and post-Hajj samples was higher in the vaccinated group compared to the unvaccinated group (3.4 versus 1.3) [17]. In the 2012 French pilgrim cohort, 19.1% of the PPV23-vaccinated pilgrims were positive for *S. pneumoniae* at the end of the Hajj, compared to 19.6% in unvaccinated pilgrims [20]. The year after, during the 2013 Hajj, 56.1% of the PPV23-vaccinated French pilgrims were positive for *S. pneumoniae* at the end of the Hajj, compared to 68.3% in the control group [19]. In the Australian cohort, the carriage of *S. pneumoniae* was 10.5% in PCV13-vaccinated pilgrims versus 15.2% in unvaccinated pilgrims during the Hajj 2014, a difference which was not significant; post-Hajj *S. pneumoniae* carriage was nil in the vaccinated group and 1.8% in controls17] [24]. However, Ismail *et al.* are the only to report a significant reduction of *S. pneumoniae* acquisition in vaccinated Malaysian pilgrims (5/388, 1.3%) compared to unvaccinated pilgrims [(5/100, 5%), (p = 0.03)] [23].

The results of the meta-analysis showed that the pooled ORs did not reach statistical significance with p = 0.48 and a high level of heterogeneity was observed between studies (I^2 = 64%) (Fig. 3).

3.3.3. Prevalence of vaccine serotype

Two studies reported a higher carriage of the vaccine pneumococcal serotype in post-Hajj pilgrims compared to pre-Hajj pilgrims [17,18]. Among the 3203 subjects enrolled during the 2011 and 2012 Hajj, Memish *et al.* observed a higher carriage of the pneumococcal serotype present in the PCV13 vaccine in the post-Hajj specimen compared to pre-Hajj specimens (1.1% versus 3.6%; PR 3.2, 95% CI 1.9–5.6). A similar observation was made for the carriage of pneumococcal serotype content in PPV23 (2.3% versus 4.1%; PR 1.8, 95% CI 1.2–2.7) and in PCV10 (0.6% versus 1.6%; PR2.6, 95% CI 1.2–5.3) [17]. The potential coverage of PCV10, PCV 13 and PPV23 vaccines were 19%, 38% and 54%, respectively [17]. These data were also confirmed during the 2013 Hajj [18]. The carriage of pneumococcal vaccine serotype has also increased between pre- and post-Hajj for PCV13 (0.3 versus 3.1%), PCV10 (0.2% versus 1.8%), PPV 23 (0.2% versus 3.4%). The potential coverage of PCV10, PCV 13 and PPV23 was 19.1%, 35.5% and 40%, respectively, which was similar to the previous study. In the Indian pilgrim study where no individual was vaccinated, a higher potential coverage of PCV10, PCV 13 and PPV23 was evaluated, accounting for 47%, 56% and 76% of positive samples, respectively during the Hajj 2016 [22].

Table 2
Pneumococcal vaccination rates in Hajj pilgrims.

Country of origin of pilgrims	Year of pilgrimage	Prevalence of pneumococcal vaccination (%)	Prevalence of pneumococcal vaccination (%) in at-risk patient[a]	References
Australia	2011	28.5	30.6	[27]
Australia	2012	28.7	45.3	[27]
Australia	2013	14.2	29	[27]
Australia	2014	27.5	NA[b]	[24]
France	2009	31.4	NA[b]	[44]
France	2010	1.7	2.2	[45]
France	2012	35.9	47.8	[20]
France	2013	51.2	NA[b]	[19]
India	2016	0	0	[22]
Iran	2004	2.5	NA[b]	[46]
Iran	2005	8.9	NA[b]	[46]
UK	2005	5	15	[14]
Malaysia	2013	79.3	NA[b]	[23]
Multinational cohort	2013	4.4	1.5% in aged ≥65 years 27.3% among diabetics	[47]
Multinational cohort	2013	1.4		[18]
Multinational cohort	2013	1.2	NA[b]	[21]
Multinational cohort	2011–2012	11.3	NA[b]	[17]

[a] At risk patients were patients aged >5 years or younger patients with chronic medical conditions, including diabetes mellitus, cardiovascular disorder, asthma, immune deficiency, chronic respiratory disease.
[b] NA: data not available.

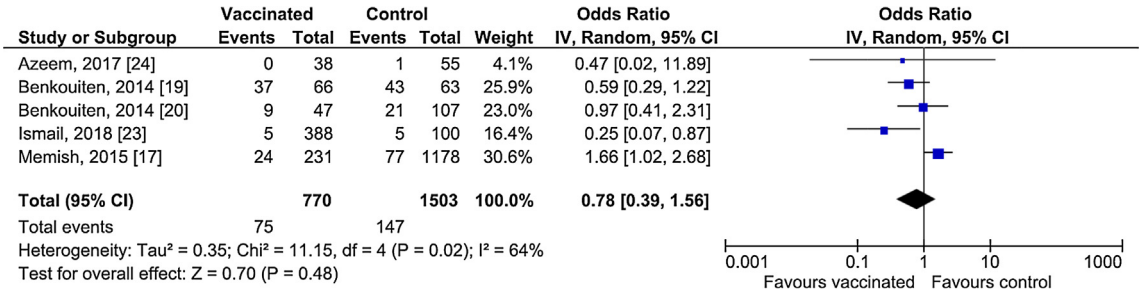

Fig. 3. The effect of pneumococcal vaccine on post-Hajj carriage of *Streptococcus pneumoniae* in pilgrims. Meta-analysis of five studies.

4. Discussion

All studies evaluated in this review support the fact that the overcrowding and mixing of populations increase pneumococcal carriage among pilgrims during the Hajj. Pneumococcal acquisition appears to be rapid after the arrival of pilgrims in Saudi Arabia, and no correlation was found between the rate of pneumococcal carriage and the duration of the Hajj stay [17]. No significant difference was found between the prevalence of *S. pneumoniae* in subjects tested at<10 days after their arrival (7.8%), tested between 11 and 20 days (8.2%) or more than 20 days (6.4%) [17]. Several factors seemed to influence the carriage of *S. pneumonia*e during the Hajj, including advanced age [17,21], Hajj dates [17,19] and the geographical origin of pilgrims [17,21]. For example, Memish *et al.* did not find a significant change in the pneumococcal carriage among Indian pilgrims sampled before and after the pilgrimage, whereas the acquisition rate in Indonesian pilgrims was high (6.8% versus 6.1%, p = 0,70, and 1.2% versus 8.3%, respectively, p < 0.0001) [17]. The carriage of antibiotic-resistant *S. pneumoniae* increases during the Hajj, but does not appear to be linked to self-antibiotic use, which suggests frequent circulation and transmission of these strains [17]. High antibiotic consumption during the pilgrimages may increase selective pressure. Several serotypes were found to be circulating in the Hajj, including serotype 3 and 19F, which were the most common. However, isolated serotypes were not all covered by vaccine. The potential coverage of the PCV10, PCV 13 and PPV23 were around 20%, 35% and 50%, respectively. These data were confirmed using Multilocus Sequence Typing, which showed that several clones of *S. pneumoniae* were present at the Hajj [17]. The most common clones included ST 700 and ST 9220, but no single invasive clonal expansion was identified. Increased carriage and serotype mixing following the Hajj may have an impact on the epidemiology of the disease in the countries of origin of the pilgrims, notably through the introduction of new serotypes and serotype replacement especially in countries that have introduced pneumococcal vaccination in their childhood vaccination programs.

The prevalence of nasopharyngeal carriage of *S. pneumoniae* varied according to the study and according to the Hajj season. Moreover, results from these studies cannot be extrapolated to all pilgrims from all countries, as only a small fraction of the over 2 million Hajj pilgrims have been investigated.

Culture remains the gold standard for pneumococcal detection. In the previous studies using culture, *S. pneumoniae* identification seems to be reliable; several phenotypic tests were used and molecular identification was performed in case of doubt [17,18]. However, the major limitation of some studies is the lack of data about antibiotic use among pilgrims during the stay, which certainly underestimates *S. pneumoniae* carriage, especially as pneumococci were evaluated by culture [17,18]. Overall, the prevalence of pneumococcal carriage was constantly lower when assessed by culture than when using qPCR. As an example, a 3 fold increase of pneumococcal detection in the nasopharynx was found using qPCR (19%) compared to culture (6.5%) in Indian pilgrims prior to the Hajj 2016 [22]. A similar pattern was observed post-Hajj; however, 45% of pilgrims received antibiotherapy during the Hajj. On other hand, some qPCR systems may lack specificity for pneumococcal detection. In fact, the qPCR system targeting the *lytA* gene of *S. pneumoniae* used by Benkouiten et al. [19,20] and Memish et al. [21] also amplified *S. pseudopneumoniae*; however, the prevalence of *S. pseudopneumoniae* in the respiratory tract is low [28–30]. *S. pseudopneumoniae* was found in 1 to 12% of patients with respiratory tract infections, but was found more frequently in sputum than in nasopharyngeal samples [31]. Of 61 *S. pseudopneumoniae* strains isolated from humans, 32 were isolated from sputum, 17 from bronchial aspirates, 4 from bronchoalveolar lavages, 1 from a nasal swab, 1 from a pharyngeal swab and 6 from other samples in one study [31].

The assessment of *S. pneumoniae* carriage is dependent on many factors, including the type of samples, their storage and laboratory techniques which may bias the results. In 2003 and 2013, the Pneumococcal Carriage Working Group from the World Health Organization (WHO) published recommendations to standardize the detection of bacteria in the upper respiratory tract [32]. In summary, for adults, both nasal and oropharyngeal samples are recommended to increase the sensitivity of pneumococcal detection [32]. If only one specimen can be collected, it is better to collect a nasopharyngeal sample than oropharyngeal specimens. Few data exists comparing sampling methods in adults, and they have shown discordant results. Some studies have shown a higher rate of nasal pneumococcus [33,34], but others have reported the superiority of oropharyngeal samples [35]. However, these recommendations are based mainly on culture detection of pneumococcus; further studies testing qPCR in nasal versus pharyngeal specimens are needed to establish if these recommendations can be applied to molecular detection. Nylon and Dacron swabs are suitable for culture-based methods and are preferred for molecular analysis, because they are less likely to inhibit DNA amplification. One milliliter of transport medium skim-milk tryptone glucose glycerol (STGG) broth is required; the swab should be transported to the laboratory within 8 h under cold conditions and frozen at -70 °C minimum as soon as possible [32]. We propose here our recommendations for future *S. pneumoniae* carriage studies in Hajj pilgrims, with a minor modification of the previous WHO recommendations (Table 3).

A high proportion of pilgrims were treated with antibiotics during the Hajj, which possibly affects the detection of pneumococcus, especially if culture is used. The prevalence of *S. pneumoniae* was probably underestimated in the studies using only a culture approach, because the bacteria are no longer viable after effective antibiotic treatment. This is less of a problem with PCR that detects bacterial DNA which is able to persist after efficient antibiotic treatment. DNA from *Streptococcus* spp. seems to be particularly persistent in clinical samples compared to DNA from others bacte-

Table 3
Recommendations for future *Streptococcus pneumoniae* carriage studies in Hajj pilgrims.

1- Study design	Longitudinal cohort design with paired samples before and after the pilgrimage
2- Sampling	Nasopharyngeal sample for culture-based methods Nasopharyngeal, pharyngeal or nasal samples[a] for qPCR
3- Clinical information required	Recording pneumococcal vaccination status and the use of antibiotics
4- Technique	qPCR is preferable for the screening of *S. pneumoniae* carriage Specific primer and probes lytA-CDC are recommended Culture is necessary for studying serotypes and antibiotic susceptibility
5-Transport and storage	Medium transport skim-milk tryptone glucose glycerol (STGG) and transport to the laboratory within 8 h in cold condition
6-Storage	Frozen at −70 °C minimum

[a] Further studies testing qPCR in nasal versus pharyngeal specimens are needed to establish the recommendations.

rial species despite appropriate antibiotherapy [36,37]. As an example, DNA can persist for as long as 7 years after the infectious episode in a cardiac valve in a patient with endocarditis. Consequently, qPCR is probably the best tool to assess the carriage of pneumococcus in the Hajj pilgrims, but the disadvantages are that qPCR does not distinguish between living and dead microorganisms and does not allow assessing antibiotic susceptibility. Moreover, the most significant advantages of qPCR are the lower limit of detection compared to culture, notably in patients receiving antibiotherapy before sampling and a detailed quantification of pneumococci in the samples. Ganaie et al., found a clear difference of the number of DNA copy in patients presenting respiratory symptoms (mean 312 copie/μl) versus asymptomatic people (mean 145 copie/μl) and a clear difference of the number of DNA copy between the pre-Hajj group (mean 264 copie/μl) and the post-Hajj group (mean 480 copie/μl) [22]. The risk of contamination of the sample is low, especially when strict validated protocols with positive and negative controls are used.

However, misidentifications have been reported between *S. pneumoniae* and other viridans group streptococci, either by phenotypic identification, mass spectrometry (MALDI-TOF MS) identification or by PCR [38]. In fact, some qPCR systems also amplified *S. pneumoniae*-related bacteria, particularly S. *pseudopneumoniae* [38]. This is especially critical in non-sterile respiratory samples such as sputum, pharyngeal or nasal specimens. The usual targets used for specific *S. pneumoniae* qPCR are *lytA*, *ply*, Spn9802 and *psaA* genes. Most qPCR systems targeting *lytA* and *psaA* genes were specific for *S. pneumoniae,* although the previously popular PCR gene target *ply* has been found to be nonspecific [38,39]. The *lytA*-CDC system presents a high specificity for *S. pneumoniae* compared to other qPCR systems and clearly discriminates *S. pneumoniae*-related bacteria including *S. pseudopneumoniae* from *S. pneumoniae* [38,40]. This system is strongly recommended by the Pneumococcal Carriage Working Group from WHO [32].

Reducing pneumococcal disease and carriage in Hajj pilgrims involves a multi-pronged prevention approach, including vaccine [26]. Consistent local or national official recommendations on the use of pneumococcal vaccine for Hajj pilgrims are lacking [14,26]. Recommendations exist only for high risk individuals, and considerable variation exists between countries [14,26]. The recent recommendations of the Saudi Thoracic Society (STS) for pneumococcal vaccination before the Hajj season are to vaccinate all persons $\geq$ 50 years with combined PCV13 and PPV23 vaccination [41]. A combination of these 2 vaccines was proposed to extend the spectrum of protections to a higher number of pneumococcal serotypes. Moreover, studies showed strong evidence of protective efficacy of PPV23 against IPD but its effect to prevent *S. pneumoniae* CAP is uncertain and no significant effect was found on nasopharyngeal carriage [42]. In contrast, PCV13 showed an efficient protection against pneumococcal bacteremic and nonbacteremic CAP, invasive pneumococcal disease and pneumococcal carriage [42,43].

However, for those planning to be vaccinated immediately before the Hajj, it is recommended to administer only one dose of PPV23. It is recommended to administer a single PPV23 dose at least 3 weeks before the beginning of pilgrimage to immunocompetent persons aged less than 50 years with risk factors. The STS does not recommend pneumococcal vaccine routinely to healthy persons aged <50 years because of the lack of evidence of benefit-risk ratio and the lack of data about epidemiology of pneumococcal disease and pneumococcal serotype circulating during Hajj. However, the vaccine clearly elicits an immune response in this population. Approximately one third of pilgrims have risk factors for pneumococcal disease, with age and comorbidities [26]. However, the WHO and CDC recommend pneumococcal vaccine for Hajj pilgrims, but only for those aged $\geq$65 years and for younger pilgrims with comorbidities [14].

The prevalence of vaccinated pilgrims has tended to increase in recent years, but still remains low in numerous countries, even in at-risk individuals [26]. Vaccination rates in at risk-French pilgrims prior inclusion were very low (<3%), comparing to overall French population of similar age range with vaccination rate varying 14.2–21.1% [48]. Following inclusion, however vaccination coverage increased to 31.4–51.2%. Vaccination rates in at risk-Australian pilgrims after inclusion (14.2–28.7%) were lower than in overall Australian population of similar age range with vaccination rate varying 50.3–72.8% [49]. Continuing medical and health education is necessary to improve the vaccination rate. To date, the effectiveness of the PPV23 vaccine on pneumococcal carriage during the pilgrimage has not been proven, but few studies have been done, and most of them included a low number of patients. Further studies with a large number of patients and collection of accurate data on their vaccination status are needed to more precisely assess the effectiveness of pneumococcal vaccination with PPV23 or PCV on carriage during the pilgrimage.

5. Conclusion

All the studies analyzed in this review showed an increased carriage of pneumococci in post-Hajj pilgrims compared to pre-Hajj pilgrims, including vaccine serotypes. However, the distribution of *S. pneumoniae* serotypes during the Hajj should be more precisely characterized in order to provide reliable data on which to base recommendations for pneumococcal vaccination in Hajj pilgrims.

Further studies are needed to investigate the possible relationships between carriage, disease and vaccine efficacy during the pilgrimage. We recommend a longitudinal cohort design with paired samples before and at the end of the pilgrimage. Culture and qPCR are complementary for surveying the density and duration of pneumococcal carriage episodes. qPCR is preferable for the screening of pneumococcal carriage because of its high sensitivity, ease, and usability in large scale studies, but culture techniques are necessary for investigating antibiotic susceptibility and serotypes.

Conflict of interest

The authors report no conflict of interest.

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