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Original article

Impact of Hajj on the *S. pneumoniae* carriage among Indian pilgrims during 2016- a longitudinal molecular surveillance study

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ABSTRACT

Background: The population flow dynamics of Hajj increases the probability of pneumococcal acquisition and amplification among Hajis. This multi-site longitudinal molecular surveillance study was designed to assess the impact and potential variations of pneumococcal carriage in a single cohort of pre and post-Hajj pilgrims from India.

Method: A total of 3228 pre and post-Hajj, nasopharyngeal and oropharyngeal swabs were collected from 807 pilgrims with an interval of 40 ± 5 days. The carriage was detected by culture and qmPCR. Quellung test, mPCR-FAF, PCRseqTyping, and MLST was used for typing. Antibiogram was performed by MIC method.

Results: An increased incidence of pneumococcal carriage was detected in post Hajj cohort by qmPCR (19% vs 21.8%) (p-value = 0.0487) and culture (6.5% vs 8.2%) (p-value = 0.0645). Fragment analysis could identify multiple serotype carriage in 76 pilgrims. Increase in drug resistance was also observed in post-hajj cohort for Tetracycline (29% vs 51%), Erythromycin (26% vs 46%) and Levofloxacin (6% vs 17%). Multidrug resistant strains in post Hajj group was 32% compared to 11% in pre Hajj group (p-value = 0.0002).

Conclusion: Our results confirm high acquisition rate of multidrug-resistant *S. pneumoniae* in Hajj pilgrims and highlight its potential spread to home countries upon their return. Surveillance studies are needed to evaluate modifiable factors associated with carriage.

1. Introduction

Hajj, the pilgrimage to Mecca, Saudi Arabia, performed in the 12th lunar month of each year is the largest and most long-standing annual mass gathering event on earth [1]. Hajj is one of the five fundamental pillars of Islam, a religious duty undertaken by Muslims at least once in their lifetime. Around 10% of the world's Muslim population (176 million) reside in India [2]. Following an exponential rise in the numbers of non-Saudi pilgrims, annually, 3–5 million of Muslims from > 180 countries with diverse medical and social background embark on Hajj pilgrimage [3]. Indian Hajj pilgrims form the second largest number (0.17 million) with 60–70% of them above 50 years of age [4]. Though its rituals last for 5 days, the period of pilgrimage is up to 40–45 days for Indians, where they reside for several nights in tent camps.

The congregation of masses in extreme climatic conditions and overcrowding of the pilgrims from diverse parts of the world within a confined area results in the intermingling of different pathogens or the subtypes of the same pathogen [5]. Exposure to these novel, emerging or re-emerging pathogens triggers many health challenges and risks [6]. The unavoidable close contact conditions heighten risk pilgrims to acquire and spread infectious organisms via air droplets, which are exacerbated by air travel [7].

During Hajj season, respiratory infections are ranked as the second leading cause of morbidity among pilgrims, with heart diseases being the chief cause [1]. Pneumonia is the foremost cause of hospitalization and admission to intensive care units among pilgrims aged over 50 years [8]. Though limited data is available on the microbiological causes of Hajj, *S. pneumoniae* appears to be a common pathogen isolated

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from patients with pneumonia and respiratory tract infections [9]. Studies have reported high acquisition of *S. pneumoniae* by pilgrims during Hajj season and highlighted the potential for spread of these infections to home countries of pilgrims upon their return [10,11]. The other bacterial etiological causes of respiratory infections during Hajj include: *H. influenzae*, *M. tuberculosis*, *B. pertussis*, Streptococci, Chlamydia, *Mycoplasma*, *Legionella*, *Klebsiella*, *Pseudomonas*, *S. aureus*, and Gram-negative bacteria [12–14].

At least one-third of Hajj pilgrims are considered “at risk” of pneumococcal disease. The risk factors for the pneumococcal disease include smoking, age ≥50 years, asthma, diabetes, chronic obstructive lung disease (COPD), alcohol use, HIV, and other immunosuppressive conditions. Comorbidities increase the risk of incidence and morbidity of pneumococcal disease [15]. The WHO and regulatory bodies from several countries have identified an unmet medical need for vaccination, to prevent pneumococcal pneumonia in adults [16]. On December 30, 2011, the US-FDA approved PCV13 for prevention of pneumonia and IPD caused by PCV13 serotypes among adults aged 50 years and older [17]. CDC recommends people with asthma, COPD, or other conditions that affect the lungs get pneumococcal vaccines-once as an adult before 65 years of age, and then two more doses at 65 years or older [18].

Lack of sensitive and specific diagnostic tools limits our ability to determine the true burden of *S. pneumoniae* in respiratory infections. Inability to detect and type *S. pneumoniae* directly from clinical samples, culture-negative samples, multiple serotypes, adds to problem [18,19]. Use of molecular techniques in addition to conventional methods could address many of these issues. Surveillance of acute respiratory infection in defined populations to monitor prevailing pathogens and to determine population groups at special risk are important for taking preventive measures [11]. To the best of our knowledge, this is the first molecular surveillance study from India, taking into account different geographical locations among Indian pre and post-Hajj cohorts.

2. Materials and methods

2.1. Study design and study population

This prospective multisite longitudinal study was conducted during the 2016 Hajj season. The study population consisted of 807 convenience sample of pilgrims from 4 cosmopolitan cities of India (Fig. 1) aged 18 years and older. They were screened for *S. pneumoniae* carriage at the beginning and at the end of the Hajj with an interval of 40 ± 5 days. The sample size was calculated to provide 80% power to detect a prevalence of 6% with 20% precision and 5% level of significance. Informed consent was obtained from all subjects prior to study enrollment. A standardized questionnaire was used to collect information from the pre and post-Hajj cohorts. Demographic data, habits, medical conditions, vaccination and antibiotics pre-treatment history was recorded. Post Hajj questionnaire included additional information related to Hajj, such as housing conditions, clinical illness, medication, hospitalization etc.

2.2. Sample collection

Paired nasopharyngeal (n = 1614) and oropharyngeal (n = 1614) swab specimens were collected from the 807 pilgrims during pre and post-Hajj sessions. The specimens were collected according to the standard procedure using Copan flocked swabs (NP swab- 502CS01 and OP swab-503CS01, Copan, Italy) [20]. Swabs were immediately inoculated into1mL skim milk-tryptone-glucose-glycerin (STGG media) and transferred to the local lab within 2 h on dry ice for storage at −80 °C.The stored specimens were transferred to Central Research Lab, KIMS, Bangalore for further processing according to the CDC Streptococcus Laboratory Protocols [21].

2.3. Microbiological identification, serotyping and antimicrobial susceptibility testing

The standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group protocols were employed [20]. Briefly, upon delivery to the laboratory, 10 µl of NP-STGG and OP-STGG inoculum were streaked onto 5% sheep blood agar plates, incubated in a 5% CO_2 incubator at 37 °C for 18–24 h. *S. pneumoniae* isolates were identified on the basis of colony morphology, Gram staining, susceptibility to optochin and bile solubility tests. Serotyping of pneumococcal isolates was performed by Quellung reaction using Pneumotest kit and type-specific antisera (SSI, Denmark), as recommended by the manufacturer [22]. Antimicrobial susceptibility testing (AST) of the isolates was performed by broth microdilution method (Vitek system- Biomerieux, France to determine the minimum inhibitory concentration MIC) as per the 2016 Clinical Laboratory Standards Institute guidelines [23].

2.4. Molecular identification and typing

2.4.1. DNA extraction

The STGG aliquots of NP and OP swabs were thawed to room temperature and vortexed for 15 s 200 µL of each sample was processed for total nucleic acid extraction on the QIAcube instrument (Qiagen, Hilden, Germany) using the QIAamp® DNA mini kit according to the manufacturer's recommendations. Quantification and quality of the extracted DNA were evaluated spectrophotometrically at 260 nm absorbance with Nanodrop 2000 (Thermo Fisher Scientific, USA).

2.4.2. Quantitative multiplex real time PCR (qmPCR)

The extracted DNA was subjected to qmPCR assay for the identification of *S. pneumoniae* targeting *ply, lytA, psaA* and *spn9802* genes as described in our previous publication [24]. Positive samples were defined as those which showed amplification for ≥3 target specific sequences with quantification cycle (Cq) value of ≤35 and lower limit of detection ≥4 genome copies/µl. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) specific primer-probe set was used to amplify the endogenous positive control in multiplex reactions. A no template control (NTC) was included in every run. Pilgrims positive for nasopharyngeal and/or oropharyngeal swabs were counted only once as positive cases.

2.4.3. PCRSeqTyping

PCR was performed targeting 1061bp, a cpsB region for detection of pneumococci and the same amplicons were subjected to sequencing. The sequence data was analyzed using NCBI database for identification and typing. For homologous strains, a second round of PCR, sequencing and data analysis was performed targeting ten group-specific genes located in capsular polysaccharide region. The PCRSeqTyping assay was performed as described in our earlier publication [25].

2.4.4. Multiplex PCR combined with fragment analysis detection by automated fluorescent capillary electrophoresis (mPCR-FAF)

Forty primer pairs were used to target serotypes 1, 2, 3, 4, 5, 6A/6B, 6C, 7F/7A, 7C/(7B/40), 8, 9V/9A, 9N/9L, 10A, 10F/(10C/33C), 11A/11D/11F, 12F/(12A/44/46), 13, 14, 15A/15F, 15B/15C, 16F, 17F, 18/(18A/18B/18C/18F), 19A, 19F, 20, 21, 22F/22A, 23A, 23B, 23F, 24/(24A/24B/24F), 31, 33F/(33A/37), 34, 35A/(35C/42), 35B, 35F/47F, 38/25F, and 39 70 serotypes. The oligonucleotide sequences were obtained from published source [26]. Some serotypes were indistinguishable from other closely related serotypes, most of which belonged to the same serogroup. The sequences for the type-specific primers are available at the CDC web page (http://www.cdc.gov/ncidod/biotech/strep/pcr.htm). Primers specific to the *cpsA* (*wzg*) gene were used as an internal positive control. In the present study,