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Etiology and epidemiology of respiratory infections in community-based influenza-like illness during the COVID-19 pandemic, Vientiane, Lao People's Democratic Republic

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ABSTRACT

Objectives: Respiratory infections pose an ongoing global public health burden, with multiple viral and bacterial etiologies. This study aimed to characterize the etiology of influenza-like illness (ILI) during the COVID-19 pandemic in a community cohort in Vientiane, Lao People's Democratic Republic (Lao PDR).
Methods: From September 2021 to April 2022, 6300 individuals from 999 households in 25 villages were enrolled in a prospective surveillance study. Oropharyngeal swabs were collected from ILI cases and tested for SARS-CoV-2 using reverse transcription-polymerase chain reaction, and for 21 additional respiratory pathogens using a multiplex panel.
Results: Among 462 samples analyzed, 360 (77.92%) were positive for at least one pathogen, including 338 viral and 79 bacterial infections. SARS-CoV-2 was predominant (67.53%), followed by *Staphylococcus aureus* (12.55%), human rhinovirus (6.93%), and *Streptococcus pneumoniae* (5.41%). Seasonal viruses, such as influenza A/B, respiratory syncytial virus, human parainfluenza virus, and human metapneumovirus were notably absent. Co-infections occurred in 21.21% of cases, with lower rates among SARS-CoV-2-positive individuals.
Conclusions: These findings highlight the dominance of SARS-CoV-2 and the suppression of typical seasonal viruses, likely due to public health measures and viral interference. The results emphasize the importance of multiplex, community-level surveillance to understand respiratory pathogen dynamics and to strengthen preparedness in resource-limited settings.

Introduction

Respiratory infections represent a major global public health challenge, encompassing a diverse spectrum of illnesses affecting both upper and lower respiratory tracts [1]. These infections range from acute, self-limiting conditions to severe, life-threatening diseases, imposing substantial economic burdens and ranking among the leading causes of morbidity and mortality, particularly in children under 5 years of age [2,3].

The etiological landscape of respiratory infections is complex, involving numerous viral and bacterial pathogens. Key viral agents include influenza viruses, parainfluenza viruses, human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), human coronaviruses (HCoV), adenoviruses, human bocavirus, and enteroviruses, while important bacterial pathogens include *Strep-*

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tococcus pneumoniae and *Staphylococcus aureus*. These endemic, emerging, and re-emerging respiratory pathogens, continuously threaten both local and global populations, as demonstrated by the emergence of SARS-CoV in 2002 [4] and, more recently, SARS-CoV-2.

SARS-CoV-2, the causative agent of COVID-19, emerged in Wuhan, China, in December 2019 [5] and rapidly spread across all continents, prompting the World Health Organization (WHO) to declare COVID-19 a pandemic on March 11, 2020 [6]. By September 2021, the pandemic had resulted in over 228 million confirmed cases and more than 4.6 million deaths globally [7]. Lao People's Democratic Republic (Lao PDR) reported its first COVID-19 case on March 24, 2020, and by May 2023, the country had documented over 218,000 cases and 671 deaths [8,9]. The pandemic response in Lao PDR, like elsewhere, involved comprehensive control measures and mobilization of health services to combat COVID-19. However, this intense focus on SARS-CoV-2 inadvertently reduced attention to other respiratory pathogens among researchers and public health authorities. The challenge was compounded by limited availability of specific diagnostic tools for comprehensive respiratory pathogen detection and the nonspecific, overlapping clinical presentations of respiratory infections, including fever, cough, and dyspnea which significantly complicated differential diagnosis during the pandemic period [10]. Despite these challenges, emerging evidence documented co-circulation and co-infection of SARS-CoV-2 with other respiratory pathogens, including influenza or RSV [11,12].

In Lao PDR, lower respiratory tract infections constitute the third leading cause of death, highlighting their significant public health impact [13]. While several studies have documented respiratory pathogens circulation in hospital settings prior to COVID-19 [14,15], community-based surveillance of SARS-CoV-2 co-circulation with other respiratory pathogens remained absent. Notably, a comprehensive household study conducted in Vientiane between 2015-2016 revealed respiratory pathogen positivity rates exceeding 65%, with influenza viruses accounting for approximately 11% of infections [16]. Although these pre-pandemic studies enhanced understanding of respiratory pathogen circulation in Lao PDR, surveillance gaps remained regarding SARS-CoV-2 co-circulation with other respiratory pathogens during the pandemic period. This study aimed to characterize the etiology and epidemiology of respiratory infections among patients presenting with influenza-like illness (ILI) in a household cohort in Vientiane, Lao PDR, during the COVID-19 pandemic, addressing this critical knowledge gap in community-based respiratory pathogen surveillance.

Material and methods

Study design

A prospective, community-based cohort study encompassing 999 households, with 6300 participants across 25 villages in Vientiane capital, Lao PDR from September 2021 to April 2022 (Figure 1). This study is built upon an existing cohort previously monitored for respiratory pathogens detection during a ILI surveillance between March 2015 and February 2019 under the LACORIS framework [16].

Study population and sample collection

The 25 participating villages were geographically distributed across three zones within Vientiane capital: the central zone comprising nine villages (Hatsadi Neua, Sibounheuang, Banfal, Mongchan, Kaonhiot, Thongkhankham, Anou, Saphangmo, Phonthan Neua); the first urbanization belt encompassing 10 villages (Thongphanthong, Sisavat Neua, Nongduang Thong, Nongduang Neua, Xhounta Thong, Savang, Phonetong Chommani, Nahe, Thongpong, Nongtha Neua) and the second urbanization belt consisting of six villages (Nalao, Nongtha Tayn Bonangua, Somvang Neua, Somvang Tay, Somsanouk) (Figure 1).

Active surveillance for ILI cases was conducted through daily telephone contact with participant households. Suspected ILI cases were identified using the WHO case definition, incorporating both ILI and COVID-19 criteria: acute onset of fever (axillary temperature >37.5°C or tympanic temperature >38°C) plus cough, or sudden onset of one or more of the following symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anosmia, or ageusia, with symptoms onset occurring within the preceding 10 days [17].

Participants were eligible for inclusion if they: (i) had resided in their respective village for $\geq$6 months; (ii) maintained physical residence in the village for more than 80% of the study period; (iii) were $\geq$6 months of age with no upper age limit; (iv) provided written informed consent (or consent from parent/guardian for participants <17 years or those with reduced capacity to consent); and (v) committed to comply with study requirements. Upon identification of cases meeting WHO-defined ILI criteria, trained healthcare personnel conducted same-day home visit to verify eligibility, administer a standardized disease investigation questionnaire, and collect biological samples. Nasopharyngeal swabs were obtained following a standardized protocol and transported under appropriate cold chain conditions to the Christophe Mérieux Center for Infectious Diseases in Lao PDR (CILM) for laboratory analysis (Supplementary Figure 1).

Molecular analysis

Detection of SARS-CoV-2 by RT-PCR

Automated extraction of nucleic acids from oropharyngeal samples for the detection of SARS-CoV-2 was performed at the CILM with Viral Nucleic Acid Extraction Kit (Magnetic Bead method) from Jiangsu Bioperfectus Technologies following the manufacturer's instructions (Bioperfectus, Taizhou, China).

For viral RNA detection, we used the Sansure Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic kit (polymerase chain reaction-fluorescence probing) which targets the ORF1ab genes and the specific sequence of the gene coding for the N protein of SARS-CoV-2 according to the manufacturer's recommendations (Sansure Biotech Inc., China).

Detection of other respiratory pathogens by multiplex RT-PCR

Following receipt of oropharyngeal samples at the Institut de Recherche pour le Développement (IRD) Montpellier, France, viral RNA extraction was performed using the QIAamp® Viral RNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols. The extracted nucleic acids were subsequently analyzed using the FTD Respiratory pathogens 21 Plus (RUO) multiplex real-time reverse transcription-polymerase chain reaction platform (Fast Track Diagnostics, Luxembourg) to detect 23 additional respiratory pathogens. The multiplex panel targeted the following viral pathogens: influenza A virus (IAV), influenza A(H1N1) virus of the swine lineage (IAV(H1N1)swl), influenza B virus (IBV), metapneumoviruses A and B (HMPV A/B), human rhinovirus (HRV), human coronaviruses NL63, 229E, OC43 and HKU1; human parainfluenza virus 1, 2, 3 and 4 (HPIV), HRSVs A and B (HRSV), human bocavirus (HBoV), human adenovirus (HAdV), enterovirus (EV) and human parechovirus (HPeV) Bacterial pathogens included: *Chlamydia pneumoniae, S. aureus, S. pneumonia* and *Haemophilus influenzae type B.*

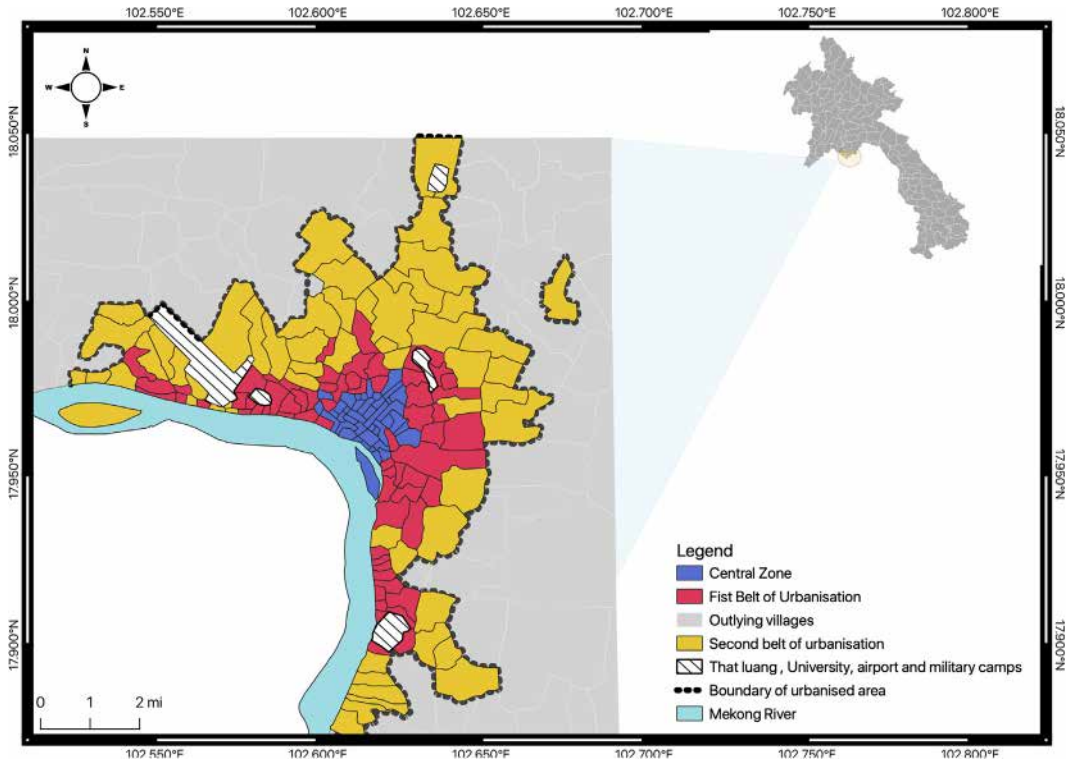

Figure 1. Location of the sampling area.

Statistical analysis

All statistical analyses were conducted using R software version 4.2.1 with appropriate statistical packages. Statistical significance was defined as $P < 0.05$, with 95% confidence intervals (CIs) calculated for all estimates. Descriptive statistics were employed to summarize demographic, clinical, and laboratory characteristics of the study population. Continuous variables were reported as mean ± standard deviation for normally distributed data, while categorical variables were expressed as frequencies and percentages with corresponding 95% CIs. We used the nonparametric Wilcoxon and Kruskal-Wallis tests to compare means between two independent groups, and binary logistic regression analysis was used to examine the association between independent variables and a binary outcome variable using the Hosmer-Lemeshow goodness-of-fit test model and area under the receiver operating characteristic (ROC) curve (AUC). Adjusted odds ratios (AORs) with their corresponding 95% CIs were calculated to quantify the strength of associations while controlling for potential confounding variables.

Results

Socio-demographic characteristics of the study population

Between September 2021 and April 2022, 462 oropharyngeal swabs were collected from individuals presenting with ILI within a cohort of 999 households encompassing 6300 participants across 25 villages. The samples were distributed among three geographic zones: the central zone (71 samples; 15.37%), first urbanization zone (201 samples; 43.51%), and second urbanization zone (190 samples, 41.12%). The study population comprised 273 women (59.09%) and 189 men (40.91%), yielding a male-to-female ratio of 0.69. Participant ages ranged from 1 to 89 years, with a mean age of 37.72 ± 20.11 years. Detailed socio-demographic characteristics of the participants are summarized in Supplementary Table S1.

Prevalence of different respiratory pathogens

Of the 462 samples analyzed, 360 (77.92%) tested positive for at least one respiratory pathogen, with 338 (73.16%) cases for at least one viral detection, and 79 (17.10%) for at least one bacterial detection. SARS-CoV-2 emerged as the predominant pathogen, accounting for 312 cases (67.53%), followed by *S. aureus* : (58 cases, 12.55%), human rhinovirus (32 cases, 6.93%), *S. pneumoniae* (25 cases, 5.41%), human adenovirus (19 cases, 4.11%), HCoV-HKU1 (9 cases, 1.95%), enterovirus (8 cases, 1.73%), HCoV-229E (6 cases, 1.30%), human bocavirus (2 cases, 0.43%), *H. influenzae* type B (2 cases, 0.43%), and HCoV-NL63 (1 case, 0.21%) (Figure 2). Notably, several respiratory pathogens showed zero prevalence throughout the study period, including influenza A and B viruses, influenza A (H1N1) virus, swine lineage (IAV(HN)swl) influenza virus, HMPV A/B, human coronavirus OC43, human parainfluenza virus 1-4, HRSV A/B, human paraechovirus (HPeV), *Mycoplasma pneumoniae, and Chlamydia pneumoniae* (Figure 2).

The overall prevalence of pathogens other than SARS-CoV-2 in this study was 29.22% (135/462), with respiratory viruses accounting for 15.42% (74/462) and respiratory bacteria for 16.46% (87/462) of cases.

Co-infection patterns

Co-infections, defined as simultaneous detection of two or more pathogens, occurred in 98 of 462 samples (21.21%, 95% CI: 17.6-25.2%). Analyses revealed a wide variety of combinations, with SARS-CoV-2 being the most frequently detected pathogen involved in co-infections.

The most prevalent co-infection pattern was SARS-CoV-2 with *S. aureus* (35 cases), followed by SARS-CoV-2/HRV and SARS-CoV-2/*S. pneumoniae*, each observed in 18 cases (see Figure S2). Less

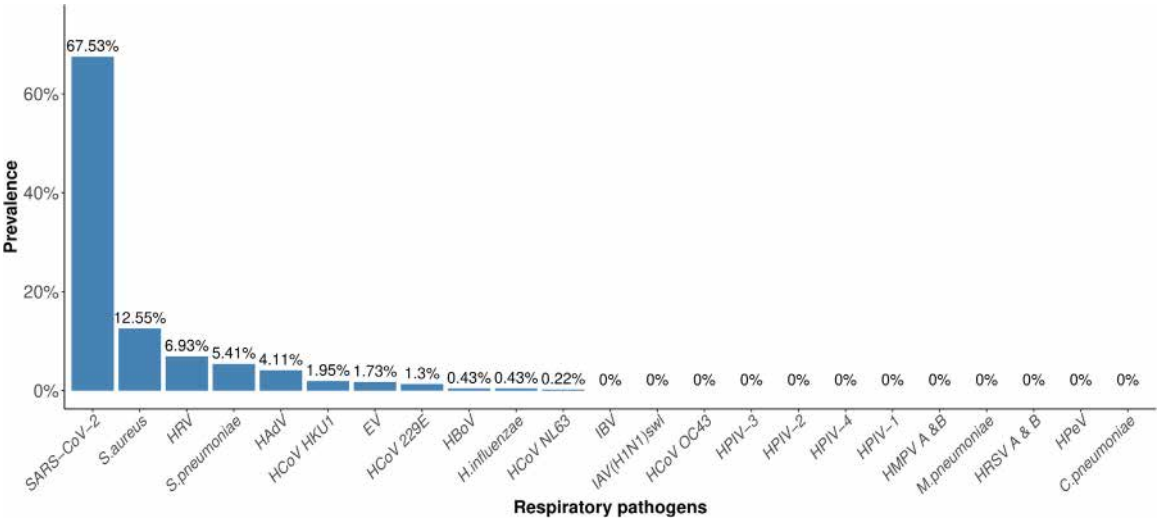

Figure 2. Prevalence of different respiratory pathogens in the study population.
EV, enterovirus; HAdV, human adenovirus; HBoV, human bocavirus; HCoV, human coronaviruses NL63, 229E, OC43 and HKU1; HMPV A/B, metapneumoviruses A and B; HRV, human rhinovirus; HPeV, human parechovirus; HPIV, human parainfluenza virus 1, 2, 3 and 4; HRSV, human respiratory syncytial virus; IAV, influenza A virus; IBV, influenza B virus; IAV(H1N1)swl, influenza A(H1N1) virus of the swine lineage.

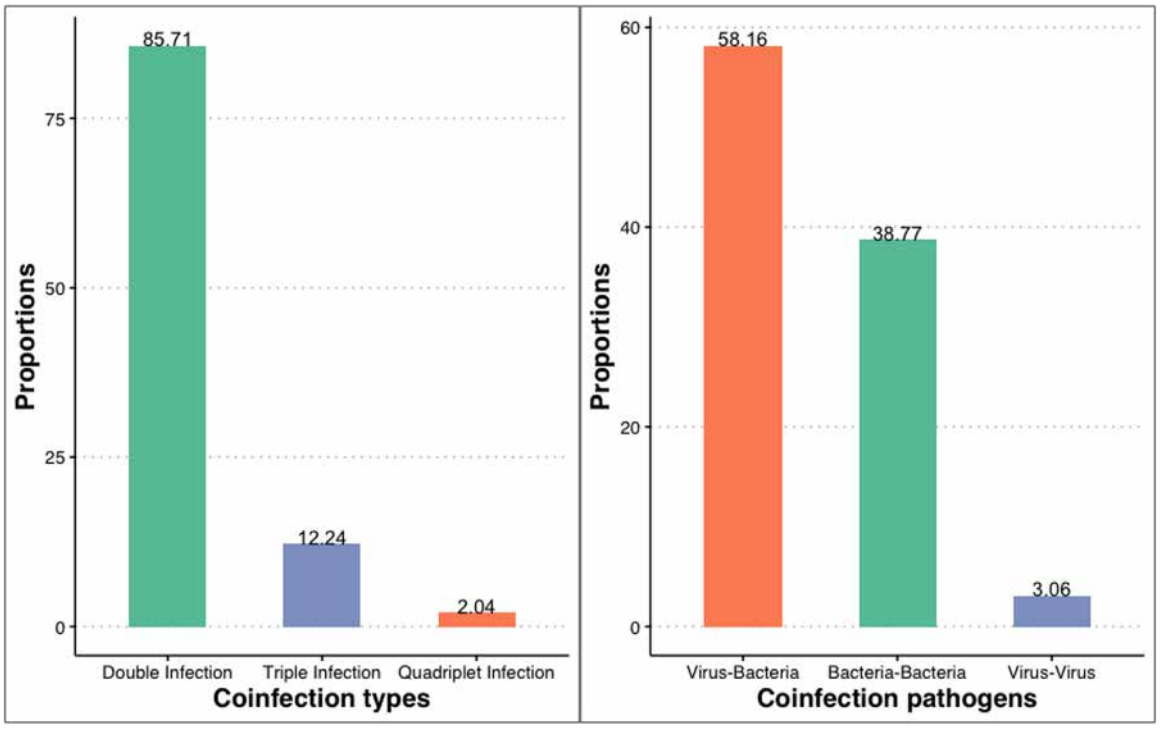

Figure 3. Distribution of co-infection types and associated pathogen combinations in the study population.

frequent associations included SARS-CoV-2/HAdV (14 cases), SARS-CoV-2/EV (6 cases), SARS-CoV-2/HCoV-HKU1 (5 cases), SARS-CoV-2/HCoV-229E (4 cases), SARS-CoV-2/HBoV (2 cases), and a single case of SARS-CoV-2/HCoV-NL63 co-infection.

Co-infections not involving SARS-CoV-2 were less common, including HRV/*S. aureus* (7 cases), HRV/*S. pneumoniae* (3 cases), HRV/HAdV (1 case), HAdV/HCoV-HKU1 (1 case), and *S. aureus*/*S. pneumoniae* (3 cases).

Double infections constituted the majority of co-infection cases (84 cases, 85.71%), with triple infections comprising the remainder (14 cases, 14.29%) (Figure 3). In addition, co-infections between viruses and bacteria were the most prevalent (57 cases, 58.16% of all co-infections) followed by virus-virus combinations (Figure 3).

Pathogen distribution in SARS-CoV-2 positive vs negative groups

Among SARS-CoV-2 positive samples (n = 312), 87 cases (27.88%) harbored additional respiratory pathogens, with bacteria detected in 50 cases (15.48%) and other viruses in 48 cases (14.86%). In contrast, among SARS-CoV-2 negative samples, (n = 150), 48 cases (32%) tested positive for other respiratory pathogens, with bacterial prevalence of 18.47% (29/150) and viral prevalence of 16.56% (27/150).

Statistical analysis revealed no significant differences in pathogen distribution between SARS-CoV-2 positive and negative groups for most pathogens, with the exception of *Haemophylus influenzae B*, which showed a statistically significant difference

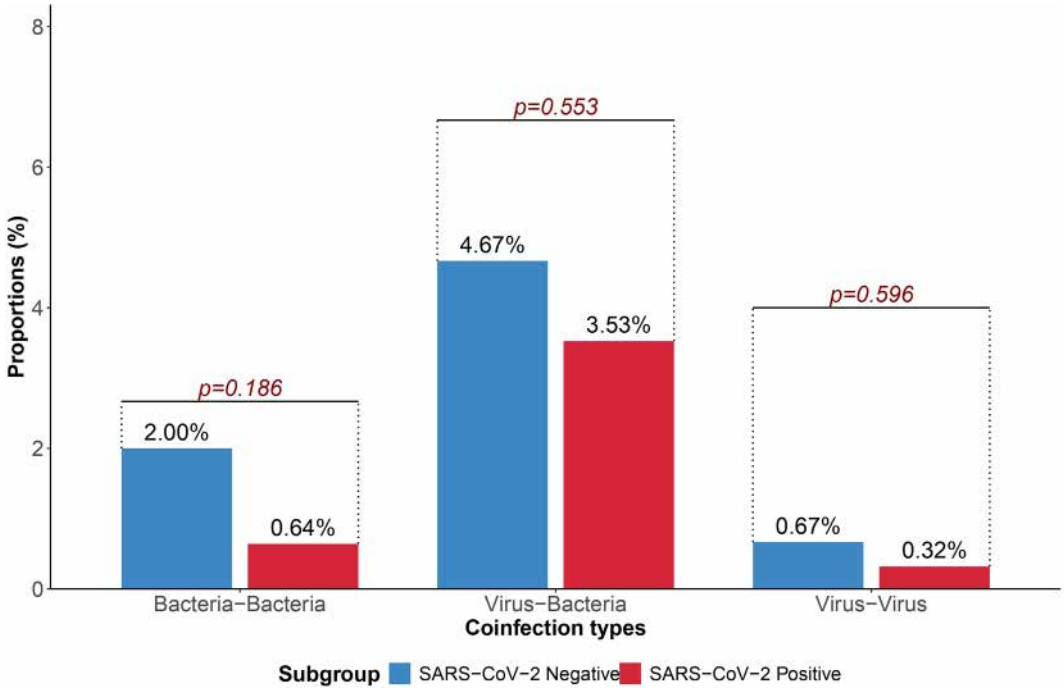

Figure 4. Different types of co-infections according to SARS-CoV-2 status.

($P = 0.04$) (Supplementary Table S2). Human rhinovirus was the most prevalent non-SARS-CoV-2 virus in both groups (5.77% in SARS-CoV-2 positive vs 9.33% in SARS-CoV-2 negative cases), while *S. aureus* was the most common bacterium (11.22% in SARS-CoV-2 positive vs 15.33% in SARS-CoV-2 negative cases).

However, in the other 10 respiratory pathogens detected in both SARS-CoV-2 groups, HCoV-NL63 and HBoV were absent in the SARS-CoV-2 negative group, while *H. influenzae B* was absent in the SARS-CoV-2 positive group (Supplementary Table S2).

Co-infection rates did not differ significantly between groups, occurring in 14 SARS-CoV-2 positive cases (4.49%) vs 11 SARS-CoV-2 negative cases (7.33%, $P > 0.05$). Virus-bacteria co-infections predominated in both groups (Figure 4).

Factors associated with pathogen detection

Gender distribution among infected individuals showed a slight female predominance (58.61%) compared to men (41.39%), although this difference was not statistically significant ($P = 0.73$). Among the 462 samples, age group analysis revealed that individuals aged 45-64 years had the highest infection rate (27.5%), while the 0-4 years age group had the lowest (4.4%). However, no statistically significant age-related differences were observed for overall pathogen detection. Similarly, no association was found between age and positivity when analyzing either specific pathogens or overall viral infections. In contrast, bacterial positivity was significantly associated with age ($P = 0.004$), with the 0-4 and 45-64 age groups being most at risk (Supplementary Table S3).

Multiple clinical symptoms demonstrated significant associations with respiratory pathogen detection, including ageusia, anosmia, coryza, cough, fever, headache/myalgia, muscle aches, respiratory symptoms, runny nose, sore throat, and weakness/fatigue (all $P < 0.05$). Conversely, no associations were found with dyspnea, smoking status, occupational factors, presence of chronic medical conditions, or COVID-19 vaccination status. For bacterial pathogen detection specifically, only sore throat showed a significant association ($P = 0.011$) (Table 1).

Temporal distribution of pathogens and co-infections

Monthly analyses revealed that October, November, and April recorded the highest number of cases, with at least one respiratory pathogen-positive case (116, 87, and 61 cases, respectively). SARS-CoV-2 maintained predominance throughout the study period, exhibiting two distinct peaks: the first in November 2021 and the second in April 2022. Similar patterns were observed for *Staphylococcus aureus* and *Streptococcus pneumoniae*, while human adenovirus peaked in October 2021 and human rhinovirus in April 2022 (Supplementary Figure S3). Co-infection patterns showed temporal variation, with the highest frequency occurring in October (31 cases). Double infections, predominantly virus-bacteria, remained the most common co-infection types throughout the study period, reaching their peak in October with 23 documented cases (Supplementary Figure S3).

Discussion

The results of this study demonstrate substantial circulation of respiratory pathogens, with 77.9% of ILI cases testing positive for at least one pathogen. This high detection rate reflects significant infectious agent circulation within the community during the pandemic period and aligns with findings from comparable studies. Our findings exceed pre-pandemic rates in the same Lao population, reported by Rudge et al. [16].

In the COVID-19 pandemic context, this high positivity rate highlights the persistence of numerous respiratory pathogens, despite the implementation of control measures (barrier gestures, social distancing, lockdowns). This residual circulation could be explained by an increase in intra-family transmission, in closed environments [18], or declining herd immunity due to an “immune debt” from successive lockdowns. The use of multiplexed tests documented not only SARS-CoV-2 prevalence, but also the diversity of other respiratory viruses and bacteria detected, underscoring the importance of maintaining broad surveillance, even during a targeted pandemic.

Table 1
Factors associated with the diagnosis of pathogens.

Characteristics		Positive for at least one pathogen			Virus			Bacteria		
		AOR	95% CI	*P*-value	AOR	95% CI	*P*-value	AOR	95% CI	*P*-value
Sex		1.09	0.68-1.76	0.73	0.90	0.58-1.40	0.66	1.34	0.80-2.24	0.25
Clinical symptoms	Ageusia	6.14	2.78-9.50	0.02[a]	9.37	1.49-10.43	0.005[a]	0.40	0.04-1.69	0.28
	Anosmia	10.44	2.68-20.62	<0.001[b]	13.2	3.40-21.84	<0.001[b]	0.58	0.21-1.35	0.27
	Coryza	2.80	1.73-4.63	<0.001[b]	3.02	1.92-4.80	<0.001[b]	0.91	0.54-1.53	0.80
	Cough	3.20	1.97-5.28	<0.001[b]	3.31	2.1-5.26	<0.001[a]	0.84	0.50-1.40	0.53
	COVID-19 vaccine status	0.92	0.49-1.67	0.88	1.22	0.70-2.10	0.42	0.38	0.21-0.68	0.1
	Fever	2.76	1.70-4.53	<0.001[b]	3.65	2.30-5.87	<0.001[b]	0.90	0.54-1.52	0.71
	Headache/myalgia	2.79	1.69-4.69	<0.001[b]	3.19	1.98-5.20	<0.001[b]	0.77	0.45-1.30	0.31
	Muscle aches	2.36	1.42-4.02	0.0005[a]	3.07	1.88-5.15	<0.001[b]	0.70	0.40-1.20	0.19
	Presence of chronic medical conditions	1.45	0.84-2.58	0.1	1.37	0.83-2.32	0.23	0.82	0.44-1.50	0.57
	Respiratory symptoms	4.18	2.57-6.86	<0.001[b]	5.38	3.36-8.67	<0.001[b]	0.61	0.36-1.07	0.07
	Runny nose	3.37	2.04-5.68	<0.001[b]	3.84	2.40-6.25	<0.001[b]	0.81	0.48-1.36	0.45
	Shortness of breath	0.90	0.42-2.04	0.85	0.92	0.45-1.98	0.86	0.71	0.24-1.77	0.53
	Smoker	0.99	0.42-2.60	0.98	0.95	0.42-2.28	0.84	1.18	0.42-2.91	0.64
	Sore throat	2.78	1.66-4.78	<0.001[a]	3.13	1.93-5.21	<0.001[b]	1.51	0.88-2.59	0.011[a]
	Weakness/fatigue	1.94	1.06-3.73	0.02[a]	2.02	1.15-3.71	0.009[a]	1.05	0.56-1.92	0.88
	Work	0.95	0.59-1.53	0.90	0.99	0.64-1.54	0.98	0.67	0.39-1.13	0.13

AOR, adjusted odds rate; CI, confidence interval.
[a] Significant p-value.
[b] Highly significant p-value.

Our results reinforce the idea that community-acquired acute respiratory infections, even in a pandemic context, retain a varied etiology. They argue for a broader syndromic approach, based on sensitive diagnostic tools and close surveillance within epidemics. Such a strategy is essential to anticipate epidemic peaks, optimize targeted vaccination campaigns, and reduce unjustified prescriptions of antibiotics in the context of viral agents circulation.

In the context of the COVID-19 pandemic, our study conducted in Lao PDR revealed a high prevalence of SARS-CoV-2 (67.5%), making this virus the main respiratory pathogen detected, while no commonly circulating seasonal viruses, such as influenza, parainfluenza, RSV, or metapneumovirus were detected. Following SARS-CoV-2, the most frequently identified agents were *S. aureus* (12.6%) and human rhinovirus (HRV, 6.9%).

The prevalence of SARS-CoV-2 observed in our study is in the upper range of rates reported in recent literature, but shows notable variations when compared to other studies, reporting prevalences from 39.1% to 66.5% [11,12,19]. The variability observed in the prevalence of SARS-CoV-2 at the regional and international levels highlights the importance of considering several contextual elements: the nature and size of the populations studied (community vs hospital setting), the inclusion criteria (presence of specific symptoms such as flu-like symptoms), the specific periods of data collection (different epidemic waves, variants in circulation), the health policies in force (restrictions, vaccination campaigns), the levels of herd immunity as well as the diagnostic tools used (sensitivity, specificity of polymerase chain reaction tests or others). Lao PDR, with a relatively late start to the epidemic compared to other Asian countries, has also benefited from significant community mobilization around prevention measures, particularly in rural areas.

A particularly striking finding of our study is the complete absence of seasonal respiratory viruses, such as influenza, RSV, metapneumovirus, and parainfluenza, contrasting sharply with pre-pandemic trends. A similar absence during the pandemic, has been reported in several countries, including Brazil and India [20,21].

The results of this study differ significantly from those from Rudge et al. [16], who studied the same Lao PDR pre-pandemic cohort and found greater viral diversity, with notable rates for influenza (approximately 15%), RSV (nearly 10%), and metapneumovirus (approximately 5%) [16]. This discrepancy reflects the fundamental difference in study periods and pandemic-imposed public health measures that significantly reduced transmission of respiratory viruses other than SARS-CoV-2 [20–22]. Additionally, SARS-CoV-2 predominance may have altered the viral landscape through viral interference mechanisms and innate immune responses that inhibit other respiratory virus replication. These results illustrate a profound upheaval in respiratory viral ecology during the pandemic [23]. This hypothesis is supported by similar observations in other Southeast Asian countries, where the circulation of seasonal respiratory viruses was significantly reduced during the pandemic [24].

Finally, the failure to detect other respiratory viruses in our study, unlike observations in comparable contexts, reinforces the hypothesis of a viral suppression or dominance effect exerted by SARS-CoV-2, supported by several experimental and epidemiological studies [25].

Besides SARS-CoV-2, 29.22% (135/462) of the samples analyzed revealed the presence of at least another pathogen, with a predominance of bacterial pathogens (16.46%) over respiratory viruses (15.42%). This epidemiological profile suggests a bacterial-dominated infectious landscape in this pandemic community setting, which contrasts with several previous studies conducted in Southeast Asia where seasonal respiratory viruses, including influenza, RSV, or rhinoviruses, predominated before the emergence of SARS-CoV-2 [16,22]. This development could reflect a residual effect of public health measures adopted during the pandemic (mask wearing, social distancing, enhanced hygiene), which had a greater impact on viral transmission than on bacterial circulation. Other international studies also reported a significant proportion of non-SARS-CoV-2 pathogens, sometimes with a bacterial predominance, particularly in community settings [26,27].

Co-infections involving multiple pathogens were detected in 21.21% of cases, SARS-CoV-2 and *S. aureus* representing the most frequent combination (35 cases) followed by SARS-CoV-2 and human rhinovirus co-infections. Virus-bacteria co-infection was most prevalent 12.34%. This prevalence corroborates with Rudge et al.'s [16] findings (24.1%) in the same cohort, but varies considerably compared to international studies ranging from 3.2-52.2% [11,12,19].

The results of this study do not allow for the determination of pathogen seasonality, as the entire annual cycle was not cov-

ered. Sample collection primarily occurred during the dry season, which accounted for 6 of the 8 months of surveillance. However, this study documented circulation of 11 respiratory pathogens throughout the study period, lower than Rudge et al.'s [16] report of 23 pathogens in the same cohort. This difference may be attributed to SARS-CoV-2 considerable impact on respiratory pathogens circulation and seasonality, with some pathogens seeing reduced or eliminated prevalence while others had delayed circulation patterns [21,28]. Non-pharmaceutical interventions also played an important role in this altered distribution of certain respiratory pathogens [29].

Comparing SARS-CoV-2 positive and negative individuals revealed no significant difference in respiratory pathogen distribution, with *S. aureus* and HRV being most prevalent in both groups. These results align with those of a study by Samal et al. in 2022, which reported prevalence rates of other respiratory pathogens of 23.9% and 39.7%, respectively, for SARS-CoV-2 positive and negative patients with liver pathology, respectively [30]. These findings suggest that SARS-CoV-2 status has minimal influence on other respiratory pathogens circulation in our cohort.

This study presents several limitations which should be recognized in the interpretation of our results. First, the study period (September 2021 to April 2022) did not include complete seasonal cycles, the predominant dry season (6 of the 8 months), which potentially limits the detection of seasonal respiratory viruses with distinct temporal patterns. Second, the uneven distribution of the sample through the months of study may not completely represent real models of circulation of pathogens, introducing a time sampling bias which could influence our understanding of the seasonality of pathogens and epidemic dynamics. Third, focusing on people with influenza-like disease may have bias of selection by missing asymptomatic infections or respiratory pathogens causing non-ILI presentations. In addition, although our community design provides valuable information at the population level, it may not fully represent the circulation of pathogens in other demographic groups or geographic environments within the Lao PDR. Finally, sequencing analyzes were not carried out in this study, which limited our ability to explore potential associations between specific co-infections and SARS-COV-2 variants. Although information on co-morbidities has been collected for patients with co-infections, we have not carried out statistical analyzes to assess their impact on clinical gravity.

Conclusion

This community-based study provides valuable insights into the etiology and epidemiology of ILI in a household cohort in Vientiane, Lao PDR, during the COVID-19 pandemic. A high positivity rate (77.92%) for respiratory pathogens was observed, with SARS-CoV-2 identified as the dominant agent (67.5%), followed by *S. aureus* and human rhinovirus. Notably, no seasonal respiratory viruses (influenza, RSV, parainfluenza, metapneumovirus) were detected, likely reflecting the combined effects of pandemic control measures (social distancing, masking, hygiene, travel restrictions) and possible viral interference. Similar findings across Asia support these interpretations.

Co-circulation with other pathogens particularly *S. aureus, S. pneumoniae*, and HRV underscores the importance of comprehensive viral and bacterial surveillance during pandemics. Our household-based approach highlights the relevance of intra-domestic transmission in shaping local respiratory disease dynamics.

These findings stress the importance of accounting for geographic and epidemiological variability when interpreting pathogen circulation, especially in Southeast Asia. The Lao context offers unique insights into pathogen interactions and the differential impacts of public health interventions.

As COVID-19 transitions toward endemicity, sustained surveillance will be critical to detect the resurgence of suppressed seasonal viruses and evolving co-infection patterns. Regional studies remain essential for guiding evidence-based strategies in resource-limited health systems.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki and received approval from the Lao National Ethics Committee for Health Research (approval number: 819/NECHR).

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Author contributions

Novy Charel Bobouaka Bonguili: Investigation, Formal analysis, Data curation, Writing-Original Draft; Illich Manfred Mombo: Investigation, Formal analysis; Léadisaelle Hosanna Lenguiya: Data statistical analysis, illustration; Eric Deharo, Vatthanapone Lattaphasavang, Phimpha PABOURIBOUNE: Investigation, Paper Revision; Matthieu Fritz: Formal analysis; Jordy Exaucé Demboux Lyelet, Félix Koukouikila-Koussounda, Pembe Issamou Mayengue: Paper Revision; Eric Elguero: Data curation and analysis; Eric M. Leroy: Conceptualization, Supervision, Writing - Review & Editing, Project administration, Funding acquisition; Roch Fabien Niama: Supervision, Writing-Review, Sabrina Locatelli: Resources, Investigation, Writing – Review & Editing, Supervision, Project administration. All authors have read and approved the manuscript.

Data availability

The data sets used and/or analyzed in the current study are available from the corresponding author on request.

Consent to participate

Informed consent was obtained from all participants via written signatures, and data confidentiality was ensured.

Consent for publication

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108079.

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