Research Article ISSN 2639-8494

Clinical Immunology & Research

SARS-CoV-2 Antigen Screening and its Clinical Characterization in Suspected Patients at Tertiary Care Hospital of Kathmandu, Nepal

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Received: 08 Sep 2024; **Accepted:** 15 Oct 2024; **Published:** 25 Oct 2024

Citation: Sanskriti Pandit, Suraj Aryal, Pabitra Bista, et al. SARS-CoV-2 Antigen Screening and its Clinical Characterization in Suspected Patients at Tertiary Care Hospital of Kathmandu, Nepal. Clin Immunol Res. 2024; 8(2): 1-7.

ABSTRACT

Background: *SARS-CoV-2 has been circulating around the world after first case identification in China.Many of the therapeutic options are being studied globally and recently, many countries have been vaccinating their people. However, its efficacy is under study and yet to be clearly defined. In this circumstance, reliable and faster diagnostic testing is critical for limiting the spread of the virus. This study is an attempt to identify SARS-CoV-2 by an antigen screening test, its effectiveness and clinical correlation in COVID cases.*

Methods: *This is laboratory based cross sectional study was performed in Manmohan Memorial Medical College and Teaching Hospital, Kathmandu from November 2020 to January 2021. A total of 150 patients were screened for SARS-CoV-2 antigen in the nasopharyngeal swab. A structured questionnaire was administered to collect information on clinico-demographic profiles and illness history of patients. Antigen screening was performed using lateral flow immunoassay. Data were analyzed according to standard statistical method using SPSS version 20.*

Results: *Among 150 participants, 11(7.3%) were SARS-CoV-2 antigen positive. Significant group differences between positive and negative were observed for age ($p= 0.002$), presence of symptoms ($p<0.001$), duration of symptom onset ($p<0.001$), presence of underlying illness ($p=0.001$) and contact history with COVID-19 infected case ($p<0.001$) but not for gender ($p=1.00$).*

Conclusion: *Adults, elderly and the individuals having close contact with COVID-19 infected patient were at high risk of acquiring infection. Majority of the infected patients presented the symptoms and had underlying disease suggesting, underlying illness could be the risk factor for SARS-CoV-2 infection.*

Keywords

SARS-CoV-2, Antigen screening, Suspected patients.

Abbreviations

SARS-CoV-2: Sub-Acute Respiratory Syndrome Corona Virus 2, COVID-19: Coronavirus Disease 2019, WHO: World Health Organization, MMTH: Manmohan Memorial Medical College and Teaching Hospital, ACE 2: Angiotensin Converting Enzyme 2, nCoV: Novel Coronavirus, NAATs: Nucleic Acid Amplification Tests, SPSS: Statistical Package for Social Sciences, IQR: Inter Quartile Range, rRT-PCR: Real Time Reverse Transcriptase Polymerase Chain Reaction, RAAS: Renin–angiotensin–aldosterone system.

Introduction

SARS-CoV-2, previously 2019 nCoV is an enveloped non segmented, positive sense single stranded RNA virus of the Coronaviridae family [1,2]. In December 2019, retrospective investigations identified human cases with onset of the symptoms of atypical pneumonia of unknown etiology in Wuhan, China [3,4]. The unknown etiology was found to be new virus, causing a new disease called COVID-19 having high infectious potential

[5,6]. Since its first case identification in China, similar cases were reported globally within a month, declaring it a pandemic by WHO in 11th March, 2020 [5]. The first confirmed case in Nepal was reported on January 5, 2020 [7]. As of 21st July,2021 there have been more than 190 million confirmed cases of Covid-19 globally, resulting more than 4 million deaths [8].

SARS-CoV-2 causes systemic and respiratory diseases with respiratory symptoms (cough, sore throat, runny nose or congestion and pneumonia), GI symptoms (loss of appetite, nausea, vomiting, diarrhea and abdominal pain), neurological symptoms (loss of smell and taste, muscle weakness, tingling or numbness in hands and feet, dizziness, confusion, delirium, seizures and stroke), fever and chills. sore throat, runny nose/congestion, nausea/vomiting, and diarrhea [9,10]. The incubation period for COVID-19 varies between 2-14 days, median time of 4-5 days from exposure to symptoms onset [11]. However, large proportion of people have been reported to be asymptomatic and symptomatic illness ranged from very mild to severe even resulting to death [9]. Severity of the infection depends upon age-group, immune status, co-morbidity, and may even be geography and gene [12]. Any of the vulnerable population with chronic illness like: diabetes, cardiovascular disease, renal disease, lung diseases, cancer, not only acquire serious illness but also develop severe clinical presentations leading to multiple organ failure and death [13].

Accurate and timely COVID-19 testing is an essential step in the management of the COVID-19 outbreak but diagnostic technique widely preferred and specified as "gold-standard test" is rRT-PCRrequiring at least four hours of operation performed by skilled techniciansand also, is a time consuming procedure to process and generate results [14-16]. This diagnostic approach seems to be challenging for resource limited settings includingNepal with limited diagnostic capabilities, because of which it has been slow to conduct screening by PCR [17]. Alternate to rRT-PCR, reliable and faster diagnostic tests for detecting antigen specific for SARS-CoV-2 infection are introduced [16]. The interim guidance of WHO on September 11, 2020, has presented rapid antigen detection, as a new technology for COVID-19 detection that is simpler and faster to perform than NAATs that directly detect the SARS-CoV-2 proteins produced by replicating viruses in the respiratory secretions within 10-30 minutes [16]. Thus, this study aims to screen SARS-CoV-2 antigen among the suspected patients using rapid antigen detection method.

Methods

Study Design and Selection Criteria

A cross sectional study was performed in Manmohan Memorial Medical College and Teaching Hospital, Kathmandu, Nepal during the period of 3 months (November 2020 to January 2021). Initially, informed consent was obtained from the patients and they were interviewed to obtain information regarding clinico-demographic profile (age, sex, symptoms) and underlying illness history using structured questionnaire. Patients fulfilling any one of the following criteria viz; any symptoms of acute respiratory illness (Fever, cough, shortness of breath), patients with history of contact with positive case, international travel history or, who were being admitted to the hospital for treatment procedureswere included in the study.

Testing Protocol

Nasopharyngeal swabs were obtained from the study subjects by inserting the sterile swab into the nostril of the posterior pharynx following specimen collection guideline of CDC and instruction manual of the kit manufacturer company.

Antigen screening was performed using STANDARD Q COVID-19 Ag test (SD Biosensor, Inc., REPUBLIC OF KOREA). Sample processing was performed according to manufacturer's instruction. Briefly, the swab was inserted into extraction buffer tube immediately after obtaining the specimen. Squeezing the buffer tube, swab was stirred more than 5 times. Then, swab was removed while squeezing the sides of the tube to extract the liquid from the swab. Nozzle cap was tightly pressed onto the tube and 3 drops of extracted specimen was applied to the specimen well of the test device. Results were read in 15-30 minutesand the results after 30 minutes were considered invalid. The test was validated by using the nasopharyngeal swab from the patient with recentSARS-CoV-2 infection, confirmedby PCR as a positive control and by using the nasopharyngeal swab from the healthy individual with PCR confirmed absence of SARS-CoV-2 infection as a negative control.

Statistical Analysis

Data analysis was done using SPSS Version 20 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2013.

Demographic characteristics, symptoms, duration of symptom/s onset, contact history and underlying illness were assessed for whether or not a suspected case tested positive for SARS-CoV-2 antigen. Age was the only continuous variable defined using median and Inter-quartile range (IQR). The Kolmogorov-Smirnov test was used to verify the normality distribution and the Mann-Whitney U test was used for assessing group difference in age. Categorical variables were expressed as frequency rates and percentages. The Fisher's exact test was used as applicable to test for association between group differences in categorical variables. p-Value< 0.05 was considered as statistically significant.

Results

A total of 150 patients with suspected COVID-19 were included in this study, among which 11 (7.3%) tested positive for SARS-CoV-2 antigen. Overall, 113(75.3%) were female and 37(24.7%) were male. Of these, 9 (81.8%) female and 2 (18.2%) male tested positive for antigen. The median age was 30 years (IQR, 25-42). Age groups were categorized as Children (1-17 years), Adult (18-64 years) and elderly ($\geq$ 65 years). There were total 10 (6.7%) children, 124(82.7%) adults and 16 (10.6%) elderly participants. None of the children tested positive for SARS-CoV-2 antigen whereas, 6 (54.5%) adults and 5 (45.5%) of the elderly were antigen positive. Among 150 individuals tested, 22 (14.7%) were symptomatic and 128 (85.3%) were asymptomatic. Of the antigen

positive individuals, 8 (72.7%) were symptomatic with ≤ 7 days history of symptom onset. Among 8 of the symptomatic antigen positive individuals, fever, dry cough and dyspnea were the predominant symptoms reported in 7 (63.6%) subjects. Similarly, fatigue and body ache being second most common, presented in 6 (54.5%) followed by sore throat and runny nose in 5 (45.5%) individuals. Headache was reported in 4(36.4%) of them. Anosmia and ageusia were least common symptoms presented only by 1 (9%) individual (Figure 1).

Twenty-seven (18%) of the total patients enrolled in the study had at least one underlying illness and remaining 123 (82%) did not have any kind of underlying illness. Diabetes mellitus was the most predominant underlying illness among antigen positive individuals as reported by 4 (36.4%) individuals. It was followed by hypertension in 3 (27%) of them. Similarly, cardiovascular disease, renal disease, liver disease and neurological disorder was reported in 1 (9%) individual (Figure 2). Among the total participant, 19 (12.7%) had positive contact history with known COVID-19 infected cases. None of the individuals were reported to have travel history. 114 (76%) of the 150 individuals were from inside of Kathmandu valley and 36 (24%) were from outside of the Kathmandu valley (Figure 3). Among antigen positive patients, 8 (72.7%) were from Kathmandu valley and 3 (27.3%) were from outside the valley.

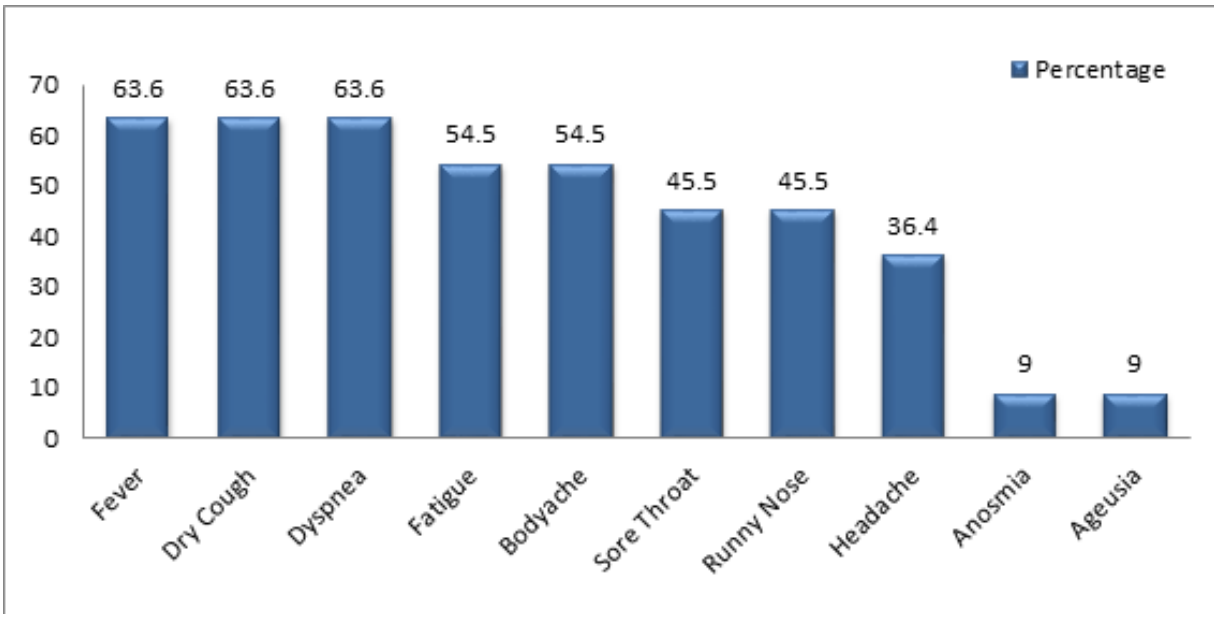

Figure 1: Reported symptoms among antigen positive individuals.

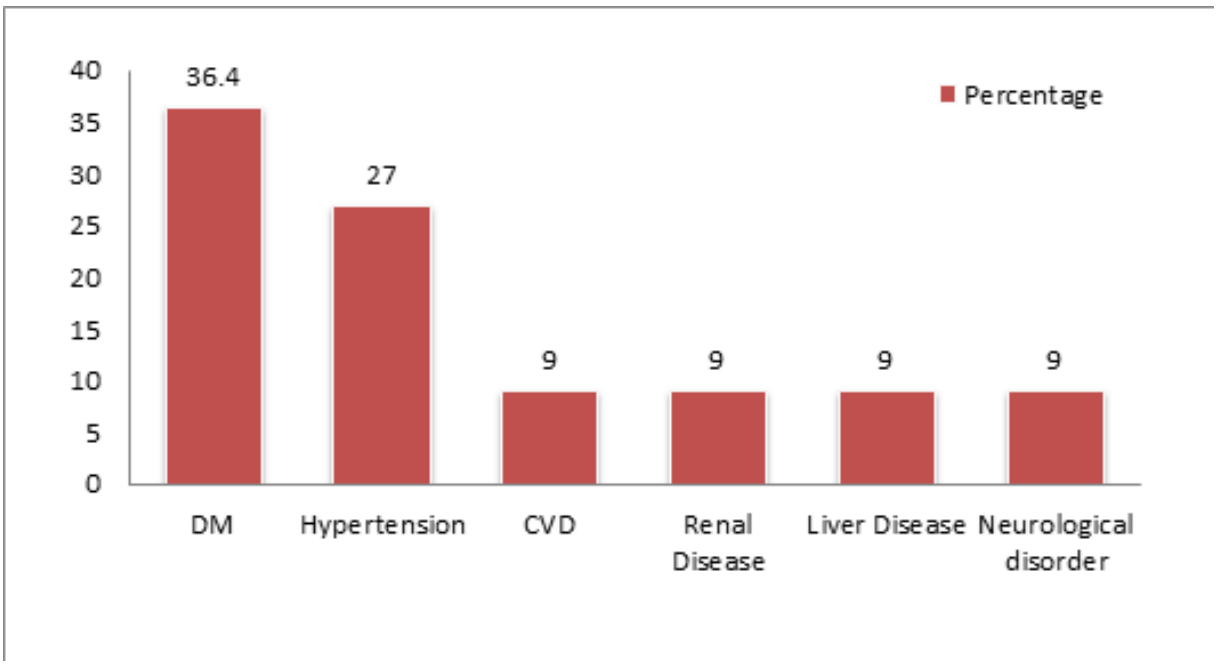

Figure 2: Reported underlying illness among antigen positive individuals.

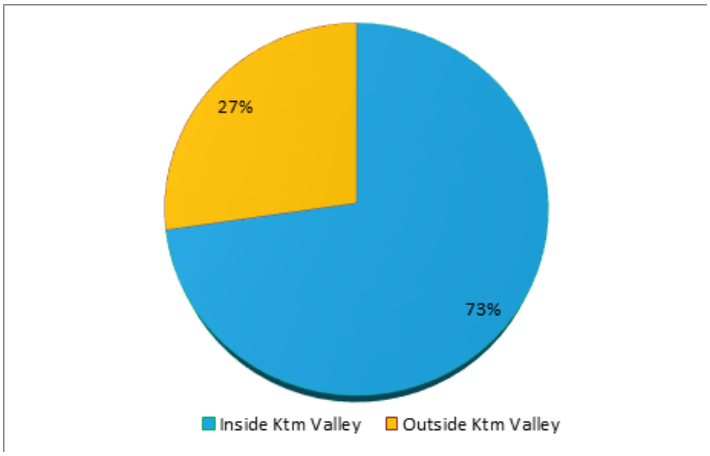

Figure 3: Area-wise distribution of the antigen positive patients.

Age (p= 0.002), presence of symptoms (p<0.001), ≤ 7 days duration of symptom onset (p<0.001), presence of underlying illness (p=0.001) and contact history with COVID-19 infected case (p<0.001) showed significant group differences between test results of antigen screening (Table 1).

Table 1: Subject characteristics and group differences.

Patient Characteristics	SARS-CoV-2 Antigen screening		Total N (%)	p-Value
	Antigen Positive N (%)	Antigen Negative N (%)		
Overall	**11 (7.3)**	**139 (92.7)**	**150**	
Age (Years) Median (IQR)	63 (35-76)	30 (25-40.5)	30 (25-42)	**0.002**
Age Groups 1-17 (Children)	0 (0)	10 (7.2)	10 (6.7)	
18-64 (Adult)	6 (54.5)	118 (84.8)	124 (82.7)	**<0.001**
≥ 65 (Elderly)	5 (45.5)	11 (8.0)	16 (10.6)	
Gender Male	2 (18.2)	35 (25.2)	37 (24.7)	1.00
Female	9 (81.8)	104 (74.8)	113 (75.3)	
Flu like symptom/s				
Yes	8 (72.7)	14 (10.0)	22 (14.7)	**<0.001**
No	3 (27.3)	125 (90.0)	128 (85.3)	
Duration of symptom/s onset (Days)				
≤ 7	8 (72.7)	4 (2.9)	12 (8.0)	**<0.001**
>7	0 (0)	10 (7.1)	10 (6.7)	
No symptoms	3 (27.3)	125 (90)	128 (85.3)	
Underlying illness				
Yes	7 (63.6)	20 (14.4)	27 (18.0)	**0.001**
No	4 (36.4)	119 (85.6)	123 (82.0)	
Contact history				
Yes	7 (63.6)	12 (8.6)	19 (12.7)	**<0.001**
No	4 (36.4)	127 (91.4)	131 (87.3)	

Discussion

After first case identification, this pandemic has completed more than one and half year. Still, millions of people are being infected worldwide with number of deaths rising daily due to infection. The stronger propagation capability has resulted faster transmission among people making difficult for the infection to bring under control. The efficacy of therapeutic options including vaccinesis under study and yet to be clearly defined.

Diagnostic testing is crucial for limiting the spread as well as managing infected patients during hospitalization [18]. Early diagnostic approach involved NAAT technique requiring high turnaround time and involving high cost [19]. So, mass screening by this adds economic burden to both the government and citizens, and delay in identifying the infected case majorly contributes to wider infection transmission. Analysis of European national PCR and contact tracing data showed that cases with high viral load are the most infectious and under laboratory conditions, the best performing lateral flow tests detect 91% of cases that lead to further transmission [20]. Low cost, faster turnaround time and acceptable performance of the available antigen testing kits plays greater role in infection control when used for mass screening of the infection. Also, antigen tests detect the replicating viral protein indicating the presence of high viral load and the infectiousness, that may be a useful indicator in determining the current infectivity unlike PCR that can detect post infectious shedders [20].

In our study, the incidence of SARS-CoV-2 infected cases was found to be 7.3% detected by the kit with 84.38% sensitivity and 100% specificity as claimed by manufacturer. The study conducted in Thailand by Chaimayo et al. reported 98.33% sensitivity with 98.73% specificity [21]. However, a prospective, multi-centre diagnostic accuracy study in Germany and the UK showed 76.6% sensitivity with 99.3% specificity [22]. Similarly, two independent studies conducted in Italy and Uganda, reported 70% sensitivity and the antigen test was more likely to be positive in samples with Qrt-PCR Ct values ≤29 reaching a sensitivity of 92% [19,23]. The low incidence of infected cases in our study might be due to low sample size, low sensitivity of the test or higher Ct values of the infected cases that was more likely to be missed by lateral flow antigen test kits. The antigen positivity in our study was higher in age group of 18 and above. According to CDC, risk for severe illness with COVID-19 increases with age, older adults at highest risk. Many studies have shown markedly low proportion of children being infected with COVID-19 and age differences in the infected cases could be explained by children having lower susceptibility to infection, lower probability of showing clinical symptoms or both [24]. As children experience more frequent infection by respiratory viruses than adults and decreased susceptibility among them might be due to cross-protection from other coronaviruses or from non-specific protection resulting from recent infection by other respiratory viruses [24]. Considering the occupational factors, younger adults are at higher risk of being infected as they make up a large proportion working in frontline where constant implementation of prevention strategies might be difficult. Similarly, analyzing the behavioral aspect, they are more likely to have group gatherings violating community mitigation strategies [25]. Old aged susceptibility to the infection might be

due to weaker immune response, obesity, decline in respiratory function, frailty and multimorbidity [26]. Majority of the antigen positive individuals in our study were female. Similarly, in South Korea, 60% females tested positive for SARS-CoV-2 [27]. The Global Health 50/50 research initiative presented an overview of sex-disaggregated data from countries worldwide demonstrated similar numbers of cases in women and men, but an increased case fatality in men [28]. The major factor resulting in increased incidence of infection in female in our study might be due to higher participation of female that was three times of the male subjects.

In our study, 72.7% of the symptomatic patients were antigen positive. All the symptomatic patients had less than 7 days duration of symptom onset at the time of testing. A study conducted at two university campuses for performance of an antigen-based test in United States performed in 227 symptomatic and 871 asymptomatic individuals identified 34 (15.0%) antigen positive among 40 (17.6%) RT-PCR positive cases with median interval from symptom onset to specimen collection being 3 days [29]. Ag-RDTs are most likely to perform well in patients in pre-symptomatic (1-3 days before symptoms onset) and early symptomatic phase (5-7 days of symptoms onset) with high viral loads (Ct values ≤25 or >106 genomic virus copies/Ml) [16]. Studies have shown high sensitivity and specificity in samples mainly obtained during the first week (within 7 days) of symptoms and with high viral loads [21,30,31]. However, asymptomatic cases have also been demonstrated to have viral loads similar to symptomatic cases [16]. High incidence of SARS-CoV-2 antigen among symptomatic individuals in our study might be due to early symptomatic phase of the tested individuals whereas relatively high incidence of asymptomatic individuals might also be due to elevated viral load corresponding to that of symptomatic patients or due to pre-symptomatic phase of the asymptomatic individuals that could be detected by lateral flow antigen. The most predominant symptoms among antigen positives in our study was fever, dry cough and dyspnea reported in 63.6% of the symptomatic individuals. This finding was similar to finding of Bajracharya et al. performed in COVID-19 confirmed individuals where 65.2% of them had fever [32]. Various meta-analysis and systematic review study of COVID-19 patients have shown fever and dry cough as the most frequently occurring symptoms among the infected people [33-35].

In this study, we reported 63.6% of the antigen positive cases had underlying illness, the most common being diabetes mellitus in 36.4% followed by hypertension in 27% of them. This result contradicts a retrospective study in China that observed hypertension as predominant underlying disease followed by diabetes [36]. A meta-analysis study on COVID-19 comorbidities in 1786 patients identified hypertension as most frequently occurring illness reported in 15.8%, cardiovascular and cerebrovascular conditions in 11.7% and diabetes in 9.4% [37]. Diabetes mellitus is important risk factor for increased COVID-19 disease severity and worse outcomes, including higher mortality due to effects on glucose homeostasis, inflammation, altered immune status and activation of the RAAS [38].

Our study showed 63.6% of the SARS-CoV-2 infected individuals had contact history with known COVID-19 positive cases. In a study by Sharma *AK* et al. among 121 children diagnosed with COVID-19, 83.4% were identified from contact tracing [39]. A study conducted by Xiahong Li et al. in 135 individuals, reported 37.88% of the infected cases had history of close contact [40]. Early epidemiology of the COVID-19 in Nepal shows the trend in rise of the infected cases from first case identification to local transmission where the tested individuals were mostly the close contact of confirmed cases [41,42]. There are various routes of transmission of SARS-CoV-2 among which the most predominant is respiratory with growing evidence indicating the infectious virus can be found in aerosols and in exhaled breath samples [43-46]. Therefore, higher proportion of positive antigen result among the study subjects with contact history might be due to easy exposure with the virus during close interaction with the infected person and the positive antigen test among the people with no reported contact history might be due to unknown contact with the infected cases.

Majority of the SARS-CoV-2 positive individuals in our study were from Kathmandu valley (72.7%). The national epidemiological situation report published by WHO in three months (November 2020- December 2021) has also shown Kathmandu valley as the hotspot of infection with more than half of the cases in the valley alone.

Conclusion

This study showed 7.3% of the suspected patients were SARS-CoV-2 antigen positive. Adults, elderly and the individuals having close contact with COVID-19 infected patient were at high risk of acquiring infection. Majority of the infected patients presented the symptoms and had any one of the underlying illness. The finding obtained also provide insight into the mass screening strategy in under-resourced setting i.e. if mass screening is to be done based on priority; adults, elderly, people with underlying illness, symptoms and contact history should be kept at first priority.

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