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RESPIRATORY PATHOGENS DETECTED BY MULTIPLEX REAL-TIME PCR IN PATIENTS WITH ACUTE RESPIRATORY INFECTIONS IN ALMATY, KAZAKHSTAN, DURING THE 2023–2024 EPIDEMIC SEASON

Salima Mamutbayeva[1], Mincheol Lee[2], Geonsang Park[3], Heesuk Min[4], Minjoong Jang[5], Seiick Joo[6], Yongha Kim[7], Seehyen Ham[8], Yoonkyung Choi[9], Amina Muradalieva[10], Abiyrova Zhazira Meyramovna[11], Utaganov Bakhyt Kustaevich[12], Umirbekova Lazzat Zhaksylykovna[13]

[1] *Main Microbiologist, Laboratory Division, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[2] *Chief Executive Officer, Openhealthcare, Inc. 280, Cheonho-daero, Seongdong-gu, Seoul, Republic of Korea;*
[3] *Chief Information and Clinical Officer, Medical Division, Openhealthcare, Inc. 280, Cheonho-daero, Seongdong-gu, Seoul, Republic of Korea;*
[4] *Medical Division Director, Medical Division, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[5] *Managing Director, Clinical Laboratory Services, Openhealthcare, Inc. 280, Cheonho-daero, Seongdong-gu, Seoul, Republic of Korea;*
[6] *General Manager, Testing Planning Office, Openhealthcare, Inc. 280, Cheonho-daero, Seongdong-gu, Seoul, Republic of Korea;*
[7] *Laboratory Division Director, Laboratory Division, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[8] *General Manager, Medical Support Department, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[9] *Lab Management Department General Manager, Lab Management Department, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[10] *Interpreter, Laboratory Division, Openhealthcare KZ. Dostyk Avenue 210, Block B, Almaty, Kazakhstan;*
[11] *Deputy Director for Medical Work, City Polyclinic #26. City Polyclinic #26, Almaty, Kazakhstan;*
[12] *Deputy Director for Medical Affairs, City Emergency Hospital. City Emergency Hospital, Almaty, Kazakhstan;*
[13] *Head of the laboratory – doctor. Dostyk Avenue 210, Block B, Almaty, Kazakhstan*

Corresponding author
Minjoong Jang | Email: michaeljmj@naver.com | ORCID: 0009-0008-0130-3499

Introduction. Acute respiratory infections remain an important public health problem, while data describing the combined spectrum of viral and bacterial respiratory pathogens in Kazakhstan remain limited. Multiplex real-time PCR enables simultaneous detection of a broad range of respiratory targets and may improve epidemiological characterization during seasonal increases in respiratory morbidity.
Objective. To investigate the distribution of viral and bacterial respiratory pathogens among patients with acute respiratory infections in Almaty, Kazakhstan, during the 2023–2024 epidemic season using multiplex real-time PCR, and to assess age-specific positivity patterns and the frequency of multiple pathogen detections.
Materials and Methods. Respiratory specimens obtained from 374 patients with clinically diagnosed acute respiratory infections between November 2023 and January 2024 were analyzed using multiplex real-time PCR assays targeting 30 respiratory pathogens, including 19 viral and 11 bacterial targets. Detection frequencies, age-specific positivity patterns, and combinations of simultaneously positive assay targets were analyzed descriptively.
Results. Viral targets were detected in 195 of 374 patients (52.1%). Rhinovirus (24.3%) and influenza A virus (14.7%) were the most frequently detected viral pathogens. Bacterial targets were detected in 240 patients (64.2%), predominantly *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*. Multiple positive assay targets were recorded in 202 patients (54.0%); among these, 94 patients had two positive targets, 67 had three, and 41 had four or more. Distinct age-related patterns of viral and bacterial positivity were also observed.
Discussion. The findings demonstrate a heterogeneous respiratory pathogen profile during the epidemic season, with frequent simultaneous detection of multiple molecular targets. However, bacterial PCR positivity does not necessarily distinguish colonization from active infection, and simultaneous positivity for a generic influenza A target and a subtype-

specific target should not automatically be interpreted as infection with two distinct viruses. Accordingly, the observed multiple-target positivity should be interpreted as molecular co-detection rather than confirmed clinical co-infection unless supported by additional clinical and microbiological information.

Conclusion. Multiplex real-time PCR provided broad characterization of respiratory pathogens circulating among patients with acute respiratory infections in Almaty during the 2023–2024 season. Rhinovirus and influenza A predominated among viral detections, whereas *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were the most frequently detected bacterial targets. The results support the potential value of multiplex molecular diagnostics for respiratory surveillance and clinical decision support, while emphasizing the need for careful interpretation of bacterial and multiple-target PCR positivity.

Keywords: acute respiratory infections; multiplex real-time PCR; respiratory pathogens; viral pathogens; bacterial pathogens; molecular co-detection.

2023 ЖЫЛҒЫ ЭПИДЕМИЯЛЫҚ МАУСЫМДА ҚАЗАҚСТАНДА КӨПТЕГЕН ИНФЕКЦИЯЛАРҒА RT-PCR ӘДІСІМЕН АНЫҚТАЛҒАН ЖЕДЕЛ РЕСПИРАТОРЛЫҚ ИНФЕКЦИЯЛАРДЫҢ ҚОЗДЫРҒЫШТАРЫ

Салима Мамутбаева[1], Mincheol Lee[2], Гео Geonsang Park[3], Heesuk Min[4], Minjoong Jang[5], Seiick Joo[6], Yongha Kim[7], Seehyen Ham[8], Yoonkyung Choi[9], Амина Мұрадәлиева[10], Абирова Жазира Мейрамовна[11], Утаганов Бахыт Кустаевич[12], Умирбекова Лаззат Жаксылыковна[13]

[1] Негізгі микробиолог, зертхана бөлімі, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[2] Бас атқарушы директор, Openhealthcare, Inc. 280, Чхонхо-даэро, Сонгдонг-гу, Сеул, Корея Республикасы;

[3] Бас ақпараттық және клиникалық директор, медицина бөлімі, Openhealthcare, Inc. 280, Чхонхо-даэро, Сонгдонг-гу, Сеул, Корея Республикасы;

[4] Медицина бөлімінің директоры, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[5] Клиникалық зертхана қызметтерінің басқарушы директоры, Openhealthcare, Inc. 280, Чхонхо-даэро, Сонгдонг-гу, Сеул, Корея Республикасы;

[6] Тестілеуді жоспарлау офисінің бас менеджері, Openhealthcare, Inc. 280, Чхонхо-даэро, Сонгдонг-гу, Сеул, Корея Республикасы;

[7] Зертхана бөлімінің директоры, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[8] Медициналық қолдау бөлімінің бас менеджері, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[9] Зертхана басқармасы бөлімінің бас менеджері, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[10] Аудармашы, зертхана бөлімі, Openhealthcare KZ. Достық даңғылы 210, В блогы, Алматы, Қазақстан;

[11] Медицина жұмысы жөніндегі директордың орынбасары, №26 қалалық емхана. №26 қалалық емхана, Алматы, Қазақстан;

[12] Медицина ісі жөніндегі директордың орынбасары, Қалалық шұғыл медициналық жәрдем ауруханасы. Алматы, Қазақстан;

[13] Зертхана меңгерушісі – дәрігер. Достық даңғылы 210, В блогы, Алматы, Қазақстан

Кіріспе. Жедел респираторлық инфекциялар қоғамдық денсаулық сақтаудың маңызды мәселелерінің бірі болып қала береді, ал Қазақстандағы вирустық және бактериялық респираторлық қоздырғыштардың бір мезгілде таралу спектрін сипаттайтын кешенді деректер шектеулі. Нақты уақыттағы мультиплексті ПТР көптеген респираторлық нысаналы бір уақытта анықтауға және маусымдық сырқаттану шылық кезеңіндегі эпидемиологиялық жағдайды жан-жақты бағалауға мүмкіндік береді.

Мақсаты. 2023–2024 жылдардағы эпидемиялық маусымда Алматы қаласында жедел респираторлық инфекциясы бар пациенттер арасында вирустық және бактериялық респираторлық қоздырғыштардың таралуын нақты уақыттағы мультиплексті ПТР әдісімен зерттеу, сондай-ақ жас ерекшеліктеріне байланысты анықталу жиілігін және бірнеше нысананың бір мезгілде оң болу жиілігін бағалау.

Материалдар мен әдістер. 2023 жылғы қараша мен 2024 жылғы қаңтар аралығында клиникалық тұрғыдан жедел респираторлық инфекция диагнозы қойылған 374 пациенттен алынған респираторлық үлгілер 19 вирустық және 11 бактериялық нысананы қамтитын 30 респираторлық қоздырғышқа арналған нақты уақыттағы мультиплексті ПТР тесттерімен зерттелді. Қоздырғыштардың анықталу жиілігі, жас ерекшеліктеріне байланысты оң нәтижелер және бір мезгілде анықталған ПТР нысаналарының комбинациялары сипаттамалық түрде бағаланды.

Нәтижелер. Вирустық нысаналар 374 пациенттің 195-інде (52,1%) анықталды. Ең жиі риновирус (24,3%) және А тұмауы вирусы (14,7%) анықталды. Бактериялық нысаналар 240 пациентте (64,2%) тіркелді; олардың ішінде *Haemophilus influenzae*, *Streptococcus pneumoniae* және *Moraxella catarrhalis* басым болды. Бірнеше ПТР нысанасының бір мезгілде оң болуы 202 пациентте (54,0%) тіркелді: 94 пациентте екі, 67 пациентте үш, 41 пациентте төрт және одан да көп оң нысана анықталды. Сонымен қатар жекелеген вирустық және бактериялық қоздырғыштардың жасқа байланысты анықталу ерекшеліктері байқалды.
Талқылау. Нәтижелер эпидемиялық маусымдағы респираторлық қоздырғыштар құрылымының әртектілігін және бірнеше молекулалық нысананың бір мезгілде анықталуының жоғары жиілігін көрсетті. Алайда ПТР арқылы бактериялық ДНҚ-ның анықталуы колонизацияны белсенді инфекциядан бірмәнді ажыратуға мүмкіндік бермейді. Сонымен қатар А тұмауы вирусының жалпы нысанасы мен оның белгілі бір қосалқы типінің бір мезгілде оң болуы екі дербес вирустық инфекция ретінде автоматты түрде қарастырылмауы тиіс. Сондықтан бірнеше оң нәтижені қосымша клиникалық және микробиологиялық деректер болмаған жағдайда расталған клиникалық коинфекция ретінде емес, молекулалық бірлескен анықталу ретінде бағалау дұрысырақ.
Қорытынды. Нақты уақыттағы мультиплексті ПТР 2023–2024 жылдардағы маусымда Алматы қаласында ЖРИ бар пациенттер арасында таралған респираторлық қоздырғыштардың спектрін кешенді сипаттауға мүмкіндік берді. Вирустық нысаналар арасында риновирус пен А тұмауы вирусы, ал бактериялық нысаналар арасында *H. influenzae*, *S. pneumoniae* және *M. catarrhalis* басым болды. Алынған нәтижелер бактериялық және бірнеше ПТР-оң нәтижелерді сақтықпен интерпретациялау қажеттілігін ескере отырып, мультиплексті молекулалық диагностиканың эпидемиологиялық қадағалау мен клиникалық шешімдерді қолдаудағы әлеуетін көрсетеді.
Түйінді сөздер: жедел респираторлық инфекциялар; нақты уақыттағы мультиплексті ПТР; респираторлық қоздырғыштар; вирустық патогендер; бактериялық патогендер; молекулалық бірлескен анықталу.

ВОЗБУДИТЕЛИ ОСТРЫХ РЕСПИРАТОРНЫХ ИНФЕКЦИЙ, ВЫЯВЛЕННЫЕ МЕТОДОМ МУЛЬТИПЛЕКСНОЙ ПЦР В РЕЖИМЕ РЕАЛЬНОГО ВРЕМЕНИ В ЭПИДЕМИЧЕСКИЙ СЕЗОН 2023 ГОДА В КАЗАХСТАНЕ

Салима Мамутбаева[1], Mincheol Lee[2], Geo Geonsang Park[3], Heesuk Min[4], Minjoong Jang[5], Seiick Joo[6], Yongha Kim[7], Seehyen Ham[8], Yoonkyung Choi[9], Амина Мұрадәлиева[10], Абирова Жазира Мейрамовна[11], Утаганов Бахыт Кустаевич[12], Умирбекова Лаззат Жаксылыковна[13]

[1] Главный микробиолог, лабораторное подразделение, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[2] Генеральный директор, Openhealthcare, Inc. 280, Чхонхо-даэро, район Сондонг-гу, Сеул, Республика Корея;
[3] Директор по информационным технологиям и клиническим вопросам, медицинское подразделение, Openhealthcare, Inc. 280, Чхонхо-даэро, район Сондонг-гу, Сеул, Республика Корея;
[4] Директор медицинского подразделения, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[5] Управляющий директор по клинико-лабораторным услугам, Openhealthcare, Inc. 280, Чхонхо-даэро, район Сондонг-гу, Сеул, Республика Корея;
[6] Генеральный менеджер, отдел планирования тестирования, Openhealthcare, Inc. 280, Чхонхо-даэро, район Сондонг-гу, Сеул, Республика Корея;
[7] Директор лабораторного подразделения, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[8] Генеральный менеджер, отдел медицинской поддержки, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[9] Генеральный менеджер отдела лабораторного управления, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[10] Переводчик, лабораторное подразделение, Openhealthcare KZ. проспект Достык 210, блок В, Алматы, Казахстан;
[11] Заместитель директора по лечебной работе, Городская поликлиника №26, Алматы, Казахстан;
[12] Заместитель директора по медицинским вопросам, Городская больница скорой помощи, Алматы, Казахстан;
[13] Заведующая лабораторией – врач, проспект Достык 210, блок В, Алматы, Казахстан

Введение. Острые респираторные инфекции остаются значимой проблемой общественного здравоохранения, при этом комплексные данные о спектре одновременно циркулирующих вирусных и бактериальных респираторных возбудителей в Казахстане ограничены. Мультиплексная ПЦР в режиме реального времени позволяет одновременно выявлять широкий спектр респираторных мишеней и расширяет возможности

эпидемиологической оценки в период сезонного подъёма заболеваемости.
Цель. Изучить распределение вирусных и бактериальных респираторных возбудителей среди пациентов с острыми респираторными инфекциями в Алматы в эпидемический сезон 2023–2024 гг. с использованием мультиплексной ПЦР в режиме реального времени, а также оценить возрастные особенности выявляемости и частоту множественных положительных результатов.
Материалы и методы. Респираторные образцы от 374 пациентов с клинически диагностированными острыми респираторными инфекциями, обследованных с ноября 2023 г. по январь 2024 г., анализировали с использованием мультиплексных ПЦР-тестов в режиме реального времени, включавших 30 респираторных мишеней — 19 вирусных и 11 бактериальных. Описательно оценивали частоту выявления возбудителей, возрастные особенности положительных результатов и комбинации одновременно выявленных ПЦР-мишеней.
Результаты. Вирусные мишени были выявлены у 195 из 374 пациентов (52,1%). Наиболее часто определялись риновирус (24,3%) и вирус гриппа А (14,7%). Бактериальные мишени выявлены у 240 пациентов (64,2%); наиболее часто обнаруживались *Haemophilus influenzae*, *Streptococcus pneumoniae* и *Moraxella catarrhalis*. Множественные положительные ПЦР-мишени зарегистрированы у 202 пациентов (54,0%): у 94 выявлены две, у 67 — три, у 41 — четыре и более мишени. Установлены также возрастные особенности выявляемости отдельных вирусных и бактериальных возбудителей.
Обсуждение. Полученные результаты свидетельствуют о гетерогенной структуре респираторных возбудителей в эпидемический сезон и высокой частоте одновременного выявления нескольких молекулярных мишеней. Вместе с тем выявление бактериальной ДНК методом ПЦР не позволяет однозначно отличить колонизацию от активной инфекции. Кроме того, одновременный положительный результат на общую мишень вируса гриппа А и его подтип не следует автоматически трактовать как наличие двух самостоятельных вирусных инфекций. Поэтому множественные положительные результаты корректнее интерпретировать как молекулярное совместное выявление возбудителей, а не как подтверждённую клиническую коинфекцию без дополнительных клинических и микробиологических данных.
Заключение. Мультиплексная ПЦР в режиме реального времени позволила комплексно охарактеризовать спектр респираторных возбудителей у пациентов с ОРИ в Алматы в сезон 2023–2024 гг. Среди вирусных мишеней преобладали риновирус и вирус гриппа А, среди бактериальных — *H. influenzae*, *S. pneumoniae* и *M. catarrhalis*. Результаты подтверждают потенциальную значимость мультиплексной молекулярной диагностики для эпидемиологического надзора и поддержки клинических решений при условии осторожной интерпретации бактериальных и множественных ПЦР-положительных результатов.
Ключевые слова: острые респираторные инфекции; мультиплексная ПЦР в режиме реального времени; респираторные возбудители; вирусные патогены; бактериальные патогены; совместное молекулярное выявление.

Introduction

Acute respiratory infections (ARIs) remain one of the leading causes of morbidity and mortality worldwide, particularly affecting vulnerable populations such as children, older adults, and individuals with chronic illnesses [1]. According to the World Health Organization (WHO), ARIs account for a substantial proportion of healthcare visits and hospitalizations during seasonal peaks, especially in winter [2]. Viral pathogens such as rhinoviruses, influenza viruses, and respiratory syncytial virus (RSV) are frequently detected in ARIs. Bacterial pathogens, including *Streptococcus pneumoniae* and *Staphylococcus aureus*, may also be identified as primary pathogens or in mixed detections [3]. Rapid and accurate identification of respiratory pathogens is important for clinical management and infection-control strategies [4].

In Kazakhstan, ARIs represent a persistent public health burden, with seasonal outbreaks placing considerable strain on healthcare systems [5]. Although ARIs are known to impose a substantial disease burden, regional epidemiological surveillance studies characterizing both viral and bacterial detections remain limited. The prevalence and clinical implications of simultaneous detection of multiple respiratory pathogens are also insufficiently characterized in the local context, despite evidence that clinically confirmed co-infections may be associated with increased disease severity and prolonged hospitalization [6, 7].

Conventional diagnostic approaches, including culture-based methods and serology, can be time-consuming and may have limited sensitivity for some pathogens [8]. Multiplex real-time polymerase chain reaction (PCR), in contrast, enables simultaneous detection of multiple respiratory targets within a short time frame. This technology has been widely used for clinical diagnostics and epidemiological

monitoring of respiratory pathogens, particularly during influenza and COVID-19 epidemics [9-15].

The 2023–2024 respiratory season in Kazakhstan was marked by increased ARI activity [5]. Despite the growing use of multiplex PCR in surveillance systems, regional data comprehensively describing circulating viral and bacterial respiratory targets and multi-pathogen detections during epidemic periods remain limited. Such data can support diagnostic strategies, antimicrobial stewardship, and public health responses [7].

This study aimed to analyze the distribution of viral and bacterial respiratory pathogens among patients with ARIs in Almaty during the 2023–2024 epidemic season using a multiplex real-time PCR panel capable of detecting 30 respiratory pathogens. Age-specific detection patterns and the frequency of simultaneous detection of multiple pathogens were also assessed.

Materials and Methods

Study design and setting. This descriptive laboratory-based study included patients with clinically diagnosed ARIs tested in Almaty, Kazakhstan, between November 2023 and January 2024. A total of 374 patients were included.

Laboratory testing. Respiratory specimens were tested at the OHKZ laboratory using Allplex™ Respiratory Panel 1–4 kits (Seegene, Republic of Korea) together with an OHKZ-developed assay for four additional bacterial targets. The combined testing scheme comprised 30 respiratory targets, described in the source manuscript as 19 viral and 11 bacterial targets (Table 1).

Variables and definitions. Outcomes included detection of at least one viral target, detection of at least one bacterial target, age-specific pathogen detection rates, and simultaneous detection of multiple targets. Multi-pathogen detections were categorized as two, three, or four or more detected targets per patient. Age categories were 0–1, 2–5, 6–10, 11–20, 21–30, 31–40, 41–50, 51–60, and ≥61 years, consistent with Figures 3 and 4.

Statistical analysis. Data were summarized descriptively using absolute counts and percentages. No inferential statistical testing is reported; therefore, between-month and between-age comparisons are interpreted descriptively rather than as statistically significant differences.

Reporting completeness. The source manuscript does not specify the respiratory specimen type(s), sampling and transport procedures, nucleic acid extraction method, PCR instrument, Ct cut-off/interpretation criteria, positive, negative and internal controls, or detailed quality-control procedures. These parameters were not reconstructed and should be supplied from the laboratory protocol by the authors before submission to ensure reproducibility.

Table 1 - Respiratory pathogens targeted by Allplex™ Respiratory Panel Kits (Seegene, Republic of Korea)

Reagents	Target category	Target pathogen	Abbreviations
AllplexTM Respiratory Panel 1 (Seegene, South Korea)	7 Viruses, including variants of Influenza A	Respiratory Syncytial Virus A Respiratory Syncytial Virus B Influenza A virus Influenza A (H1) Influenza A (H1N1 pdm09) Influenza A (H3) Influenza B virus	RSV A RSV B Flu A Flu A (H1) Flu A (H1N1) Flu A (H3) Flu B
AllplexTM Respiratory Panel 2 (Seegene, South Korea)	7 Viruses	Human Adenovirus Human Enterovirus Parainfluenza virus 1 Parainfluenza virus 2 Parainfluenza virus 3 Parainfluenza virus 4 Metapneumovirus	HADV HEV PIV 1 PIV 2 PIV 3 PIV 4 MNV
AllplexTM Respiratory Panel 3	5 Viruses Including 3 Variants of Coronavirus	Bocavirus Rhinovirus	BoV RhV

Reagents	Target category	Target pathogen	Abbreviations
(Seegene, South Korea)		Coronavirus NL63 Coronavirus 229E Coronavirus OC43	CoV NL63 CoV 229E CoV OC43
AllplexTM Respiratory Panel 4 (Seegene, South Korea)	7 Bacteria	*Streptococcus pneumoniae* *Mycoplasma pneumoniae* *Haemophilus influenzae* *Legionella pneumophila* *Chlamydophila pneumoniae* *Bordetella pertussis* *Bordetella parapertussis*	*S. pneumoniae* *M. pneumoniae* *H. influenzae* *L. pneumophila* *C. pneumoniae* *B. pertussis* *B. parapertussis*
Respiratory Bacteria, 4 types (The test was developed in the OHKZ Laboratory)	4 Bacteria	*Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Moraxella catarrhalis*	*K. pneumoniae* *P. aeruginosa* *S. aureus* *M. catarrhalis*

Results

I. Respiratory viral detections

Among 374 examined patients, at least one viral pathogen was detected in 195 (52.1%). The most frequently detected viruses were rhinovirus (24.3%), influenza A virus (14.7%), and adenovirus (5.9%). In December 2023, relatively high detection rates were observed for influenza A virus and respiratory syncytial virus A (RSV A), whereas in January 2024 the detection rates of rhinovirus, adenovirus, and RSV A increased (Figure 1).

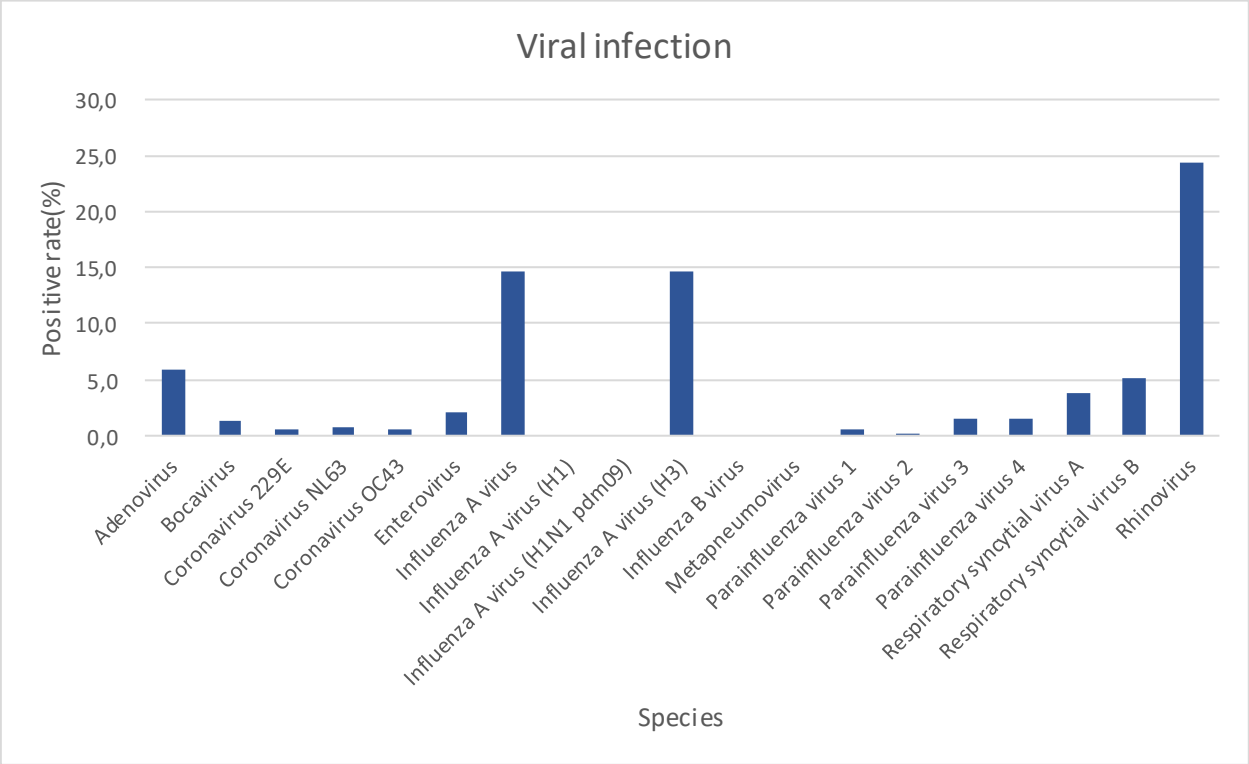


Figure 1 - Detection rates of respiratory viruses among ARI patients in Almaty, Kazakhstan, from November 2023 to January 2024 using multiplex real-time PCR

II. Respiratory bacterial detections

Respiratory bacterial targets were detected in 240 of 374 patients (64.2%). The most common bacterial detections were *Haemophilus influenzae* (32.9%), *Streptococcus pneumoniae* (24.9%), *Moraxella catarrhalis* (23.8%), *Staphylococcus aureus* (12.8%), *Klebsiella pneumoniae* (3.5%), and *Pseudomonas aeruginosa* (1.6%). *Mycoplasma pneumoniae* was not detected. *Bordetella pertussis* was detected in 2.4% of cases (Figure 2).

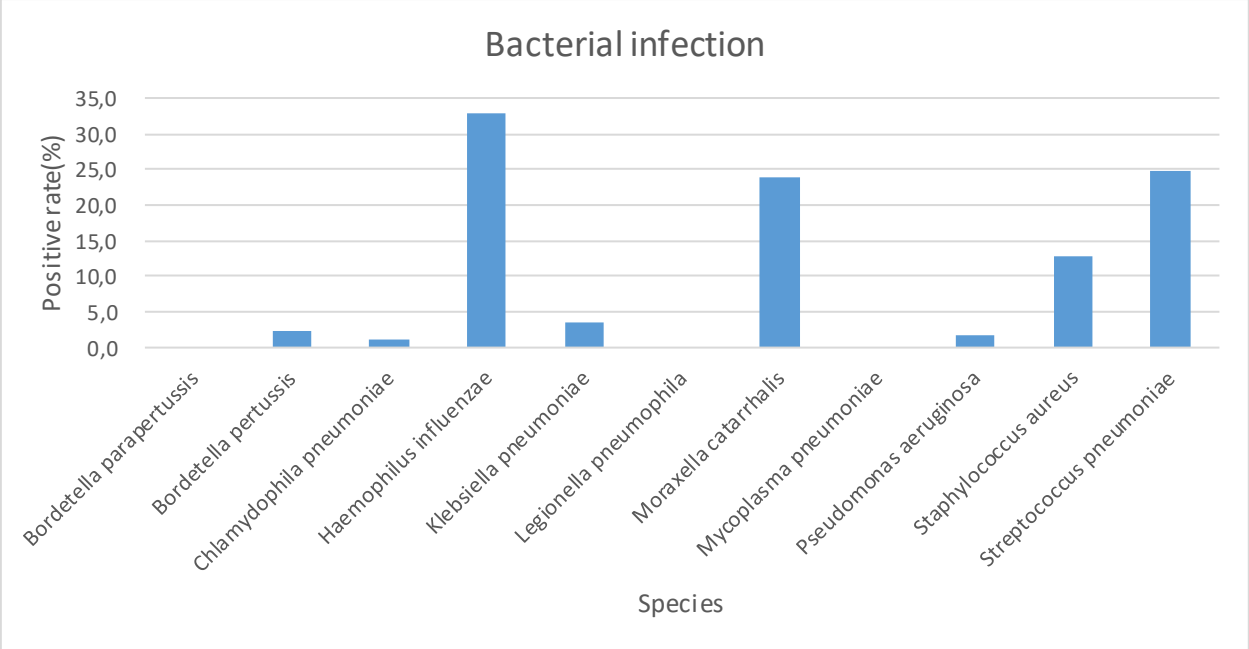


Figure 2 - Detection rates of respiratory bacterial pathogens among ARI patients in Almaty, Kazakhstan, from November 2023 to January 2024.

III. Positivity rates of respiratory pathogens by age

Positivity rates of respiratory viruses by age

Age-specific viral detection rates were evaluated descriptively. Most viral targets were detected more frequently in children younger than 5 years than in adults, with the exception of influenza A virus. In the 0–1-year age category, adenovirus (14.3%), RSV A (11.4%), and RSV B (14.3%) showed higher positivity rates than in several older age categories (Figure 3). The overall positivity rate for influenza A virus was 14.7%, with the highest observed rate in the 31–40-year age group; among patients aged ≥61 years, the rate was 15.9%. In children aged ≤10 years, the average positivity rate for rhinovirus was 35.2%, the highest among the reported viral detections (Figure 3).

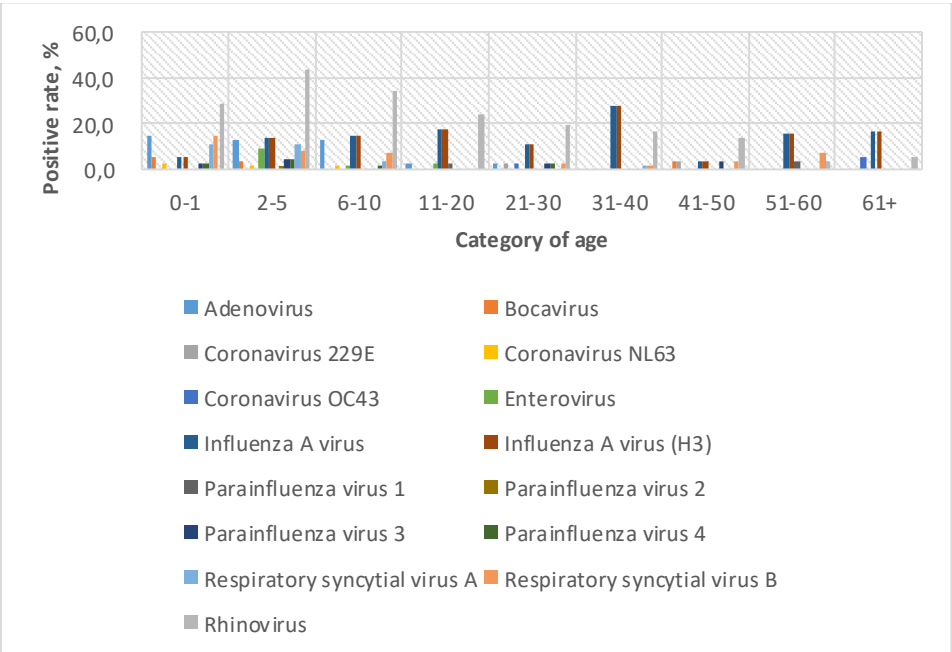


Figure 3 - Positivity rates of respiratory viruses by age

Positivity rates of respiratory bacteria by age

In children younger than 10 years, *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were among the most frequently detected bacterial targets, whereas *H. influenzae* and *S. aureus* predominated among adults. *B. pertussis* was detected across several age categories (Figure 4).

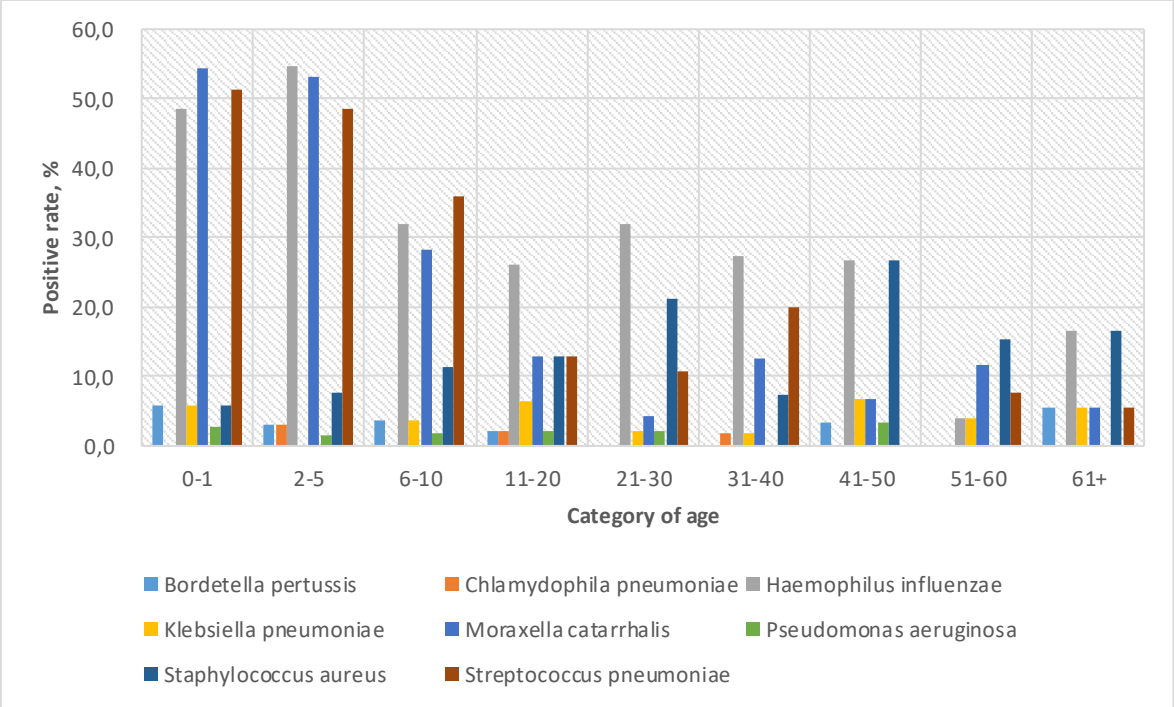


Figure 4 - Positivity rates of respiratory bacteria by age

IV. Multiple respiratory pathogen detections

Among the 374 patients tested, a single viral target was detected in 33 patients (8.8%), a single bacterial target in 63 patients (16.8%), and no investigated target was detected in 76 patients (20.3%). Multiple positive assay targets were recorded in 202 patients (54.0%). Among the 202 patients with multiple positive targets, two targets were detected in 94 patients (46.5%), three in 67 patients (33.2%), and four or more in 41 patients (20.3%) (Figure 5).

Among the bacterial targets represented in the multiple-detection profiles, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Klebsiella pneumoniae* were among the most frequently detected (Table 2).

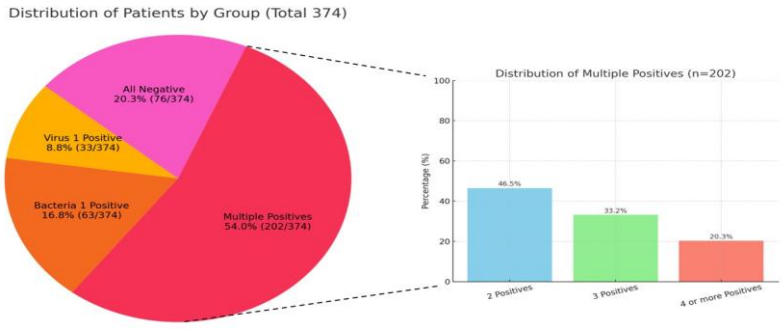


Figure 5 - Distribution of single and multiple respiratory pathogen detections among ARI patients

Table 2 - Combinations of multiple respiratory pathogen targets detected by multiplex real-time PCR

Multiple detection (2 targets)			Multiple detection (3 targets)		
Category	**Pathogens**	**N. of case**	**Category**	**Pathogens**	**N. of case**
Bacteria +Bacteria	*H.influenzae*+*S.pneumoniae*	8	Bacteria only	*H.influenzae*+*M.catarrhalis*+*S.pneumoniae*	6
	H.influenzae+*M.catarrhalis*	6		*H.influenzae*+*K.pneumoniae*+*S.aureus*	2
	M.catarrhalis+*S.pneumoniae*	5		*M.catarrhalis*+*S.aureus*+*S.pneumoniae*	1
	K.pneumoniae+*S.aureus*	3	Mixed (Virus +Bacteria)	*H.influenzae*+S.pneumoniae+Rhinovirus	8
	H.influenzae+*S.aureus*	2		*M.catarrhalis*+Influenza A virus+Influenza A virus H3	7
	S.aureus+*S.pneumoniae*	2		*H.influenzae*+Influenza A virus+Influenza A virus H3	5
	B.pertussis+*H.influenzae*	1		*H.influenzae*+*M.catarrhalis*+Adenovirus	3
	B.pertussis+*S.aureus*	1		*H.influenzae*+*M.catarrhalis*+Rhinovirus	3
	H.influenzae+*K.pneumoniae*	1		*S.pneumoniae*+Influenza A virus+Influenza A virus H3	3
	H.influenzae+*P.aeruginosa*	1		*H.influenzae*+*M.catarrhalis*+Respiratory syncytial virus A	2
	K.pneumoniae+*M.catarrhalis*	1		*H.influenzae*+*S.pneumoniae*+Respiratory syncytial virus B	2
Virus +Bacteria	*M.catarrhalis*+*Rhinovirus*	8		*H.influenzae*+Adenovirus+Rhinovirus	2
	H.influenzae+*Rhinovirus*	6		*M.catarrhalis*+S.pneumoniae+Rhinovirus	2
	S.pneumoniae+*Rhinovirus*	4		*S.aureus*+*S.pneumoniae*+Rhinovirus	2
	M.catarrhalis+Respiratory syncytial virus B	3		*S.aureus*+Influenza A virus+Influenza A virus H3	2
	H.influenzae+Respiratory syncytial virus A	2		*H.influenzae*+*S.aureus*+Respiratory syncytial virus B	1
	H.influenzae+Respiratory syncytial virus B	2		*H.influenzae*+*S.aureus*+Rhinovirus	1
	M.catarrhalis+Adenovirus	2		*H.influenzae*+*S.pneumoniae*+Adenovirus	1
	S.pneumoniae+Respiratory syncytial virus A	2		*H.influenzae*+*S.pneumoniae*+Parainfluenza virus 4	1
	S.pneumoniae+Respiratory syncytial virus B	2		*H.influenzae*+*S.pneumoniae*+Respiratory syncytial virus A	1
	B.pertussis+Adenovirus	1		*H.influenzae*+Bocavirus+Rhinovirus	1
	B.pertussis+Coronavirus 229E	1		*M.catarrhalis*+*S.aureus*+Rhinovirus	1
	B.pertussis+Rhinovirus	1		*M.catarrhalis*+*S.pneumoniae*+Coronavirus NL63	1
	C.pneumoniae+Adenovirus	1		*M.catarrhalis*+*S.pneumoniae*+Respiratory syncytial virus A	1
	H.influenzae+Adenovirus	1		*S.aureus*+*S.pneumoniae*+Parainfluenza virus 4	1
	H.influenzae+Parainfluenza virus 2	1		*S.aureus*+*S.pneumoniae*+Respiratory syncytial virus B	1
	H.influenzae+Parainfluenza virus 3	1		*S.aureus*+Enterovirus+Parainfluenza virus 4	1

	K.pneumoniae+Respiratory syncytial virus B	1		*S.pneumoniae*+Enterovirus+Rhinovirus	1
	M.catarrhalis+Bocavirus	1	Virus only	Influenza A virus+Influenza A virus H3+Rhinovirus	3
	M.catarrhalis+Enterovirus	1		Influenza A virus+Influenza A virus H3 virus 3 +Parainfluenza	1
	P.aeruginosa+Rhinovirus	1			
	S.aureus+Parainfluenza virus 1	1			
	S.aureus+Rhinovirus	1			
Virus+Virus	Influenza A virus+Influenza A virus H3	18			
	Coronavirus NL63+Parainfluenza virus 4	1			
	Total	94		**Total**	67

Multiple detection (4 or more targets)

Pathogens	**N. of case**
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Rhinovirus	4
M.catarrhalis+*S.pneumoniae*+Influenza A virus+Influenza A virus H3	2
B. pertussis+*H.influenzae*+*M. catarrhalis*+*S.pneumoniae*+Adenovirus+Rhinovirus	1
B.pertussis+*H.influenzae*+*M.catarrhalis*+*S.pneumoniae*+Respiratory syncytial virus B	1
B.pertussis+*H.influenzae*+*M.catarrhalis*+*S.pneumoniae*+Rhinovirus	1
B.pertussis+*H.influenzae*+*S.pneumoniae*+Adenovirus	1
C.pneumoniae+*H.influenzae*+*M. catarrhalis*+*S.pneumoniae*+Adenovirus+Respiratory syncytial virus A+Rhinovirus	1
H.influenzae+*K.pneumoniae*+*S.aureus*+Influenza A virus+Influenza A virus H3	1
H.influenzae+*M.catarrhalis*+*P.aeruginosa*+Adenovirus+Rhinovirus	1
H.influenzae+*M.catarrhalis*+*S.aureus*+*S.pneumoniae*+Influenza A virus+Influenza A virus H3	1
H.influenzae+*M.catarrhalis*+*S.aureus*+*S.pneumoniae*+Rhinovirus	1
H.influenzae+*M.catarrhalis*+*S.aureus*+Influenza A virus+Influenza A virus H3+Rhinovirus	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Adenovirus+Respiratory syncytial virus A	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Bocavirus	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Enterovirus	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Parainfluenza virus 3+Rhinovirus	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Parainfluenza virus 4	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Respiratory syncytial virus B	1
H.influenzae+*M.catarrhalis*+*S.pneumoniae*+Respiratory syncytial virus B+Rhinovirus	1
H.influenzae+*M.catarrhalis*+Enterovirus+Influenza A virus+Influenza A virus H3+Rhinovirus	1
H.influenzae+*M.catarrhalis*+Enterovirus+Parainfluenza virus 3+Rhinovirus	1
H.influenzae+*M.catarrhalis*+Influenza A virus+Influenza A virus H3	1

H.influenzae+*P.aeruginosa*+*S.pneumoniae*+Influenza A virus+Influenza A virus H3	1
H.influenzae+*S.aureus*+Influenza A virus+Influenza A virus H3	1
H.influenzae+*S.pneumoniae*+Adenovirus+Respiratory syncytial virus A+Rhinovirus	1
H.influenzae+*S.pneumoniae*+Influenza A virus+Influenza A virus H3	1
H.influenzae+*S.pneumoniae*+Influenza A virus+Influenza A virus H3+Parainfluenza virus 3	1
H.influenzae+*S.pneumoniae*+Influenza A virus+Influenza A virus H3+Rhinovirus	1
H.influenzae+Adenovirus+Bocavirus+Respiratory syncytial virus B+Rhinovirus	1
H.influenzae+Influenza A virus+Influenza A virus H3+Rhinovirus	1
K.pneumoniae+*S.aureus*+Influenza A virus+Influenza A virus H3	1
M.catarrhalis+*S.pneumoniae*+Adenovirus+Respiratory syncytial virus B	1
M.catarrhalis+*S.pneumoniae*+Influenza A virus+Influenza A virus H3+Rhinovirus	1
M.catarrhalis+Adenovirus+Respiratory syncytial virus A+Rhinovirus	1
S.aureus+*S.pneumoniae*+Enterovirus+Respiratory syncytial virus A+Rhinovirus	1
S.pneumoniae+Adenovirus+Coronavirus NL63+Rhinovirus	1
Adenovirus+Bocavirus+Influenza A virus+Influenza A virus H3+Respiratory syncytial virus A	1
Total	41

Discussion

This study describes the distribution of respiratory pathogen detections during the 2023–2024 epidemic season in Almaty, Kazakhstan, using multiplex real-time PCR. Among 374 patients with ARIs, viral targets were detected in 52.1%, with rhinovirus (24.3%) and influenza A virus (14.7%) the most common. Bacterial targets were detected in 64.2%, most often *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis*. Multiple targets were reported in 54.0% of patients, indicating a high frequency of mixed molecular detections in the study sample.

These findings are broadly consistent with international evidence showing that rhinovirus, RSV, influenza viruses, and major bacterial respiratory pathogens contribute substantially to the burden of acute and lower respiratory infections [1]. The relative frequency of individual pathogens, however, varies by age, clinical setting, geography, season, sampling strategy, and diagnostic platform.

In a multicenter study of adults hospitalized with community-acquired pneumonia in the United States, rhinovirus, influenza virus, and RSV were among the most common viral detections, while *S. pneumoniae* and *H. influenzae* were also identified [3]. Jiang et al. demonstrated the feasibility of a three-tube multiplex real-time PCR assay for simultaneous detection of nine microorganisms causing ARIs, supporting the broader diagnostic principle of multiplex molecular testing [15]. In an Italian hospital series, rhinovirus/enterovirus, RSV, and influenza viruses were among the leading respiratory viral detections, with age-related differences and co-detections particularly relevant in pediatric samples [16]. Longitudinal surveillance in Moscow likewise documented persistent seasonal circulation of influenza viruses, RSV, rhinovirus, and other respiratory viruses [17].

In Kazakhstan, the present findings are broadly aligned with previous observations. A metagenomic study of adult ARI patients identified human rhinovirus (16.3%), betaherpesvirus 7 (14.3%), and Epstein-Barr virus (8.2%) among prevalent viral agents, with *Streptococcus spp.*, *Pseudomonas aeruginosa*, and *Burkholderia spp.* among bacterial

contributors [18]. A media report citing national sanitary-epidemiological surveillance data for the same epidemic season indicated that rhinovirus accounted for the largest proportion of non-influenza respiratory virus detections, followed by RSV, adenovirus, coronavirus, parainfluenza virus, bocavirus, and metapneumovirus [5]. These observations are directionally consistent with the prominence of rhinovirus and RSV in the present dataset, although differences in population, sampling, and testing methods limit direct comparison.

Taken together, the results underscore the complexity of respiratory pathogen detection in ARIs and the presence of age-specific patterns. Multiplex PCR provides rapid, broad detection and may support diagnostic decision-making and antimicrobial stewardship when interpreted together with clinical findings. Prior studies have associated clinically confirmed viral-bacterial co-infections with greater disease severity and worse outcomes in selected pediatric and adult hospitalized populations [19, 20]. However, the present study did not collect clinical outcome data and therefore cannot establish an association between multi-target detection and disease severity. In addition, bacterial PCR positivity may reflect colonization rather than active infection; detection alone should not be used as a stand-alone indication for antibiotic therapy [21].

Evidence from interventional studies also supports cautious interpretation of molecular diagnostics. In a randomized trial of hospitalized patients with community-acquired pneumonia, adding multiplex real-time PCR to conventional microbiological testing increased etiological detection but did not produce a statistically significant reduction in total antibiotic days; the authors did not support routine implementation of the strategy as an initial test for all hospitalized CAP patients [21]. Thus, the value of multiplex PCR is greatest when results are integrated with clinical assessment, sample quality, and antimicrobial stewardship rather than interpreted in isolation.

Limitations of the study

This study has several limitations. First, it was conducted in a single city over a three-month epidemic-season period, which limits the generalizability of the findings to other regions of Kazakhstan and to other respiratory seasons. Second, clinical outcome data were not available; therefore, associations between detected pathogens, multiple detections, and disease severity could not be assessed. Third, detection of bacterial DNA by PCR does not distinguish colonization from active bacterial infection, particularly for organisms that may colonize the upper respiratory tract. Fourth, the multiplex panel contains both generic influenza A and subtype-specific targets. Consequently, simultaneous positivity for these assay targets may represent a single influenza A infection rather than infection with two independent viruses, and target-level counting may overestimate the number of distinct pathogens in some multi-positive profiles. Finally, the analysis was descriptive and did not include inferential or multivariable statistical testing. These limitations should be considered when interpreting the reported positivity and multiple-detection rates.

Conclusion

Multiplex real-time PCR provided broad characterization of respiratory pathogens detected among patients with acute respiratory infections in Almaty during the 2023–2024 season. Rhinovirus and influenza A virus predominated among viral detections, whereas *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were the most frequently detected bacterial targets. Multiple assay targets were reported in more than half of patients, but these findings should be interpreted as molecular co-detection rather than confirmed clinical co-infection without additional clinical and microbiological evidence. The results support the potential value of multiplex molecular diagnostics for respiratory surveillance and clinical decision support, while emphasizing cautious interpretation of bacterial and multiple-target PCR positivity.

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DECLARATIONS / ДЕКЛАРАЦИИ / ДЕКЛАРАЦИЯЛАР

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.
Финансирование. Внешнее финансирование исследования не заявлено.
Этическое одобрение и информированное согласие. Исследование основывалось на анализе данных, полученных в рамках рутинной лабораторной диагностики, и не предусматривало изменения стандартного объема медицинской помощи или дополнительных вмешательств в отношении пациентов. В соответствии с применимыми локальными требованиями отдельное одобрение этического комитета и получение дополнительного информированного согласия для данного вида анализа не требовались.
Доступность данных. В рукописи представлены агрегированные результаты исследования. Условия доступа к исходному обезличенному набору индивидуальных данных в исходной версии не указаны и должны быть уточнены автором для корреспонденции до подачи статьи.
Вклад авторов. Концептуализация - Heesuk Min; методология - Yongha Kim; валидация - Yoonkyung Choi; формальный анализ - Seiick Joo, Умирбекова Лаззат Жаксылыковна; проведение исследования - Yongha Kim, Yoonkyung Choi, Абирова Жазира Мейрамовна, Утаганов Бахыт Кустаевич; ресурсы - Seehyen Ham; курирование данных - Seiick Joo, Амина Мурадалиева; написание первоначального варианта рукописи - Салима Мамутбаева, Minjoong Jang; рецензирование и редактирование рукописи - Mincheol Lee, Minjoong Jang; визуализация - Амина Мурадалиева, Seehyen Ham; научное руководство - Mincheol Lee; администрирование проекта - Geonsang Park. Все авторы ознакомились с окончательной версией рукописи и одобрили ее.

Мүдделер қақтығысы. Авторлар мүдделер қақтығысының жоқ екенін мәлімдейді.
Қаржыландыру. Зерттеуге сыртқы қаржыландыру көрсетілмеген.
Этикалық мақұлдау және ақпараттандырылған келісім. Зерттеу күнделікті зертханалық диагностика барысында алынған деректерді талдауға негізделді және пациенттерге көрсетілетін стандартты медициналық көмектің көлемін өзгертуді немесе зерттеу мақсатында қосымша араласулар жүргізуді көздеген жоқ. Қолданыстағы жергілікті талаптарға сәйкес, мұндай талдау үшін этикалық комитеттің жеке мақұлдауы және қосымша ақпараттандырылған келісім алу талап етілмеді.
Деректердің қолжетімділігі. Қолжазбада зерттеудің жинақталған нәтижелері ұсынылған. Бастапқы дербестендірілмеген жеке деңгейдегі деректерге қол жеткізу шарттары бастапқы қолжазбада көрсетілмеген және мақала жіберілгенге дейін хат-хабарға жауапты автор тарапынан нақтылануы тиіс.

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Data availability. The manuscript presents aggregated study results. The conditions for access to the underlying de-identified individual-level dataset were not specified in the source manuscript and should be clarified by the corresponding author before submission.
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Сведения об авторах:
Мамутбаева Салима - главный микробиолог, лабораторное подразделение, Openhealthcare KZ, Алматы, Республика Казахстан.
Mincheol Lee - генеральный директор, Openhealthcare, Inc., Сеул, Республика Корея.
Geonsang Park - директор по информационным технологиям и клиническим вопросам, медицинское подразделение, Openhealthcare, Inc., Сеул, Республика Корея.
Heesuk Min - директор медицинского подразделения, Openhealthcare KZ, Алматы, Республика Казахстан.
Minjoong Jang - управляющий директор по клинико-лабораторным услугам, Openhealthcare, Inc., Сеул, Республика Корея.
ORCID: 0009-0008-0130-3499.
E-mail: michaeljmj@naver.com.
Автор для корреспонденции.
Seiick Joo - генеральный менеджер отдела планирования тестирования, Openhealthcare, Inc., Сеул, Республика Корея.
Yongha Kim - директор лабораторного подразделения, Openhealthcare KZ, Алматы, Республика Казахстан.
Seehyen Ham - генеральный менеджер отдела медицинской поддержки, Openhealthcare KZ, Алматы, Республика Казахстан.
Yoonkyung Choi - генеральный менеджер отдела лабораторного управления, Openhealthcare KZ, Алматы, Республика Казахстан.

Амина Мурадалиева - переводчик, лабораторное подразделение, Openhealthcare KZ, Алматы, Республика Казахстан.
Абирова Жазира Мейрамовна - заместитель директора по лечебной работе, Городская поликлиника № 26, Алматы, Республика Казахстан.
Утаганов Бахыт Кустаевич - заместитель директора по медицинским вопросам, Городская больница скорой медицинской помощи, Алматы, Республика Казахстан.
Умирбекова Лаззат Жаксылыковна - заведующая лабораторией, врач, Алматы, Республика Казахстан.

Авторлар туралы мәліметтер:
Мамутбаева Салима - бас микробиолог, зертхана бөлімі, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Mincheol Lee - бас атқарушы директор, Openhealthcare, Inc., Сеул, Корея Республикасы.
Geonsang Park - ақпараттық технологиялар және клиникалық мәселелер жөніндегі директор, медициналық бөлім, Openhealthcare, Inc., Сеул, Корея Республикасы.
Heesuk Min - медициналық бөлімнің директоры, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Minjoong Jang - клиникалық-зертханалық қызметтер жөніндегі басқарушы директор, Openhealthcare, Inc., Сеул, Корея Республикасы.
ORCID: 0009-0008-0130-3499.
E-mail: michaeljmj@naver.com.
Хат-хабарға жауапты автор.
Seiick Joo - тестілеуді жоспарлау бөлімінің бас менеджері, Openhealthcare, Inc., Сеул, Корея Республикасы.
Yongha Kim - зертхана бөлімінің директоры, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Seehyen Ham - медициналық қолдау бөлімінің бас менеджері, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Yoonkyung Choi - зертхананы басқару бөлімінің бас менеджері, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Амина Мурадалиева - аудармашы, зертхана бөлімі, Openhealthcare KZ, Алматы, Қазақстан Республикасы.
Абирова Жазира Мейрамовна - емдеу жұмысы жөніндегі директордың орынбасары, № 26 қалалық емхана, Алматы, Қазақстан Республикасы.
Утаганов Бахыт Кустаевич - медициналық мәселелер жөніндегі директордың орынбасары, Қалалық жедел медициналық жәрдем ауруханасы, Алматы, Қазақстан Республикасы.
Умирбекова Лаззат Жаксылыковна - зертхана меңгерушісі, дәрігер, Алматы, Қазақстан Республикасы.

Information about the Authors:
Salima Mamutbayeva - Main Microbiologist, Laboratory Division, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Mincheol Lee - Chief Executive Officer, Openhealthcare, Inc., Seoul, Republic of Korea.
Geonsang Park - Chief Information and Clinical Officer, Medical Division, Openhealthcare, Inc., Seoul, Republic of Korea.
Heesuk Min - Medical Division Director, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Minjoong Jang - Managing Director, Clinical Laboratory Services, Openhealthcare, Inc., Seoul, Republic of Korea.
ORCID: 0009-0008-0130-3499.E-mail: michaeljmj@naver.com.

Corresponding author.
Seiick Joo - General Manager, Testing Planning Office, Openhealthcare, Inc., Seoul, Republic of Korea.
Yongha Kim - Laboratory Division Director, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Seehyen Ham - General Manager, Medical Support Department, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Yoonkyung Choi - General Manager, Lab Management Department, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Amina Muradalieva - Interpreter, Laboratory Division, Openhealthcare KZ, Almaty, Republic of Kazakhstan.
Zhazira Meyramovna Abiyrova - Deputy Director for Medical Work, City Polyclinic No. 26, Almaty, Republic of Kazakhstan.
Bakhyt Kustaevich Utaganov - Deputy Director for Medical Affairs, City Emergency Hospital, Almaty, Republic of Kazakhstan.
Lazzat Zhaksylykovna Umirbekova - Head of Laboratory, Physician, Almaty, Republic of Kazakhstan.