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Sentinel surveillance of acute respiratory infections and distribution of SARS-CoV-2, influenza A and B, and respiratory syncytial virus in the post-COVID-19 period

Sentinelni nadzor nad akutnim respiratornim infekcijama i distribucija SARS-CoV-2, virusa gripa tipa A i B i respiratornog sincicijalnog virusa u post-COVID-19 periodu

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Abstract

Background/Aim. Acute respiratory infections (ARIs) remain a major global health concern. The aim of this study was to analyze the epidemiological and clinical characteristics of ARIs and detect the distribution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, influenza B, and respiratory syncytial virus (RSV) among outpatients during the 2023/24 and 2024/25 seasons. **Methods.** A prospective study was conducted through sentinel surveillance, enrolling only patients with ARI symptoms/signs. Identification of one of the four viruses was performed using the CerTest SARS-CoV-2 + Flu A + Flu B + RSV rapid combined test. **Results.** In the 2023/24 season, 26.57% of samples tested positive for at least one virus, while in the 2024/25 season, a decrease in positivity for the tested viruses was observed, dropping to 14.24%. Influenza A dominated during the 2023/24 season, while the incidence of influenza B increased in early 2025, peaking in late February. SARS-CoV-2 showed moderate fluctuations throughout the study period, with a pronounced peak in September 2024, while RSV remained low during both seasons. The age distribution of patients varied: in the 2023/24 season, RSV and influenza B were most frequently recorded in young children aged 0–4 years, influenza A in children aged 0–4 and 5–14 years, while SARS-CoV-2 was most prevalent among adults aged 30–64 years. In the 2024/25 season, RSV and influenza A were most commonly diagnosed in children aged 0–4 years, influenza B in children aged 5–14 years, and SARS-CoV-2 in individuals over 30 years old. Elevated body temperature was the most common symptom regardless of the type of confirmed viral infection but was significantly less present in SARS-CoV-2 infections compared to other viruses. **Conclusion**. The results of this study indicate a shift in the dominance of viral causative agents between the observed seasons, with influenza A prevailing in 2023/24, while an increase in the incidence of influenza B virus was observed in 2024/25. SARS-CoV-2 circulated continuously but at a low level, while the impact of RSV was minimal in both seasons.

Key words:
epidemiology; influenza a virus; influenza b virus; respiratory syncytial viruses; respiratory tract infections; sars-cov-2.

Apstrakt

Uvod/Cilj. Akutne respiratorne infekcije (ARI) i dalje predstavljaju značajan globalni zdravstveni problem. Cilj rada bio je da se analiziraju epidemiološke i kliničke karakteristike ARI i detektuje distribucija koronavirusa 2 izazivača teškog akutnog respiratornog sindroma (*severe acute respiratory syndrome coronavirus 2* - SARS-CoV-2), virusa gripa tipa A, virusa gripa tipa B i respiratornog sincicijalnog virusa (RSV) među ambulantno lečenim bolesnicima tokom sezona 2023/24 i 2024/25. **Metode**. Sprovedena je prospektivna studija putem sentinelnog nadzora, a obuhvaćeni su samo bolesnici sa simptomima/znacima ARI. Identifikacija jednog od četiri virusa izvršena je korišćenjem brzog kombinovanog testa *CerTest SARS-CoV-2 + Flu A + Flu B + RSV*. **Rezultati**. U sezoni 2023/24, 26,57% uzoraka bilo je pozitivno na najmanje jedan virus, dok je u sezoni 2024/25 zabeležen pad pozitivnosti ispitivanih virusa na 14,24%. Infekcije virusom gripa A dominirale su tokom sezone 2023/24, dok je učestalost virusa gripa B porasla početkom 2025. godine, dostižući vrhunac krajem februara. SARS-CoV-2 pokazao je umerene fluktuacije tokom studijskog

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perioda, sa izraženim pikom u septembru 2024, dok je aktivnost RSV ostala niska tokom obe sezone. Uzrasna distribucija bolesnika je varirala: u sezoni 2023/24, RSV i virus gripa B najčešće su registrovani kod dece uzrasta 0–4 godine, virus gripa A kod dece uzrasta 0–4 i 5–14 godina, dok je SARS-CoV-2 bio najzastupljeniji među odraslima uzrasta 30–64 godine. U sezoni 2024/25, RSV i virus gripa A najčešće su dijagnostikovani kod dece uzrasta 0–4 godine, virus gripa B kod dece uzrasta 5–14 godina, a SARS-CoV-2 kod osoba starijih od 30 godina. Povišena telesna temperatura bila je najčešći simptom bez obzira na vrstu dokazane virusne infekcije, ali je bila značajno ređe prisutna kod infekcija SARS-CoV-2 u poređenju sa ostalim virusima. **Zaključak**. Rezultati ove studije ukazuju na promenu u dominaciji virusnih uzročnika između posmatranih sezona, pri čemu je virus gripa A bio dominantan u 2023/24, dok je u sezoni 2024/25 zabeležen porast učestalosti virusa gripa B. SARS-CoV-2 cirkulisao je kontinuirano, ali na niskom nivou, dok je uticaj RSV bio minimalan u obe sezone.

Ključne reči:
epidemiologija; grip a virus; grip b virus; respiratorni sincicijalni virusi; respiratorni trakt, infekcije; sars-cov-2.

Introduction

Acute respiratory infections (ARIs) are a major global health burden, contributing to medical costs, lost productivity, and antimicrobial resistance due to frequent antibiotic prescriptions [1–4]. Viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A and B, and respiratory syncytial virus (RSV) are key ARI pathogens, with overlapping clinical presentations that complicate diagnosis and highlight the need for timely testing [1, 5]. The coronavirus disease 2019 (COVID-19) pandemic accelerated molecular diagnostics, focusing on SARS-CoV-2 detection to curb transmission. Public health measures also reduced the circulation of other respiratory viruses, raising concerns about future outbreaks [5, 6]. SARS-CoV-2 has underscored the need to differentiate between viral pathogens due to its unique severity [7]. While fever and cough are common ARI symptoms, age-specific patterns vary: RSV predominantly affects young children, whereas influenza A is prevalent in school-aged children and young adults [1, 6, 7]. Coinfections, particularly with SARS-CoV-2 and influenza, may intensify disease burden, making rapid detection essential for effective management [8].

Seasonal variations shape the epidemiology of ARIs, with influenza and RSV peaking in winter [1, 9]. Understanding these patterns, alongside demographic and clinical characteristics, helps optimize preventive strategies, such as patient isolation and antiviral use [5–8]. Surveillance of respiratory infections is crucial for public health [9, 10].

The aim of this study was to analyze the epidemiology of ARI and the circulation patterns of four respiratory viruses in primary care across two consecutive seasons, to inform clinical practice and guide public health policies.

Methods

This study was designed as an observational, prospective clinical-epidemiological investigation analyzing the characteristics of patients presenting with one or more symptoms/signs of ARIs. A subset of patients treated by six physicians [three pediatricians and three general practitioners (GPs)] was included, comprising a total of 10,194 patients during the 2023/24 season and 8,726 patients during the 2024/25 season. In both seasons, patients were categorized into five age groups: 0–4 years, 5–14 years, 15–29 years, 30–64 years, and ≥ 65 years. The study was conducted at the Health Center Novi Sad, Novi Sad, Serbia. The 2023/24 season covered the period from December 21, 2023, to March 29, 2024 (15 consecutive weeks), while the 2024/25 season extended from September 18, 2024, to February 28, 2025 (24 consecutive weeks).

Inclusion/exclusion criteria

Patients of any age and gender presenting with one or more symptoms/signs of ARI – including fever (or subjective feverishness), cough, headache, malaise, myalgia, sore throat, or loss of appetite within the past seven days – during the two surveillance seasons and seeking medical care from paediatricians or GPs at their first visit, were included in the study. Given that six physicians in both seasons expressed willingness to participate, we included 2.8% and 2.4% of the total population under surveillance during the 2023/24 and 2024/25 seasons, respectively (Table 1) [11].

Patients were excluded if they did not present with at least one of the specified symptoms/signs during the

Table 1

Population under sentinel surveillance according to age groups

Season/Population	Age (years)					Total
	0–4	5–14	15–29	30–64	≥ 65	
Season 2023/24						
population of Novi Sad according to the 2023 estimated census	20,533	38,778	67,322	178,299	65,691	370,623
population under sentinel surveillance	1,184 (5.8)	2,165 (5.6)	1,958 (2.9)	3,086 (1.7)	1,801 (2.7)	10,194 (2.8)
Season 2024/25						
population of Novi Sad according to the 2024 estimated census	20,459	38,389	67,666	177,136	65,093	368,743
population under sentinel surveillance	1,503 (7.3)	2,606 (6.8)	1,436 (2.1)	1,839 (1.0)	1,342 (2.1)	8,726 (2.4)

All values are given as numbers (percentages).

surveillance periods, if their symptoms had persisted for more than seven days, if they sought medical care outside the designated study periods, or if they had consulted pediatricians or GPs more than once.

Patient survey

Patients were interviewed by a physician to complete the remaining sections of the questionnaire, which included information on age, gender, symptoms/signs associated with ARIs during the physician's visit, and the date of laboratory sampling.

Laboratory examination

Education on proper sampling techniques and sample handling procedures was provided to all participating physicians before the start of the study and lasted for five days. Nasopharyngeal swabs were collected for combined testing of SARS-CoV-2, Influenza A, Influenza B, and RSV to confirm or rule out infection with any of these four viruses. Since these were point-of-care tests, sample analysis was performed at the Health Center Novi Sad at the physicians' outpatient clinics.

CerTest SARS-CoV-2 + Flu A + Flu B + RSV (CerTest Rapid Test)

A total of 1,690 patients with one or more symptoms/signs of ARI were tested using the CerTest Rapid Test, with patient data collected prospectively. The CerTest Rapid Test one-step combo card is a chromatographic immunoassay designed for the simultaneous qualitative detection of SARS-CoV-2, influenza A, influenza B, and RSV antigens in nasopharyngeal or nasal samples from patients suspected of having these infections. The test contains four strips, each dedicated to detecting one of the target viruses: strip A – SARS-CoV-2, strip B – influenza A, strip C – influenza B, and strip D – RSV. Each strip contains monoclonal antibodies on the test (T) line to capture the virus-specific antigen and polyclonal antibodies on the control (C) line to verify test validity. If the sample contains viral antigens, they bind to a red-colored conjugate and form a visible red line at the T line, indicating a positive result. If no antigens are present or if levels are too low, only the C line appears. Testing is most effective within 5–7 days of symptom onset. A negative result does not rule out infection, and additional testing [e.g., polymerase chain reaction (PCR)] may be needed. The performance of the CerTest Rapid Test is shown in Table 2 [12].

Statistical analysis

The population under sentinel surveillance at the primary care level at the Health Center Novi Sad (Table 1) served as the denominator for calculating the weekly incidence of ARIs *per* 10,000 inhabitants, both in total and stratified by age group. The numerator represented the number of ARI cases recorded within this population during two distinct surveillance periods: from December 21, 2023, to March 29, 2024, and from September 18, 2024, to February 28, 2025.

Due to the small number of positive cases, the weekly incidence rates of SARS-CoV-2, influenza A, influenza B, and RSV were calculated *per* 100,000 inhabitants, based on the number of laboratory-confirmed cases of each virus, with adjustments made according to the denominator representing the population under sentinel surveillance.

Numerical data were expressed as measures of central tendency (arithmetic mean, median) and variability (interquartile range, mean ± standard deviation), while categorical data were presented as frequencies and percentages. The Chi-square test or Fisher's exact test was used to assess associations between categorical variables. Analysis of variance (ANOVA) was applied to compare mean values across multiple independent groups. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 21. Statistical significance was set at $p < 0.05$.

Ethical considerations

This investigation was classified as routine public health surveillance; therefore, approval from the Ethics Committee and written informed consent were not required in Serbia. However, before enrollment, oral informed consent was obtained from each participant or, in the case of participants under 15 years of age, from their parents or legal guardians. During the process of obtaining oral consent from participants or the parents/guardians of a child, a physician (either a GP or pediatrician) and a team nurse were present. Personal and confidential information was excluded, except for demographic data, including age, gender, symptoms/signs associated with ARI, and the date of sample collection. All data were anonymized before being accessed by the authors.

Results

The course of research and the laboratory testing results for four respiratory viruses over two consecutive seasons are presented in Figure 1. During the 2023/24 season, 186

Table 2

Evaluations for CerTest Rapid Test

Variable	SARS-CoV-2	Influenza type A	Influenza type B	RSV
Sensitivity	93.0 (86.1–97.1)	80.7 (73.8–86.5)	84.3 (75.0–91.1)	94.7 (74.0–99.9)
Specificity	99.8 (98.8–100.0)	99.2 (98.6–99.6)	99.5 (98.9–99.8)	100.0 (69.2–100.0)
PPV	98.9 (94.2–10.0)	92.2 (86.5–96.0)	92.6 (84.6–97.2)	100 (81.5–100.0)
NPV	98.5 (96.9–99.4)	97.8 (96.9–98.5)	98.8 (98.0–99.4)	90.9 (58.7–99.8)

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; RSV – respiratory syncytial virus; PPV – positive predictive value; NPV – negative predictive value.
All values are given as mean values (95% confidence interval).

(26.57%) out of 700 tested samples were positive for at least one of four viral pathogens. Influenza A was the most frequently detected virus, accounting for 113 (60.75%) cases. In the 2024/25 season, the proportion of positive samples decreased to 14.24% (141/990), with influenza B being the predominant virus [57 (40.43%)].

In the 2023/24 season, the incidence of ARI increased at the beginning of the year, peaking at 105 cases *per* 10,000 inhabitants from January 29 to February 2, 2024, followed by a subsequent decline. In the 2024/25 season, a gradual increase in incidence was observed starting in September, with peaks recorded between September 23 and 27, 2024 (75.6 cases *per* 10,000 inhabitants) and in the final weeks of January 2025 (76.8 cases *per* 10,000 inhabitants) (Figure 2).

Overall, across both seasons, the highest incidence rates of ARIs were observed in the youngest age group (0–4 years), while the lowest incidence rates were recorded in the oldest age group (≥ 65 years). Among children aged 0–4 years, a

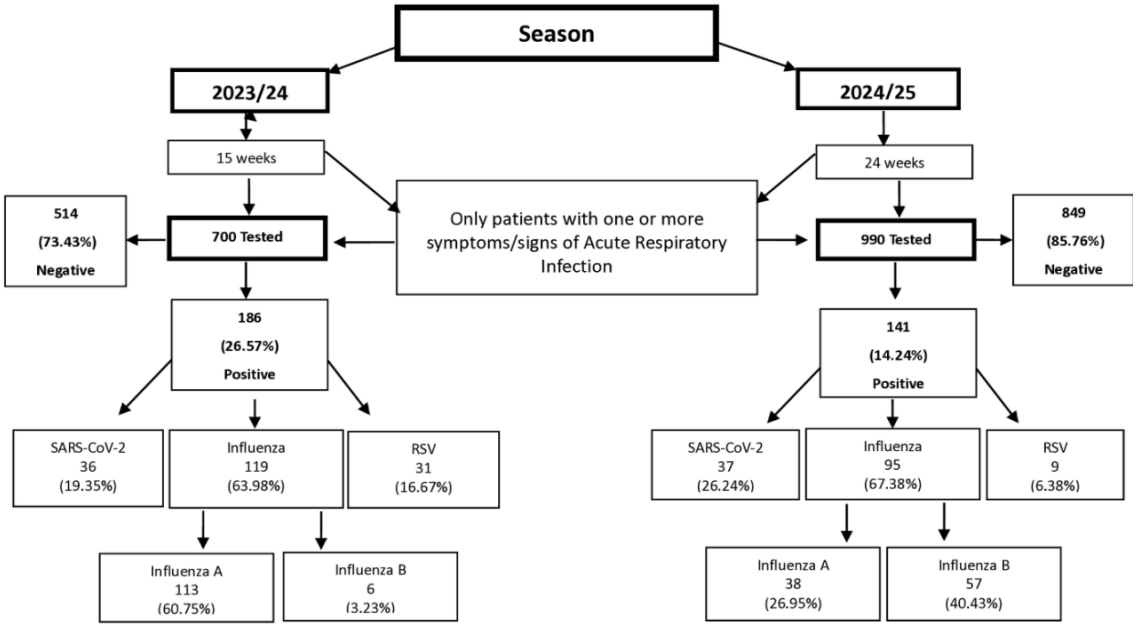


Fig. 1 – Algorithm for the sentinel surveillance and laboratory diagnosis of four different viruses using CerTest Rapid Test. For abbreviations, see Table 2.

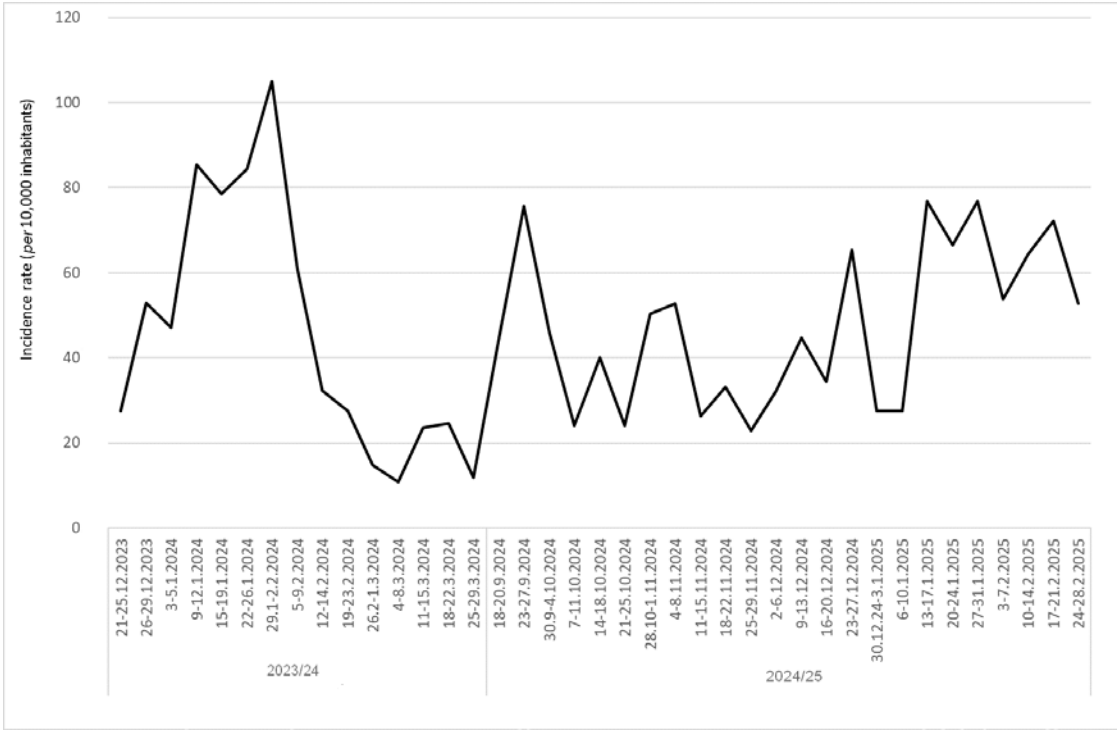


Fig. 2 – Weekly incidence rate of acute respiratory infection during the two seasons.

rapid increase in incidence was observed early in the 2023/24 season, peaking at over 200 cases *per* 10,000 inhabitants in early January 2024, followed by a sharp decline. In contrast, the 2024/25 season exhibited a different pattern, with a lower initial incidence but multiple peaks throughout the season, particularly in December 2024 and January 2025 (Figure 3a). Among school-aged children, incidence rates in the 2023/24 season peaked early, reaching approximately 140 cases *per* 10,000 inhabitants at the end of January 2024, followed by a marked decline. Conversely, the 2024/25 season exhibited a more gradual increase in incidence within this age group, characterized by multiple fluctuations and a peak in early 2025 (Figure 3b). In comparison, adults (15–29 years and 30–64 years) and the elderly population (≥ 65 years) consistently exhibited lower incidence rates across both seasons compared to younger age groups (Figures 3c–e).

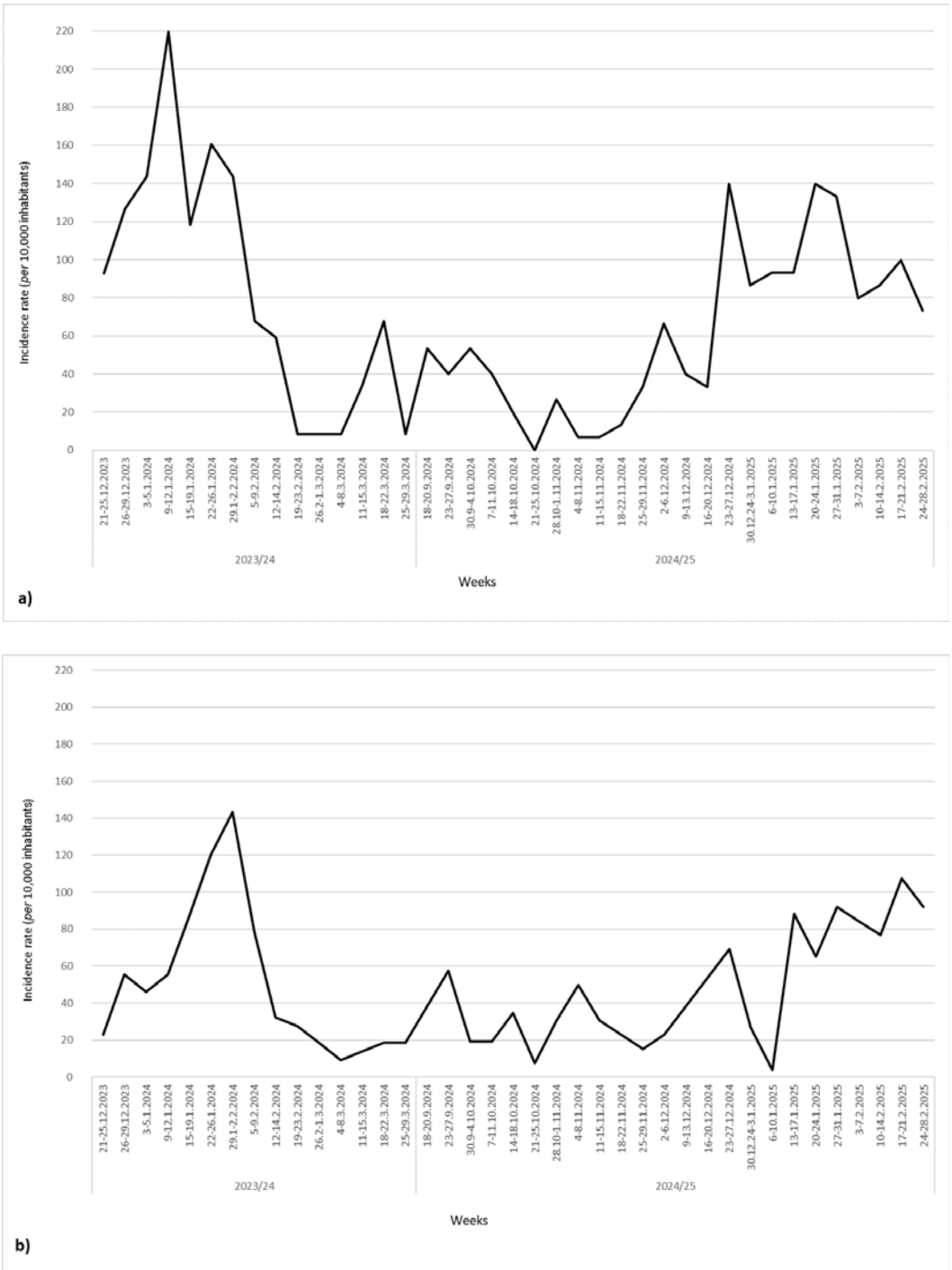
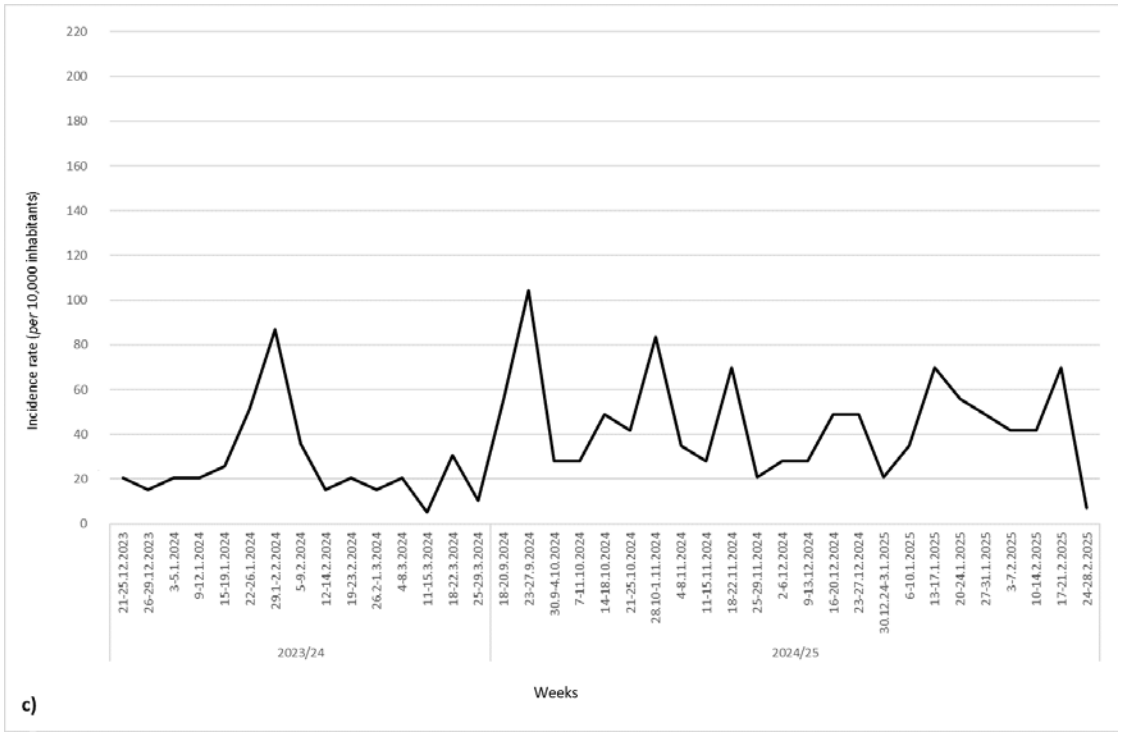


Fig. 3 – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

***Note*: Fig. 3 continued on next page.**



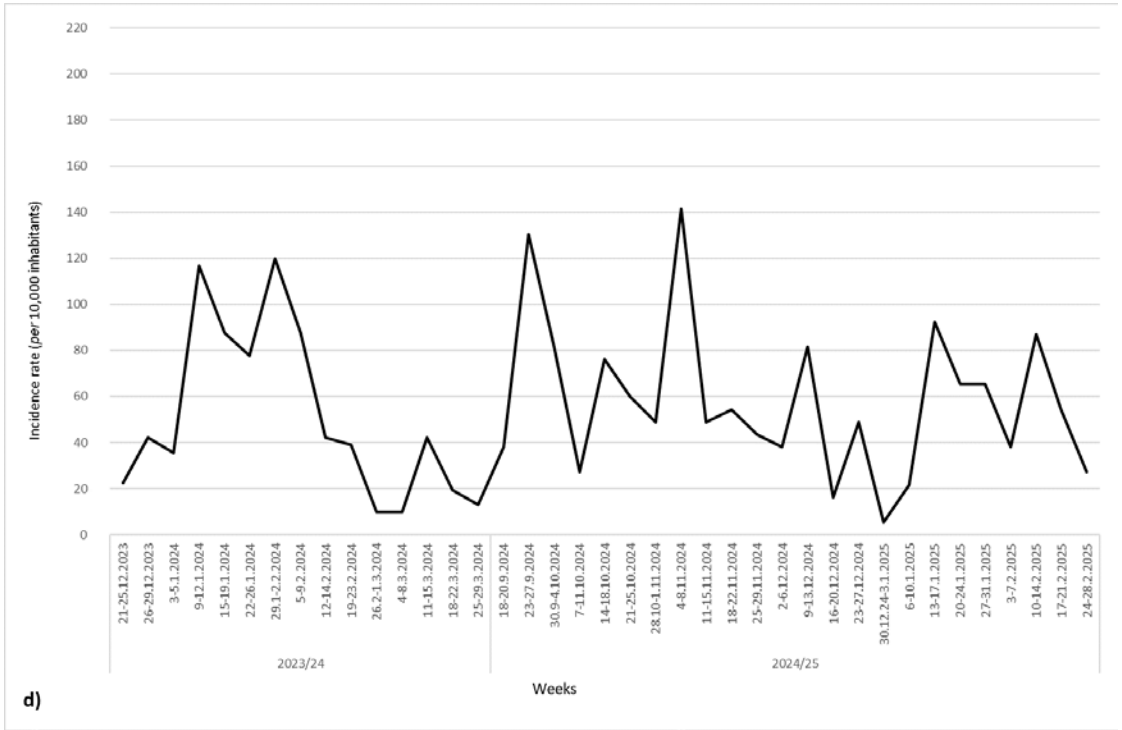


Fig. 3 (Continued) – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

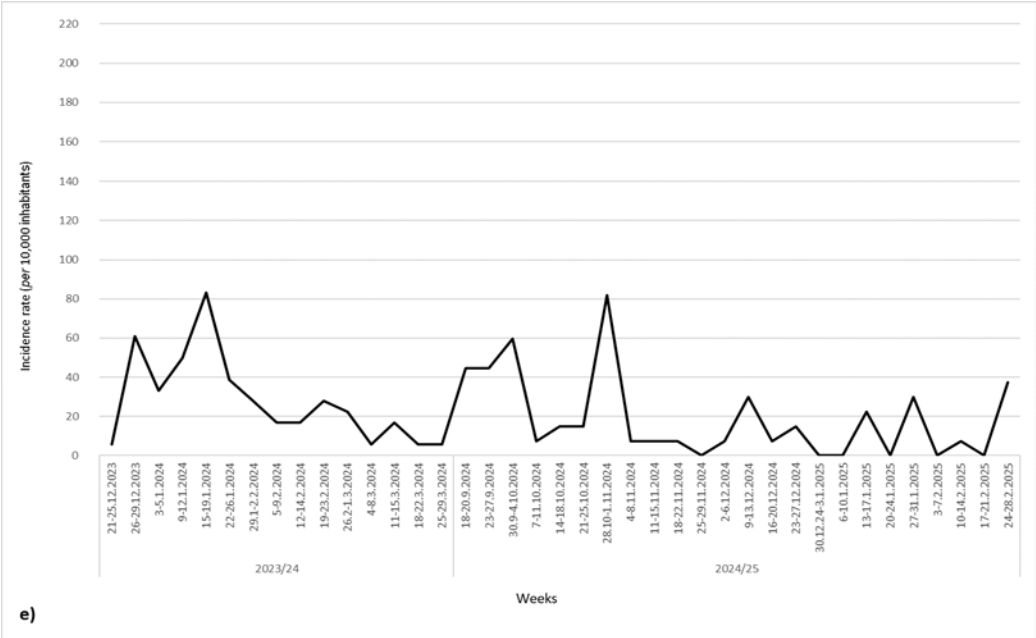


Fig. 3 (Continued) – Incidence rates of acute respiratory infections *per* 10,000 inhabitants, stratified by surveillance week, for two consecutive seasons, and age: a) 0–4, b) 5–14, c) 15–29, d) 30–64, and e) ≥ 65 years.

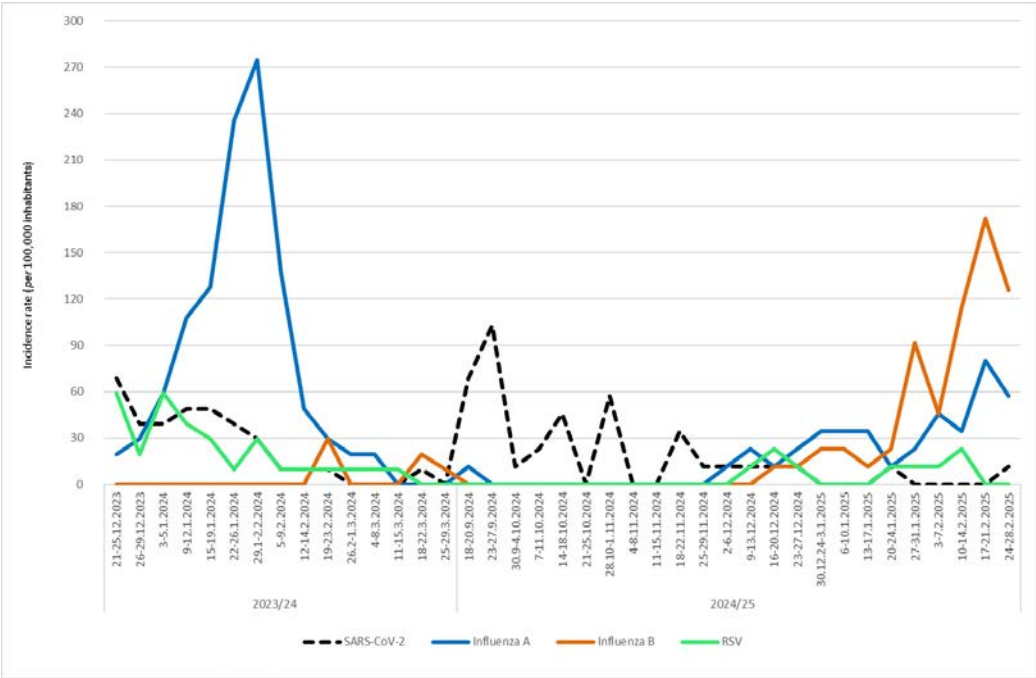


Fig. 4 – Weekly incidence rates of respiratory viruses (SARS-CoV-2, influenza A, influenza B, and RSV) during the two seasons. For abbreviations, see Table 2.

During the 2023/24 season, influenza A was the predominant virus, with a sharp increase in incidence observed from mid-January 2024, peaking in early February at approximately 270 cases *per* 100,000 inhabitants. Following this peak, the incidence of influenza A declined rapidly, reaching minimal levels by March 2024. Influenza B, SARS-CoV-2, and RSV exhibited relatively low activity during the same period, with sporadic detections and no significant peaks. In the 2024/25 season, influenza B showed a gradual increase in incidence starting from January 2025, peaking in late February with rates exceeding 170 cases *per* 100,000 inhabitants. Influenza A activity also rose during this period, though to a lesser extent compared to the previous season. SARS-CoV-2 exhibited moderate fluctuations throughout the season, with a notable peak (103.1 cases *per* 100,000 inhabitants) in late September 2024, while RSV activity remained consistently low, without any marked surges (Figure 4).

Figure 5a depicts the incidence rates of SARS-CoV-2 *per* 100,000 inhabitants during the 2023/24 and 2024/25 seasons. The data show fluctuations in incidence rates, with distinct peaks and variations across age groups. During the 2023/24 season, incidence rates were relatively low initially but increased in late January and early February 2024, particularly among young children (0–4 years) and the elderly (≥ 65 years). A sharp peak occurred in mid-February, predominantly among the elderly, followed by a decline. In the 2024/25 season, a resurgence occurred in late September, mostly affecting the youngest age group, followed by a peak in October 2024 among the 15–29 and ≥ 65 age groups. Sporadic increases were observed in the subsequent weeks, accompanied by minor fluctuations across age groups. A pronounced peak in influenza A incidence was recorded during the 2023/24 season, with the highest rates observed between late December 2023 and mid-February 2024. Children aged 0–4 and 5–14 years were the most affected groups, while incidence rates among individuals aged ≥ 65 years remained relatively low throughout this season. Following this peak, incidence rates declined sharply, with only sporadic detections in the later weeks of the season. During the 2024/25 season, in-

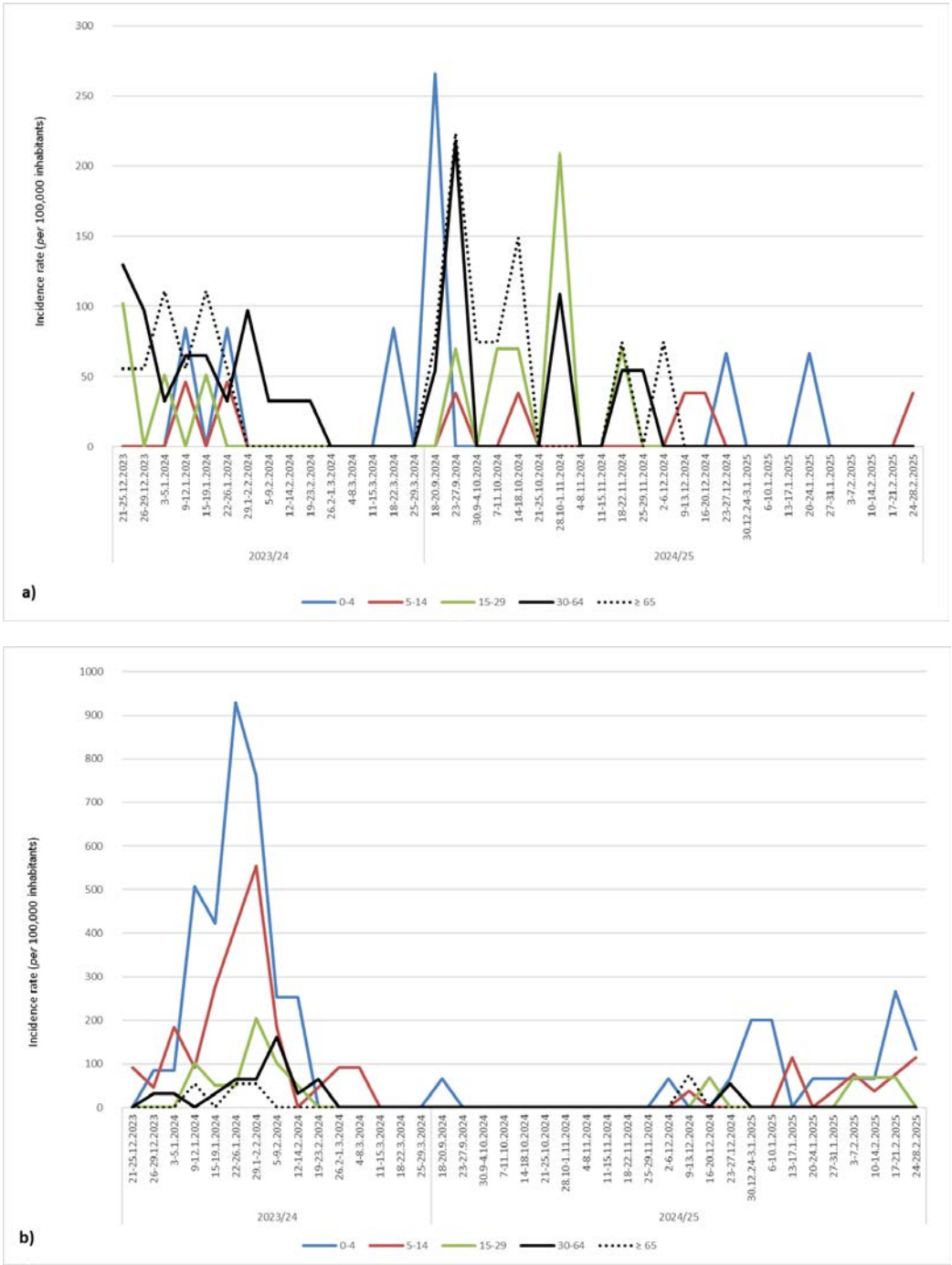


Fig. 5 – Incidence rates of: a) SARS-CoV-2, b) influenza A, c) influenza B, and d) RSV across age groups and surveillance week for two consecutive seasons. For abbreviations, see Table 2.

***Note*: Fig. 5 continued on next page.**

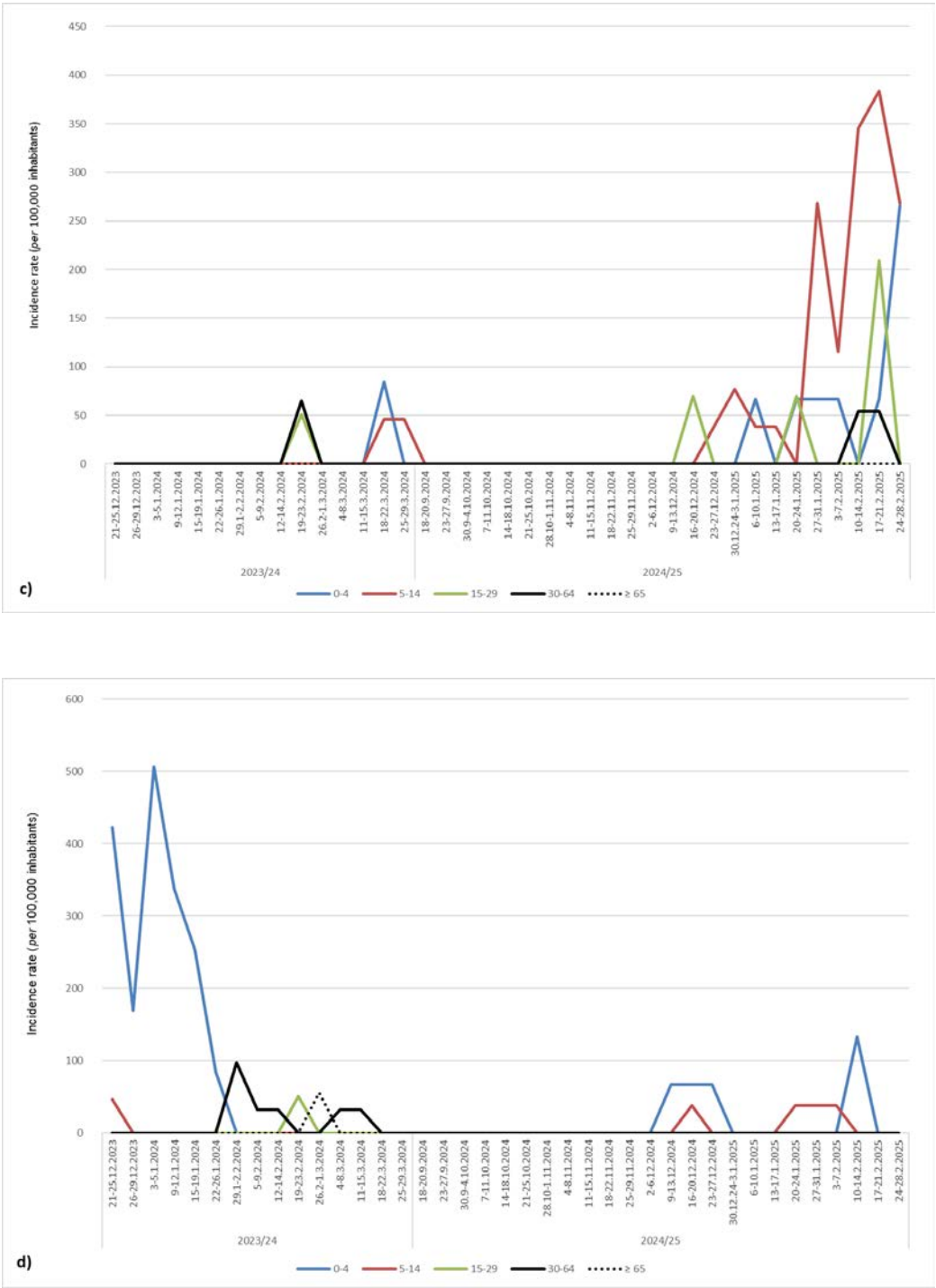


Fig. 5 (Continued) – Incidence rates of: a) SARS-CoV-2, b) influenza A, c) influenza B, and d) RSV across age groups and surveillance week for two consecutive seasons. For abbreviations, see Table 2.

fluenza A activity was markedly lower, with only minor increases observed in late December 2024 and early January 2025. Cases were detected across all age groups but with lower levels of incidence than in the previous season (Figure 5b). Figure 5c illustrates influenza B incidence rates across different age groups during the 2023/24 and 2024/25 seasons. Influenza B activity across age groups was minimal during the 2023/24 season, with only sporadic detections. In contrast, a significant increase in influenza B incidence was observed in the 2024/25 season, with a notable rise beginning in late December 2024, initially among individuals aged 15–29 years. The incidence peaked in February 2025, with the highest rates recorded among school-aged children (5–14 years), followed by young adults (15–29 years) and the youngest age group (0–4 years). Moderate incidence rates were reported among middle-aged adults (30–64 years), whereas the elderly population (≥ 65 years) exhibited the lowest incidence. During the 2023/24 season, the highest burden of RSV was observed in

the 0–4-year age group, with a peak in December 2023 and early January 2024, followed by a sharp decline. Incidence rates in other age groups were significantly lower, with only sporadic detections throughout the season. In the 2024/25 season, RSV activity remained low across all age groups, with low incidence rates primarily observed among children aged 0–4 and 5–14 years. No substantial RSV activity was detected in older age groups (≥ 15 years) (Figure 5d).

In the 2023/24 season, gender distribution did not differ significantly among the groups ($p = 0.4789$). However, a significant difference was observed in age distribution ($p < 0.0001$). RSV infections were predominantly observed in children aged 0–4 years (67.74%), whereas influenza A was most frequently detected in the 5–14-year age group (38.94%). SARS-CoV-2 infections were most prevalent among adults aged 30–64 years (52.78%) and those aged ≥ 65 years (25%). The highest mean age was recorded in SARS-CoV-2 patients (44.56 ± 23.14 years), while the lowest mean age was observed in patients with influenza B infection (9.17 ± 12.12 years) cases ($p < 0.0001$). The distribution of infections varied significantly by month of testing ($p < 0.0001$). SARS-CoV-2 cases peaked in January 2024 (50%), similar to influenza A (66.37%) and RSV (45.16%). Notably, 66.67% of influenza B cases were recorded in March 2024, whereas no SARS-CoV-2 cases were identified during that month. The median time from symptom onset to testing was comparable across the groups, ranging from 2 to 3 days, with no statistically significant difference ($p = 0.0810$). Fever (≥ 38 °C) was the most common symptom across all groups, with the highest prevalence in influenza A and influenza B cases (100.00% each). Fever was significantly less frequent in SARS-CoV-2 cases (80.56%) ($p = 0.0289$). Cough was prevalent across all infections, particularly in RSV patients (96.77%). Headache was most frequently reported in influenza B (83.33%) and SARS-CoV-2 (66.67%) cases, while it was rare in RSV infections (19.35%). Malaise and myalgia were most common in influenza cases, whereas sore throat was present in approximately half of the SARS-CoV-2 and influenza B cases. Loss of appetite was most frequently reported in RSV patients (58.06%) and was less common in other groups ($p < 0.05$) (Table 3).

Table 3

Demographic and clinical characteristics of patients with laboratory-confirmed SARS-CoV-2, influenza A, influenza B, and RSV infections during the 2023/24 season

Parameters	SARS-CoV-2 (n = 36)	Influenza A (n = 113)	Influenza B (n = 6)	RSV (n = 31)	*p*-value
	n (%)	n (%)	n (%)	n (%)	
Gender					
male	18 (50.00)	46 (40.71)	4 (66.67)	15 (48.39)	0.4789
female	18 (50.00)	67 (59.29)	2 (33.33)	16 (51.61)	
Age, years					
0–4	2 (5.56)	34 (30.09)	3 (50.00)	21 (67.74)	
5–14	2 (5.56)	44 (38.94)	2 (33.33)	1 (3.23)	
15–29	4 (11.11)	11 (9.73)	0 (0.00)	1 (3.23)	**< 0.0001**
30–64	19 (52.78)	15 (13.27)	1 (16.67)	7 (22.58)	
≥ 65	9 (25.00)	9 (7.96)	0 (0.00)	1 (3.23)	
mean ± standard deviation	44.56 ± 23.14	18.54 ± 23.68	9.17 ± 12.12	16.19 ± 23.85	**< 0.0001****
median (interquartile range)	43 (31–64)	9 (3–15)	5 (2–14)	2 (1–34)	
Month of testing					
December 2023	13 (36.11)	5 (4.42)	0 (0.00)	9 (29.03)	
January 2024	18 (50.00)	75 (66.37)	1 (16.67)	14 (45.16)	**< 0.0001**
February 2024	5 (13.89)	31 (27.43)	1 (16.67)	6 (19.35)	
March 2024	0 (0.00)	2 (1.77)	4 (66.67)	2 (6.45)	
Period of symptom onset to testing					
mean ± standard deviation	2.31 ± 1.05	2.61 ± 1.16	3.33 ± 1.70	2.90 ± 1.06	0.0810**
median time (interquartile range)	2 (2–3)	2 (2–4)	3 (2–5)	3 (2–4)	
Symptoms and signs*					
fever (≥ 38 °C)	29 (80.56)	113 (100.00)	6 (100.00)	27 (87.10)	
cough	27 (75.00)	99 (87.61)	4 (66.67)	30 (96.77)	
headache	24 (66.67)	64 (56.64)	5 (83.33)	6 (19.35)	
malaise	14 (38.89)	53 (46.90)	3 (50.00)	4 (12.90)	**0.0289**
myalgia	11 (30.56)	33 (29.20)	2 (33.33)	4 (12.90)	
sore throat	19 (52.78)	46 (40.71)	3 (50.00)	9 (29.03)	
loss of appetite	12 (33.33)	35 (30.97)	0 (0.00)	18 (58.06)	

n – number. For other abbreviations, see Table 2.

***Note*: *one patient could have one or more symptoms and signs; **ANOVA analysis of variance; values that differ significantly ($p < 0.05$) are marked in bold.**

In the 2024/25 season, gender distribution did not differ significantly among the groups ($p = 0.5098$). However, a statistically significant difference was observed in age distribution ($p < 0.0001$). RSV and influenza A infections were most prevalent among children aged 0–4 years (55.56% and 47.37%, respectively), whereas influenza B was predominantly detected in the 5–14-year age group (71.93%). SARS-CoV-2 infections primarily affected older individuals, with 27.03% of cases occurring in patients aged ≥ 65 years and 24.32% in those aged 30–64 years. The highest mean age was recorded among SARS-CoV-2 patients (37.97 ± 29.59 years), while the lowest was observed in RSV cases (4.11 ± 4.04 years) ($p < 0.0001$). The distribution of infections varied significantly by month of testing ($p < 0.0001$). SARS-CoV-2 cases were primarily detected in September (43.24%) and October 2024 (24.32%), whereas the majority of influenza A (50.00%) and influenza B (68.42%) cases were registered in February 2025. RSV cases were most frequently recorded in December 2024 (44.44%) and February 2025 (33.33%). The median time from symptom onset to testing was similar across all groups, ranging from 2 to 3 days, with no statistically significant difference ($p = 0.3650$). Analysis of clinical symptoms revealed that fever (≥ 38 °C) was highly prevalent in all groups, occurring in 100.00% of influenza A, influenza B, and RSV cases, and in 75.68% of SARS-CoV-2 cases, without statistically significant differences ($p = 0.2550$). Cough was most frequently reported in patients with influenza B (80.70%) and influenza A (78.95%). Headache was most common in SARS-CoV-2 (62.16%) and influenza B (59.65%) cases, but was absent in RSV infections. Malaise and myalgia were reported across all groups, with the highest frequency observed in SARS-CoV-2 and influenza B cases. Sore throat was present in approximately one-third of SARS-CoV-2 cases (37.84%) and 40.35% of influenza B cases, while it was rare in RSV infections (11.11%). Loss of appetite was most frequently reported in influenza A (55.26%) and influenza B (49.12%) cases, compared to 24.32% in SARS-CoV-2 and 33.33% in RSV cases (Table 4).

Discussion

To our knowledge, this is the first study using rapid combination tests for SARS-CoV-2, influenza A and B, and RSV in ARI sentinel surveillance in our country. The findings reveal seasonal patterns, demographic variations, and clinical characteristics over two consecutive seasons. Despite differences in surveillance duration (15 weeks in 2023/24 vs. 24 weeks in 2024/25), the latter showed a slower onset but a sharper rise later.

Table 4

Demographic and clinical characteristics of patients with laboratory-confirmed SARS-CoV-2, influenza A, influenza B, and RSV infections during the 2024/25 season

Parameters	SARS-CoV-2 (n = 37)	Influenza A (n = 38)	Influenza B (n = 57)	RSV (n = 9)	*p*-value
	n (%)	n (%)	n (%)	n (%)	
Gender					
male	14 (37.84)	17 (44.74)	30 (52.63)	5 (55.56)	0.5098
female	23 (62.16)	21 (55.26)	27 (47.37)	4 (44.44)	
Age, years					
0–4	6 (16.22)	18 (47.37)	9 (15.79)	5 (55.56)	
5–14	5 (13.51)	14 (36.84)	41 (71.93)	4 (44.44)	
15–29	7 (18.92)	3 (7.89)	5 (8.77)	0 (0.00)	**< 0.0001**
30–64	9 (24.32)	2 (5.26)	2 (3.51)	0 (0.00)	
≥ 65	10 (27.03)	1 (2.63)	0 (0.00)	0 (0.00)	
mean ± standard deviation	37.97 ± 29.59	10.79 ± 16.19	8.89 ± 6.54	4.11 ± 4.04	**< 0.0001****
median age (interquartile range)	30 (14–69)	5 (3–10)	7 (6–10)	2 (1–7)	
Month of testing					
September 2024	16 (43.24)	0 (0.00)	0 (0.00)	0 (0.00)	
October 2024	9 (24.32)	0 (0.00)	0 (0.00)	0 (0.00)	
November 2024	7 (18.92)	1 (2.63)	0 (0.00)	0 (0.00)	**< 0.0001**
December 2024	3 (8.11)	6 (15.79)	4 (7.02)	4 (44.44)	
January 2025	1 (2.70)	12 (31.58)	14 (24.56)	2 (22.22)	
February 2025	1 (2.70)	19 (50.00)	39 (68.42)	3 (33.33)	
Period of symptom onset to testing					
mean ± standard deviation	2.22 ± 0.93	2.16 ±1.31	2.53 (±1.11)	2.44 ± 0.68	0.3650**
median (interquartile range)	2 (2–3)	2 (1–3)	2 (2–3)	3 (2–3)	
Symptoms and signs*					
fever (≥ 38 °c)	28 (75.68)	38 (100.00)	57 (100.00)	9 (100.00)	
cough	20 (54.05)	30 (78.95)	46 (80.70)	7 (77.78)	
headache	23 (62.16)	16 (42.11)	34 (59.65)	0 (0.00)	
malaise	18 (48.65)	11 (28.95)	21 (36.84)	1 (11.11)	0.2550
myalgia	12 (32.43)	9 (23.68)	21 (36.84)	1 (11.11)	
sore throat	14 (37.84)	10 (26.32)	23 (40.35)	1 (11.11)	
loss of appetite	9 (24.32)	21 (55.26)	28 (49.12)	3 (33.33)	

n – number. For other abbreviations, see Table 2.

***Note:* *one patient could have one or more symptoms and signs; **ANOVA analysis of variance; values that differ significantly ($p < 0.05$) are marked in bold.**

The highest ARI incidence in the 2023/24 season (105 *per* 10,000 inhabitants) was recorded in late January, coinciding with school reopenings and cold weather. In contrast, the 2024/25 peak incidence (76.8 *per* 10,000 inhabitants) occurred in mid-to-late January [13], with a lower rate possibly influenced by school closures during public protests in Serbia. In the 2023/24 season, 73.43% of tested samples were negative, increasing to 85.76% in 2024/25. This suggests many ARI cases were unrelated to the tested viruses, possibly due to test limitations, other viral or bacterial pathogens, low viral loads, non-infectious conditions, or preexisting immunity [1, 2, 8, 10, 12]. The widespread circulation of SARS-CoV-2 significantly impacted the epidemiology of respiratory viruses [8–10, 14]. A study across 27 countries found SARS-CoV-2 in over 70% of hospitalized patients with viral infections between 2020 and 2022 [9]. Its dominance may be due to a higher basic reproduction number than influenza and RSV. While public health measures implemented during the COVID-19 pandemic reduced influenza and RSV transmission, they were less effective against the spread of SARS-CoV-2 [15, 16]. Rhinoviruses and enteroviruses persisted despite interventions like masking and school closures, likely due to their greater surface stability and different transmission routes [17, 18]. These viruses accounted for about 75% of viral detections in pediatric healthcare visits during the first year of the pandemic [17].

During the pandemic, SARS-CoV-2 dominated, with other respiratory viruses detected sporadically due to the implementation of widespread public health control measures [5, 14, 19]. Our findings indicate a shift in viral dominance during the 2023/24 and 2024/25 seasons. Influenza A, the most common virus in 2023/24 (60.75%), declined to 26.95% in 2024/25, while influenza B rose from 3.23% to 40.43%, suggesting a strain shift. SARS-CoV-2 remained stable, increasing slightly from 19.35% to 26.24%. RSV decreased from 16.67% to 6.38%, possibly due to seasonal dynamics or population immunity. These trends align with the resurgence of pre-pandemic influenza and RSV patterns in the European Union [20, 21]. Our findings emphasize seasonal variability in respiratory viruses and the need for ongoing surveillance. The earlier circulation of SARS-CoV-2 in 2024/25 and shifting influenza strains highlight the importance of timely public health interventions, including vaccination and preparedness measures [2, 4, 5, 7–10].

As the sentinel surveillance mainly covered preschool and school-aged children, ARIs disproportionately affected those aged 0–4 years, who had the highest incidence rates. RSV was most frequently detected in this group in both seasons. During the 2023/24 season, predominant influenza A suppressed other respiratory viruses. In 2024/25, SARS-CoV-2 initially dominated but was later overtaken by influenza. Throughout the study, influenza had a higher weekly incidence than SARS-CoV-2 and RSV, whose trends were similar. A study of 25 European countries found a similar pattern, with influenza peaking separately from SARS-CoV-2 and RSV [22].

In the 2023/24 season, influenza A was most common in preschool- and school-aged children (0–4 and 5–14 years), while influenza B was more frequent in children aged 0–4 years. The following season saw an inverse pattern. SARS-CoV-2 affected all age groups, especially adults (30–64 years) and the elderly, with a higher frequency in 2024/25. Similar age distribution trends have been observed in previous studies [5, 23, 24]. Once again, the distribution of respiratory viruses aligns with the restoration of pre-pandemic epidemiological patterns after the relaxation of COVID-19 preventive measures [2, 5, 7, 16, 18, 23].

The clinical presentations of ARIs showed significant overlap, with fever, cough, and headache as the most common symptoms. Fever was present in all cases of influenza A, influenza B, and RSV, but less so in SARS-CoV-2 infections. Similar patterns have been observed in other studies, where influenza and RSV were more strongly associated with fever than SARS-CoV-2 [1, 2, 8]. RSV infections also had lower frequencies of headache and malaise, but a higher prevalence of loss of appetite [17, 25], highlighting distinct clinical profiles.

The median time from symptom onset to testing was 2 to 3 days for all four viruses in both seasons, indicating consistent diagnostic practices. This highlights the importance of timely detection for effective clinical management and infection control. Patients with symptoms of any of the four viruses seek medical care at the primary healthcare level around the same time.

The shift in viral dominance and age-specific incidence trends highlights the need for targeted prevention, especially for young children. Enhanced RSV surveillance should be considered in both primary healthcare and hospital settings. Differentiating these viruses remains challenging due to overlapping symptoms, but multiplex molecular testing could aid early diagnosis, optimize antiviral use, and reduce unnecessary antibiotics. Seasonal variations emphasize the need for rapid, adaptive public health interventions, including timely vaccination and non-pharmaceutical measures to ease healthcare system burdens.

Our study had several limitations. First, it was conducted in a specific healthcare setting, limiting generalizability. ARI incidence rates were based on sentinel surveillance, which may miss mild or asymptomatic cases, leading to an underestimation of the true disease burden. The sample also had a lower participation rate among those aged 15 and above, especially in the 30–64 and ≥ 65 age groups. Future studies should include more sentinel physicians to better represent the age distribution. Second, we did not perform PCR testing to confirm rapid test results, and the sensitivity of the CerTest Rapid Test varied across pathogens, with lower sensitivity for influenza A and B, increasing the likelihood of false negatives. The test's performance for RSV also showed wide confidence intervals, suggesting variability in its accuracy. PCR remains essential for confirmation. Additionally, we lacked data on the test's limit of detection, which impacts its ability to detect low viral loads. Third, the study did not analyze viral co-detection, which may affect disease severity and transmission. Fourth, conducted over two seasons, the study offers short-term insights but limits conclusions on long-term trends. Finally, we did not assess vaccination status, which could influence infection risk and severity. Future

research should include vaccination data for a more comprehensive understanding. Despite the limitations, our study shows that ARI incidence of common respiratory viruses during the autumn-winter period can be effectively assessed with minimal burden on physicians and resources in the sentinel surveillance system.

Conclusion

Our data show a shift in viral dominance between the seasons, with influenza A prevailing in 2023/24 and influenza B becoming more prominent in 2024/25. SARS-CoV-2 circulated at low levels, while RSV had minimal impact. Despite using a limited set of indicators, our findings can be cautiously compared to broader research on viral pathogens. These results emphasize the need for ongoing surveillance, timely diagnostics, and targeted prevention strategies. Future research should explore the long-term effects of shifting viral patterns and the integration of rapid tests for multiple viruses in surveillance.

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