OSONG PUBLIC HEALTH
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Vol. 17, No. 3, June 2026

Pages 193-285

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OSONG PUBLIC HEALTH
AND RESEARCH PERSPECTIVES

Vol. 17, No. 3, June 2026

pISSN 2210-9099
eISSN 2233-6052

Vol. 17, No. 3, June 2026

Korea Disease Control and
Prevention Agency

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

https://ophrp.org

pISSN 2210-9099
eISSN 2233-6052

Vol. 17, No. 3, June 2026

Published on 30 June 2026

Aims & Scope

Osong Public Health and Research Perspectives (PHRP) is the international bimonthly (published at the end of February, April, June, August, October, and December) journal founded in 2010 by the Korea Disease Control and Prevention Agency (KDCA). With the mission of the KDCA, to create a disease-free world, PHRP encourages sharing medical information and knowledge in the areas of public health.
PHRP publishes original articles, review articles, guidelines, data profiles (including cohort profiles), special articles, short communications, viewpoints, editorials, commentaries, and correspondence, and book reviews, with a focus on the following areas of expertise: emerging infectious diseases, vaccinology, zoonotic diseases, non-communicable diseases, intractable and rare diseases, and human genomics.

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Publisher: The Korea Disease Control and Prevention Agency

Editor-in-Chief: Jong-Koo Lee, MD, PhD

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♾ This paper meets the requirements of KS X ISO 9706, ISO 9706-1994 & ANSI/NISO Z39.48-1992 (Permanence of Paper).

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

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https://ophrp.org
pISSN 2210-9099 eISSN 2233-6052

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OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2026.0475
Osong Public Health Res Perspect 2026;17(3):193-195

Editorial

Learning from the past, moving toward Health for All: reflections on WHA79 and emerging priorities in global public health

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This year marks the 20th anniversary of the death of Dr. Lee Jong-wook, former Director-General of the World Health Organization (WHO), who died shortly before the World Health Assembly in 2006. During the Seventy-ninth World Health Assembly (WHA79), the Government of the Republic of Korea organized commemorative events honoring his legacy. I have long believed that the 3 principles Dr. Lee articulated in his inaugural address to WHO staff on July 21, 2003—"We must do the right things, we must do them in the right places, and we must do them the right way"—should continue to guide public health practice [1].

Several decisions and resolutions adopted at WHA79 deserve particular attention in light of this journal's editorial scope [2]. Although the WHO Pandemic Agreement was adopted last year, negotiations on the Pathogen Access and Benefit-Sharing Annex—one of its key components—had not reached a final agreement after 6 rounds of formal meetings in 2025–2026. Discussions will therefore continue, and the outcome is expected to be submitted to the Eightieth World Health Assembly or to a special session. Meanwhile, implementation of the International Health Regulations (IHR) after coronavirus disease 2019 (COVID-19) is shifting from emergency response toward a broader framework emphasizing preparedness, resilience, equity, and whole-of-society approaches. This shift underscores the growing interdependence of health security, community engagement, and strengthened health systems [3]. WHA79 also reaffirmed antimicrobial resistance (AMR) as a major global health priority by endorsing the AMR Global Action Plan 2026–2036 and strengthening the Global Antimicrobial Resistance Surveillance System. AMR is increasingly understood as a long-term threat whose impact may rival or exceed that of many infectious diseases. WHO also formally incorporated precision medicine into the global health agenda by adopting a resolution that recognized the importance of personalized and targeted therapies in the era of artificial intelligence, genomics, and multi-omics technologies, while emphasizing equitable access to these innovations. The Assembly also highlighted growing concerns about global health workforce shortages and the international migration of health personnel. The WHO Global Code of Practice on the International Recruitment of Health Personnel was reaffirmed in response to post-COVID-19 workforce shortages and brain drain, with implications for regional disparities in health workforce distribution in the Republic of Korea. Another important resolution addressed stroke prevention and health system readiness. Beyond acute stroke management,

Received: June 22, 2026
Accepted: June 24, 2026

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the resolution emphasized rehabilitation and preparedness across the continuum of care. This is relevant to the Republic of Korea, where emergency referral gaps, regional disparities, and delayed access to care have become increasingly evident. The decision to strengthen pharmacovigilance and establish smarter, more efficient medicine safety systems is also noteworthy. For countries such as the Republic of Korea, which continue to address issues related to large-scale COVID-19 vaccination programs, adverse events, and compensation mechanisms, the resolution underscores the need to invest in digital technologies and artificial intelligence for medicine safety surveillance.

The international health landscape continues to change. In 2026, the Public Health Emergency of International Concern for poliomyelitis remained in effect, while Ebola outbreaks caused by Bundibugyo virus in the Democratic Republic of the Congo and Uganda again demonstrated the persistent threat of emerging infectious diseases. These events reaffirm that community-based surveillance, risk communication, and rapid mobilization of field personnel remain central to global health security.

The articles in this issue reflect these priorities. The review on climate change and older adults emphasizes that climate change is a public health issue. Older adults are particularly vulnerable to heat-related illness, infectious diseases, and mental health effects associated with climate change. The review supports WHO priorities on healthy aging and resilience and has implications for the Republic of Korea's expanding community-based integrated care initiatives.

Although malaria elimination remains achievable, recent progress has slowed, and gains have proven fragile in several settings. Population displacement, emerging resistance, and weaknesses in surveillance continue to threaten elimination efforts. In this context, the review on the genetic diversity of *Plasmodium* species in Southeast Asia demonstrates the increasing importance of molecular surveillance during the elimination phase.

The article on community-based surveillance in Malaysia provides practical evidence supporting community engagement, which has gained renewed attention after the Pandemic Agreement and the revised IHR. The study on scrub typhus in Busan, the Republic of Korea, demonstrates how climate and environmental changes are altering the distribution of vector-borne diseases and underscores the need for locally tailored prevention strategies.

Two papers in this issue address AMR, one of the major themes discussed at WHA79. The Indonesian study on the tolC efflux pump provides molecular insights into multidrug resistance mechanisms in *Klebsiella pneumoniae*, while the Malaysian study on carbapenem-resistant *K. pneumoniae* demonstrates the value of genomic epidemiology and One Health approaches in controlling hospital-associated transmission.

The article on EQ-5D measurement invariance in the Korea Health Panel confirms the instrument's long-term stability and provides a valuable foundation for evaluating healthy aging, health equity, and integrated care policies. The review on personalized medicine for autoimmune diseases illustrates the growing convergence of public health and precision medicine in the era of artificial intelligence and multi-omics technologies.

Finally, the Perspective article on integrating Ayush practitioners into outbreak investigations in India addresses the growing challenges of health workforce shortages and community engagement. Although the proposal is consistent with WHO's emphasis on whole-of-society approaches, outbreak investigations require specialized epidemiological competencies, and further evidence is needed to determine whether such integration improves outbreak detection and response.

The articles in this issue reflect several emerging priorities in global public health, including climate resilience, elimination-era surveillance, community engagement, AMR, healthy aging, health system strengthening, and precision medicine. As the discussions at WHA79 highlighted, resilient health systems require sustained investment in preparedness, equity, workforce development, community engagement, and innovative technologies.

Twenty years after Dr. Lee Jong-wook's death, the inscription on his memorial stone offers a timely reminder in the post-COVID-19 era: "Learning from the past, we can change the future of global public health and continue moving forward toward the goal of Health for All" [4]. His vision continues to guide efforts to build resilient, equitable, people-centered health systems.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
Jong-Koo Lee has been the editor-in-chief of *Osong Public Health and Research Perspectives* since October 2021.

Funding
None.

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https://doi.org/10.24171/j.phrp.2026.0475

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0324
Osong Public Health Res Perspect 2026;17(3):196-205

Review Article

Climate change and older adults: mapping health impacts and intervention strategies: a scoping review

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ABSTRACT

Objectives: This scoping review aimed to examine the impact of climate change on the health of older adults and to evaluate existing interventions targeting this population.

Methods: The review followed Arksey and O'Malley's framework and the PRISMA-ScR guidelines. A comprehensive search was conducted across PubMed, Google Scholar, Scopus, and Web of Science. Eligible studies included those focusing on adults aged 60 years and older that examined the physical and mental health impacts of climate change across any geographic setting or level of care. Descriptive and thematic analyses were performed to identify key findings and knowledge gaps.

Results: Climate change adversely affects the physical and mental health of older adults through increased exposure to infectious diseases, extreme temperatures, and poor air and water quality. Older adults are particularly vulnerable to heat-related illnesses, cardiovascular events, and respiratory conditions, with women and individuals with comorbidities at higher risk. Climate change is also associated with increased anxiety, insomnia, and other mental health concerns in this population. Social support and targeted interventions—such as community awareness programs and subsidized cooling costs—are associated with reduced risk. Community-based initiatives have demonstrated potential in reducing mortality and enhancing resilience among older adults during extreme weather events.

Conclusion: Healthcare professionals should be educated about climate-related health outcomes affecting older adults. Protecting this vulnerable population requires urgent, inclusive, and targeted strategies, including education, improved healthcare access, and tailored interventions.

Keywords: Aging; Climate change; Environmental exposure; Vulnerable population

Received: August 18, 2025
Revised: December 8, 2025
Accepted: February 10, 2026

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Introduction

Climate change refers to persistent shifts in the Earth's typical weather patterns, including long-term changes in temperature, precipitation, and other climatic variables occurring over

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extended periods [1]. It is widely recognized as a major global challenge that poses significant threats to public health. Over the past 4 decades, global warming has emerged as a principal driver of climate change [2]. The World Health Organization estimates that between 2030 and 2050, climate change may contribute to approximately 250,000 additional deaths annually, primarily due to undernutrition, malaria, diarrheal diseases, and heat-related stress [3].

Climate change affects not only the general population but also disproportionately impacts older adults [4]. By 2050, approximately 16% of the global population will be aged 65 years or older, with Europe and North America projected to have nearly 1 in 4 individuals in this age group [5]. Older adults are particularly vulnerable to climate-related hazards because of reduced physiological reserve, functional limitations, social disadvantage, and environmental constraints [6]. Previous research has examined how aging, quality of life, and coping strategies influence older adults' well-being in the context of climate change [7].

The association between climate change and infectious diseases is becoming increasingly evident, as rising temperatures and altered rainfall patterns create favorable conditions for pathogens and vectors to proliferate [8]. Heat waves and extreme weather events are also associated with increased cardiovascular risk, particularly among older adults [9]. Recent evidence indicates a 53.7% increase in heat-related deaths among adults aged 65 years and older over the past decade [10]. However, relatively few studies have examined these risks in tropical countries such as India, where summer heat tends to be more intense and prolonged than in temperate regions [11].

Both heat waves and sustained high temperatures are becoming more frequent and severe [11–15]. Many older adults live with chronic conditions—including cardiovascular, pulmonary, and renal diseases, as well as diabetes—that may be exacerbated by heat exposure, making these conditions particularly concerning in the context of climate-related health risks [16–18].

Extreme cold also poses significant health risks. Cold waves may contribute to seasonal influenza outbreaks [19]. Despite the heightened vulnerability of older adults in tropical countries such as India, region-specific research remains limited. In these settings, prolonged and intense summer heat further increases exposure risks compared with temperate climates. The growing severity of both chronic heat exposure and acute heat waves underscores the need for context-specific research and targeted interventions [20].

Multidisciplinary strategies that integrate weather-related health risk information into comprehensive health warning systems may improve preparedness and response, enhance public awareness, and support coordinated local and national measures to mitigate temperature-related morbidity and mortality [21]. Although attention to climate-related health impacts has increased, consolidated evidence focusing specifically on older adults remains limited. Their vulnerability is compounded by comorbidities, limited mobility, social isolation, and reduced adaptive capacity. In this scoping review, we aimed to examine the health impacts of climate change on older adults and to map existing interventions and coping strategies used to address these challenges.

HIGHLIGHTS

- Climate change increases older adults' vulnerability to heat-related illnesses, cardiovascular events, respiratory problems, and infectious diseases.
- Older adults—particularly women and those with comorbidities—face heightened risks of anxiety, insomnia, and other climate-related mental health conditions.
- Social support, targeted interventions, and community-based programs enhance resilience and reduce mortality among older adults during extreme weather events.

Research Questions

(1) What are the documented health impacts of climate change on older adults?; (2) What intervention strategies and coping mechanisms are currently employed by older adults to mitigate or adapt to these impacts?; (3) What gaps remain in the literature regarding climate change and geriatric health?

Materials and Methods

Eligibility Criteria

We included studies focusing on adults aged 60 years and older. The concept of interest was the impact of climate change on the physical and mental health of older adults. All global contexts were eligible, regardless of geographic region or healthcare setting. We excluded studies that did not focus on older adults (younger than 60 years) or did not provide age-stratified data for this population. We also excluded publications not written in English and

studies lacking sufficient methodological detail or full-text availability.

Eligible sources included original quantitative and qualitative studies, gray literature, policy documents, and organizational reports addressing climate change, health, and aging. Only publications from the past 15 years (2010–2025) were considered. (1) Population: adults aged 60 years and older. (2) Exposure: climate change and related environmental stressors affecting physical and mental health. (3) Outcomes: health outcomes and intervention strategies associated with climate-related effects.

All global contexts and study designs were eligible. The review followed Arksey and O'Malley's scoping framework and adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) reporting guidelines. Two independent reviewers conducted study selection and data extraction to ensure methodological transparency and reproducibility.

Information Sources

A comprehensive search was conducted across PubMed, Scopus, Google Scholar, and other relevant databases and resources. Both qualitative and quantitative studies reporting the effects of climate change on older adults were included. Gray literature and reports from organizations addressing climate change and aging were also reviewed.

Search Strategy

The following keywords and combinations were used: "climatic changes," "older adults," "interventions," "natural disaster," "vulnerable older population," "health impact," "coping strategies," "mortality," "morbidity," "global warming," "disaster preparedness," and "policy response." Boolean operators were applied as appropriate. Duplicate records were removed using Zotero ver. 7.0 (Corporation for Digital Scholarship).

Selection Process

Two reviewers independently screened titles, abstracts, and full-text articles for eligibility according to the predefined inclusion criteria. Disagreements were resolved through discussion and consensus. The review followed Arksey and O'Malley's scoping framework and adhered to PRISMA-ScR reporting standards (Figure 1).

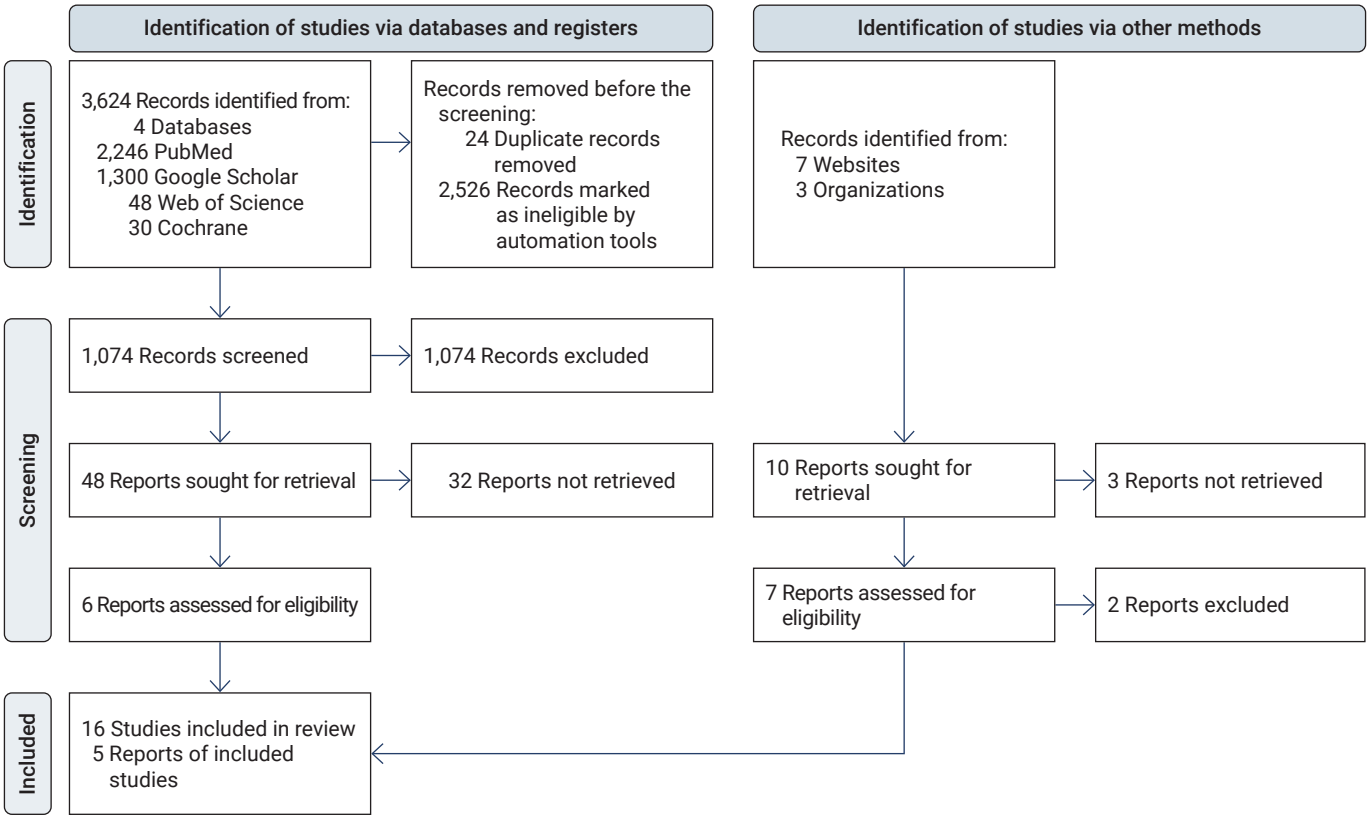


Figure 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 flow diagram of new systematic reviews which included searches of databases, registers, and other sources.

https://doi.org/10.24171/j.phrp.2025.0324

Data Collection Process

Two reviewers independently extracted data from each eligible study. Extracted variables included study characteristics (e.g., country, design, population, and setting), outcomes, and key findings. Discrepancies were resolved through discussion and consensus. The Rayyan ver. 1.6.1 (Rayyan Systems Inc.) platform was used to facilitate study management and screening.

A predominantly inductive thematic content analysis approach was employed (Table 1). Two researchers first familiarized themselves with the data through repeated review. Initial codes were generated manually to capture meaningful segments relevant to the research questions. These codes were subsequently organized into potential themes through iterative discussion. Themes were refined by comparing coded extracts to ensure internal consistency and conceptual distinctiveness. Disagreements were resolved by consensus, enhancing methodological rigor and reliability. Rayyan software supported data management; however, coding and theme development were conducted manually and collaboratively.

Data Items

Data extracted included (1) physical health outcomes: heat-related illness, cardiovascular events, respiratory diseases, and infectious disease burden; (2) mental health outcomes: anxiety, depression, insomnia, and related psychological effects; (3) additional variables: population characteristics, geographic context, type of intervention or coping strategy, and policy-related factors. When data were unclear or incomplete, contextual assumptions were made based on reported information and explicitly documented.

Table 1. Themes and subthemes

Theme	Subthemes
1. Barriers and challenges to adaptation	Resource limitations
	Knowledge and information gaps
	Institutional and policy support
2. Knowledge systems and practices	Traditional/indigenous knowledge use
	Integration of knowledge types
	Knowledge application and transmission
3. Community resilience and coping mechanisms	Individual/household strategies
	Community-level resilience
	Implementation barriers
4. Healthcare system and professional perspectives	Healthcare provider knowledge and awareness
	Clinical risk identification
	System-level preparedness
5. Disaster risk reduction and management	Primary hazards addressed
	Disaster prediction and early warning
	Mitigation and coping strategies
6. Information, education, and support systems	Information access and provision
	Awareness and education programs
	Capacity building and support

Study Risk of Bias Assessment

Because the primary objective of this scoping review was to map the breadth of existing literature rather than evaluate causal relationships, a formal risk of bias assessment was not conducted, consistent with scoping review methodology.

Effect Measures

Effect measures were not calculated because this review did not include a meta-analysis. Findings were synthesized narratively and descriptively.

Synthesis Methods

Data were analyzed using descriptive methods to summarize study characteristics (e.g., design, location, and population) (Table 2) and thematic content analysis to synthesize findings across studies [21–36]. Results were tabulated and grouped under major domains, including physical health impacts, mental health impacts, interventions, coping strategies, and policy responses. This approach facilitated the identification of recurring patterns, knowledge gaps, and areas requiring further research.

Reporting Bias Assessment

Given the scoping nature of this review, no formal assessment of reporting bias (e.g., funnel plots or Egger's test) was performed.

Results

Overview

This scoping review included 16 peer-reviewed articles and 5 grey literature sources, most of which were published in the past 15 years.

Influence of Climate Change on Older Adults' Health

Analysis of the included studies identified several key themes related to the health impacts of climate change on older adults (Figure 2). Findings were categorized according to major domains, highlighting the detrimental effects of climate-related exposures on both physical and mental health.

Infectious Diseases Associated with Climate Change

Climate-sensitive infectious diseases are conditions

Table 2. Study characteristics

Study	Publication year	Study design	Country	Sample size	Study population	Key findings
Sagy et al. [22]	2016	Observational study	Israel	26,286	Older adults	Persistent association between higher temperatures and decreased renal function, particularly among older adults receiving antihypertensive medications.
Kim et al. [23]	2020	Cross-sectional study	Republic of Korea	104	Older adults	Diastolic blood pressure generally decreased with increasing indoor temperature. Strategies to mitigate heat-related health stress are needed.
Zheng et al. [24]	2016	Observational study	China	22,069	Older adults	Short-term increases in DTR were significantly associated with emergency admissions for cause-specific cardiovascular disease. Prevention programs targeting high-risk subgroups may reduce DTR-related health impacts.
Mukhopadhyay et al. [25]	2022	Cross-sectional study	India	310	Older adults	Heat-coping behaviors were influenced more by building characteristics and geographic location than by individual factors, reflecting culturally shaped adaptive decision-making.
Kınay et al. [26]	2021	Cross-sectional study	China	3,466	Older adults	Findings inform development of adaptation strategies for older adults. Government-led awareness initiatives may support physical health adaptation to climate change.
Wang et al. [27]	2024	Cross-sectional study	China	54,690	Adults	Older individuals demonstrated greater sensitivity to diurnal temperature range. Weather forecasting services should highlight DTR values, and targeted interventions are recommended.
Herrmann and Sauerborn [28]	2018	Qualitative study	Germany	24	General practitioners	General practitioners' knowledge of heat-related health impacts and climate change requires strengthening. Strong nursing care and social networks were identified as critical components of climate-resilient health systems.
Kemen et al. [29]	2021	Quantitative survey	Germany	258	Older adults	Heat health action plans are urgently needed to support high-risk individuals in adopting effective coping strategies.
Vanasse et al. [30]	2017	Population-based cohort	Canada	112,793	Older adults with HF	Temperature and meteorological parameters exhibited lagged associations with heart failure events. Preventive measures are recommended due to the high burden and management costs of heart failure.
Nyahunda and Tirivangasi [31]	2019	Qualitative study	Zimbabwe	26	Rural population	Adaptation to climate change requires timely information dissemination, rapid response to community needs, and climate-smart agricultural policies.
Macnight Ngwese et al. [32]	2018	Mixed methods	Ghana	3,814	Two rural communities	Integrating traditional disaster risk reduction practices with scientific approaches may reduce community vulnerability; systematic evaluation of effectiveness is recommended.
Jouvencel et al. [33]	2025	Observational study	France	157	Older adults	Older adults also suffer more from climate change effects; this study further adds to the demand for clinical monitoring and housing adaptations for older adults in the future.
Corley et al. [34]	2022	Observational study	USA	411	Older adults	Findings support disaster preparedness interventions tailored to the mental health needs of older adults.
Orlando et al. [35]	2021	Retrospective ecological study	Italy	42	Older adults >80 years of age	Reducing social isolation may mitigate the impact of heat waves on mortality among older adults.
Ibrahim et al. [36]	2023	Quasi-experimental	Egypt	74	Older adults	Awareness programs improved knowledge, attitudes, and practices regarding climate change and health.
Rodrigues et al. [21]	2021	Observational study	Portugal	2,846,332	Lisbon metropolitan area (including older adults)	The proportion of deaths attributed to exposure to extreme heat was higher than that attributed to moderate heat. As with cold temperatures, people aged ≥85 years were the most vulnerable age group to heat, mostly due to extreme heat.

DTR, diurnal temperature range; HF, heart failure; USA, United States of America.

https://doi.org/10.24171/j.phrp.2025.0324

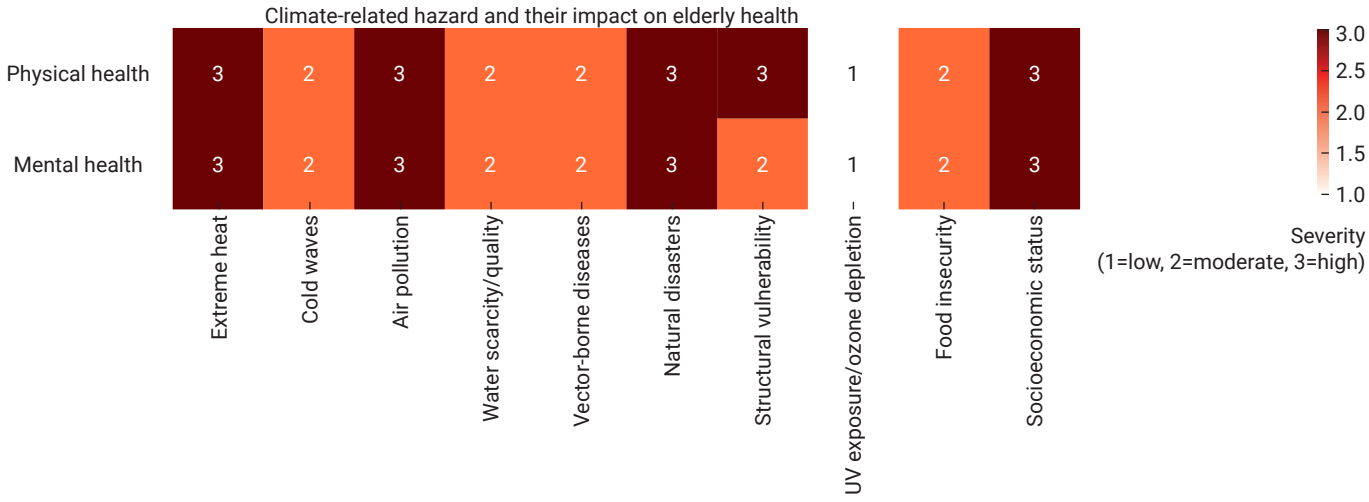


Figure 2. Climate hazard–health impact matrix. UV, ultraviolet.

whose transmission and distribution are influenced by environmental factors such as temperature, rainfall, and humidity [37]. Population aging has resulted in a growing proportion of older adults, particularly in high-income countries, thereby increasing the number of individuals vulnerable to climate-sensitive infections. Age-related reductions in physiological reserve, slower metabolic processes, and diminished immune responsiveness contribute to increased susceptibility in this population [38]. The reviewed studies identified several infectious diseases influenced by climate variability and described their transmission pathways and public health implications [39]. Air pollution and atopic conditions may increase susceptibility to infections, while poor water quality elevates the risk of exposure to waterborne pathogens. Vector-borne diseases, particularly those transmitted by mosquitoes and ticks, also pose substantial risks to older adults [40,41]. Policy responses addressing climate-sensitive infectious diseases should prioritize enhanced surveillance systems that integrate climate and epidemiological data to enable early detection and rapid response [42]. Additional preventive measures include improving drainage systems to reduce mosquito breeding sites, increasing community awareness of infection risks, and implementing targeted prevention strategies [43].

Extreme Temperatures and Health Effects

Exposure to extreme heat has been associated with reduced kidney function among older adults receiving antihypertensive medications, leading to increased rates of prolonged hospitalization [22]. Rising indoor temperatures have also been correlated with decreases in blood pressure among older adults [23]. Individuals with pre-existing myocardial dysfunction appear to face elevated risks of cardiac ischemia and cardiogenic shock during heat waves, rendering older adults particularly vulnerable to heat-related cardiovascular events [24,44]. Heat stroke further exacerbates morbidity among older adults, especially those with severe disabilities [4]. Overall, increased ambient temperatures are associated with higher mortality rates in this population [40].

Older women are more likely to report heat-related symptoms during periods of elevated temperatures, and individuals with comorbid conditions tend to experience more severe and numerous symptoms [25]. Heat waves are also associated with worsening respiratory conditions [26]. Projections of heatwave-related mortality underscore the urgent need for effective adaptation and mitigation strategies [37]. Long-term exposure to temperature variability has been associated with increased prevalence of chronic diseases in older adults [17]. Additionally, older adults demonstrate increased vulnerability to diurnal temperature range, which has been linked to elevated rates of emergency admissions for cardiovascular conditions [27].

Enhancing general practitioners' understanding of heat-related health impacts and climate change is essential. Social support and nursing care have been identified as key factors influencing vulnerability to heat exposure among older adults [28]. Coping strategies reported by older adults include body-related measures (e.g., hydration, clothing adjustment), home-protection practices, and activity modification. Socioeconomic status and culturally influenced decision-making processes also shape adaptive behaviors. Comprehensive and targeted awareness campaigns for high-risk populations are strongly recommended [25,29].

Cold waves likewise pose significant health risks. Older adults are more susceptible to cold-related morbidity and

mortality than younger populations. Notably, countries with milder winters may experience higher rates of cold-related deaths than those with more severe winters, possibly reflecting differences in adaptation and infrastructure. Among individuals with a history of congestive heart failure, the risk of hospitalization or death is significantly higher during winter months compared with summer, independent of air pollution levels [30].

Air and Water Quality

Changes in climatic conditions also affect air quality. More than half of air pollution–related deaths occur among older adults, with approximately half attributable to cardiorespiratory conditions. Ozone depletion and increased ultraviolet radiation exposure have been associated with rising incidence of skin cancers, including melanoma and basal cell carcinoma [45]. Climate-related environmental stressors may also accelerate the progression of geriatric syndromes, particularly frailty and cognitive decline [44]. Alterations in rainfall patterns and temperature can increase contamination of water sources and modify their chemical composition, thereby affecting water quality and facilitating the proliferation of microbial pathogens [46]. These changes contribute to elevated risks of infection among vulnerable older adults.

Older Adults' Vulnerability During Disasters

Older adults often face financial constraints that limit their ability to repair homes or recover following climate-related disasters [31]. Limited income and reduced access to resources restrict adaptive capacity and delay recovery [47]. Women frequently assume caregiving responsibilities for older adults during disasters, further compounding vulnerability [47]. Inadequate social support and limited assistance during emergencies have been associated with increased mortality among older individuals. Pre-existing poor health status further increases disaster-related mortality risk. In one setting, residents anticipated flooding of the Chietanga River by observing changes in wildlife behavior, including the movement of hippopotamuses. In response, community members constructed mud barriers to protect agricultural land and stored food and essential supplies in preparation for the flood [32]. These examples illustrate the importance of local knowledge and anticipatory coping mechanisms in disaster response.

Mental Health and Climate Change

Elevated night-time temperatures can disrupt sleep, as discomfort may prevent individuals from maintaining regular sleep patterns. One study reported significant seasonal variation in sleep–wake cycles among an older rural cohort in France [33]. Such disturbances are associated with increased rates of insomnia among older adults [23]. Older adults who survived wildfires reported high prevalence of anxiety, depression, insomnia, nightmares, and post-traumatic stress disorder [34]. Climate-related disasters may therefore have lasting psychological consequences in this population. Addressing climate-associated mental health outcomes in older adults is increasingly important, given their heightened vulnerability to environmental stressors. Healthcare professionals should receive training on the mental health implications of climate change to provide appropriate assessment, support, and intervention [20].

Interventions and Climate Change

In Italy, participation in the Long Live the Elderly intervention program was associated with reduced mortality during summer heat waves, particularly among individuals aged 80 years and older. Programs designed to reduce social isolation were linked to smaller increases in mortality during periods of extreme heat [46]. In Egypt, a community-based awareness program significantly improved knowledge, attitudes, and practices related to climate change and health among older adults [47]. Financial strategies to subsidize electricity costs for cooling devices during heat waves have also been proposed as essential measures to protect heat-vulnerable populations [23].

Best practice recommendations emphasize that adaptation plans should be grounded in local epidemiological data and aligned with available emergency management resources. Strengthening monitoring and alert systems, including clearly defined temperature exposure thresholds, may enhance the effectiveness of cold weather and heatwave action plans [21]. Effective adaptation requires coordinated improvements in early warning systems, healthcare infrastructure, urban planning, social support networks, and community engagement [48]. Increasing general practitioners' awareness of heat-related health risks may promote greater involvement in preventive strategies. Social support and nursing care remain central determinants of vulnerability among older adults during extreme temperature events [28].

Discussion

Climate change poses a substantial public health threat to older adults, who are disproportionately affected due to age-related physiological vulnerability and social determinants of health. Consistent with prior reviews, this scoping review identified increased morbidity and mortality associated

https://doi.org/10.24171/j.phrp.2025.0324

with extreme temperatures, air pollution, and other climate-related hazards in this population. Beyond acute morbidity and mortality, broader determinants—including functional decline, quality of life, healthcare access, and social isolation—play significant roles in shaping overall vulnerability and warrant further investigation [20,49,50].

The current evidence base remains limited regarding the effects of climate-related hazards beyond heat waves, such as floods, droughts, cold spells, and vector-borne diseases, on older populations. This limitation constrains the development of comprehensive, tailored interventions. Mental health consequences—including anxiety, depression, and trauma-related disorders—have historically received less attention but are increasingly recognized as integral components of climate-related health risk in older adults [20,51,52].

Healthcare providers play a central role in recognizing and managing both the physical and psychological consequences of climate-related events in older adults. However, targeted education and training are needed to equip clinicians and caregivers with the competencies required to address these complex challenges effectively. Age-appropriate mental health support strategies should be integrated into routine clinical practice and disaster response frameworks [20,48].

Despite the existence of policies and educational resources addressing climate change and health, implementation remains inconsistent. There is a notable lack of older adult–specific programs focused on climate-related health risks. Public health authorities should prioritize targeted education and outreach initiatives for older adults, particularly those with lower socioeconomic status, emphasizing adaptive behaviors such as protection from temperature extremes and mitigation of social isolation. Financial support for infrastructural adaptations—including access to adequate cooling and heating systems—is essential to reduce vulnerability and promote healthy aging in the context of climate change [44,48,49].

Theoretical models, including resilience theory, offer valuable frameworks for conceptualizing adaptive capacity among older adults. The findings of this review align with resilience-based approaches that emphasize the importance of social networks, community engagement, and institutional support in strengthening adaptive responses to climate stressors [53].

Strengths and Limitations of the Study

This scoping review synthesizes evidence from multiple countries, providing a broad overview of the health impacts of climate change on older adults and the range of interventions implemented internationally. By integrating findings across diverse geographic and socioeconomic contexts, the review highlights common patterns while underscoring the need for localized, context-specific research to inform tailored interventions at national and regional levels.

However, this review is limited by its reliance exclusively on previously published literature. Consequently, the findings reflect only the available evidence base and may be subject to publication bias. Confounding factors—such as comorbidities and socioeconomic status—were not systematically evaluated because formal risk of bias assessment was not conducted. Additionally, given the global scope of included studies, the review does not provide detailed analysis of country-specific interventions, which may vary substantially depending on geographic, climatic, and socioeconomic conditions.

Conclusion

As the frequency and intensity of climate-related hazards continue to increase, comprehensive mitigation and adaptation strategies are urgently required. These strategies should be inclusive, evidence-informed, and tailored to the specific vulnerabilities of older adults. Protecting this population requires coordinated efforts across healthcare systems, public health agencies, and community organizations to ensure that older adults are not disproportionately affected by the evolving health impacts of climate change.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions
Conceptualization: PP, MT; Data curation: PP, MT; Formal analysis: all authors; Investigation: PP, MT; Methodology: PP, MT; Project administration: PP, MT; Resources: all authors; Software: all authors; Supervision: PP, MT; Validation: PP, MT; Visualization: PP, MT; Writing–original draft: PP, MT; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0616
Osong Public Health Res Perspect 2026;17(3):206-215

Review Article

Genetic diversity and multiplicity of *Plasmodium* spp. infections in Southeast Asia: a scoping review

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ABSTRACT

Objectives: This scoping review synthesizes molecular evidence on *Plasmodium* genetic diversity and multiplicity of infection (MOI) in Southeast Asia and describes variation by species and epidemiological setting.

Methods: Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews guidelines, we searched PubMed, Scopus, ProQuest, and EBSCO for English language, open-access observational studies published between January 1, 2015 until October 11, 2025. Included studies involved laboratory-confirmed human malaria and reported MOI and genetic diversity data derived from appropriate molecular genotyping methods. Findings were summarized by species, geography, markers, and clinical categories.

Results: Sixteen studies were included, comprising 1,609 genotyped *Plasmodium falciparum* infections, 1,526 *Plasmodium vivax* infections, 37 *Plasmodium malariae* infections, and 188 human *Plasmodium knowlesi* infections. *P. falciparum* exhibited marked heterogeneity, with polyclonal infection rates ranging from 3.9% to 73.9% and mean MOI ranging from 1.05 to 4.9. *P. vivax* consistently showed high diversity, with polyclonal infection rates ranging from 16.9% to 71.4% and mean MOI ranging from 1.1 to 1.91, alongside expected heterozygosity values of 0.66–0.87. *P. malariae* displayed low MOI, whereas *P. knowlesi* showed polyclonal infection rates ranging from 7.3% to 21% and mean MOI ranging from 1.04 to 1.06. No data were available for *Plasmodium ovale*.

Conclusion: *P. falciparum* and *P. vivax* remain genetically diverse across Southeast Asia, with heterogeneous transmission patterns, particularly in border regions. Limited data on *P. malariae*, human *P. knowlesi*, and *P. ovale* highlight the need for standardized molecular surveillance to support targeted elimination efforts.

Keywords: Genetic variation; Malaria; *Plasmodium*; Southeast Asia

Received: December 28, 2025
Revised: March 13, 2026
Accepted: March 25, 2026

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The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

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Introduction

Malaria remains a significant public health concern in Southeast Asia despite notable progress in reducing disease burden over the past 2 decades. The region experienced a marked decline in cases, from 22.8 million in 2000 to 5.2 million in 2022; however, it still accounted for more than 8,000 malaria-related deaths and 18% of the global burden in 2022. Although countries such as Vietnam and Cambodia have achieved substantial reductions in transmission, others, including Myanmar, Laos, and Indonesia, continue to experience high disease prevalence and persistent transmission [1,2]. Malaria is a vector-borne infectious disease transmitted by female Anopheles mosquitoes and caused by *Plasmodium* parasites [3–5]. Five species are known to infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and the zoonotic *Plasmodium knowlesi*. Among these, *P. falciparum* and *P. vivax* are the primary drivers of morbidity [6].

According to the 2024 World Health Organization report, the Southeast Asia Region recorded approximately 4 million malaria cases across 8 endemic countries. Although decreases were observed in Bangladesh, India, Indonesia, and Nepal, substantial increases were reported in Myanmar (45.1%) and Thailand (46.4%). Notably, the number of reported indigenous cases in Thailand more than tripled between 2021 and 2023, largely driven by cross-border transmission and displaced populations from Myanmar, necessitating increased resources for diagnosis and treatment [7].

Understanding the genetic diversity and population structure of malaria parasites is essential for guiding and evaluating elimination strategies. Central to molecular surveillance is multiplicity of infection (MOI), defined as the number of genetically distinct parasite strains co-infecting a single host. Multiclonal infections increase the likelihood of genetic recombination during the sexual cycle in mosquitoes, generating sporozoites with novel genotypes and contributing to parasite diversification [8]. These infections may arise through cotransmission of multiple clones in a single mosquito bite or through superinfection following repeated exposure to infectious bites [9–11]. Consequently, MOI has been widely used as a surrogate marker of transmission intensity, whereas the presence of polyclonal infections reflects ongoing exposure to genetically diverse parasite populations. In endemic settings, frequent polyclonal infections are often accompanied by higher MOI values, together indicating sustained transmission and a large parasite reservoir [12,13].

Beyond serving as indicators of transmission intensity, polyclonal infections also have broader epidemiological and clinical relevance. They reflect ongoing exposure to genetically diverse parasite populations and are commonly observed in endemic settings in which individuals experience repeated infectious mosquito bites [11–13]. Importantly, minority clones within polyclonal infections may be preferentially transmitted to mosquitoes, including drug-resistant strains, thereby potentially facilitating the spread of resistance and complicating malaria control efforts [14]. Moreover, measures of genetic diversity derived from multiclonal infections can be used to assess geographic differentiation, infer parasite movement, and support surveillance efforts aimed at identifying the origin of infections in elimination settings [15]. From a clinical perspective, greater infection complexity has also been associated with increased disease severity, as patients with severe or cerebral malaria often harbor a greater number of genetically distinct parasite clones than those with uncomplicated infections [16].

Several population genetic studies from Southeast Asia have shown that *P. vivax* often maintains high genetic diversity even in low-transmission settings, with expected heterozygosity (He) frequently exceeding 0.7 and substantial proportions of polyclonal infections despite intensive control efforts [17–20]. In contrast, *P. falciparum* populations in the same areas tend to show more pronounced reductions in diversity and stronger linkage disequilibrium as incidence declines [21]. Recent studies have also begun to characterize *P. knowlesi* populations in Malaysia and Indonesia, suggesting relatively low infection complexity in humans but important reservoirs in macaques [22,23]. However, these studies remain scattered, use heterogeneous molecular markers and analytical approaches, and typically focus on single species

HIGHLIGHTS

- This scoping review mapped molecular estimates of multiplicity of infection and genetic diversity in Southeast Asia from 2015 to 2025.
- Sixteen studies reported genotyped infections for *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and human *Plasmodium knowlesi* infections.
- *P. falciparum* showed wide variation in infection complexity across settings, while *P. vivax* more consistently exhibited high diversity and frequent multiclonality.
- Evidence for *P. malariae* and *P. knowlesi* was limited, and no eligible data were found for *Plasmodium ovale*.
- Standardized genotyping and reporting are needed to support elimination-focused molecular surveillance.

and localized settings.

Understanding how genetic diversity and MOI vary by species, host population, and epidemiological context is therefore critical for interpreting molecular surveillance data during the malaria elimination phase. Accordingly, this scoping review aims to synthesize the available evidence on genetic diversity and MOI across 4 human-infecting *Plasmodium* species and to examine how these metrics vary in relation to transmission intensity across Southeast Asia.

Materials and Methods

Scoping Review Approach

We conducted a scoping review to identify studies relevant to our research question. Scoping review methodology is a structured approach to evidence synthesis that aims to systematically map the breadth and depth of the literature on a given topic and to identify key concepts, types of evidence, and research gaps [24]. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR) guidelines [25]. The review protocol was defined a priori, and the eligibility criteria, search strategy, and data extraction items were specified before study selection. The review questions were framed using the Population, Concept, and Context (PCC) structure (Table 1).

Eligibility Criteria

Inclusion criteria

The review considered (1) reported original research with primary data; (2) included human participants with laboratory-confirmed malaria (microscopy and/or polymerase chain reaction [PCR]), regardless of age, sex, or clinical severity; (3) used molecular genotyping to determine *Plasmodium* spp. genetic diversity and MOI, including mean expected He, allele frequencies, and mean MOI or percentage of multiple infections; (4) employed observational (cross sectional/survey, case–control, cohort); (5) were published in English and available as full-text open-access articles; and (6) were published in the last 10 years.

Exclusion criteria

(1) Experimental studies using only animal models or *in vitro* cultures; (2) review papers, case reports, case series, comments, or editorials; (3) studies from non-Southeast Asian settings; (4) studies without any relevant keywords related to *Plasmodium* genetic diversity or MOI in the title or abstract; and (5) studies using unclear or inappropriate molecular methods that did not allow reliable estimation of MOI or genetic diversity.

Information Sources and Search Strategy

We searched major electronic databases, including PubMed, Scopus, ProQuest, and EBSCO, from January 1, 2015, to October 11, 2025. The search strategy combined controlled vocabulary and free-text terms related to *Plasmodium* species, genetic diversity, and MOI. The search string used in the database search engines was as follows: ("Plasmodium falciparum" OR "Plasmodium vivax" OR "Plasmodium malariae" OR "Plasmodium ovale" OR "Plasmodium knowlesi" OR "malaria") AND ("genetic variation" OR "genetic diversity") AND ("multiplicity of infection" OR "multiple infection" OR "MOI").

Searches were limited to English-language publications from the previous decade. Duplicate records were removed.

Selection Process

All records retrieved from the database searches were imported into Mendeley Reference Manager (https://www.mendeley.com/). Duplicate records were identified and removed manually by the reviewer. Titles and abstracts were then screened against the predefined inclusion and exclusion criteria. Studies that clearly did not meet the eligibility criteria, such as those conducted outside Southeast Asia, involving non-human hosts, lacking genetic diversity or MOI outcomes, consisting of review articles, or lacking accessible full texts, were excluded at this stage. Potentially relevant studies were retrieved in full and assessed in detail for eligibility. Full-text screening also led to the exclusion of studies that, on closer inspection, did not contain appropriate molecular genetic data, were conducted outside Southeast Asia, or did not report the required outcome measures. Only studies that fully met all PCC-based criteria were included in the data extraction.

Table 1. Population, Concept, and Context (PCC) structure

Element	Description
Population	Human participants of any age and sex with laboratory-confirmed malaria caused by *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium knowlesi*, or *Plasmodium ovale*
Concept	Genetic diversity assessed through marker-specific allelic richness and frequencies; and infection complexity measured by the prevalence of polyclonal infections and mean MOI.
Context	Countries in Southeast Asia and contiguous border regions within the Greater Mekong Subregion

MOI, multiplicity of infection.

https://doi.org/10.24171/j.phrp.2025.0616

Data Collection Process

Data extraction was conducted by a single reviewer using a predesigned and piloted Excel extraction template. For each included study, the reviewer extracted information on study characteristics, the number of genotyped samples, species investigated, molecular markers used, measures of genetic diversity (e.g., expected He and allele frequencies), and indicators of MOI (e.g., mean MOI and the prevalence of polyclonal infections). No automation tools were used during data extraction. All data were extracted directly from the published reports, and no contact was made with study authors or investigators to obtain missing information. To minimize errors and bias, the extracted data were cross-checked by the co-authors for internal consistency before synthesis.

Data Items

All included articles were charted in a prepiloted Excel extraction form with separate sheets for each species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*). For each study, we extracted (1) first author and publication year; (2) country or border region; (3) malaria clinical category (symptomatic, asymptomatic, mixed, severe/cerebral); (4) number of infections genotyped; (5) molecular markers used; (6) measures of genetic diversity; (7) measures of MOI; (8) any notable stratifications.

Data extraction was done separately by *Plasmodium* species, which allowed cross-comparison of marker choice, diversity and MOI patterns within and between species.

Synthesis Methods

Data were synthesized narratively in accordance with the aims of a scoping review. No meta-analysis or quantitative pooling was performed because of substantial heterogeneity in study designs, molecular markers, genotyping methods, and outcome definitions across the included studies. Extracted data were organized by *Plasmodium* species and summarized descriptively in terms of the number of genotyped infections, types of molecular markers used, measures of genetic diversity, and MOI indices. Patterns were described across countries, transmission settings, and species, and no statistical comparisons or effect estimates were generated.

Results

Study Selection

The database search identified 198 records. After removal of 33 duplicates and screening of 165 titles and abstracts, 29 full-text articles were sought for retrieval. Before eligibility assessment, 3 articles could not be retrieved, leaving 26 articles for full-text review. Ten articles that appeared eligible based on title and abstract were excluded during full-text review because they lacked MOI or multilocus genetic diversity outcomes, used inadequate molecular markers, focused on non-human hosts, or were conducted outside Southeast Asia without epidemiological relevance to the China-Myanmar border or Greater Mekong Subregion. Sixteen studies met the inclusion criteria and were included in the review. Among studies that appeared eligible at the abstract stage but were excluded after full-text review, the most common reasons were the absence of MOI outcomes and insufficient genotyping detail. A PRISMA-style flow diagram is shown in Figure 1.

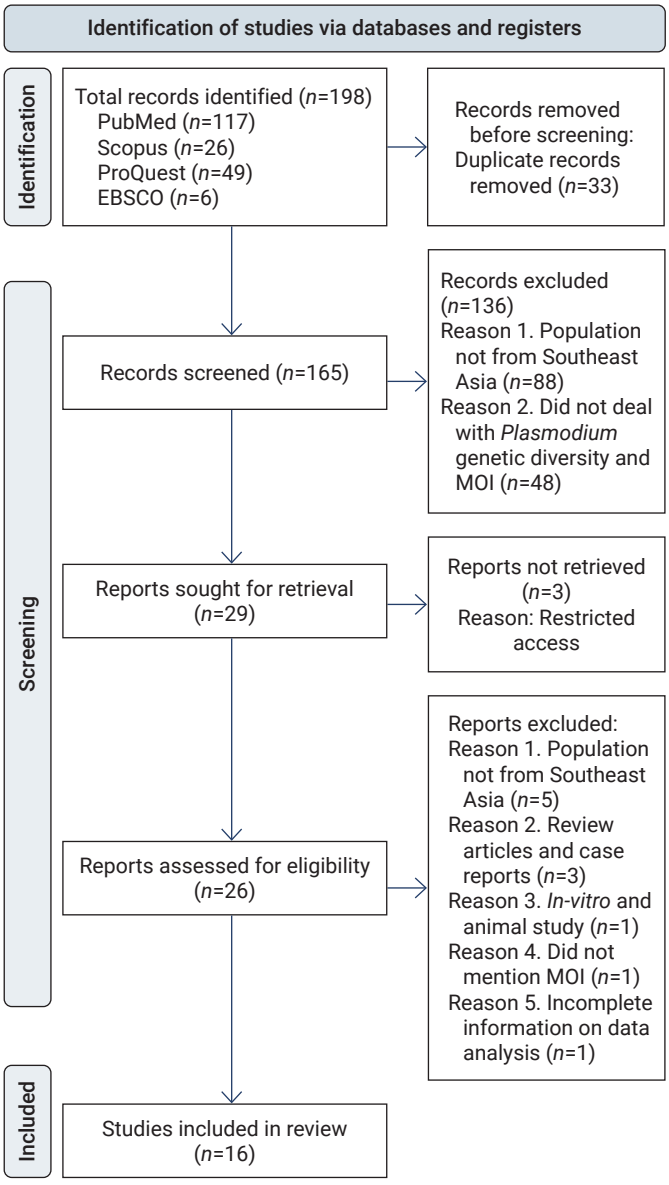


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram. MOI, multiplicity of infection.

Study Characteristics

Most included studies were cross-sectional, facility-based surveys that primarily enrolled patients presenting with symptomatic malaria. Several studies also included asymptomatic or mixed symptomatic/asymptomatic populations to capture broader transmission patterns. Notably, 1 *P. knowlesi* study conducted in Malaysia genotyped isolates from both humans and long-tailed macaques, providing insight into zoonotic transmission dynamics [23].

The geographic distribution of the included studies reflected major malaria-endemic zones in Southeast Asia. Most datasets originated from Indonesia, Thailand, Myanmar, Malaysia, and Vietnam. Additional studies were conducted in epidemiologically important border regions, including the China-Myanmar and Thailand-Myanmar borders.

Across all studies, a total of 1,609 *P. falciparum* and 1,526 *P. vivax* infections were successfully genotyped using established polymorphic markers, including msp1, msp2, glurp, pvmsp-3α/β, pvcsp, and microsatellites. In addition, 37 *P. malariae* infections and 188 human *P. knowlesi* infections (41 from Indonesia and 147 from Malaysia) were included.

The characteristics of the included studies are summarized in Table 2 [10,16–23,26–32].

Cross-Setting Pattern Synthesis

Across the included studies, greater infection complexity tended to be reported in border and high-mobility settings, particularly along the China-Myanmar and Thailand-Myanmar borders, than in more localized non-border sites, consistent with the concentration of datasets from epidemiologically important border zones [20,21,26]. However, direct quantitative comparisons remained limited because sampling frames and genotyping approaches differed across studies. Mixed clinical cohorts indicated that multiclonality was not confined to symptomatic cases. Because heterogeneous marker systems were used, results are presented by species and study context, with emphasis on within-study patterns rather than direct cross-study comparisons.

Results for *P. falciparum*

Nine studies from Indonesia, Malaysia, Thailand, Myanmar, Vietnam, and the China-Myanmar border assessed genetic variation and MOI using msp1, msp2, glurp, or microsatellite

Table 2. Characteristics of included studies on *Plasmodium* spp. genetic diversity and multiplicity of infection

Study	Year	Country/region	Species[a]	Malaria clinical category	Number genotyped	Genotyped markers
Zhang et al. [26]	2019	China-Myanmar border	*Pf*	Symptomatic	215	msp1 and msp2
Jamil et al. [27]	2021	Indonesia	*Pf*	Symptomatic	90	msp1 and msp2
Manu et al. [28]	2021	Indonesia	*Pf*	Symptomatic	51	glurp
Pava et al. [10]	2017	Indonesia	*Pf*	Asymptomatic and symptomatic	414	Microsatellite
Mohd Abd Razak et al. [16]	2016	Malaysia	*Pf*	Asymptomatic	60	msp1, msp2, microsatellite, and glurp
Goh et al. [21]	2019	Malaysia and Thailand	*Pf*	Symptomatic	173	msp1
Soe et al. [29]	2017	Myanmar	*Pf*	Acute *Plasmodium falciparum* infections	184	msp1, msp2, and glurp
Chaorattanakawee et al. [30]	2018	Thailand	*Pf*	Severe and cerebral malaria	471	msp2
Van Long et al. [31]	2020	Vietnam	*Pf*	Uncomplicated	166	msp1 and msp2
Wang et al. [20]	2023	China-Myanmar border	*Pv*	Symptomatic	442	Pvmsp-3α and Pvmsp-3β
Li et al. [17]	2020	Greater Mekong Subregion	*Pv*	Symptomatic	206	Microsatellite
Pava et al. [10]	2017	Indonesia	*Pv*	Asymptomatic and symptomatic	390	Microsatellite
Maneerattanasak et al. [19]	2016	Thailand-Myanmar border	*Pv*	Symptomatic	254	Pvcsp, Pvmsp1, and Pvmsp3α
Hong et al. [18]	2016	Vietnam	*Pv*	Asymptomatic and symptomatic	234	Microsatellite
Mathema et al. [32]	2020	Myanmar	*Pv*	Symptomatic	37	Microsatellite
Kariodimedjo et al. [22][b]	2025	Indonesia	*Pk*	Asymptomatic	41	Microsatellite
Saleh Huddin et al. [23]	2019	Malaysia	*Pk*	Symptomatic	147[a]	Microsatellite

[a]Species abbreviations: Pf, *P. falciparum*; Pv, *Plasmodium vivax*; Pm, *Plasmodium malariae*; Pk, *Plasmodium knowlesi*. [b]One *P. knowlesi* study also genotyped long-tailed macaques; macaque data are not shown here.

https://doi.org/10.24171/j.phrp.2025.0616

markers (Table 3). All 3 classical msp1 allele families (K1, MAD20, and RO33) and both msp2 families (FC27 and 3D7) were reported.

Allelic distributions indicated substantial genetic variation, with several studies reporting dominant alleles such as MAD20 or K1 in Indonesia and Malaysia. High allelic richness was observed in border regions, particularly along the China-Myanmar border, where 38 msp1 alleles and 52 msp2 alleles were identified. The prevalence of polyclonal infections ranged from 3.9% to 73.9%, whereas mean MOI ranged from 1.05 to 4.9, with the highest values reported in Myanmar and at China-Myanmar border sites (Table 3) [10,16,21,26–31].

Results for *P. vivax*

Five studies demonstrated consistently high allelic diversity in *P. vivax*, characterized by multiple alleles per locus for pvmsp-3α/β, pvmsp1-F3, pvcsp, and microsatellite markers (Table 4) [10,17–20].

Allelic richness was particularly high along the China-Myanmar border, with 19–26 alleles reported for pvmsp3α/β. The reported prevalence of polyclonal infections ranged from 16.9% to 71.4%, with the highest values observed in Vietnam. Mean MOI ranged from 1.1 to 1.91, confirming that *P. vivax* infections frequently contained multiple clones and often showed greater complexity than co-endemic *P. falciparum* infections. Multiple studies reported high expected He estimates, ranging from 0.66 to 0.86 in the Greater Mekong Subregion and reaching 0.867 in Indonesia.

Results for *P. malariae*

Only 1 eligible study from Myanmar reported multilocus diversity for *P. malariae*, genotyping 37 symptomatic infections (Table 5). Polyclonal infections accounted for 20%–22% of cases, with mean MOI values ranging from 1.05 to 1.21, indicating that most infections were monoclonal and that multiclonal infections were relatively uncommon.

Table 3. Genetic diversity and MOI for *Plasmodium falciparum*

Country/region	Genetic diversity (heterozygosity/alleles)	Polyclonal infections (%)	Mean MOI
China-Myanmar border [26]	Alleles: MAD20 (39.1%) and K1 (30.2%) dominant	64.19–72.09	1.76–2.21
Indonesia [10,27,28]	Alleles: MAD20 (46.7%) and K1 (37.7%) dominant in Aceh; Variant II (18%) in Papua He: 0.591 (Papua)	12.0–45.1	1.2–2.69
Malaysia [16]	Alleles: MAD20 dominant (53.2%). High diversity in Kalabakan vs. Marudu	13.3	1.05–1.2
Malaysia-Thailand [21]	Alleles: MAD20 (53.2%) and RO33 (38.7%) dominant	13.3	1.1–1.2
Myanmar [29]	Alleles: 3D7 (69.1%) and MAD20 (48.9%) dominant	73.9	1.94
Thailand [30]	Alleles: FC27 (46.4% for severe, 24.1% for mild) and 3D7 (23.9% for cerebral and 12.5% for mild)	35	1.2–1.4 (mild/cerebral)
Vietnam [31]	Alleles: MAD20 (99%) and 3D7 (97%) nearly fixed.	7–46	2.5–4.9

MOI, multiplicity of infection; He, heterozygosity.

Table 4. Genetic diversity and MOI for *Plasmodium vivax*

Country/region	Genetic diversity (heterozygosity/alleles)	Polyclonal infections (%)	Mean MOI
China-Myanmar border [20]	msp-3*α*: 19 alleles; msp-3*β*: 19–26 alleles	28.5	1.48
Greater Mekong Subregion [17]	He: 0.66–0.86	23.7–48	1.21–1.47
Indonesia [10]	He: 0.867	50–52	1.7
Thailand-Myanmar border [19]	He(msp1): 0.846; He(csp): 0.709	16.9	1.1
Vietnam [18]	He: 0.68	71.4	1.91

MOI, multiplicity of infection; He, heterozygosity.

Table 5. Genetic diversity and MOI for *Plasmodium malariae* and *Plasmodium knowlesi*

Species	Country/region	Genetic diversity (heterozygosity/alleles)	Polyclonal infections (%)	Mean MOI
P. malariae	Myanmar [32]	0.53–0.92	20–22	1.05–1.21
P. knowlesi	Indonesia [22]	0.634	7.3	1.06
	Malaysia [23]	0.71	21	1.04

MOI, multiplicity of infection.

Results for *P. knowlesi*

Two studies reported human *P. knowlesi* infections in Indonesia and Malaysia (Table 5) [22,23,32]. Allelic variation was moderate, consistent with limited human-to-human transmission and predominantly zoonotic spillover. In humans, the prevalence of polyclonal infections ranged from 7.3% to 21%, with mean MOI values between 1.04 and 1.06, indicating that most infections were monoclonal. In contrast, substantially higher complexity, with polyclonal infections of up to 54%, was documented in macaques, suggesting that parasite diversity was maintained primarily within the reservoir host.

Absence of data for *P. ovale*

No eligible studies from Southeast Asia or the Greater Mekong Subregion reported MOI and genetic diversity data for *P. ovale*. This represents a clear gap in the regional molecular epidemiology of malaria.

Discussion

Genetic diversity and MOI are widely used molecular indicators of *P. falciparum* transmission intensity and parasite population structure. However, findings from this scoping review indicate that their epidemiological interpretation in Southeast Asia and the Greater Mekong Subregion is highly context-dependent and shaped by transmission heterogeneity, population movement, and methodological variation. In high-transmission settings, elevated MOI, high He, and frequent polyclonal infections are commonly observed [33–35], whereas low-transmission and elimination settings exhibit more variable and focal patterns. In Southeast Asia and the Greater Mekong Subregion, malaria transmission occurs across highly heterogeneous ecological, socioeconomic, and programmatic contexts. These range from low-transmission elimination settings to persistent foci concentrated along international borders and in forested areas, despite overall regional declines in incidence. The epidemiology is further complicated by the coexistence of *P. falciparum* and *P. vivax*, with *P. vivax* showing notable resilience to control efforts, alongside the emergence and spread of artemisinin-resistant *P. falciparum* strains that threaten treatment efficacy [36,37]. Consequently, substantial variation in parasite genetic diversity and infection complexity is expected both between countries and within subnational settings.

This scoping review synthesizes the available evidence and shows that *P. falciparum* exhibits wide variation in the prevalence of polyclonal infections. Across studies, polyclonal infection prevalence ranged from 7% to 74%, and mean MOI ranged from 1.05 to 4.9, reflecting differences in transmission intensity, population movement, and study setting across countries and border regions. Notably, the China-Myanmar border dataset reported high polyclonal infection rates, ranging from 64.19% to 72.09%, and mean MOI values from 1.76 to 2.21, consistent with intensified parasite mixing and genetic diversity in this border setting [26]. Evidence from the Malaysia-Thailand border further illustrates moderate infection complexity in certain *P. falciparum*-endemic settings, where MAD20 (53.2%) and RO33 (38.7%) were the dominant msp1 allelic families and the reported prevalence of polyclonal infection was 13.3%, with mean MOI ranging from 1.1 to 1.2 [21].

This pattern contrasts with the higher MOI and polyclonal infection rates reported in some studies from Myanmar and the China-Myanmar border, highlighting substantial spatial heterogeneity in *P. falciparum* transmission intensity across Southeast Asia. Overall, these findings indicate that *P. falciparum* transmission in Southeast Asia is highly focal and spatially heterogeneous, with border regions consistently showing greater infection complexity than non-border settings. This focality has important implications for elimination strategies, as residual transmission in border areas may sustain parasite diversity and undermine national-level gains achieved in lower-transmission non-border settings.

For *P. vivax*, multiple studies consistently reported high allelic diversity and frequent multiclonality across settings including the China-Myanmar border, the Greater Mekong Subregion, Indonesia, the Thailand-Myanmar border, and Vietnam. Polyclonal infection prevalence ranged from 16.9% to 71.4%, with mean MOI values of 1.1–1.91 and expected He estimates of approximately 0.66–0.87. Multiple infections with genetically distinct parasite clones are common in many malaria-endemic settings, and MOI has been widely used as a proxy for transmission intensity, with higher MOI more frequently observed in areas of intense transmission [20]. Analyses of *P. falciparum* populations similarly indicate that MOI is broadly correlated with transmission intensity [19]. In border areas, proximity to regions with higher malaria endemicity, such as Myanmar relative to neighboring Thailand, likely contributes to increased parasite diversity through frequent cross-border population movement, resulting in the continual introduction of genetically distinct parasite strains [20]. Importantly, the consistently high genetic diversity and multiclonality observed in *P. vivax* across diverse countries and marker systems indicate that substantial infection complexity is a common feature of *P. vivax* in the reviewed

https://doi.org/10.24171/j.phrp.2025.0616

literature. However, because most estimates were derived from different studies and settings, these findings should not be interpreted as direct within-site comparisons with *P. falciparum*, but rather as evidence that *P. vivax* frequently maintains high infection complexity across a range of epidemiological contexts. Collectively, these observations suggest that high genetic diversity and multiclonality are intrinsic features of *P. vivax* populations in Southeast Asia and may persist even in settings with declining transmission and ongoing elimination efforts. In contrast with *P. falciparum*, which tends to show marked reductions in diversity as transmission declines, *P. vivax* appears to maintain high genetic diversity and multiclonality even in settings approaching elimination, highlighting fundamental differences in transmission biology and resilience to control.

Compared with *P. falciparum* and *P. vivax*, molecular epidemiological data on other *Plasmodium* species are far more limited. Only 1 study assessed *P. malariae*, reporting predominantly monoclonal infections, mean MOI values of 1.05–1.21, and moderate genetic diversity. Human *P. knowlesi* infections in Indonesia and Malaysia showed low mean MOI values (1.04–1.06) and low prevalence of polyclonal infections (7.3%–21%), consistent with limited human-to-human transmission. In contrast, the higher complexity reported in macaques suggests that parasite diversity is maintained primarily within the reservoir host, with important implications for surveillance strategies in zoonotic malaria [23]. These findings highlight the need to interpret human MOI estimates for zoonotic malaria cautiously and underscore the importance of incorporating reservoir host data into molecular surveillance frameworks.

Interpretation of findings across studies is constrained by methodological heterogeneity, particularly variation in the molecular markers used to assess genetic diversity and MOI. Antigenic markers such as msp1, msp2, and glurp are commonly used to estimate MOI and genetic diversity, whereas microsatellite markers provide complementary insights into population structure and gene flow relevant to malaria control and elimination efforts [27,38–43].

Crucially, MOI estimates derived from antigenic markers and microsatellites are not directly comparable because they differ substantially in genomic coverage, resolution, sensitivity to minority clones, and allele-calling thresholds. Antigenic markers are typically under strong immune selection, which may maintain higher allelic diversity than neutral markers, whereas microsatellites offer higher resolution for detecting fine-scale population structure but may use different thresholds for distinguishing true additional clones from PCR artifacts. Consequently, a substantial proportion of the observed between-study variability in MOI and polyclonality likely reflects these methodological differences rather than true epidemiological shifts. Therefore, comparisons of MOI and genetic diversity metrics should be interpreted primarily within studies rather than across settings [35,44].

However, variation in marker selection, allele-calling thresholds, and analytical approaches produces MOI and He estimates that are not directly comparable across studies. Genetic diversity is commonly quantified using polymorphic markers such as msp1, msp2, and glurp, with He reflecting the degree of allelic variation, where higher values indicate greater diversity [34,45]. Despite these methodological differences and the limited comparability across studies, metrics such as MOI and He remain critical for understanding malaria epidemiology. They provide essential information for monitoring transmission dynamics, evaluating the impact of control interventions, and supporting molecular surveillance in settings characterized by residual transmission or imported malaria [12,45–47].

Several gaps were identified in the molecular epidemiology literature across Southeast Asia and the Greater Mekong Subregion. Evidence remains unevenly distributed across countries, transmission settings, and *Plasmodium* species, with a predominance of cross-sectional studies and limited longitudinal data to assess temporal changes in genetic diversity and MOI in response to interventions. In addition, substantial variability in molecular marker selection, analytical approaches, and reporting practices limits cross-study comparability and constrains regional-level synthesis. Addressing these gaps will require more standardized molecular surveillance frameworks, including harmonized marker panels and reporting guidelines, as well as closer integration of genetic diversity and MOI metrics with routine epidemiological, entomological, and mobility data. Such approaches are particularly relevant in border and forest-associated transmission settings, where parasite importation and population mixing may undermine elimination efforts despite declining incidence.

The absence of eligible molecular epidemiology data for *P. ovale* represents a notable surveillance gap in Southeast Asia. *P. ovale* infections are frequently underdetected or misclassified as *P. vivax* because of morphological similarity and shared relapse patterns, which may obscure residual transmission in settings approaching elimination [28,29,48]. Without enhanced molecular surveillance, the true burden and transmission dynamics of *P. ovale* in the region will remain poorly understood, posing challenges for malaria control and elimination strategies.

Strengths and Limitations of the Study

A primary limitation of this review is the methodological

heterogeneity across the included studies. MOI estimates derived from antigenic markers (msp1/msp2/glurp) and microsatellites are not directly comparable because they differ in genomic coverage, sensitivity to minority clones, and analytical thresholds. Consequently, observed variation in genetic diversity may partly reflect these methodological differences rather than true epidemiological shifts. Furthermore, the restriction to open-access articles may have introduced selection bias by excluding relevant data published in subscription-based journals.

Conclusion

This scoping review shows that *P. falciparum* and *P. vivax* in Southeast Asia remain genetically diverse, with high allelic variation, substantial proportions of polyclonal infections, and elevated MOI indicating persistent and heterogeneous transmission, particularly in border regions. *P. vivax* consistently exhibited greater infection complexity than *P. falciparum*, whereas *P. malariae* and *P. knowlesi* were less extensively studied but still demonstrated meaningful diversity. Human *P. knowlesi* infections were largely monoclonal, consistent with zoonotic spillover. The absence of molecular data for *P. ovale* highlights a major surveillance gap. Collectively, these findings underscore the need for strengthened, standardized molecular monitoring to better detect transmission hotspots and support targeted malaria elimination strategies in Southeast Asia.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
This study was supported by DAPT-EQUITY Universitas Diponegoro 2025/2026 (No. 463/UN7.A/HK/IX/2025).

Availability of Data
All data generated or analyzed during this study are included in this published article.

Authors' Contributions
Conceptualization: APH, FM; Data curation: MM, BYJ; Formal analysis: APH, FM, JHH; Methodology: APH; Project administration: FM; Resources: APH; Supervision: FM, JBY, JHH; Validation: MM, JHH; Writing–original draft: APH, FM; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0430
Osong Public Health Res Perspect 2026;17(3):216-228

Review Article

Personalized medicine as a novel therapeutic approach for autoimmune diseases: new insights and future prospects

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Received: October 13, 2025
Revised: January 27, 2026
Accepted: February 5, 2026

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ABSTRACT

Autoimmune diseases are caused by dysfunction of the immune system, leading to inappropriate attacks on healthy tissues. Because patients have diverse genetic predispositions and heterogeneous responses to therapy, personalized medicine (PM) offers an opportunity to improve treatment effectiveness. PM uses diagnostic assessments to tailor treatment through individualized medical interventions. PM may improve therapeutic precision beyond traditional trial-and-error approaches, reduce adverse consequences, and improve outcomes by integrating genomic and transcriptomic data. PM considers genetic and molecular landscapes, immunologic factors, epigenetic influences, and environmental exposures to assess treatment response. However, challenges remain related to diagnostic access, the slow pace of biomarker identification, technological limitations, sustained patient engagement, data management, and computational requirements. Nevertheless, continued efforts to improve understanding of disease pathophysiology, gene expression, and immune regulation—together with the application of novel technologies and machine learning—may advance PM-based therapies. Additional opportunities include drug–target modeling and exploratory single-cell–based approaches to clarify patient-specific therapeutic mechanisms. This review briefly introduces the potential of PM for type 1 diabetes, rheumatoid arthritis, and multiple sclerosis.

Keywords: Autoimmune diseases; Multiple sclerosis; Personalized medicine; Rheumatoid arthritis; Type 1 diabetes

Introduction

Autoimmune diseases (ADs) represent an important and growing global health burden. In these conditions, the immune system, rather than protecting the body, inappropriately damages healthy tissues, leading to diverse clinical phenotypes. The complexity and

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heterogeneity of ADs pose major challenges to effective treatment. Despite advances in medical science, many patients experience little to no therapeutic benefit. The incidence of ADs is increasing, and genetic predisposition, environmental exposures, and other unidentified factors likely contribute. Key environmental factors associated with ADs include lifestyle, nutrition, air pollution, vector-borne diseases, toxin exposures, and chronic stress [1,2]. Approximately 4% of the global population is estimated to have one of more than 80 distinct ADs, underscoring the need for safer therapies that are better targeted to individual disease mechanisms. The limitations of single-therapy approaches further support the need for personalized treatment strategies. In addition, the absence of dependable predictive markers at initiation of AD therapy contributes to uncertainty in treatment response. Given interpatient variability in signaling pathways, individualized treatment plans are warranted. The Personalized Medicine Coalition describes personalized medicine as an emerging area that uses diagnostic tests to identify the most appropriate medical therapy for each patient to maximize outcomes [3]. Proponents of personalized medicine emphasize tailoring treatment strategies to each patient's genetic, epigenomic, and proteomic profiles [4]. This approach departs from the traditional "one-size-fits-all" model and aims to improve the effectiveness of preventive and therapeutic interventions. The core principle of personalized medicine is the delivery of individualized interventions. By incorporating a patient's genetic makeup and personal context, personalized medicine seeks to move beyond trial-and-error clinical decision-making. This approach may reduce adverse effects and improve treatment outcomes [5]. The concept of personalized medicine gained traction in the work of Boguski et al. [6], which emphasized understanding disease etiology, identifying causative factors, and targeting key mechanisms for more effective management. This framework was further strengthened by the National Research Council of the US National Academies, which emphasized integrating genetic data with clinical information to reclassify diseases and enable more precise treatments. Their emphasis on developing data platforms underscores the importance of linking genetic data with clinical observations, which remains a cornerstone of the personalized medicine framework [7]. In recent years, the term precision medicine has sometimes been used interchangeably with personalized medicine [8]. However, because personalized medicine also reflects patient–clinician tailoring at the individual level, the term personalized medicine may be more appropriate in this context [9,10]. Access to high-quality genomic and transcriptomic data can improve the quality of personalized medicine–based therapies [11]. The potential of personalized medicine is particularly promising for ADs, which arise from complex interactions between genetic and environmental factors. Although biologics have enabled more targeted approaches, treatment response remains highly heterogeneous across patients. Personalized medicine may help address these limitations by supporting more precise and effective therapies for ADs [12,13]. In general, patients with autoimmune dysfunction may share similar symptoms despite different underlying immunogenic mechanisms [14]. Personalized medicine aims to characterize environmental and immunologic context, epigenetic influences, and genetic and molecular landscapes that shape treatment response. The aim of this review is to summarize the role of personalized medicine as a therapeutic approach in major ADs, including multiple sclerosis (MS), rheumatoid arthritis (RA), and type 1 diabetes (T1D).

HIGHLIGHTS

- Autoimmune diseases are initiated by immune system dysfunction, leading to inappropriate attacks on healthy tissue.
- Personalized medicine presents an opportunity for more effective therapies.
- Personalized medicine uses diagnostic assessments to tailor treatment.

An Overview of ADs

Autoimmunity, defined as the presence of self-reactive antibodies or T lymphocytes, occurs when the immune system mistakenly targets normal host components. The immune system is designed to defend against infectious agents, but its complexity can also lead to pathology. These pathologies can take 2 broad forms: immunodeficiency syndromes, in which the immune system fails to respond adequately to pathogens, and ADs, in which the immune system mistakenly attacks host tissues. A hallmark of ADs is a breakdown of immune tolerance that prevents the immune system from distinguishing self from non-self [15]. The global frequency of ADs is increasing and currently affects approximately 4% of the population, spanning more than 80 distinct disorders. Although the mechanisms contributing to AD development remain incompletely understood, there is growing consensus that ADs result from interactions between genetic risk and environmental exposures [16,17].

Genetic Factors Affecting ADs

Although ADs share some broad patterns (including increasing prevalence with age), they remain highly heterogeneous. ADs vary in etiology, clinical features, age at onset, sex distribution, geographic patterns, genetic architecture, and ethnic predisposition. Compounding these challenges, the cause of many ADs remains unknown. Further investigation of disease-specific biological mechanisms is necessary to identify and develop effective, targeted therapeutic interventions [18]. Immune dysregulation also contributes to AD progression, including altered Th1/Th2 balance and cytokine production [19–21]. Genetic factors implicated in ADs—including those discussed here for T1D, RA, and MS—are summarized in Figure 1.

Role of genetic factors in T1D

T1D is a major global public health concern and accounts for approximately 10% of diabetes cases [22]. The etiopathogenesis of T1D is complex and involves genetic and environmental factors. Susceptibility to T1D is partly hereditary, supported by well-established genetic risk at human leukocyte antigen (HLA) loci—particularly DR4, DQ8, and DQ2 [23–25]. The presence of a single islet autoantibody confers a moderate risk of developing T1D, whereas the presence of additional autoantibodies increases risk substantially [24]. Autoantigens are presented by major histocompatibility complex (MHC) class I and II molecules (HLA) on antigen-presenting cells (APCs) to autoreactive T cells, contributing to T1D development [24]. T1D is characterized by loss of pancreatic β-cells responsible for insulin production. Evidence supporting its autoimmune nature includes: (1) inflammatory infiltrates (insulitis) within islets, (2) strong associations with specific MHC alleles, and (3) the presence of autoantibodies targeting islet-cell autoantigens [26]. Genetic, epigenetic, and environmental factors contribute to disease manifestation, and more than 60 genes have been implicated in T1D susceptibility [27,28]. Approximately 30%–50% of T1D genetic risk is attributed to HLA class II alleles [28]. Reported genetic risk factors include HLA-DR3/DQ2, HLA-DR4/DQ8, HLA-A*02:01, variants in protein tyrosine phosphatase non-receptor type 22 (PTPN22), insulin (INS) polymorphisms, interleukin-2 receptor subunit alpha (IL2RA) variants, and increased expression associated with a common IFIH1 variant [29,30]. Individuals heterozygous for HLA-DRB1*04 and HLA-DRB1*03 are at particularly high risk [31,32]. Although

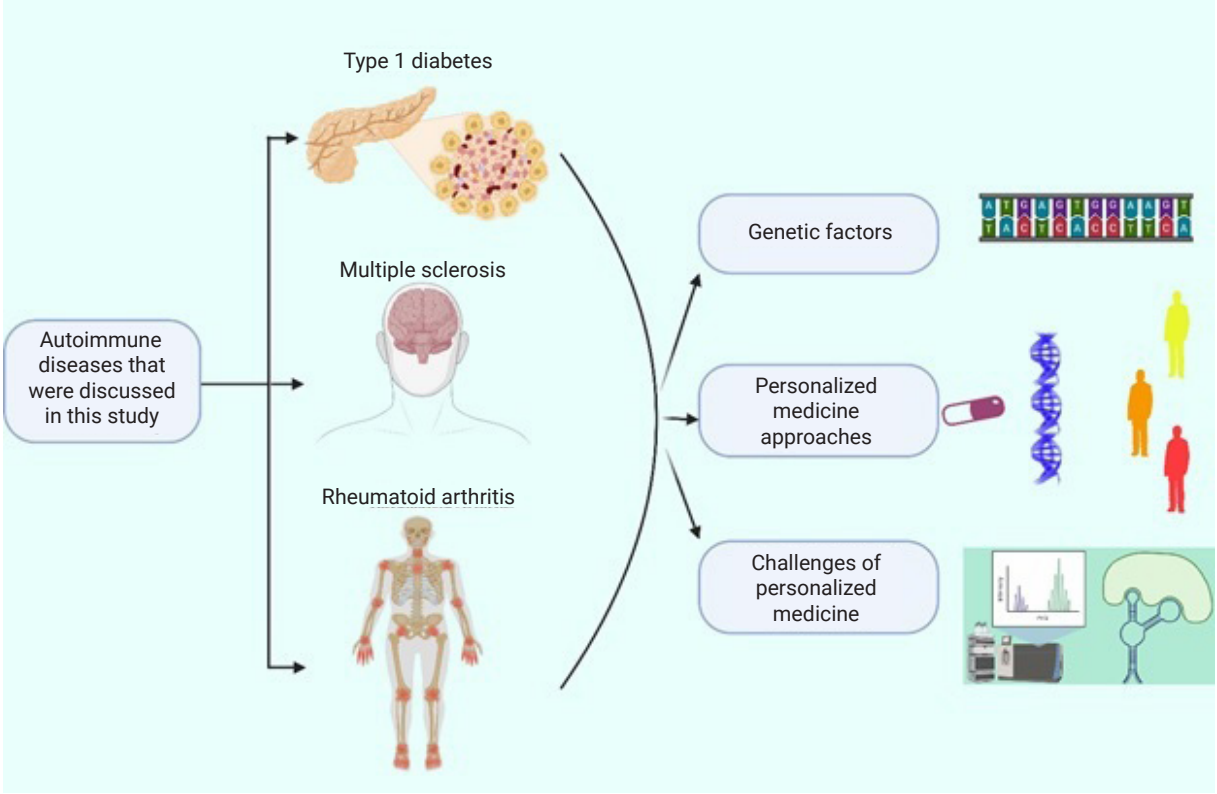


Figure 1. Autoimmune diseases discussed in this study including type 1 diabetes, multiple sclerosis and rheumatoid arthritis. Genetic factors affecting personalized medicine and personalized medicine approaches and challenges have been also included.

https://doi.org/10.24171/j.phrp.2025.0430

genetic heterogeneity and modest effect sizes for individual variants present challenges, these approaches have improved understanding of T1D biology and have informed risk prediction, prevention, and targeted therapeutics [30,31]. The HLA region on chromosome 6p21 is a major contributor to T1D risk [33,34] and accounts for approximately 50% of familial aggregation [35]. A recent study reported that DR3 homozygous carriers of HLA-DRB3*02:02 were at significantly higher risk of developing T1D than DR3 homozygous carriers of HLA-DRB3*01:01 [35]. Siblings with the high-risk DR3/DR4-DQ8 genotype who share both haplotypes with their probands face an estimated 85% risk of T1D by age 15 years [36]. Associations between HLA and T1D likely reflect polymorphisms that influence peptide binding and the repertoire of antigens presented to T cells [37]. PTPN22, located on chromosome 1p13 and encoding lymphoid tyrosine phosphatase, a negative regulator of T-cell activation, has also been associated with T1D [38]. Mechanisms of T1D progression are depicted in Figure 2.

Role of genetic factors in RA

RA is a polygenic disease with a substantial genetic contribution (estimated heritability of approximately 60%) and is a systemic inflammatory disorder that primarily affects small joints of the hands and feet. RA is associated with reduced life expectancy (approximately 3–10 years), and family history can substantially increase RA risk [39]. RA has an estimated prevalence of approximately 1% in European populations [40]. Notably, approximately half of patients with RA have absent or low levels of synovial CD20+ B cells, which may contribute to heterogeneous response to rituximab [41]. HLA-DRB1 alleles represent the strongest genetic association with RA and account for at least 30% of the genetic contribution to the disorder [42]. Many associated alleles share a conserved 5–amino acid sequence known as the “shared epitope” [43]. The shared-epitope hypothesis proposes that these alleles contribute to RA pathogenesis through T-cell–mediated autoimmune responses [42]. Studies of RA heritability

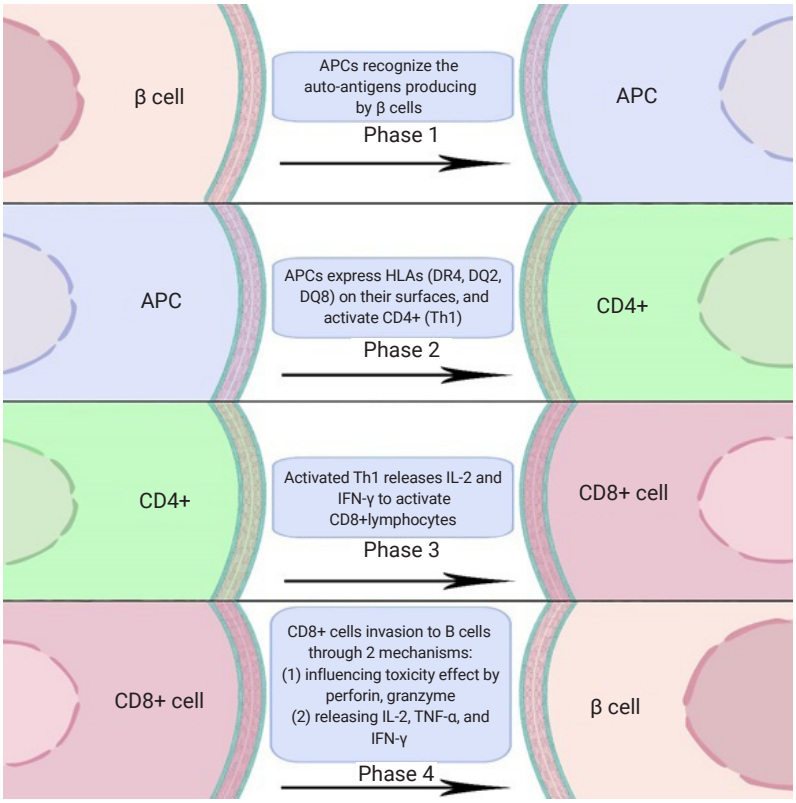


Figure 2. T1D pathway. Beta-cell proteins can serve as autoantigens once processed by antigen-presenting cells (APCs), such as macrophages and dendritic cells, and presented alongside major histocompatibility complex (MHC)-II molecules on the APC surface. This presentation triggers immunogenic signals activating CD4+ T cells, mainly from the Th1 subset. Activated Th1 cells release cytokines, including interleukin (IL)-2 and interferon (IFN)-γ, which facilitate the activation of macrophages and cytotoxic CD8+ T cells. These effector cells may eliminate islet beta cells through 2 mechanisms: (1) direct interaction between antigen-specific cytotoxic T cells and a beta-cell autoantigen-MHC-I complex, and (2) the involvement of non-specific inflammatory mediators like free radicals, oxidants, and cytokines (IL-1, tumor necrosis factor [TNF]-α, TNF-β, IFN-γ).

have also identified associations outside the HLA region, including PTPN22, cytotoxic T-lymphocyte–associated protein 4 (CTLA4), and peptidyl arginine deiminase 4 (PAD4) [44]. Among non-HLA loci, PTPN22 has been linked to RA susceptibility, and combined effects may account for a substantial proportion of genetic risk [45]. Key genes associated with RA susceptibility are summarized in Table 1 [46–49]. Mechanisms of RA progression are shown in Figure 3.

Role of genetic factors in MS

MS is a chronic inflammatory disease of the central nervous system characterized by demyelination and axonal degeneration. MS causes progressive neurologic impairment and affects more than 2 million people worldwide, posing substantial challenges for health systems and society. Understanding disease mechanisms and treatments is important for reducing disability and improving outcomes [50,51]. MS is influenced by both genetic and environmental factors [52]. The HLA gene cluster on the short arm of chromosome 6 (6p21) is the strongest genetic locus associated with MS among identified candidates [53]. With advances in molecular methods, the HLA-DR2 locus was refined to 2 molecular allotypes (HLA-DR15 and HLA-DR16), and subsequent work further localized association signals to HLA-DRB1*15 and later HLA-DRB1*15:01 [53,54]. Early association studies in MS were often underpowered and did not consistently identify reproducible genetic associations beyond the HLA region [55]. It is now recognized that common complex diseases with heritable risk typically have a different genetic architecture than Mendelian disorders. The "common disease–common variant" (CDCV) hypothesis proposes that common diseases arise from multiple frequent variants, each conferring small effects on disease risk [56]. Applied to MS, this model suggests that many common variants contribute modestly to risk, alongside loci with larger effects such as HLA-DRB1*15:01 [57]. Genome-wide association studies (GWAS) have identified the primary genome-wide signal at the HLA-DRB1 locus within the MHC region on chromosome 6p21.3 [58]. Associations between HLA loci and MS risk have been described extensively in recent years [59,60].

Table 1. Important genes associated with rheumatoid arthritis susceptibility

Gene name	Protein product	References
HLA-DRB1	HLA class II histocompatibility antigen, DRB1 beta chain	[47]
HLA-DPB1	HLA class II histocompatibility antigen, DP beta 1 chain	[48]
HLA-DOA	HLA class II histocompatibility antigen, DO alpha chain	[49]
PAD4	Protein-arginine deiminase type 4	[47]
PTPN22	Tyrosine-protein phosphatase non-receptor type 22	[46]
CTLA4	Cytotoxic T-lymphocyte protein-4	[47]

HLA, human leukocyte antigen.

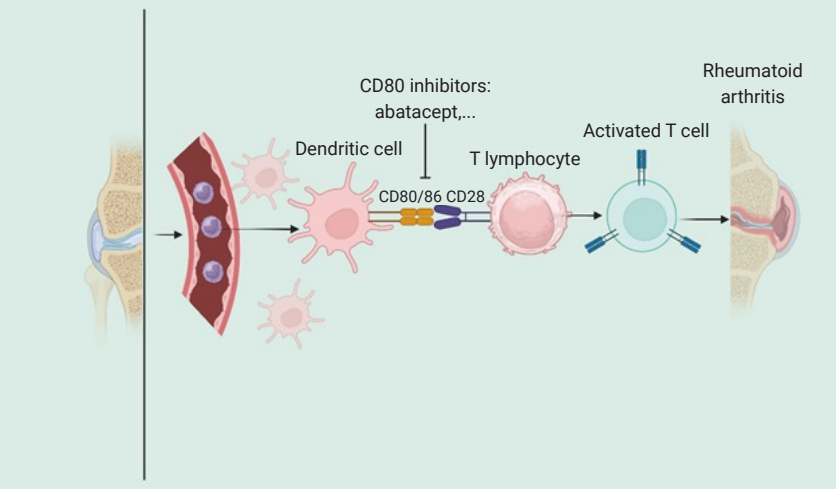


Figure 3. The process of T-cell activation through the interaction between CD80/86 on dendritic cells and the CD28 receptor on T lymphocytes, which plays a pivotal role in the development of rheumatoid arthritis. Dendritic cells, as antigen-presenting cells, present costimulatory signals via CD80 and CD86 that bind to CD28 on T cells, facilitating their activation. Once activated, T cells contribute to the inflammatory response characteristic of rheumatoid arthritis. The diagram also highlights the therapeutic role of CD80/86 inhibitors, such as abatacept, which block this costimulatory pathway, thereby mitigating T-cell activation and reducing inflammation in rheumatoid arthritis.

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Personalized Medicine in T1D

Personalized medicine approaches T1D through individualized care plans for treatment delivery and long-term disease management [61,62]. The personalized medicine framework is often described in 4 stages. The first stage is prediction: identifying individuals who are genetically and immunologically predisposed to developing T1D. The second stage is prevention: implementing interventions that delay or prevent onset (e.g., immunomodulation). The third stage is diagnosis: using biomarkers for early detection. The fourth stage is treatment: individualizing insulin regimens and adding adjunctive therapies based on a person's response profile. Sequential implementation of these stages may improve outcomes, reduce T1D-related complications, and shift management from reactive care toward individualized prevention. Uniform "one-size-fits-all" approaches are often inadequate given interpatient heterogeneity [63]. Genetic factors are central to T1D risk and may also influence response to therapy [64]. Incorporating patient feedback can support informed decisions regarding exercise and diet. Advances in personalized strategies rely, in part, on improved understanding of genetic pathways and may facilitate development of treatments tailored to an individual's genetic profile [65]. Personalized medicine may improve quality of life for populations affected by metabolic disorders, supported by advances in genetics [66]. Biomarkers are important in diabetes for identifying individuals at risk and monitoring disease progression. Glycemic biomarkers such as hemoglobin A1c are essential for assessing long-term glycemic control [67]. Beta-cell function measures are also used to predict progression in type 2 diabetes [68]. Machine learning methods may enable the extraction of actionable patterns from complex datasets to support tailored therapy [67]. Genetic factors and beta-cell function play important roles in diabetes prediction and treatment [69]. Patient age, disease duration, and comorbid cardiovascular or kidney disease also influence management decisions. Recent work has supported the feasibility of individualized glycemic targets in diabetes management [69]. Pharmacogenomics evaluates how genetic variation affects responses to medications and may help predict reactions to specific drugs [70]. Personalized lifestyle interventions are another relevant factor. Clinical plans that incorporate clinical variables, genetics, and lifestyle may optimize therapy selection. Telemedicine may also support diabetes diagnosis and management [71]. HLA typing for T1D risk prediction is a well-established approach. Certain HLA class II haplotypes (e.g., HLA-DR3-DQ2 and HLA-DR4-DQ8) are strongly associated with T1D susceptibility. When incorporated into newborn screening programs, HLA genotyping can help stratify individuals into risk categories. HLA risk profiles combined with immunologic biomarkers (e.g., islet autoantibodies) can facilitate earlier monitoring, trial enrollment, and preclinical intervention before overt symptoms. This provides a clear example of how genomic data can be used to predict disease risk prior to clinical onset.

While T1D is primarily defined by autoimmune destruction of pancreatic β-cells, there is increasing evidence that genetic variation contributes to interindividual responses to insulin therapy, complicating treatment decisions and adding complexity to personalized medicine strategies. Several loci associated with type 2 diabetes and metabolic regulation—including peroxisome proliferator-activated receptor gamma (PPARG), transcription factor 7-like 2 (TCF7L2), insulin receptor (INSR) substrate 1 (IRS1), and FTO (fat mass and obesity-associated gene)—have been reported to explain differences in insulin sensitivity among individuals with T1D. These variants may influence peripheral insulin resistance and thereby affect basal insulin requirements, glucose variability, and overall glycemic control. In addition, polymorphisms affecting INSR signaling or hepatic glucose metabolism may influence insulin utilization and clearance, thereby affecting dose adjustment and treatment effectiveness.

Polymorphisms in cytokine-related genes (e.g., IL2RA and PTPN2) and innate immune factors have been reported to influence residual inflammation and β-cell survival, thereby modulating endogenous insulin production and long-term insulin needs. Some single-nucleotide polymorphisms (SNPs) may also influence autoimmune activity and, consequently, the rate of progression from partial remission to full dependence on exogenous insulin.

Genetic variability may affect insulin therapy response in T1D through effects on insulin sensitivity, immune processes, and metabolic handling. For example, nonsynonymous variants in INSR or IRS1 may reduce INSR signaling efficiency, contributing to insulin resistance even in patients with T1D. Polymorphisms in TCF7L2 or PPARG may alter glucose uptake and tissue responsiveness, influencing overall insulin requirements. On the immune side, variants in HLA and IL2RA, as well as variation in PTPN22, may influence autoimmune activity and preservation of residual β-cell function, thereby affecting glycemic variability and dependence on exogenous insulin. Additional variants in SLC22A1 (solute carrier family 22 member 1), which influences drug transport, or IGF2BP2 (insulin-like growth factor 2 mRNA-binding protein 2), a regulator of metabolism, may affect insulin absorption, clearance, and distribution. Collectively, these differences may contribute to interindividual variation in total daily insulin dose,

hypoglycemia risk, and response to insulin formulations. These principles underpin precision approaches that aim to optimize insulin selection, dosing, and potential adjunct therapies based on genetic and physiologic profiles to improve long-term outcomes in T1D.

Personalized Medicine in RA

Personalized medicine offers new opportunities for RA management [72]. As in T1D, interindividual pharmacogenetic variability can influence treatment response; understanding these factors can support more individualized protocols and potentially reduce adverse events [73,74]. In RA, a treat-to-target approach with tight disease control can help prevent joint damage and disability [75]. Targeted therapies—including inhibitors directed at pathways involving CD80 and CD20—are used in some clinical contexts to improve long-term outcomes. The presence or absence of anticitrullinated protein antibodies (ACPAs) is an important marker for defining RA subsets that differ in environmental risk factors, genetic susceptibility, disease history, and outcomes. Nonsteroidal anti-inflammatory drugs can be effective in conditions where cyclooxygenase pathways contribute to the clinical phenotype. Personalized medicine aims to identify more homogeneous patient subgroups in which specific pathogenic pathways drive disease manifestations. Diagnostic testing can support pathway identification and help tailor healthcare practices and therapeutic decisions to the individual patient. In this model, diagnostic tests are used to select the most appropriate therapies for each patient [76]. RA is characterized by swelling and joint pain that can progress to disability, and persistent synovial inflammation is a central feature. Disease-modifying antirheumatic drugs have substantially improved treatment outcomes [77]. Targeting synovitis is another strategy to achieve disease control [78]. Methotrexate in patients with undifferentiated arthritis may delay progression to established RA [79]. RA risk is higher in women than in men, likely reflecting effects of sex hormones on autoimmune processes [80]. Differences in drug response reflect a combination of environmental and genetic influences and have been explored in -omics studies (genomics, epigenetics, transcriptomics, and proteomics). Interactions between methotrexate and other medications may affect outcomes and warrant consideration when optimizing personalized medicine-based treatment [81]. SNPs that alter methotrexate metabolism may also contribute to adverse effects. Variants in SLC19A1 (reduced folate transporter), ABCB1 (P-glycoprotein efflux transporter), methylenetetrahydrofolate reductase (MTHFR), and AICAR transformylase (ATIC) have been associated with differences in therapeutic response [82]. Overall, personalized medicine may support dose optimization, earlier screening and diagnosis, and selection of appropriate biologic therapy.

Predictive markers are important for personalizing RA treatment by identifying patients most likely to benefit from specific therapies. Serologic markers such as rheumatoid factor and ACPA can indicate disease severity and may predict response to B-cell–targeted therapy such as rituximab. Elevated inflammatory markers, including C-reactive protein and erythrocyte sedimentation rate, reflect active inflammation and can support treatment-response assessment. Genetic factors, such as HLA-DRB1 shared-epitope alleles and polymorphisms in TNF or IL6R, may influence responses to TNF or IL-6 inhibitors. Molecular signatures, including cytokine profiles and synovial tissue phenotypes, may further refine predictions; for example, myeloid-rich synovium has been associated with better response to TNF inhibitors, whereas lymphoid-dominant synovium may predict benefit from IL-6 or B-cell–targeted therapies. High anti-drug antibody (ADA) levels with low drug concentrations may suggest immunogenic treatment failure and support switching biologic therapy, whereas low drug levels without ADAs may suggest underdosing and inform dose adjustment.

Personalized Medicine in the Treatment of MS

Personalized medicine in MS uses advanced approaches such as metabolomics, proteomics, genomics, and imaging to identify biomarkers and disease subtypes. Personalized approaches may include immune pathway modulators and targeted treatments relevant to MS pathogenesis. Overall, personalized medicine may help improve diagnosis, prognosis, and treatment outcomes, and targeted strategies may reduce the risk of adverse events [57]. Despite these promising developments, challenges remain, including the high cost of biomarker and subtype diagnostics, the need for sophisticated and frequently updated technologies, and time-consuming laboratory workflows that vary by disease stage and patient characteristics [58,59]. Diagnosis is a key phase of personalized medicine in MS [83]. Disease monitoring is also important for MS characterization [84]. Magnetic resonance imaging (MRI) is used to evaluate lesion extent and severity in the brain and spinal cord [85]. Biomarker panels incorporating epigenetic, genetic, proteomic, and metabolic factors can include assessment of genetic variants, DNA methylation, protein expression, and metabolic profiles [84]. Anti-CD20 antibodies such as ocrelizumab and rituximab reduce B-cell activity in MS [86]. Tailored therapy involves adapting treatment strategies based on individual factors,

https://doi.org/10.24171/j.phrp.2025.0430

including environmental exposures, lifestyle, and genetics [87]. Personalized medicine may improve outcomes, reduce adverse effects, and optimize healthcare resource utilization [83]. However, legal and ethical concerns remain, including the acquisition and handling of genetic information and other personal data [57]. Despite these challenges, progress in targeted therapies may drive new strategies and innovations in clinical practice. Combination therapy may offer higher efficacy than monotherapy in some contexts. Standardization of diagnostic criteria and treatment guidelines may also be required for effective implementation of personalized medicine in MS [57]. Gene expression signatures and transcriptomic profiling in MS—often using peripheral blood mononuclear cells—have been developed to identify molecular patterns that differentiate relapsing from progressive disease. Such signatures may help predict relapse activity and, in some scenarios, treatment response (e.g., to IFN-β or natalizumab). Gene expression–based classifiers represent an early step toward individualized treatment selection and may inform disease monitoring strategies. Although many signatures remain under validation, these approaches exemplify the potential of personalized medicine to improve prognostication and minimize ineffective treatment in complex AD. The authors emphasize the need to develop individualized management plans for MS that consider disease severity, comorbidities, and patient lifestyle and preferences when selecting treatment options [88,89]. An Italian Delphi consensus highlighted the importance of shared decision-making between patients and clinicians to maximize adherence and outcomes. The panel identified a trend toward more personalized approaches in MS care, aiming to improve efficacy and quality of life by tailoring therapies to clinical features and biomarker data. The panel also emphasized moving beyond a "one-size-fits-all" strategy and supported developing expert consensus to refine personalized medicine paradigms for MS care. The early initiation of high-efficacy disease-modifying therapies has been reported to improve outcomes in patients with highly active MS, as outlined in the expert consensus. The panel recommended tailoring treatment to disease activity and patient profile, including a more aggressive approach for highly active disease. Close monitoring of disease progression and timely escalation of therapy following clinical or radiologic evidence of disease activity were emphasized. MRI activity and relapse rates are expected to remain central to treatment decisions and may support a proactive, individualized approach to MS management [88,89].

Personalized Medicine Challenges in AD Therapy

RA is characterized by destructive arthritis driven by autoimmune mechanisms and remains a complex and heterogeneous disease. Inadequate treatment can lead to long-term disability, and a substantial proportion of patients may experience persistent disease activity over many years [90]. Biologic therapies are often more effective than some conventional options in RA, yet response varies considerably across individuals [86,91]. Many factors contribute to immunity and inflammation, including diverse immunocompetent cells and mediators (e.g., multiple cytokines), and personalized medicine approaches remain insufficiently standardized in this setting. For some biologics, studies have explored genetic correlates of treatment response; however, clear, clinically actionable mutations or polymorphisms have not been consistently identified. For example, predictive biomarkers for response to TNF-α inhibitors remain limited [83]. Despite established associations between HLA-DRB1 shared-epitope alleles and RA susceptibility, relationships with disease severity and clinical phenotype remain complex [92,93]. Many studies have reported associations between HLA-DRB1 and ACPA-positive RA, supporting consideration of HLA-DRB1 as a potential biomarker [94]. GWAS meta-analyses in rheumatic disorders suggest that major RA-associated polymorphisms often involve pathways targeted by current therapies, supporting meta-analysis as a useful approach for exploring novel drugs and biomarkers [11,46,95,96].

Despite the potential of personalized medicine in MS, implementation faces challenges such as difficulty identifying robust biomarkers and disease subtypes and the need for high-cost, advanced technologies [97]. In addition, developing targeted therapies can be lengthy and expensive, and treatment effectiveness may depend on disease stage and patient characteristics [98,99]. Key challenges in applying personalized medicine to AD therapy are summarized in Table 2.

Future Directions and Guidelines for Personalized Medicine on ADs

Future directions for personalized medicine in AD therapy should emphasize a deeper understanding of disease-specific pathophysiology, gene expression profiles, and immune regulation and dysregulation. Advanced technologies and machine learning may support the development of this knowledge base. Building databases, strengthening data-analytic capacity, and training physicians and nurses in key personalized medicine concepts may also

Table 2. Challenges of using personalized medicine in therapy for autoimmune diseases

Challenges	Summary of challenges
Challenges in classification and stratification	Many diagnostic tests are not easily accessible
	Many biomarkers utilized in distinguished "clusters" change over time
	Most methods utilized for clustering require variables to be distributed; this can have negative effects on the accuracy of the results
Challenges in prevention	Lack of proper technologies
	Motivation of patients can be challenging
Challenges in monitoring and management	Inadequate information on drug response in underrepresented racial and ethnic groups
	Difficulties with data storage and computational analysis
	Variability of ADs among races and sexes
	Lack of data for exact approaches
	Need for effective communication strategies when conveying complex or sensitive information to patients

AD, autoimmune disease.

support implementation. Integrating genetic profiles with proteomic and transcriptomic information may help uncover actionable targets for personalized therapy. Clinical translation of personalized approaches is essential for improving AD management. Single-cell analyses of tissues, including single-cell RNA sequencing, can contribute to disease characterization and target discovery. Machine learning may also facilitate identification of therapeutic targets through drug–disease modeling. Preventive medicine is another promising strategy for predicting disease development. The outlook for personalized medicine in ADs will likely build on integrating multi-omics data to guide individualized treatment (e.g., genomics, proteomics, and metabolomics). In addition to biomarker discovery, increasing use of artificial intelligence (AI) may support earlier diagnosis, disease stratification, and prediction of therapeutic response. Standardized guidelines that incorporate genetic, environmental, and lifestyle factors will likely be needed to optimize precision-based approaches. Ethical standards for data privacy and equitable access will also need to be addressed. Successful implementation will depend on collaboration among researchers, clinicians, and policymakers to translate personalized strategies into routine practice and improve outcomes while minimizing adverse effects in AD management.

AI is increasingly supporting precision medicine applications in ADs by enabling analysis of complex biomarker patterns. Machine learning algorithms can predict disease behavior and treatment responses using serologic markers, genetic variants, imaging, and clinical history, potentially surpassing traditional approaches in some settings. For example, in RA, AI-based methods have been used to identify biomarker signatures associated with response to TNF or IL-6 inhibitors and to support early diagnosis or molecular subtyping using synovial tissue samples. AI may also support therapeutic drug monitoring by identifying patterns in drug concentrations and ADA development to optimize biologic therapy. More broadly, AI tools may accelerate biomarker discovery, automate imaging scoring (e.g., ultrasound or MRI) for inflammatory activity, and support precision pathways in conditions such as lupus, psoriasis, and inflammatory bowel disease. Through these capabilities, AI can help transform large, heterogeneous datasets into clinically useful information to improve individualized care.

Therefore, the future of personalized medicine in AD treatment will increasingly depend on integrative data analytics that combine genomics, transcriptomics, proteomics, metabolomics, microbiome-related data, and real-world clinical data to generate multidimensional disease signatures. Continued advances in machine learning and AI may enable predictive models that better capture disease heterogeneity, forecast therapeutic response, and identify patient subgroups more likely to benefit from specific interventions. This direction supports a shift from single-biomarker strategies toward system-level precision therapeutics. As large-scale genetic and molecular datasets expand, ethical considerations surrounding genetic data use become increasingly important. Key issues include data privacy, risks of re-identification, responsible data sharing, transparency of consent processes, and potential for insurance or employment discrimination. Strong governance frameworks will be needed to sustain patient trust and support the integration of personalized medicine technologies into clinical care. Ensuring equitable access to personalized medicine worldwide is also a major challenge. Many available genomic and transcriptomic datasets disproportionately represent individuals of European ancestry, which can limit generalizability to diverse populations. In addition, unequal

https://doi.org/10.24171/j.phrp.2025.0430

access to laboratory infrastructure, digital health capacity, and costly therapies may constrain implementation in resource-limited settings.

Conclusion

To improve the effectiveness of personalized medicine in ADs, there is a need for stratification techniques and molecular profiling technologies to improve response to treatment. Furthermore, additional cellular and molecular subgroups remain to be identified. More trials and longitudinal outcomes investigations are also needed for therapy optimization in subgroups. In ADs such as T1D, RA, and MS, personalized medicine has exhibited heterogeneous outcomes mainly due to disease types and insufficient data on individual molecular targets. Several further challenges persist, such as the inaccessibility of various diagnostic tests, gradual identification of biomarkers over time, lack of proper technologies, patient engagement, and inadequacies in data collection and processing and computational analyses. The expanding understanding of the disease pathophysiology, gene expression profiles, immune regulation and dysfunction, novel technologies, and machine learning (omics-based stratification) will improve the effectiveness of personalized medicine. Drug-target modeling and single-cell analyses will also contribute to improving therapeutic outcomes.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
No underlying data was collected or produced in this study.

Authors' Contributions
Conceptualization: SM, AG; Data curation: SM, AG; Formal analysis: SM, AG, MNG, YF; Investigation: MNG, YF; Methodology: SM, AG, MNG, YF; Project administration: SM, AG; Resources: MNG, YF; Supervision: SM, AG; Validation: SM, AG; Visualization: SM, AG, MNG, YF; Writing–original draft: SM, AG, MNG, YF; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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https://doi.org/10.24171/j.phrp.2025.0430

phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0447
Osong Public Health Res Perspect 2026;17(3):229-241

Original Article

Modeling social and behavioral factors associated with community participation in community-based surveillance of communicable diseases: a cross-sectional study in Kelantan, Malaysia

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ABSTRACT

Objectives: This study examined the social and behavioral factors influencing community leaders' participation in community-based surveillance (CBS) of infectious diseases. We hypothesized that knowledge, attitudes, and perceptions significantly predict the behavioral likelihood of engaging in CBS activities.

Methods: A cross-sectional study was conducted among 470 schoolteachers in Kelantan, Malaysia, from March to June 2024. Participants were selected using multistage sampling, with random selection of schools followed by convenience sampling of teachers. Data were collected using the validated KAP-CBS-ID questionnaire, which was based on the Theory of Reasoned Action and Health Belief Model. Structural equation modeling was used to analyze relationships among the study variables with the maximum likelihood robust estimator.

Results: The final model demonstrated good fit (comparative fit index, 0.921; standardized root mean square residual, 0.071; root mean square error of approximation, 0.040 [90% confidence interval, 0.036–0.044]). Ten significant path relationships were identified, explaining 46.1% of the variance in behavioral likelihood. Knowledge of infectious diseases (β = 0.419, $p < 0.001$), subjective norms (β = 0.235, $p = 0.038$), and negative attitudes (β = −0.432, $p < 0.001$) significantly influenced intention. Behavioral likelihood was directly predicted by intention (β = 0.347, $p < 0.001$), perceived susceptibility (β = 0.310, $p < 0.001$), perceived benefits (β = 0.198, $p = 0.001$), and perceived barriers (β = −0.132, $p = 0.008$). Self-efficacy showed significant indirect effects through perceived benefits (β = 0.785, $p < 0.001$) and perceived susceptibility (β = 0.765, $p < 0.001$).

Conclusion: Knowledge and psychosocial factors significantly influence community engagement in CBS of infectious diseases. Public health interventions should address these interconnected determinants to strengthen early disease detection and reporting systems.

Keywords: Communicable diseases; Community participation; Health Belief Model; Public health surveillance; Theory of Planned Behavior

Received: October 22, 2025
Revised: December 9, 2025
Accepted: March 24, 2026

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The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Korea Disease Control and Prevention Agency and Korea National Institute of Health.

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Introduction

Background/Rationale

Infectious diseases continue to pose major global public health threats, with emerging and re-emerging diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome, severe acute respiratory syndrome, H1N1 influenza, and coronavirus disease 2019 (COVID-19) causing substantial morbidity and mortality [1,2]. The rise of antimicrobial resistance further compounds these threats by complicating treatment and control efforts [2]. Without early detection, infectious diseases can rapidly escalate from localized outbreaks to epidemics or pandemics [3]. Because these diseases often originate within communities, community members are typically the first to recognize changes in local health patterns. Timely reporting of such changes to health authorities enables prompt intervention and may help contain outbreaks before they become unmanageable.

Community-based surveillance (CBS) has emerged as a critical strategy for the early detection and reporting of health events [4]. The World Health Organization defines CBS as "the systematic detection and reporting of events of public health significance within a community by the community members" [5]. Successful CBS systems depend on community willingness to participate and on participatory engagement principles, including acceptability, collaboration, communication, local ownership, and trust [6]. Key informants—individuals with extensive community connections, such as schoolteachers, village leaders, traditional healers, and religious leaders—serve as primary sources of health signals, thereby reducing noise while maintaining sensitivity [7]. However, previous studies have documented several barriers to effective community engagement in CBS, including limited awareness, low trust in health systems, cultural beliefs, time constraints, and inadequate feedback mechanisms [8,9]. These barriers underscore the need to systematically examine the psychological and social factors that influence participation.

In Malaysia, delays in reporting health events to the authorities have persisted, with events often being reported within communities or by the media before health officials are informed [10]. These delays hinder timely intervention and increase the risk of outbreaks. Malaysia presents several unique challenges for CBS implementation, including a diverse multiethnic population, varying levels of health literacy across rural and urban areas, and a dual healthcare system comprising both public and private sectors. In addition, the country's tropical climate and biodiversity create favorable conditions for the emergence of vector-borne and zoonotic diseases, making early community detection particularly important [11]. Limited community participation in health event reporting suggests a need to better understand the underlying factors.

HIGHLIGHTS

- Knowledge of infectious diseases, subjective norms, and attitudes significantly predict intention to participate in community-based surveillance (CBS) activities.
- Behavioral likelihood of engaging in CBS is directly influenced by intention, perceived susceptibility, perceived benefits, and perceived barriers.
- Self-efficacy operates indirectly through perceived benefits and perceived susceptibility rather than directly affecting CBS participation.
- The integrated theoretical model explains 46.1% of the variance in behavioral likelihood, demonstrating the multidimensional nature of CBS participation.

Although the importance of CBS is increasingly recognized, substantial gaps remain in understanding the behavioral determinants of community participation, particularly in Southeast Asian settings. Although previous studies have examined challenges in CBS implementation [12], few have systematically investigated the interplay among knowledge, attitudes, and perceptions using integrated theoretical frameworks. Furthermore, the mechanisms through which psychosocial factors influence participation intentions and engagement behaviors remain poorly understood. Several psychological theories have been developed to understand and predict health behaviors, including the Health Belief Model (HBM) [13], the Theory of Reasoned Action (TRA) [14], and the Theory of Planned Behavior (TPB) [15]. These theories identify factors that influence intentions and behaviors related to specific health actions. Recent studies in Southeast Asia have demonstrated the applicability of these frameworks to health behaviors related to disease prevention and surveillance [16], yet their integration in CBS contexts remains limited. In this study, we integrated the TRA and HBM to evaluate factors associated with community participation in CBS, with a focus on schoolteachers as community leaders. The study used a validated questionnaire comprising 3 domains: knowledge (infectious diseases, CBS, and community-level case definitions), attitudes (based on the TRA, including subjective norms, negative attitudes, intention to participate, and behavioral likelihood), and perceptions (based on the HBM, including perceived benefits, perceived barriers, perceived susceptibility, and self-efficacy) [17].

https://doi.org/10.24171/j.phrp.2025.0447

Objectives

This study aimed to examine the interrelationships among social and health behavioral factors and their influence on the behavioral likelihood that community leaders would engage in CBS activities. Specifically, we hypothesized that (1) knowledge, subjective norms, and attitudes influence intention to participate in CBS; (2) intention, perceived benefits, perceived barriers, perceived susceptibility, and self-efficacy influence the behavioral likelihood of engaging in CBS; and (3) these factors have both direct and indirect effects on CBS participation. The findings are expected to inform the development of evidence-based educational programs and policy interventions to strengthen CBS implementation in Malaysia and similar settings, particularly through targeted training curricula for community leaders and strategic approaches to addressing identified barriers.

Materials and Methods

Study Design and Setting

A cross-sectional study design was used. Although this design allows efficient examination of relationships among multiple variables, causal inferences cannot be drawn from cross-sectional data, and temporal relationships among knowledge, attitudes, perceptions, and behavioral likelihood cannot be established definitively. The study was conducted in Kelantan, Malaysia, from March to June 2024. Four districts were selected: Kota Bharu and Bachok represented urban areas, whereas Kuala Krai and Pasir Puteh represented rural areas. Data were collected in both Islamic and public schools across these districts. Teachers in both school types serve as respected community leaders and regularly interact with families and community members, making them valuable key informants for CBS. Including both school types allowed representation of different educational structures and community engagement patterns that may influence CBS participation.

Participants

Participants were schoolteachers from Islamic and public schools in Kelantan. Schools were randomly selected from each district, whereas teachers within each school were selected by convenience sampling based on their availability during researcher visits. This approach was adopted for logistical feasibility because teachers' availability varied considerably according to teaching schedules, administrative duties, and school activities. Although this method facilitated implementation and may have improved response rates, it may also have introduced selection bias, as participating teachers could have differed systematically from non-participating teachers in motivation, interest in health topics, or available time. This potential bias should be considered when interpreting the generalizability of the findings to the broader teacher population.

Eligibility criteria were as follows: (1) active employment as a teacher in a selected school, (2) willingness to participate voluntarily, (3) provision of informed consent, and (4) completion of the questionnaire. Teachers who were absent during data collection or declined participation were excluded.

Participants who met the inclusion criteria were briefed on the study, and both verbal and written informed consent were obtained before questionnaire completion. Schools involved in earlier stages of the study, including pilot testing and exploratory factor analysis, were excluded from this phase to minimize response bias.

Variables

Outcome variables

- Intention to participate in CBS (INT): motivation and conscious decision to engage in CBS behaviors
- Behavioral likelihood of engaging in CBS (BL): probability of actual CBS engagement

Predictor variables

- Knowledge of infectious diseases (KID1): understanding of transmission, prevention, and control measures
- Knowledge of CBS (KID2): awareness of CBS concepts and reporting systems
- Community-level case definition (KID3): ability to recognize reportable disease signals
- Negative attitudes (ATT): unfavorable beliefs about CBS activities
- Subjective norms (SN): perceived social pressure from influential others
- Perceived susceptibility (SUS): beliefs about disease likelihood without CBS participation
- Perceived benefits (BEN): beliefs about the effectiveness of CBS in disease prevention
- Perceived barriers (BARR): obstacles to CBS participation
- Self-efficacy (SE): confidence in performing CBS activities

Data Sources/Measurement

Data were collected using the validated KAP-CBS-ID questionnaire [17,18], a self-administered instrument comprising 3 main sections. The questionnaire was originally developed in English and underwent rigorous cultural adaptation and validation for the Malaysian context, including forward and backward translation by bilingual experts, cognitive

interviewing with representatives of the target population, and pilot testing to ensure cultural appropriateness and comprehensibility [17].

Knowledge domains

- Knowledge of infectious diseases (KID1): 18 dichotomous items assessing understanding of infectious diseases
- Knowledge of CBS (KID2): 3 dichotomous items measuring awareness of CBS
- Community-level case definition (KID3): 10 dichotomous items assessing the ability to recognize reportable signals

Attitudes domains (TRA-based)

- Negative attitudes (ATT): 6 items on a 5-point Likert scale (5 items retained after factor analysis)
- Subjective norms (SN): 4 items on a 5-point Likert scale
- Intention to participate (INT): 6 items on a 5-point Likert scale
- Behavioral likelihood (BL): 7 items on a 5-point Likert scale (6 items retained after factor analysis)

Perception domains (HBM-based)

- Perceived susceptibility (SUS): 5 items on a 5-point Likert scale
- Perceived benefits (BEN): 5 items on a 5-point Likert scale
- Perceived barriers (BARR): 3 items on a 5-point Likert scale (reduced from 7 after confirmatory factor analysis [CFA])
- Self-efficacy (SE): 4 items on a 5-point Likert scale

The questionnaire underwent rigorous validation, including content validation by 11 experts in public health and epidemiology, face validation, pilot study with 30 participants, exploratory factor analysis, and CFA.

Bias

To minimize selection bias, schools were randomly selected from each district. To reduce response bias, researchers emphasized confidentiality and encouraged honest responses during briefing sessions. Social desirability bias was addressed by including reverse-coded items and ensuring anonymous responses. However, residual bias related to self-report may have remained despite these measures, as participants may still have provided socially desirable responses or had limited insight into their own attitudes and behaviors. Schools involved in the pilot testing and exploratory factor analysis phases were excluded to prevent familiarity bias.

Study Size

Sample size was determined using 2 methods. First, applying the rule of 5 participants per item (N:P = 5:1) to the 75-item questionnaire yielded a required sample of 375 participants. After accounting for an anticipated dropout rate of 20%, the adjusted sample size was 468 using Arifin's formula [19]:

$$Sample\ size + dropouts = \frac{Calculated\ sample\ size}{1 - Proportion\ of\ dropouts}$$

$$= \frac{357}{1 - 0.2} = 468.7$$

sample size with dropouts = (calculated sample size)/(1–proportion of dropouts) = 375/(1–0.2) = 468.7. Second, Arifin's web-based calculator (2017) estimated a required sample of 463 participants [20]. On the basis of these calculations, 470 participants were recruited. According to Kline [21], although 200 participants would generally suffice, the required sample size depends on model complexity. The final sample of 470 exceeded the minimum requirement for structural equation modeling (SEM) as recommended by Hair et al. [22]. SEM was selected because it allows simultaneous examination of multiple interrelated pathways among constructs, accounts for measurement error, and enables testing of both direct and indirect effects within a single comprehensive model. Given the complexity of the hypothesized relationships among knowledge, attitudes, perceptions, intentions, and behaviors, SEM was more appropriate than traditional regression approaches.

Quantitative Variables

Knowledge items were scored dichotomously (correct = 1, incorrect = 0). Items from the 3 knowledge scales were grouped into 3 parcels for SEM analysis to achieve a more parsimonious model and more stable parameter estimates [23]. The 3 knowledge domains (KID1, infectious disease knowledge; KID2, CBS knowledge; and KID3, community-level case definitions) were modeled as a single second-order latent construct representing overall knowledge because they theoretically reflect different facets of the comprehensive knowledge base required for effective CBS participation. This approach is consistent with hierarchical conceptualizations of health knowledge and reduces model complexity while maintaining theoretical coherence. The 3 parcels (PK1, PK2, and PK3) were formed by assigning items from each respective knowledge domain: PK1 comprised items from KID1, PK2 comprised items from KID2, and PK3 comprised items from KID3, thereby preserving the domain-specific content within each parcel. Attitude and perception items were measured on 5-point Likert scales (1 = strongly disagree to 5 = strongly agree). Reverse-coded items were recoded as appropriate. Factor scores were computed for each construct on the basis of the CFA results.

https://doi.org/10.24171/j.phrp.2025.0447

Statistical Methods

Descriptive statistics were generated using JAMOVI version 2.4.11 (https://www.jamovi.org/). Numerical variables were summarized as means and standard deviations (SDs), whereas categorical variables were summarized as frequencies and percentages.

CFA was conducted in R within the RStudio (R Foundation for Statistical Computing) integrated development environment to validate the measurement model. Model fit was evaluated using the following criteria: comparative fit index (CFI) >0.92, Tucker-Lewis index (TLI) >0.92, root mean square error of approximation (RMSEA) <0.07, and standardized root mean square residual (SRMR) <0.08 [22].

Structural equation modeling was performed using Mplus version 7.4 (Muthen & Muthen) to examine interrelationships among the study variables. Multivariate normality was assessed using Mardia's test. Because the assumption of multivariate normality was not met, the maximum likelihood robust (MLR) estimator was used, as proposed by Yuan and Bentler [24]. MLR was selected because it provides robust standard errors and chi-square test statistics corrected for non-normality, making it appropriate when data violate the assumption of multivariate normality. Unlike standard maximum likelihood estimation, MLR yields accurate parameter estimates and valid inference even with nonnormal data, which are common in social and behavioral research using Likert-scale measures.

The initial hypothesized model included 11 variables: KID1, KID2, KID3, ATT, SN, INT, BL, BARR, BEN, SUS, and SE. We hypothesized that KID1–KID3, SN, and ATT would influence INT, whereas INT, BARR, BEN, SUS, and SE would influence BL, as guided by the TRA and HBM (Figure 1). Model modifications were made on the basis of modification indices and theoretical justification. Error correlations were added between items within the same factors only when the modification indices suggested substantial improvement and when a theoretical rationale supported shared method variance or conceptual overlap. All modifications were theory-driven to ensure that the final model remained interpretable and theoretically coherent rather than simply achieving optimal statistical fit.

Direct, indirect, and total effects were calculated to examine mediation pathways. Statistical significance was set at $p < 0.05$ (2-tailed).

Ethics Statement

The study protocol was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (Reference No: USM/JEPeM/22050317). Participants were briefed about the study objectives, procedures, and their rights. Written informed consent was obtained from all participants prior to data collection. Participation was voluntary, and

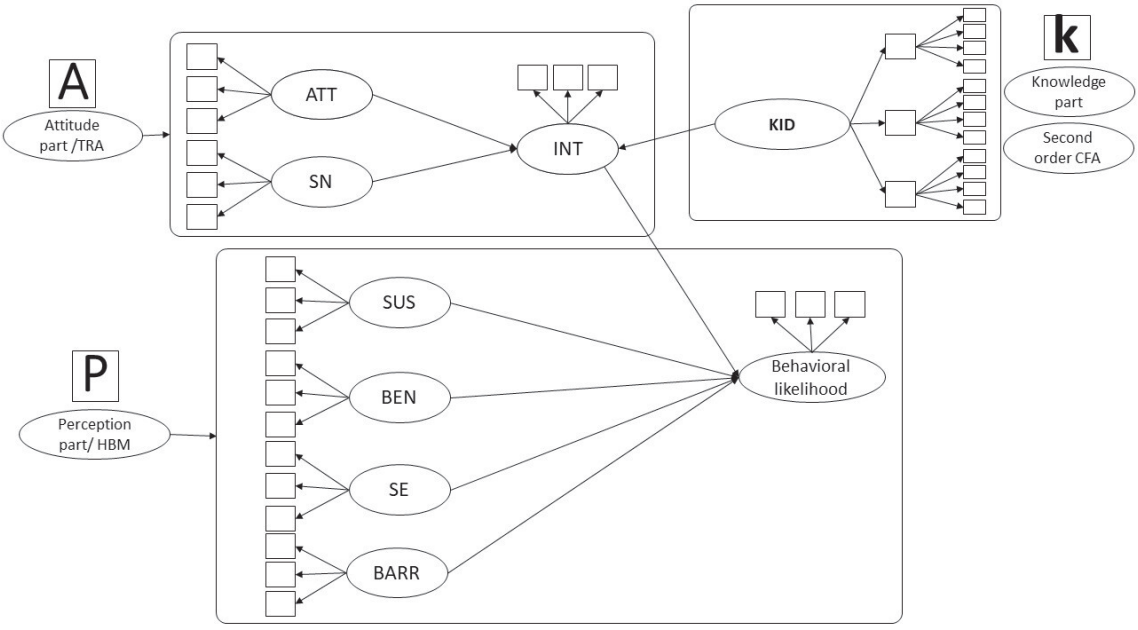


Figure 1. Proposed structural framework based on the Theory of Reasoned Action (TRA) and Health Belief Model (HBM) used in this study.

SN, subjective norms; ATT, negative attitudes; INT, intention to participate in community-based surveillance; KID, knowledge of infectious diseases; CFA, confirmatory factor analysis; SUS, perceived susceptibility; BEN, perceived benefits; SE, self-efficacy; BARR, perceived barriers.

participants were assured of confidentiality and anonymity. All completed questionnaires and electronic data files are stored securely in password-protected computers and locked filing cabinets accessible only to the research team. Data will be retained for 5 years following publication, after which they will be securely destroyed. No personal identifiers were collected, ensuring participant anonymity is maintained throughout data storage and analysis. Questionnaire completion required approximately 20–25 minutes.

Results

Participants

A total of 470 schoolteachers participated in the study. All 470 participants who completed the questionnaires were included in the analysis, with no loss to follow-up and no missing data requiring exclusion. Table 1 presents the demographic and background characteristics of the participants.

The mean age of the participants was 43.3 years (SD, 9.5). Most participants were female (59.6%, *n*=280), of Malay ethnicity (99.4%, *n*=467), and married (79.4%, *n*=373), and a large proportion were government servants (85.5%, *n*=402). Regarding educational attainment, 87.2% (*n*=410) had a university education or higher, reflecting the educational requirements for teaching positions. In terms of role, 52.8% (*n*=248) were Islamic schoolteachers and 47.2% (*n*=222) were public schoolteachers. In addition, 64.0% (*n*=301) resided in urban areas, whereas 36.0% (*n*=169) resided in rural areas. These demographic characteristics have several implications for CBS participation. The relatively high educational level may facilitate understanding of infectious disease concepts and accurate reporting. The predominance of government-employed teachers may reflect greater exposure to structured communication channels and public health messaging. The inclusion of both Islamic and public schoolteachers provides insight into participation across different educational environments, whereas the urban-rural distribution helps contextualize potential differences in access to health information and surveillance systems. Overall, these characteristics suggest a sample with a strong baseline capacity to engage in CBS activities effectively.

Table 1. Socio-demographic characteristics of community representatives (*n*=470)

Variable	Value
Age (y)	43.3±9.5
Sex	
Male	190 (40.4)
Female	280 (59.6)
Ethnicity	
Malay	467 (99.4)
Others	3 (0.6)
Marital status	
Single	73 (15.5)
Married	373 (79.4)
Divorced	6 (1.3)
Widowed	18 (3.8)
Occupation	
Government servant	402 (85.5)
Private sector	60 (12.8)
Pensioner	3 (0.6)
Other	5 (1.1)
Level of education	
Primary school	3 (0.6)
Secondary school (PMR/SRP)	2 (0.4)
Secondary school (SPM)	16 (3.4)
Religious school (pondok)	2 (0.4)
Diploma	37 (7.9)
University and higher education	410 (87.2)
Role of community leader	
Public schoolteacher	222 (47.2)
Islamic schoolteacher	248 (52.8)
Residency	
Rural	169 (36.0)
Urban	301 (64.0)

Data are presented as mean±standard deviation or *n* (%).
PMR, Penilaian Menengah Rendah (Lower Secondary Assessment); SRP, Sijil Rendah Pelajaran (Lower Certificate of Education); SPM, Sijil Pelajaran Malaysia (Malaysian Certificate of Education).

Descriptive Data

Table 2 presents the CFA results across all domains. In the knowledge section, information on infectious diseases showed excellent composite reliability (CR, 0.92) but slightly low convergent validity (average variance extracted [AVE]=0.45), whereas CBS knowledge (CR, 0.78; AVE, 0.58) and community-level case definition (CR, 0.93; AVE, 0.50) demonstrated adequate psychometric properties. In the attitude section, subjective norms showed the strongest performance (CR, 0.895; AVE, 0.69), followed by behavioral likelihood (CR, 0.860; AVE, 0.58). Intention (CR, 0.714; AVE, 0.31) and negative attitudes (CR, 0.546; AVE, 0.20) showed suboptimal convergent validity but were retained because of their theoretical importance. The lower convergent validity of intention and negative attitudes suggests that these constructs may capture somewhat heterogeneous aspects of attitudes and intentions related to CBS participation. This may reflect the multifaceted nature of behavioral intentions and the complexity of attitudes in real-world contexts. Although these metrics were below ideal thresholds, the constructs were retained because they are theoretically central to the

https://doi.org/10.24171/j.phrp.2025.0447

Table 2. Results of confirmatory factor analysis across all domains ($n = 470$)

Domain/factor	Items	λ	AVE	CR
Knowledge				
Information on infectious diseases (KID1)	Q1	0.33	0.45	0.92
	Q3	0.55		
	Q4	0.67		
	Q7	0.55		
	Q8	0.65		
	Q9	0.49		
	Q10	0.91		
	Q11	0.96		
	Q12	0.98		
	Q14	0.61		
	Q15	0.92		
	Q16	0.90		
	Q17	0.62		
	Q18	0.71		
	Q22	0.61		
	Q23	0.62		
	Q24	0.65		
	Q25	0.60		
Knowledge of CBS (KID2)	Q33	0.68	0.58	0.78
	Q34	0.85		
	Q35R	0.74		
Community-level case definition (KID3)	Q36	0.69	0.50	0.93
	Q37	0.74		
	Q38	0.81		
	Q39	0.78		
	Q40	0.79		
	Q41	0.87		
	Q42	0.91		
	Q43	0.63		
	Q44	0.70		
	Q45	0.79		
Attitude				
Behavioral likelihood (BL)	Q45F1	0.441	0.58	0.860
	Q46F1	0.529		
	Q49F1	0.879		
	Q50F1	0.835		
	Q51F1	0.906		
	Q52F1	0.870		
Subjective norms (SN)	Q39F2	0.728	0.69	0.895
	Q40F2	0.815		
	Q41F2	0.905		
	Q42F2	0.886		
Intention to engage in CBS (INT)	Q32F3	0.651	0.31	0.714
	Q33F3	0.628		
	Q34F3	0.642		
	Q36F3	0.332		
	Q38F3	0.490		
	Q43F3	0.387		
Negative attitudes (ATT)	Q35RF4	0.366	0.20	0.546
	Q37RF4	0.475		
	Q44RF4	0.364		
	Q53RF4	0.415		
	Q54RF4	0.658		
Perception				
Perceived susceptibility (SUS)	Q56F1	0.621	0.545	0.816
	Q57F1	0.594		
	Q61F1	0.787		
	Q62F1	0.799		
	Q63F1	0.873		
Perceived benefits (BEN)	Q71F2	0.702	0.65	0.904
	Q72F2	0.804		
	Q73F2	0.889		
	Q74F2	0.835		
	Q75F2	0.810		
Perceived barriers (BARR)	Q58RF3	0.731	0.58	0.808
	Q59RF3	0.765		
	Q60RF3	0.796		
Self-efficacy (SE)	Q64F4	0.791	0.53	0.820
	Q65F4	0.777		
	Q67F4	0.630		
	Q68F4	0.745		

λ, standardized factor loading; AVE, average variance extracted; CR, composite reliability; CBS, community-based surveillance; R, reverse-coded items.

TRA and demonstrated acceptable reliability. Findings related to these constructs should therefore be interpreted with this measurement limitation in mind. In the perception domain, psychometric properties were generally good, with perceived benefits showing the strongest performance (CR, 0.904; AVE, 0.65), followed by perceived barriers (CR, 0.808; AVE, 0.58), self-efficacy (CR, 0.820; AVE, 0.53), and perceived susceptibility (CR, 0.816; AVE, 0.545).

The CFA models demonstrated acceptable to good fit across all domains: knowledge (RMSEA, 0.028; CFI, 0.945; TLI, 0.941), attitude (SRMR, 0.067; RMSEA, 0.055; CFI, 0.937; TLI, 0.927), and perception (SRMR, 0.055; RMSEA, 0.059; CFI, 0.962; TLI, 0.954). Factor loadings ranged from 0.33 to 0.98.

Main Results

Table 3 summarizes the model fit indices from the initial model (M0) to the final model (M3), showing clear improvement across iterations. Models M0 and M1 showed poor fit (CFI < 0.86, TLI < 0.85), whereas M2 and M3 achieved acceptable fit (CFI,

0.921; TLI, 0.913; RMSEA, 0.040 [90% CI, 0.036–0.044]; SRMR, 0.071).

The initial model (M0) included 3 separate knowledge factors but showed suboptimal fit and therefore required respecification. In M1, knowledge was treated as a single construct measured by 3 parcels (PK1, PK2, and PK3), which improved fit but remained inadequate. M2 incorporated error correlations between items within the same factors on the basis of modification indices and achieved acceptable fit. M3, the final model, added theoretically justified pathways to improve coherence and parsimony while maintaining acceptable fit. Figure 2 presents the final structural model (M3) with standardized path coefficients, illustrating the relationships among all study variables.

Table 3. Summary of the findings of the fitness test for models 1 to 4 ($n = 470$)

Model	RMSEA (90% CI)	RMSEA *p*	CFI	TLI	SRMR
M0	0.055 (0.05–0.058)	0.010	0.848	0.835	0.093
M1	0.054 (0.050–0.057)	0.032	0.856	0.843	0.075
M2	0.040 (0.036–0.044)	>0.950	0.921	0.913	0.071
M3	0.040 (0.036–0.044)	>0.950	0.921	0.913	0.071

RMSEA, root mean square error of approximation; CFI, comparative fit index; TLI, Tucker-Lewis index; SRMR, standardized root mean square residual.

Table 4 presents the path relationships in the final model. Knowledge significantly increased intention (β = 0.419; 95% CI, 0.249–0.622; $p < 0.001$). Subjective norms positively influenced intention (β = 0.235; 95% CI, 0.049–0.457; $p = 0.038$), whereas negative attitudes reduced intention (β = −0.432; 95% CI, −0.667 to −0.235; $p < 0.001$). Intention positively predicted

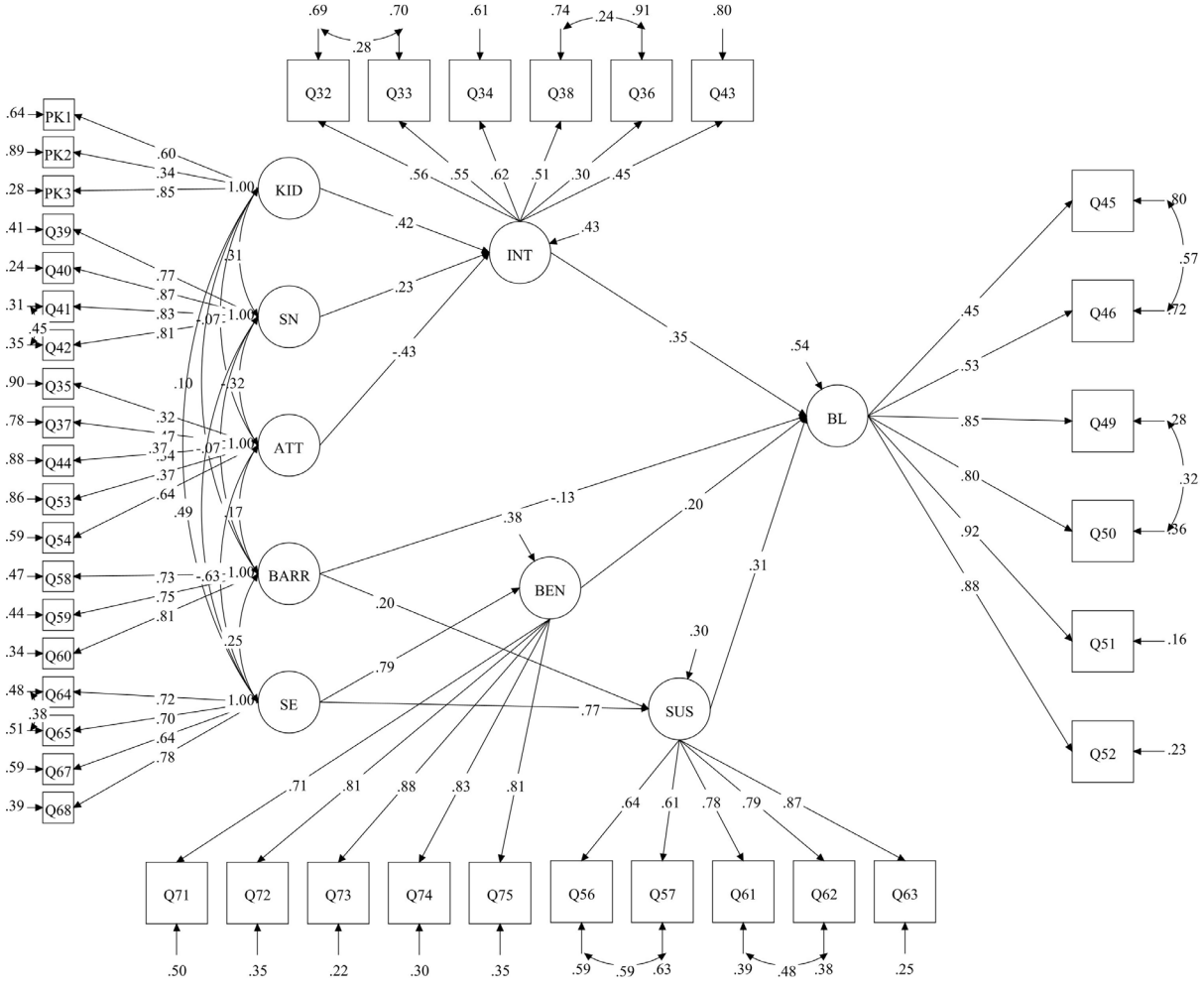


Figure 2. Final model (M3) showing standardized path coefficients among knowledge, attitudes, intention, and behavioral likelihood among community leaders ($n = 470$).

PK1, parcel of knowledge of infectious diseases; PK2, parcel of knowledge of CBS; PK3, parcel of community-level case definition; KID, knowledge of infectious diseases; SN, subjective norms; ATT, negative attitudes; INT, intention to participate in CBS; BL, behavioral likelihood; SUS, perceived susceptibility; BEN, perceived benefits; BARR, perceived barriers; SE, self-efficacy; CBS, community-based surveillance.

https://doi.org/10.24171/j.phrp.2025.0447

behavioral likelihood (β = 0.347; 95% CI, 0.230–0.486; $p < 0.001$). Perceived susceptibility (β = 0.310; 95% CI, 0.175–0.471; $p < 0.001$) and perceived benefits (β = 0.198; 95% CI, 0.097–0.319; $p = 0.001$) were positively associated with behavioral likelihood, whereas perceived barriers were negatively associated with behavioral likelihood (β = −0.132; 95% CI, −0.229 to −0.034; $p = 0.008$).

Additional significant paths included a positive association between perceived barriers and perceived susceptibility (β = 0.205; 95% CI, 0.128–0.296; $p < 0.001$). Self-efficacy significantly increased perceived benefits (β = 0.785; 95% CI, 0.732–0.850; $p < 0.001$) and perceived susceptibility (β = 0.765; 95% CI, 0.673–0.874; $p < 0.001$).

The final model explained 46.1% of the variance in behavioral likelihood, 57.0% of the variance in intention, 70.4% of the variance in perceived susceptibility, and 61.7% of the variance in perceived benefits.

Table 5 presents the direct and indirect relationship results. Perceived barriers showed a significant negative direct effect on behavioral likelihood (β = −0.132, $p = 0.008$) and a positive indirect effect through perceived susceptibility (β = 0.063, $p = 0.004$), resulting in a non-significant total effect. Self-efficacy demonstrated a strong positive total effect on behavioral likelihood (β = 0.393, $p < 0.001$), mediated through perceived susceptibility (β = 0.237, $p = 0.001$) and perceived benefits (β = 0.156, $p = 0.002$). Negative attitudes exerted a significant negative indirect effect on behavioral likelihood

Table 4. Path relationships of the final model ($n = 470$)

Relationship	β (95% CI)	CR	Standard error	*p*
KID→INT	0.419 (0.249–0.622)	4.054	0.103	< 0.001
SN→INT	0.235 (0.049–0.457)	2.079	0.113	0.038
ATT→INT	−0.432 (−0.667 to −0.235)	−3.600	0.120	< 0.001
INT→BL	0.347 (0.230–0.486)	4.887	0.071	< 0.001
SUS→BL	0.310 (0.175–0.471)	3.780	0.082	< 0.001
BEN→BL	0.198 (0.097–0.319)	3.226	0.061	0.001
BARR→BL	−0.132 (−0.229 to −0.034)	−2.649	0.050	0.008
Additional paths				
BARR→SUS	0.205 (0.128–0.296)	4.386	0.047	< 0.001
SE→BEN	0.785 (0.732–0.850)	23.972	0.033	< 0.001
SE→SUS	0.765 (0.673–0.874)	13.687	0.056	< 0.001

β, standardized regression coefficient; CI, confidence interval; CR, critical ratio; KID, knowledge of infectious diseases; SN, subjective norms; ATT, attitudes; INT, intention; BL, behavioral likelihood; SUS, perceived susceptibility; BEN, perceived benefits; BARR, perceived barriers; SE, self-efficacy.

Table 5. Standardized direct, total indirect, and total effects ($n = 470$)

Predictor variable	Path	Outcome variable	Effect type	β	*p*
Perceived barriers (BARR)	BARR→BL	Behavioral likelihood (BL)	Total effect	−0.068	0.124
	BARR→SUS→BL		Total indirect effect	0.063	0.004
	BARR→SUS→BL		Specific indirect (via SUS)	0.063	0.004
	BARR→BL		Direct effect	−0.132	0.008
Self-efficacy (SE)	SE→BL	Behavioral likelihood (BL)	Total effect	0.393	< 0.001
	SE→SUS→BL		Total indirect effect	0.393	< 0.001
	SE→SUS→BL		Specific indirect (via SUS)	0.237	0.001
	SE→BEN→BL		Specific indirect (via BEN)	0.156	0.002
Negative attitudes (ATT)	ATT→BL	Behavioral likelihood (BL)	Total effect	−0.150	0.005
	ATT→INT→BL		Total indirect effect	−0.150	0.005
	ATT→INT→BL		Specific indirect (via INT)	−0.150	0.005
Subjective norms (SN)	SN→BL	Behavioral likelihood (BL)	Total effect	0.082	0.076
	SN→INT→BL		Total indirect effect	0.082	0.076
	SN→INT→BL		Specific indirect (via INT)	0.082	0.076
Knowledge of infectious diseases (KID)	KID→BL	Behavioral likelihood (BL)	Total effect	0.145	0.001
	KID→INT→BL		Total indirect effect	0.145	0.001
	KID→INT→BL		Specific indirect (via INT)	0.145	0.001

β, standardized regression coefficient; BARR, perceived barriers; BL, behavioral likelihood; SUS, perceived susceptibility; SE, self-efficacy; BEN, perceived benefits; ATT, attitude; INT, intention; SN, subjective norms; KID, knowledge of infectious diseases.

through intention (β = –0.150, p = 0.005). Knowledge positively affected behavioral likelihood through a significant indirect effect via intention (β = 0.145, p = 0.001). Subjective norms showed non-significant total and indirect effects (p = 0.076).

Discussion

This study examined the interrelationships among social and health behavioral factors and the behavioral likelihood that community leaders would engage in CBS activities. The structural model revealed 10 significant hypothesized path relationships, explaining 46.1% of the variance in the behavioral likelihood of participating in CBS activities.

The CFA showed satisfactory psychometric properties for most constructs, supporting the multidimensional nature of CBS-related behaviors. The knowledge factors demonstrated excellent CR, particularly for information on infectious diseases, although convergent validity was slightly low. This finding is consistent with previous research suggesting that knowledge constructs can be difficult to measure because they often comprise diverse and partly independent components [25]. The attitude and perception domains generally showed stronger psychometric properties, with subjective norms and perceived benefits demonstrating particularly robust measurement characteristics, consistent with the TRA and HBM [13,14].

Knowledge of infectious diseases significantly and positively influenced intention, emphasizing the importance of informed community representatives in promoting CBS engagement. This finding is consistent with studies identifying knowledge as a key determinant of health behavior intentions [26]. However, this relationship may differ in resource-limited settings where access to health information is substantially lower than in Malaysia's educational context. In contrast with studies from sub-Saharan Africa, where knowledge gaps were reported as major barriers to participation in disease surveillance [27], the highly educated sample in this study (87.2% with university education) suggests that knowledge may be necessary but not sufficient for behavioral engagement. These findings support the value of educational programs that focus on disease awareness and the benefits of CBS in strengthening community participation [28].

Subjective norms also positively influenced intention to participate in CBS, suggesting that social expectations and encouragement from peers or authorities promote participation. This finding is consistent with the TRA and TPB, which emphasize the role of subjective norms in shaping intentions [13,14]. Leveraging social influence through campaigns and endorsements may therefore strengthen CBS participation [29–31].

Negative attitudes were negatively associated with intention, underscoring the importance of addressing unfavorable perceptions to improve CBS participation. This finding is also consistent with the TRA, which emphasizes the influence of attitudes on health behavior intentions [32]. The relatively strong magnitude of this association (β = –0.432) is consistent with findings from other Southeast Asian settings, where cultural beliefs and trust in reporting systems substantially influenced willingness to participate [33]. Negative attitudes have similarly been shown to hinder public health initiatives in other contexts [27].

Intention emerged as a direct predictor of behavioral likelihood. Notably, intention had a stronger direct effect than the individual perception variables of perceived susceptibility and perceived benefits, confirming its central mediating role and aligning with the TPB, which positions intention as a primary determinant of behavior [34]. This finding suggests that intervention strategies should prioritize intention formation as a key leverage point. In practical terms, motivational messaging and communication campaigns designed to strengthen intentions to participate in CBS may be more effective than approaches that focus solely on perceptions of disease risk or surveillance benefits.

Perceived susceptibility and perceived benefits were positively associated with behavioral likelihood, whereas perceived barriers were negatively associated with behavioral likelihood (β = –0.132, p = 0.008). These direct effects are consistent with the predictions of the HBM. The negative association of perceived barriers with behavioral likelihood highlights the importance of reducing obstacles such as time constraints and perceived burden. This finding is consistent with previous studies reporting adverse effects of barriers on health behaviors [13]. Conversely, the positive association between perceived benefits and behavioral likelihood suggests that recognizing the advantages of CBS encourages participation.

A notable finding was that self-efficacy influenced behavioral likelihood indirectly rather than directly. Although self-efficacy often shows direct associations with health behaviors in diverse populations [35], this study suggests that it operates indirectly through perceived benefits and perceived susceptibility. This pattern indicates that confidence in performing CBS activities does not automatically translate into behavioral engagement; rather, self-efficacy may first strengthen beliefs about disease risk and the effectiveness of surveillance, which in turn increase behavioral likelihood. This indirect mechanism has important implications for intervention design. Rather than addressing self-efficacy in isolation through skills-based training alone, interventions may need to combine confidence-building activities with

https://doi.org/10.24171/j.phrp.2025.0447

messaging that clearly explains how such capabilities can reduce disease risk and enhance community protection. This interpretation is consistent with Koerniawan [36], who reported that self-efficacy had no direct effect on health-promoting behavior during the COVID-19 pandemic.

Perceived barriers demonstrated a complex relationship with behavioral likelihood, with both negative direct effects and positive indirect effects through perceived susceptibility. This counterintuitive finding suggests that individuals who perceive greater barriers to CBS participation may simultaneously report higher perceived susceptibility to disease, possibly because awareness of such obstacles heightens concern about surveillance gaps and disease risk. This pathway suggests that interventions aimed at reducing barriers should also acknowledge and address these heightened perceptions of risk so that barriers do not ultimately discourage participation.

Several indirect pathways influenced behavioral likelihood through intention: negative attitudes and subjective norms affected behavioral likelihood through intention, consistent with studies using the TRA and TPB [26,37]. Knowledge also indirectly influenced behavioral likelihood through intention, highlighting the role of awareness in engagement. Collectively, these indirect pathways account for a substantial proportion of the model's explanatory power, underscoring that CBS participation is shaped by multifactorial pathways rather than isolated direct effects. For intervention design, this finding suggests the need for integrated approaches that simultaneously strengthen knowledge, reshape attitudes, mobilize social support, and build confidence in ways that influence intention and downstream behavior.

Limitations

This study has several limitations. First, the cross-sectional design precludes causal inferences regarding the relationships among knowledge, attitudes, perceptions, and behavioral likelihood. Longitudinal studies are needed to establish temporal relationships. Second, convenience sampling of teachers within schools may limit generalizability to other teachers and to other types of community leaders. Third, the self-reported nature of the data may have introduced social desirability bias, although researchers emphasized confidentiality and encouraged honest responses during briefing sessions. In addition, self-reported Likert-scale and knowledge items may be subject to measurement error, acquiescence bias, and variation in how participants interpret response options. Dichotomous knowledge items may also fail to capture the nuanced understanding required for effective CBS participation and may be influenced by guessing. Fourth, the study focused exclusively on schoolteachers in Kelantan, Malaysia, which limits generalizability to other community leaders, such as village leaders, religious leaders, and traditional healers. Fifth, the study did not account for potential confounders, including prior CBS training, personal health experiences, or organizational support, that might influence CBS participation. Sixth, the absence of qualitative data is a limitation because interviews or focus groups could have provided richer contextual understanding of the attitudes, barriers, and facilitators identified quantitatively. Such insights could help clarify the cultural, social, and institutional factors underlying the observed relationships and strengthen interpretation of the findings.

Generalizability

The findings are most applicable to schoolteachers in similar cultural and geographic contexts, particularly in Southeast Asian countries with comparable educational systems and community structures. The high educational level of the participants (87.2% with university education) may limit generalizability to community leaders with lower educational attainment. This high educational level may reflect stronger baseline knowledge, critical thinking, and health risk perception than would be expected among community leaders with less formal education. As a result, the relationships among knowledge, attitudes, and behaviors may differ in other populations; for example, leaders with lower educational attainment may rely more on subjective norms than on knowledge. Caution is therefore warranted when generalizing these findings, and interventions should be adapted to varying levels of health literacy and education. However, the TRA and HBM have demonstrated cross-cultural validity, suggesting potential applicability to other settings with appropriate cultural adaptation. Future research should include a wider range of community leaders across Malaysia and Southeast Asia and should examine CBS participation in non-school settings, such as villages, religious institutions, and healthcare facilities, to improve external validity and practical relevance.

Conclusion

This study identified significant relationships between social and health behavioral factors and CBS participation among community leaders. Knowledge, subjective norms, attitudes, perceived benefits, perceived barriers, perceived susceptibility, and self-efficacy influenced behavioral likelihood through direct and indirect pathways. Intention served as a strong mediator, whereas self-efficacy operated indirectly through perceived benefits and perceived susceptibility. Public health interventions should focus on enhancing knowledge through targeted training workshops, addressing negative attitudes

through community engagement campaigns, leveraging social influence through endorsements from educational authorities, reducing barriers by providing simplified reporting mechanisms, and building self-efficacy through hands-on practice sessions. These findings provide actionable guidance for CBS implementation policy in Malaysia and support the development of evidence-based educational programs and implementation strategies that integrate CBS training into the professional development frameworks of teachers and other community leaders. Future research should include longitudinal designs, more diverse groups of community leaders beyond teachers, and implementation studies evaluating the effectiveness of CBS interventions based on these identified factors.

Notes

Ethics Approval

The study protocol was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (ref. no. USM/JEPeM/22050317). Informed consent was obtained from all participants. Questionnaire completion required approximately 20–25 minutes.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: AAH, AKG, NSB, NMY, SMH; Data curation: AAH; Formal analysis: AAH, AKG; Investigation: AAH, NAMN, FMS; Methodology: AAH, AKG; Supervision: AKG, NSB, NMY, SMH; Validation: AAH, AKG, NSB, NMY; Writing–original draft: AAH; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2026.0085
Osong Public Health Res Perspect 2026;17(3):242-253

Original Article

Epidemiological trends and regional risk factors for scrub typhus in Busan, Republic of Korea: 2019–2023

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ABSTRACT

Objectives: Scrub typhus, an acute disease caused by *Orientia tsutsugamushi* and transmitted by larval chigger mites, is a representative autumn febrile illness in the Republic of Korea. We examined epidemiological characteristics underlying spatial heterogeneity in Busan Metropolitan City to inform region-specific prevention and control policies.

Methods: We analyzed 1,645 confirmed and suspected scrub typhus cases reported in Busan from 2019 to 2023. Crude incidence rates and age-standardized incidence rates (ASIRs) per 100,000 population were calculated using the 2021 mid-year population. The chi-square test assessed associations between disease occurrence and exposure activities within the preceding 30 days. Correlation analyses examined the relationships of ASIR with environmental indicators, including per capita urban forest area, and epidemiological indicators, including prior infectious disease education.

Results: The overall mean ASIR in Busan was 8.35 per 100,000 population, below the national average. However, incidence was higher in peri-urban districts, including Gijang-gun (26.12/100,000) and Gangseo-gu (15.02/100,000), than in central urban districts such as Jung-gu (2.20/100,000). Higher incidence was observed among women and older adults (≥60 years). ASIR correlated strongly with per capita urban forest area ($r = 0.92$; 95% confidence interval, 0.79–0.97; $p < 0.001$). Regional differences were significant for agriculture/forestry, gardening, and general outdoor activities ($p < 0.001$). Districts with higher incidence also displayed higher rates of infectious disease education, although this may reflect reverse causation.

Conclusion: In Busan, scrub typhus incidence was concentrated in peri-urban districts. These findings support region-specific prevention strategies, evaluation of infectious disease education, enhanced mite surveillance, and practical protective measures during fieldwork.

Keywords: Busan; Epidemiology; *Orientia tsutsugamushi*; Personal protective equipment; Risk factors; Scrub typhus

Received: January 24, 2026
Revised: March 13, 2026
Accepted: March 19, 2026

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Introduction

Scrub typhus is a vector-borne zoonotic disease caused by infection with *Orientia tsutsugamushi* following the bite of larval mites (chiggers). It is particularly endemic in the Asia–Pacific region, commonly referred to as the "tsutsugamushi triangle" [1,2]. After an incubation period of 6–21 days, patients typically present with nonspecific symptoms, including fever, headache, myalgia, chills, and vomiting; the presence of a rash and eschar provides key diagnostic clues [1,3]. Because its clinical presentation closely resembles that of other febrile illnesses, delayed diagnosis may lead to rapid progression to severe complications, including multiple organ failure, and case fatality rates as high as 70% have been reported [1].

Recent increases in case reports from newly affected areas have renewed attention to the geographic distribution and evolving global epidemiology of scrub typhus. In particular, changes in climate and human activity patterns are influencing vector habitats and transmission dynamics, suggesting that infection may occur even in previously non-endemic regions [4,5]. Scrub typhus has become a major public health concern in several Asian countries, including India, China, Japan, and Thailand; for example, India has reported recurrent large-scale outbreaks and high mortality over the past several decades [5]. In addition, a recent meta-analysis reported high global average seroprevalence, highlighting both the hidden burden of infection and substantial regional heterogeneity [6]. Collectively, these findings indicate that scrub typhus is not confined to traditionally endemic areas and that its epidemiological patterns continue to evolve in response to climatic and environmental factors.

In the Republic of Korea, the incidence of scrub typhus has increased markedly over the past decade [6], underscoring the need for proactive infectious disease control policies that extend beyond responses to climatic and environmental factors alone. This increase may be partly explained by rapid land-use change and the resulting fragmentation and expansion of chigger habitats. Recent long-term time-series analyses in neighboring China have shown that land-use changes, such as deforestation and agricultural fragmentation, may amplify infection risk by creating an "edge effect" that increases contact between humans and wild vectors [7,8]. Human exposure also increases during agricultural work, grass cutting (e.g., *beolcho*), and outdoor leisure activities in shrublands and grassy fields near villages and farmlands, which provide abundant food sources for rodents. This mechanism likely contributes to the pronounced seasonal peak observed in autumn [9]. In addition, a recent climate change vulnerability assessment found that coastal areas of Jeolla Province, characterized by extensive farmland and large agricultural populations, were more vulnerable to scrub typhus than other regions [10].

HIGHLIGHTS

- Scrub typhus incidence in Busan showed marked spatial heterogeneity. Incidence was higher in peri-urban districts. Age-standardized incidence was positively associated with per capita urban forest area. Urban-rural transitional environments and outdoor activities were key risk factors. Region-specific prevention and strengthened vector surveillance are needed.

These epidemiological patterns are strongly shaped by climatic and environmental factors. Domestic studies have reported nonlinear associations between scrub typhus incidence and changes in average temperature and rainfall [11]. In addition, socioenvironmental conditions, such as living near forests, engaging in agricultural activities, and residing in traditional houses, have been identified as key risk factors for infection [5]. The effective control of scrub typhus therefore requires a multidimensional strategy that extends beyond patient treatment to incorporate climate and environmental change, demographic characteristics, risk-related behaviors, disease awareness, and relevant educational experience. However, when interpreting the correlation between education and disease incidence, reverse causation cannot be excluded, because public health education efforts may be concentrated preferentially in high-incidence areas. Accordingly, caution is warranted when evaluating the true preventive effectiveness of these educational interventions.

Scrub typhus is one of the major mite-borne infectious diseases that recurs annually in the southern regions of the Republic of Korea, including Busan, and it imposes a substantial disease burden, particularly on older adults and residents of rural and peri-urban areas. Although Busan Metropolitan City is the second-largest city in the Republic of Korea, it also includes urban–rural mixed settings, such as Gijang-gun, resulting in pronounced spatial heterogeneity. Accordingly, this study analyzed scrub typhus cases reported in Busan Metropolitan City from 2019 to 2023. We focused specifically on the associations of incidence with demographic factors (age and sex), regional characteristics, risk-related behaviors, urban forest environments, and prior educational experience. Recognizing the potential ecological fallacy inherent in aggregated data, we also conducted sensitivity analyses to assess the influence of extreme outliers, such as Gijang-gun, and thereby evaluate the statistical robustness

of our findings. Ultimately, this study sought to identify the key epidemiological and environmental factors underlying regional disparities in incidence, emphasize the need for targeted prevention strategies for high-risk populations, and provide evidence to inform region-specific vector-borne disease control policies in Busan.

Materials and Methods

Data Source and Case Definition

To analyze the incidence and epidemiological characteristics of scrub typhus in Busan Metropolitan City, we used data on confirmed and suspected cases reported to the Korea Disease Control and Prevention Agency (KDCA) through the Integrated Disease and Health Management System. The study period extended from January 1, 2019, to December 31, 2023. A total of 1,797 cases were reported, of which 152 were excluded because of missing epidemiological investigation records or incomplete data entries. Therefore, the final analytical dataset included 1,645 cases. The epidemiological data collected included general characteristics (sex, age, and residential district), clinical symptoms, and exposure history during the 30 days before illness onset, specifically participation in forestry-, agriculture-, or livestock-related activities.

Case Definition

Case definitions were based on the KDCA National Notifiable Infectious Diseases Diagnostic and Reporting Standards (2024). A confirmed case was defined as a patient with clinical symptoms in whom pathogen infection was verified by laboratory testing. A suspected case was defined as a patient with compatible clinical symptoms and epidemiological links in whom infection was suspected on the basis of a presumptive diagnosis, such as the detection of specific antibodies in blood specimens.

Data Analysis

Crude incidence rates were calculated per 100,000 population using the mid-year population of each year as the denominator and were stratified by region, sex, and age group. Age-standardized incidence rates (ASIRs) were calculated by the direct standardization method using 5-year age intervals and the 2021 mid-year population as the standard population. As an indicator of vector habitat, per capita urban forest area was obtained from the National Urban Forest Statistics (2021) through the e-Nara Indicator portal. Regional differences in epidemiological characteristics were compared using the chi-square test. Correlations between ASIRs and environmental or epidemiological factors were evaluated using Pearson or Spearman correlation analyses. Statistical analyses were performed using jamovi (ver. 2022) and Microsoft Excel 2016 (Microsoft Corp.), and the results were presented in tables and figures.

Ethics Statement

This study used de-identified public health surveillance data and was deemed exempt from KDCA Institutional Review Board review (Exemption No: KDCA-2025-10-09).

Results

General Characteristics

During 2019–2023, Busan Metropolitan City reported 1,645 cases of scrub typhus (confirmed and suspected), corresponding to an average incidence rate of 9.85 per 100,000 population. Incidence peaked in 2022 at 13.02 per 100,000 and then declined to 8.20 per 100,000 in 2023. Female patients accounted for 56.2% of all cases, and the incidence rate was consistently higher among female residents (10.84 per 100,000) than among male residents (8.80 per 100,000). The age distribution showed a clear concentration among older adults: individuals aged 60–69 years accounted for 35.4% of cases, and those aged ≥70 years accounted for 36.2%, indicating that more than half of cases occurred in persons aged 60 years or older (Table 1).

Regional Incidence

From 2019 to 2023, the national average ASIR was 10.25 per 100,000 population, whereas the overall ASIR in Busan was 8.35 per 100,000. However, substantial variation was observed across Busan's 16 districts. Gijang-gun had the highest ASIR, at 26.12 per 100,000, over 2 and 3 times the national and city averages, respectively (Figure 1). Other districts with elevated incidence included Gangseo-gu (15.02), Haeundae-gu (13.28), and Geumjeong-gu (11.13). In contrast, central districts such as Dong-gu (3.97), Yeongdo-gu (3.92), and Jung-gu (2.20) had relatively low incidence. Regarding annual trends, Busan's ASIR peaked in 2022 at 10.77 per 100,000 and then declined markedly to 6.50 per 100,000 in 2023. This pattern paralleled the national trend, which decreased from 11.74 to 10.31 per 100,000 between 2022 and 2023 (Table 2).

Correlation Between Urban Forest Area and ASIR

To evaluate the relationship between scrub typhus incidence and the surrounding green-space environment, we performed a Pearson correlation analysis between per capita total urban forest area (2021) and ASIR in each district of Busan. The analysis showed a very strong positive correlation ($r = 0.92$; 95% confidence interval [CI], 0.79–0.97; $p < 0.001$) (Figure 2). Because the sample size was small ($n = 16$) and certain

https://doi.org/10.24171/j.phrp.2026.0085

Table 1. Annual, sex-specific, and age-specific incidence rates of confirmed and suspected scrub typhus cases reported in Busan Metropolitan City (per 100,000 population)

Region/district	2019–2023 (total)		2019		2020		2021		2022		2023	
	n (%)	IR	*n* (%)	IR	*n* (%)	IR	*n* (%)	IR	*n* (%)	IR	*n* (%)	IR
Korea (total)	26,297	10.26	4,005	7.80	4,479	8.72	5,915	11.52	6,235	12.16	5,663	11.07
Busan (total)	1,645 (100)	9.85	289 (100)	8.52	345 (100)	10.25	309 (100)	9.24	432 (100)	13.02	270 (100)	8.20
Sex												
Male	720 (43.77)	8.80	118 (40.83)	7.09	152 (44.06)	9.21	138 (44.66)	8.43	179 (41.44)	11.05	133 (49.26)	8.29
Female	925 (56.23)	10.84	171 (59.17)	9.91	193 (55.94)	11.25	171 (55.34)	10.02	253 (58.56)	14.91	137 (50.74)	8.12
Age (y)												
≤19	34 (2.07)	1.37	4 (1.38)	0.7	9 (2.61)	1.75	10 (3.24)	2.02	7 (1.62)	1.46	4 (1.48)	0.86
20–29	37 (2.25)	1.78	6 (2.08)	1.4	6 (1.74)	1.39	4 (1.29)	0.95	11 (2.55)	2.72	10 (3.70)	2.59
30–39	32 (1.95)	1.55	8 (2.77)	1.8	8 (2.32)	1.89	4 (1.29)	0.98	8 (1.85)	2.01	4 (1.48)	1.02
40–49	84 (5.11)	3.35	20 (6.92)	3.9	22 (6.38)	4.33	18 (5.83)	3.59	14 (3.24)	2.83	10 (3.70)	2.06
50–59	279 (16.96)	10.02	60 (20.76)	10.4	60 (17.39)	10.57	43 (13.92)	7.75	69 (15.97)	12.67	47 (17.41)	8.72
60–69	583 (35.44)	22.07	98 (33.91)	20.0	113 (32.75)	22.06	117 (37.86)	21.89	156 (36.11)	28.43	99 (36.67)	17.83
≥70	596 (36.23)	21.22	93 (32.18)	18.5	127 (36.81)	23.88	113 (36.57)	20.19	167 (38.66)	28.25	96 (35.56)	15.40

Incidence rates (IRs) per 100,000 population were calculated using the mid-year population for each corresponding year as the denominator. The average incidence rate per 100,000 population for the entire study period (2019–2023) was calculated by pooling the data as follows: average incidence (2019–2023) = [total number of cases (2019–2023)/sum of mid-year populations (2019–2023)]×100,000.

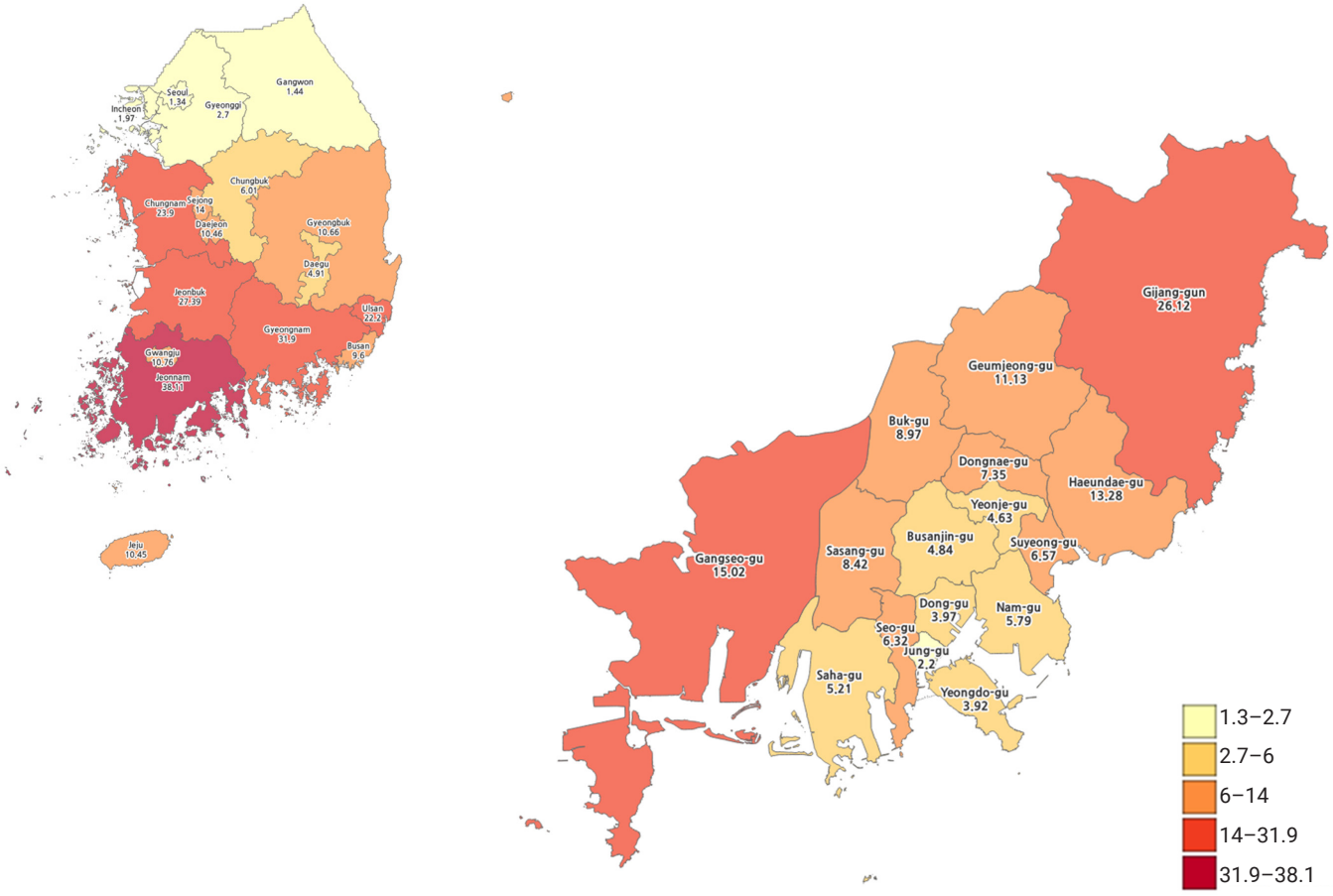


Figure 1. Average age-standardized incidence rate (ASIR) for the Nationwide and 16 Districts/Counties of Busan, 2019–2023. ASIR = age-standardized incidence rate. ASIR = Σ(crude incidence rate by age × standard population ratio by age group) × 100,000 people. The standard population by age group (5-year intervals) is calculated based on the national mid-year population of 2021.

Table 2. ASIRs of confirmed and suspected scrub typhus cases reported via notifiable disease surveillance in Busan (2019–2023)

Region/district	2019–2023		2019		2020		2021		2022		2023	
	n	ASIR	*n*	ASIR	*n*	ASIR	*n*	ASIR	*n*	ASIR	*n*	ASIR
Korea	26,297	10.25	4,005	8.33	4,479	9.02	5,915	11.52	6,235	11.74	5,663	10.31
Busan	1,645	8.35	289	7.68	345	8.91	309	7.87	432	10.77	270	6.50
Jung-gu	6	2.20	1	2.42	0	0.00	1	1.57	4	6.79	0	0.00
Seo-gu	40	6.32	10	8.62	5	3.94	12	9.95	6	4.81	7	4.44
Dong-gu	24	3.97	8	6.91	1	0.72	2	1.75	11	9.03	2	1.56
Yeongdo-gu	33	3.92	7	4.17	10	6.50	4	2.99	8	3.98	4	2.06
Busanjin-gu	99	4.84	20	5.16	32	8.04	3	0.71	23	5.20	21	4.85
Dongnae-gu	112	7.35	20	6.44	7	2.15	25	8.23	44	14.54	16	5.03
Nam-gu	90	5.79	19	6.13	14	4.35	23	7.55	21	6.44	13	4.29
Buk-gu	150	8.97	30	9.40	47	14.25	4	1.44	44	12.57	25	7.15
Haeundae-gu	295	13.28	48	11.61	65	15.14	88	19.69	53	11.77	41	8.58
Saha-gu	96	5.21	25	6.84	12	3.48	20	5.40	29	8.00	10	2.60
Geumjeong-gu	155	11.13	28	10.62	29	11.39	15	4.76	48	16.89	35	11.85
Gangseo-gu	86	15.02	11	10.13	22	20.70	22	19.42	15	11.75	16	13.63
Yeonje-gu	57	4.63	12	5.16	0	0.00	6	2.29	27	11.07	12	4.59
Suyeong-gu	68	6.57	3	1.65	17	7.94	20	9.28	11	5.69	17	7.69
Sasang-gu	110	8.42	16	6.11	30	11.74	21	8.47	26	8.89	17	6.66
Gijang-gun	448	26.12	62	21.04	108	32.15	86	24.65	124	34.90	68	17.78

Age-standardized incidence rate (ASIR) calculation: ASIR = ∑(age-specific crude incidence rate×proportion of the standard population in each age group)×100,000. The standard population was based on the 2021 national mid-year population, stratified by 5-year age intervals.

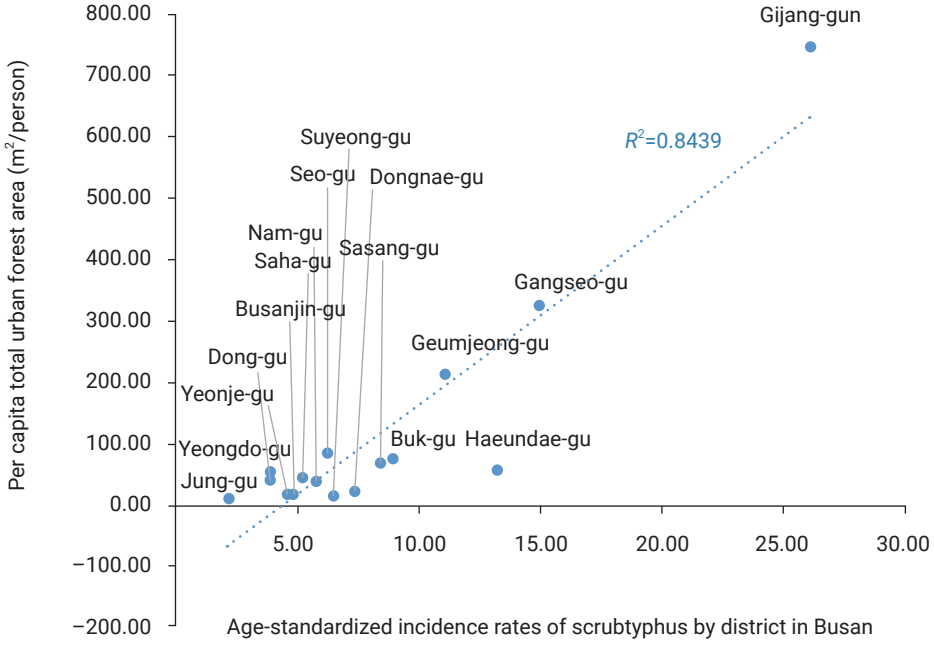


Figure 2. Correlation analysis between age-standardized incidence rates of scrub typhus and per capita total urban forest area (m²/person) across the 16 districts of Busan (2019–2023), showing a strong positive correlation ($r = 0.92$).

https://doi.org/10.24171/j.phrp.2026.0085

districts had disproportionately high values, an additional sensitivity analysis was conducted after excluding Gijang-gun as a potential outlier. After this exclusion, the correlation coefficient decreased slightly to $r = 0.86$ (95% CI, 0.63–0.95; $p = 0.004$), but the positive association remained statistically significant. This finding suggests that the observed association between urban forest distribution and scrub typhus incidence was not driven solely by extreme values, supporting the robustness of this association.

In general, districts with larger per capita green space had higher incidence rates. For example, Gijang-gun, which had the highest ASIR (26.1 per 100,000), also had the largest per capita urban forest area (747.9 m^2), followed by Gangseo-gu (ASIR, 15.0; 324.8 m^2) and Geumjeong-gu (ASIR, 11.1; 215.2 m^2). By contrast, densely populated metropolitan cities with smaller green areas, such as Seoul (ASIR, 1.3; 19.7 m^2) and Incheon (ASIR, 2.0; 52.6 m^2), showed substantially lower incidence rates. A similar pattern was observed across the 8 metropolitan cities nationwide, including Ulsan (ASIR, 22.2; 366.2 m^2), Sejong (ASIR, 14.0; 49.9 m^2), and Daejeon (ASIR, 10.5; 186.9 m^2) (Figure 3). In addition, to address the potential temporal mismatch between the baseline year of the environmental indicator (2021) and the disease incidence analysis period (2019–2023), we conducted supplementary comparative analyses using biennial forest statistics from 2019, 2021, and 2023. These analyses showed that temporal variation in forest coverage and urban forest area in Busan was minimal over the 5-year study period. This supports the use of the single time point of 2021 as representative of environmental characteristics during the entire study period.

Risk-Related Exposure Activities

Based on epidemiological investigation data from the 1,645 scrub typhus cases reported in Busan from 2019 to 2023, exposure activities during the 30 days before illness onset were analyzed to identify potential risk factors. These activities were classified into 5 categories: (1) agriculture and forestry/livestock work, (2) kitchen garden work, (3) weekend farming, (4) one-time agricultural/forestry work, and (5) non-agricultural outdoor activities. Chi-square analyses showed statistically significant regional differences for most activities, including agriculture and forestry/livestock activities ($\chi^2 = 91$, $p < 0.001$), kitchen garden work ($\chi^2 = 59$, $p < 0.001$), one-time agricultural/forestry work ($\chi^2 = 46$, $p < 0.001$), and non-agricultural outdoor activities ($\chi^2 = 33$, $p = 0.004$). Weekend farming was the only activity that did not differ significantly by region ($\chi^2 = 23$, $p = 0.082$).

Regional analysis of these activities showed distinct patterns. Exposure to agriculture and forestry/livestock activities was highest in Gijang-gun (21.74%), followed by Haeundae-gu and Suyeong-gu (15.65% each) and Nam-gu and Gangseo-gu (12.17% each). Kitchen garden work was most prevalent in Gijang-gun (18.96%) and Haeundae-gu (14.81%). One-time agricultural or forestry-related activities were reported most frequently in Buk-gu and Haeundae-gu

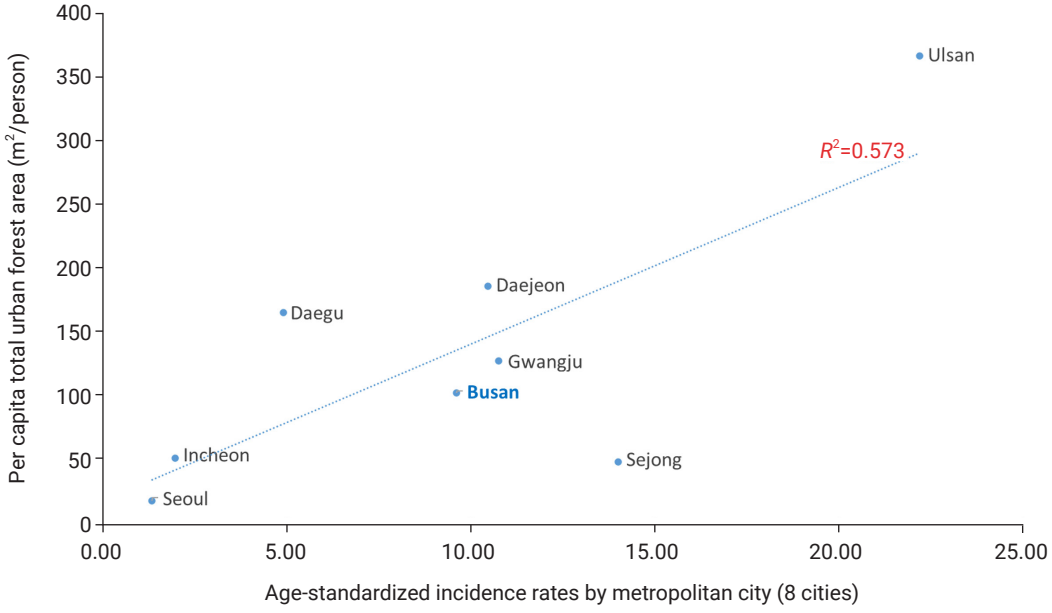


Figure 3. Correlation analysis between age-standardized incidence rates of scrub typhus and per capita total urban forest area (m²/person) across 8 metropolitan cities in Korea (2019–2023), showing a positive correlation ($r = 0.76$).

(16.66% each), followed by Dongnae-gu and Nam-gu (10.00% each). Non-agricultural outdoor activities were reported most often in Haeundae-gu (16.34%), followed by Gijang-gun (13.10%), Geumjeong-gu (9.86%), and Buk-gu (9.15%) (Figure 4).

Kitchen garden work was a notable risk-related exposure, with significant findings regarding both specific tasks and geographic distribution. By activity type, statistically significant regional differences were observed for crop harvesting ($\chi^2 = 41.8$, $p < 0.001$), weeding ($\chi^2 = 67.7$, $p < 0.001$), grass cutting ($\chi^2 = 25.6$, $p = 0.042$), and other related tasks ($\chi^2 = 59.3$, $p < 0.001$) (Table 3). Among these, weeding and crop harvesting were reported relatively frequently in suburban districts with extensive green space and farmland, such as Haeundae-gu, Gijang-gun, and Gangseo-gu. These findings suggest that tasks involving direct contact with vegetation were important contributors to infection risk. In contrast, crop planting (e.g., transplanting seedlings) did not differ significantly by region ($\chi^2 = 23.4$, $p = 0.075$), which may reflect seasonal constraints and the timing of data collection.

Analysis of kitchen garden activities by location further showed statistically significant regional differences for both work in rice paddies ($\chi^2 = 56.2$, $p < 0.001$) and direct contact with grass ($\chi^2 = 56.3$, $p < 0.001$) (Table 4). Collectively, these findings suggest that specific types and settings of agricultural activity, particularly those involving vegetation contact, were key factors associated with scrub typhus infection.

Awareness and Educational Experience

The proportion of patients who reported prior education on scrub typhus was low, at approximately 10%. However, statistically significant regional variation was observed ($\chi^2 = 58.1$, $p < 0.001$). Higher rates of prior educational experience were reported in Geumjeong-gu (23.1%), Haeundae-gu (19.0%), Gijang-gun (16.9%), and Gangseo-gu (14.6%). In contrast, Jung-gu (0%), Buk-gu (4.7%), and Saha-gu (4.3%) had very low levels of such experience (Figure 5).

Correlation analysis between the rate of scrub typhus educational experience (per 100,000 population, based on the 2021 mid-year population) and the ASIR in each district showed a strong positive correlation ($r = 0.70$, $p < 0.001$) (Figure 6). This finding indicates that districts with higher infection burden also tended to report higher levels of educational experience. For example, Gijang-gun (ASIR, 26.1 per 100,000) had a relatively high educational experience rate (16.9%). Similarly, Geumjeong-gu (ASIR, 11.1; educational experience rate, 23.1%) and Haeundae-gu (ASIR, 13.3; educational experience rate, 19.0%) were characterized by both high incidence and high levels of educational outreach.

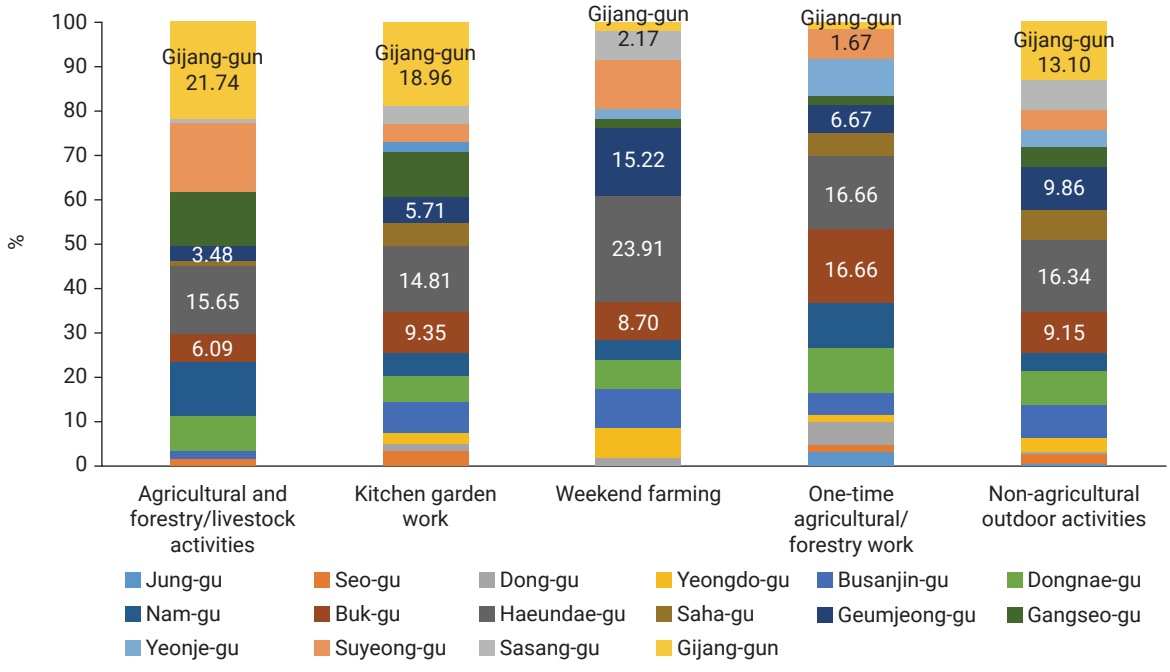


Figure 4. Regional distribution of risk-related exposure activities among scrub typhus cases in Busan (2019–2023). Labels indicate the primary high-risk districts displayed: Gijang-gun, Haeundae-gu, Buk-gu, and Geumjeong-gu. Statistical results of chi-square (χ^2) tests for regional differences in exposure activities: agricultural/forestry/livestock activities: $\chi^2 = 91$, $p < 0.001$; kitchen garden work: $\chi^2 = 59$, $p < 0.001$; weekend farming: $\chi^2 = 23$, $p = 0.082$; one-time agricultural/forestry work: $\chi^2 = 46$, $p < 0.001$; non-agricultural outdoor activities: $\chi^2 = 33$, $p = 0.004$.

https://doi.org/10.24171/j.phrp.2026.0085

Table 3. Types of kitchen garden activities among confirmed and suspected scrub typhus cases in Busan (2019–2023)

Region/ district	Crop planting ($\chi^2 = 23.41$, $p = 0.075$)		Crop harvesting ($\chi^2 = 41.8$, $p < 0.001$)		Weeding ($\chi^2 = 67.7$, $p < 0.001$)		Grass cutting ($\chi^2 = 25.6$, $p = 0.042$)		Other activities ($\chi^2 = 59.3$, $p < 0.001$)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Total	105 (100)	1,540 (100)	227 (100)	1,418 (100)	160 (100)	1,485 (100)	87 (100)	1,558 (100)	55 (100)	1,590 (100)
Jung-gu	0 (0.00)	6 (0.39)	0 (0.00)	6 (0.42)	0 (0.00)	6 (0.40)	0 (0.00)	6 (0.39)	0 (0.00)	6 (0.38)
Seo-gu	4 (3.81)	36 (2.34)	6 (2.64)	34 (2.40)	2 (1.25)	38 (2.56)	1 (1.15)	39 (2.50)	5 (9.09)	35 (2.20)
Dong-gu	1 (0.95)	23 (1.49)	3 (1.32)	21 (1.48)	3 (1.88)	21 (1.41)	2 (2.30)	22 (1.41)	1 (1.82)	23 (1.45)
Yeongdo-gu	5 (4.76)	28 (1.82)	6 (2.64)	27 (1.90)	5 (3.13)	28 (1.89)	3 (3.45)	30 (1.93)	1 (1.82)	32 (2.01)
Busanjin-gu	7 (6.67)	92 (5.97)	20 (8.81)	79 (5.57)	1 (0.63)	98 (6.60)	4 (4.60)	95 (6.10)	5 (9.09)	94 (5.91)
Dongnae-gu	4 (3.81)	108 (7.01)	17 (7.49)	95 (6.70)	3 (1.88)	109 (7.34)	2 (2.30)	110 (7.06)	1 (1.82)	111 (6.98)
Nam-gu	4 (3.81)	86 (5.58)	5 (2.20)	85 (5.99)	5 (3.13)	85 (5.72)	4 (4.60)	86 (5.52)	12 (21.82)	78 (4.91)
Buk-gu	8 (7.62)	142 (9.22)	16 (7.05)	134 (9.45)	15 (9.38)	135 (9.09)	10 (11.49)	140 (8.99)	7 (12.73)	143 (8.99)
Haeundae-gu	20 (19.05)	275 (17.86)	46 (20.26)	249 (17.56)	37 (23.13)	258 (17.37)	21 (24.14)	274 (17.59)	2 (3.64)	293 (18.43)
Saha-gu	8 (7.62)	88 (5.71)	12 (5.29)	84 (5.92)	6 (3.75)	90 (6.06)	4 (4.60)	92 (5.91)	0 (0.00)	96 (6.04)
Geumjeong-gu	6 (5.71)	149 (9.68)	11 (4.85)	144 (10.16)	9 (5.63)	146 (9.83)	1 (1.15)	154 (9.88)	6 (10.91)	149 (9.37)
Gangseo-gu	13 (12.38)	73 (4.74)	25 (11.01)	61 (4.30)	22 (13.75)	64 (4.31)	9 (10.34)	77 (4.94)	1 (1.82)	85 (5.35)
Yeonje-gu	4 (3.81)	53 (3.44)	5 (2.20)	52 (3.67)	1 (0.63)	56 (3.77)	1 (1.15)	56 (3.59)	3 (5.45)	54 (3.40)
Suyeong-gu	2 (1.90)	66 (4.29)	12 (5.29)	56 (3.95)	6 (3.75)	62 (4.18)	1 (1.15)	67 (4.30)	0 (0.00)	68 (4.28)
Sasang-gu	5 (4.76)	105 (6.82)	8 (3.52)	102 (7.19)	8 (5.00)	102 (6.87)	7 (8.05)	103 (6.61)	1 (1.82)	109 (6.86)
Gijang-gun	14 (13.33)	210 (13.64)	35 (15.42)	189 (13.33)	37 (23.13)	187 (12.59)	17 (19.54)	207 (13.29)	10 (18.18)	214 (13.46)

Data are presented as *n* (%).

Table 4. Kitchen garden activity locations and grass contact exposure among confirmed and suspected scrub typhus cases in Busan (2019–2023)

District	Rice paddies ($\chi^2 = 56.2$, $p < 0.001$)		Fields ($\chi^2 = 16.6$, $p = 0.343$)		Orchards ($\chi^2 = 15.8$, $p = 0.396$)		Mountains ($\chi^2 = 17.9$, $p = 0.27$)		Grass contact ($\chi^2 = 56.3$, $p < 0.001$)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Total	338 (100)	1,307 (100)	7 (100)	1,638 (100)	15 (100)	1,630 (100)	35 (100)	1,610 (100)	366 (100)	1,279 (100)
Jung-gu	0 (0.00)	6 (0.46)	0 (0.00)	6 (0.37)	0 (0.00)	6 (0.37)	0 (0.00)	6 (0.37)	0 (0.00)	6 (0.47)
Seo-gu	12 (3.55)	28 (2.14)	0 (0.00)	40 (2.44)	1 (6.67)	39 (2.39)	1 (2.86)	39 (2.42)	14 (3.83)	26 (2.03)
Dong-gu	5 (1.48)	19 (1.45)	0 (0.00)	24 (1.47)	0 (0.00)	24 (1.47)	1 (2.86)	23 (1.43)	5 (1.37)	19 (1.49)
Yeongdo-gu	9 (2.66)	24 (1.84)	0 (0.00)	33 (2.01)	0 (0.00)	33 (2.02)	0 (0.00)	33 (2.05)	9 (2.46)	24 (1.88)
Busanjin-gu	22 (6.51)	77 (5.89)	1 (14.29)	98 (5.98)	0 (0.00)	99 (6.07)	4 (11.43)	95 (5.90)	22 (6.01)	77 (6.02)
Dongnae-gu	19 (5.62)	93 (7.12)	0 (0.00)	112 (6.84)	1 (6.67)	111 (6.81)	2 (5.71)	110 (6.83)	21 (5.74)	91 (7.11)
Nam-gu	16 (4.73)	74 (5.66)	0 (0.00)	90 (5.49)	2 (13.33)	88 (5.40)	3 (8.57)	87 (5.40)	19 (5.19)	71 (5.55)
Buk-gu	31 (9.17)	119 (9.10)	3 (42.86)	147 (8.97)	4 (26.67)	146 (8.96)	1 (2.86)	149 (9.25)	34 (9.29)	116 (9.07)
Haeundae-gu	55 (16.27)	240 (18.36)	1 (14.29)	294 (17.95)	2 (13.33)	293 (17.98)	1 (2.86)	294 (18.26)	55 (15.03)	240 (18.76)
Saha-gu	17 (5.03)	79 (6.04)	0 (0.00)	96 (5.86)	1 (6.67)	95 (5.83)	3 (8.57)	93 (5.78)	19 (5.19)	77 (6.02)
Geumjeong-gu	17 (5.03)	138 (10.56)	0 (0.00)	155 (9.46)	1 (6.67)	154 (9.45)	4 (11.43)	151 (9.38)	22 (6.01)	133 (10.40)
Gangseo-gu	35 (10.36)	51 (3.90)	0 (0.00)	86 (5.25)	2 (13.33)	84 (5.15)	3 (8.57)	83 (5.16)	37 (10.11)	49 (3.83)
Yeonje-gu	6 (1.78)	51 (3.90)	1 (14.29)	56 (3.42)	1 (6.67)	56 (3.44)	2 (5.71)	55 (3.42)	8 (2.19)	49 (3.83)
Suyeong-gu	16 (4.73)	52 (3.98)	0 (0.00)	68 (4.15)	0 (0.00)	68 (4.17)	0 (0.00)	68 (4.22)	16 (4.37)	52 (4.07)
Sasang-gu	13 (3.85)	97 (7.42)	1 (14.29)	109 (6.65)	0 (0.00)	110 (6.75)	1 (2.86)	109 (6.77)	14 (3.83)	96 (7.51)
Gijang-gun	65 (19.23)	159 (12.17)	0 (0.00)	224 (13.68)	0 (0.00)	224 (13.74)	9 (25.71)	215 (13.35)	71 (19.40)	153 (11.96)

Data are presented as *n* (%).

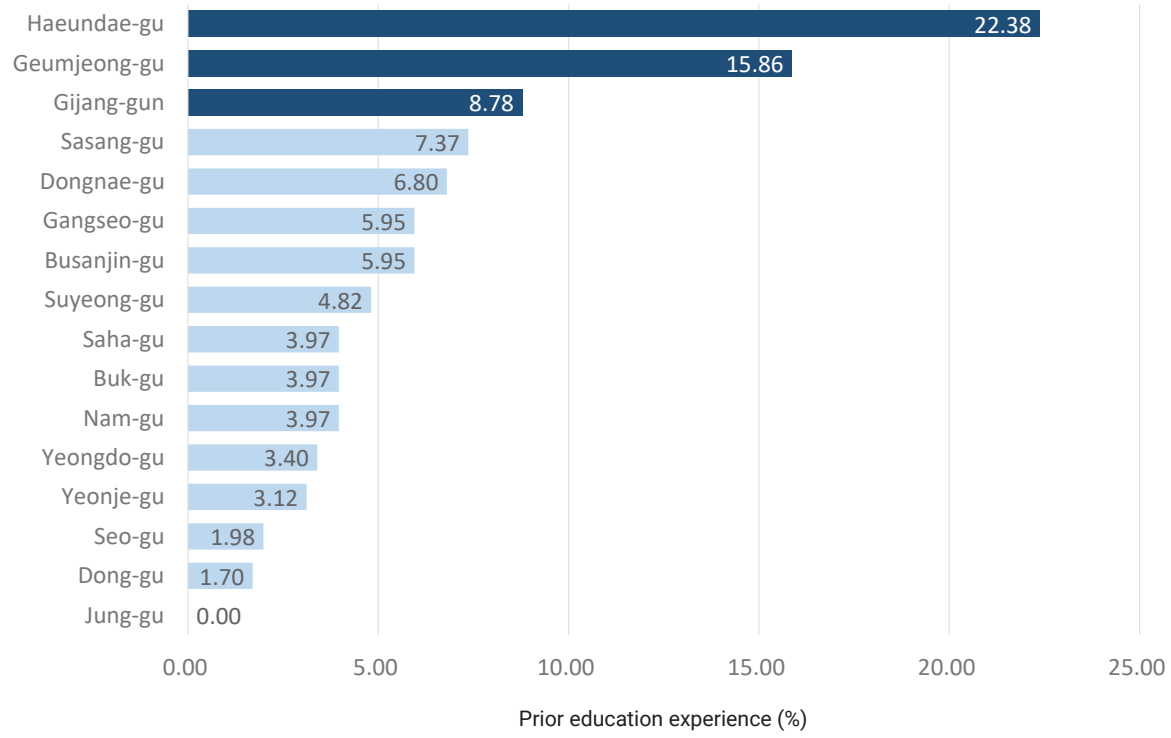


Figure 5. Regional distribution of prior education on scrub typhus in Busan (2019–2023).

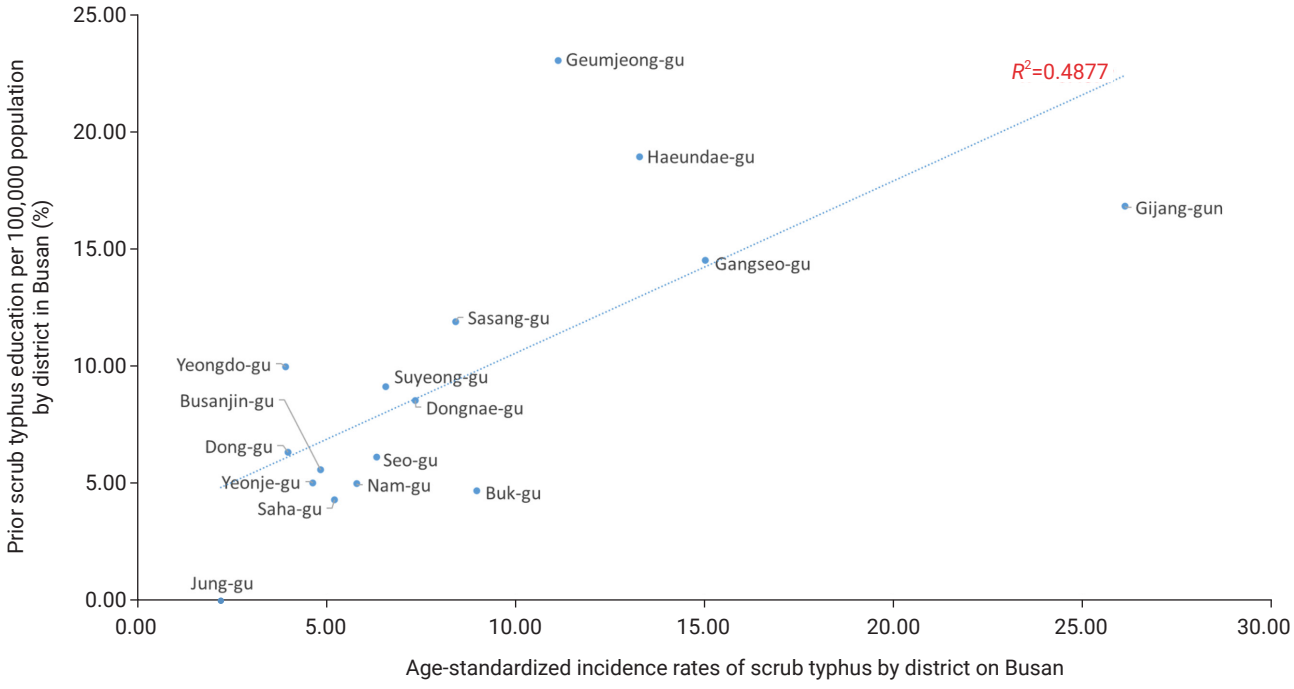


Figure 6. Correlation analysis between the age-standardized incidence rate of scrub typhus and the rate of prior scrub typhus education per 100,000 population (2019–2023), showing a strong positive correlation ($r = 0.70$).

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Discussion

Analysis of scrub typhus cases (confirmed and suspected) reported in Busan Metropolitan City from 2019 to 2023 identified several key epidemiological features. Although Busan's average ASIR was lower than the national average, relatively high incidence rates were observed in suburban districts such as Gijang-gun, Gangseo-gu, Haeundae-gu, and Geumjeong-gu. Notably, Gijang-gun recorded 26.1 cases per 100,000 population, over 2 and 3 times the national and city averages, respectively, confirming its status as a major high-risk area. In contrast, central urban districts, including Jung-gu, Dong-gu, and Yeongdo-gu, had very low incidence. With respect to temporal trends, incidence peaked in 2022 and then declined sharply in 2023. Although a nationwide decline was also observed, the decrease was more pronounced in Busan. These temporal variations may reflect the combined effects of fluctuations in vector density related to climatic and environmental conditions, strengthened public vector-control interventions, and improved personal preventive behaviors. However, because this study did not include direct vector-density data or quantitative indicators of vector-control effectiveness, these interpretations should be regarded as plausible hypotheses based on the available epidemiological context rather than as direct evidence.

With respect to sex distribution, female patients accounted for 56.2% of cases, and the incidence rate among female residents exceeded that among male residents in every study year. By age group, individuals aged 60–69 years (35.4%) and those aged ≥70 years (36.2%) collectively accounted for a large proportion of cases, indicating a concentration among older adults. This pattern may reflect more frequent participation by older adults in agricultural and forestry-related outdoor activities, as well as the larger proportion of women in older age groups. These findings are consistent with previous reports showing high incidence rates among individuals in their 60s and 70s.

Differences in incidence across Busan's districts were closely associated with local ecological and environmental characteristics. Correlation analysis between per capita total urban forest area (2021) and ASIR showed a very strong positive correlation ($r=0.92$; 95% CI, 0.79–0.97; $p<0.001$), indicating that districts with more extensive green space tended to have higher incidence. For example, Gijang-gun had both the largest per capita urban forest area (747.9 m^2) and the highest incidence. Elevated incidence was also observed in other suburban districts with extensive forest and farmland, including Gangseo-gu, Haeundae-gu, and Geumjeong-gu. In contrast, central urban districts with high population density and limited green space had lower incidence rates. A similar pattern was observed in comparisons across the 8 metropolitan cities nationwide: incidence tended to be higher in areas with larger green space (e.g., Ulsan, Sejong, Daejeon, and Gwangju) and lower in urban centers with less green space (e.g., Seoul and Incheon). Together, these findings suggest that urban green areas may increase opportunities for human contact with mite vectors and thus may be associated with increased infection risk. Consistent with this interpretation, recent studies have reported that forest area, rodent habitat indices, and regional socioeconomic conditions are important predictors of vector-mite distribution and that high-risk populations tend to be concentrated in rural or suburban areas at the urban periphery [11,12]. However, given the limited sample size ($n=16$ districts) and the presence of extreme values such as Gijang-gun, the correlation coefficient may have been overestimated. Although the sensitivity analysis excluding Gijang-gun still showed a statistically significant positive correlation ($r=0.86$, $p=0.004$), future studies should use more sophisticated spatial regression models to account more rigorously for potential spatial autocorrelation among adjacent districts. In addition, because this was an ecological analysis based on aggregated area-level data, caution is required to avoid ecological fallacy; district-level environmental indicators cannot be used to infer individual-level infection risk directly.

The analysis of exposure activities further supported these findings. Participation in agricultural, kitchen garden, and outdoor activities during the preceding 30 days was more common in suburban districts with higher proportions of forest and farmland, such as Gijang-gun, Haeundae-gu, Geumjeong-gu, and Gangseo-gu, than in central urban districts. In particular, kitchen garden activities involving direct contact with vegetation, such as crop harvesting and weeding, were associated with infection. Work in rice paddies and contact with grassy fields also showed significant regional differences. These findings suggest that wet rice paddies and grassy fields may provide favorable habitats for chigger mites and that behaviors such as sitting or squatting during agricultural tasks may increase exposure risk. Collectively, these results support the interpretation that agricultural and kitchen garden activities in suburban areas were important factors associated with scrub typhus transmission.

The analysis of disease awareness and educational experience showed that only about 10% of patients had received prior education on scrub typhus, indicating a low overall level of awareness. However, marked regional

differences were observed ($\chi^2 = 58.1$, $p < 0.001$), with relatively high rates of educational experience in high-risk areas such as Geumjeong-gu (23.1%), Haeundae-gu (19.0%) and Gijang-gun (16.9%). The strong positive correlation between educational experience and ASIR ($r = 0.70$, $p < 0.001$) should not be interpreted as evidence that education is ineffective in reducing incidence. Rather, this pattern may reflect reverse causation, whereby public health authorities preferentially direct educational programs toward high-risk communities. The indicator used in this study reflects an ecological correlation at the population level and does not measure the preventive effectiveness of education at the individual level. It is therefore plausible that educational interventions were implemented more intensively in high-incidence areas. To evaluate the infection-reducing effect of health education more rigorously, future prospective studies should control carefully for the timing and intensity of educational interventions. For example, despite multiple educational and promotional activities, Gijang-gun continued to record the highest incidence rate, underscoring the limitations of education alone in reducing community transmission. In addition, some high-risk areas lacked sufficiently tailored educational materials and programs, and residents often relied on family members, neighbors, or mass media for information [13,14]. Therefore, practical prevention strategies are needed in addition to strengthened education for high-risk groups. These strategies should emphasize personal protective equipment (PPE), post-exposure hygiene, recognition of early symptoms, and prompt medical consultation [13,15]. Accordingly, the current educational framework should move beyond a knowledge-centered approach toward more practical, action-oriented guidance. Specifically, during high-risk agricultural activities involving direct vegetation contact, such as weed clearing, practical guidance on PPE use should be emphasized to minimize skin exposure. Such guidance should include wearing long-sleeved shirts, long pants, and boots; securing sleeves and trouser hems inside arm covers or socks; and applying insect repellents containing DEET or picaridin before and during fieldwork [4,5].

The distribution of chigger mites, particularly *Leptotrombidium pallidum* (the principal vector of scrub typhus), is closely associated with disease incidence [15]. Effective prevention of vector-borne diseases requires proactive surveillance strategies, including monitoring vector distribution and managing vector habitats. The KDCA currently operates the Regional Climate Change Vector Surveillance Centers project, which collects, identifies, and tests mosquitoes, chiggers, and hard ticks across 8 designated regions nationwide. However, the Busan–Gyeongnam region is not included.

In this study, Gijang-gun had an incidence rate 2 to 3 times higher than the national average and represented a substantial disease burden within eastern Busan. Establishment of a dedicated vector-surveillance hub in eastern Busan, including Gijang-gun, should therefore be considered to enable systematic monitoring of chigger density and pathogen carriage. Both domestic and international studies have shown that coastal suburban areas with dense forest cover provide favorable habitats for chigger larvae and may therefore have increased transmission risk [16–19]. In China, ecological modeling has been used to predict high-risk regions, with corresponding recommendations to strengthen mite surveillance and control in those areas [20]. Integrating predictive approaches for high-risk areas that combine land-use change and vegetation-fragmentation modeling, as proposed in recent studies [7,8], into Busan's vector-control policy could provide a stronger scientific basis for predicting risk zones and for strengthening targeted surveillance and habitat-management strategies.

Conclusion

Future infectious disease control policies should extend beyond education-centered approaches to include multidimensional strategies, such as environmental improvement (e.g., grass cutting and removal of mite habitats around farmland, urban forest management), vector control, and the distribution and promotion of PPE. Further studies are needed to quantify the effectiveness of tailored regional management programs. Although this study was limited by the use of urban forest area data from a single reference year (2021), which introduces a temporal limitation, our supplementary review of biennial statistics indicated that forest coverage in Busan remained relatively stable throughout the study period, thereby minimizing potential bias due to temporal mismatch. Nevertheless, future research should develop multiyear, high-resolution environmental time-series datasets to characterize more precisely the dynamic temporal relationships between exposure and disease outcomes.

Because this study used aggregated area-level data across 16 districts within an ecological study design, it is inherently subject to ecological fallacy. Accordingly, the ecological associations observed between district-level incidence and urban forest area cannot be extrapolated directly to infer individual-level infection risk, and causal interpretations should be made with caution. To examine these associations more rigorously, future analyses should use multivariable spatial regression models that control for potential confounders, such as age structure, the proportion of agricultural workers, and population density.

https://doi.org/10.24171/j.phrp.2026.0085

In addition, direct vector-habitat indicators highlighted in the Introduction, including vegetation type, soil moisture, and rodent density, as well as microclimatic variables such as temperature and precipitation, were not incorporated directly into the present analytical model. Future studies should therefore attempt to integrate high-resolution satellite remote-sensing data, localized meteorological data, and field-based vector-surveillance data to develop a more comprehensive environmental risk index. Ultimately, scrub typhus control in Busan Metropolitan City should shift toward a multidimensional strategy that integrates the ecological risks of peri-urban areas with the behavioral patterns of older adults. Targeted distribution of PPE to high-risk groups and establishment of a Regional Climate Change Vector Surveillance Center would represent important policy measures to strengthen the city's infectious disease response capacity and promote public health equity. Through implementation of such region-specific vector-borne disease management strategies, disease prevention could be improved and health equity strengthened over time.

Notes

Ethics Approval

This study was confirmed to be exempt from institutional review board review at its 20th meeting in 2025 (Exemption No: KDCA-2025-10-09).

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

None.

Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: KK, HL, SEL; Data curation: KK; Formal analysis: KK; Investigation: KK; Methodology: KK, HL; Project administration: SP, GOK; Supervision: SL; Writing–original draft: KK; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0573
Osong Public Health Res Perspect 2026;17(3):254-261

Original Article

Genetic profiling of the *tolC* component of the *acrAB-TolC* efflux pump in clinical *Klebsiella pneumoniae* isolates: a cross-sectional study in Pontianak, Indonesia

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ABSTRACT

Objectives: *Klebsiella pneumoniae* is a leading cause of hospital-acquired infections. Its increasing prevalence poses substantial challenges to both hospital and public health systems due to the emergence of multidrug-resistant strains. Understanding the epidemiology of *K. pneumoniae* and its antimicrobial resistance characteristics may support antimicrobial stewardship and infection control programs. A cross-sectional study was conducted from June to November 2025.

Methods: A total of 62 isolates underwent phenotypic identification and antimicrobial susceptibility testing using the BD Phoenix system (Becton Dickinson), followed by molecular detection of *K. pneumoniae* and efflux pump genes. Sanger sequencing was performed on isolates positive for the *tolC* gene.

Results: Among the isolates, 66.1% were classified as extended-spectrum β-lactamase (ESBL) producers, 6.5% as carbapenem-resistant Enterobacterales (CRE), and 6.5% as both ESBL and CRE producers. Most clinical isolates were resistant to ampicillin, cefazolin, ceftazidime, ceftriaxone, ciprofloxacin, and cefepime. The *acrAB* and *tolC* genes were detected in all 62 isolates. One isolate showed a genetic profile similar to that of the *K. pneumoniae* KP 52.145 strain.

Conclusion: This study demonstrated a high prevalence of antibiotic resistance among *K. pneumoniae* isolates and confirmed the presence of efflux pump genes, including *tolC*, with observable genetic variability. Further investigation of *tolC* gene evolution is essential, as these genes play critical roles in antibiotic resistance mechanisms.

Keywords: *acrAB-TolC* efflux pump; Antimicrobial stewardship; Bacterial genetic profile; Drug resistance; *Klebsiella pneumoniae*

Received: December 12, 2025
Revised: February 23, 2026
Accepted: March 9, 2026

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Introduction

Klebsiella pneumoniae is an opportunistic Gram-negative pathogen commonly found in the bloodstream, respiratory tract, and urinary tract. It can cause bloodstream infections, pneumonia, and urinary tract infections and is a frequent cause of healthcare-associated infections [1–4]. Over the past 3 decades, the emergence of antibiotic-resistant *K. pneumoniae* has increased substantially and has been associated with more invasive disease and higher mortality rates [3,5]. *K. pneumoniae* is known to exhibit resistance to several antibiotic classes, including penicillins, cephalosporins, and quinolones [3]. Antibiotic resistance can arise through multiple mechanisms, including point mutations in target proteins, production of β-lactamases, and multidrug resistance mediated by the overexpression of efflux pumps [6,7].

In Gram-negative bacteria, the resistance–nodulation–division (RND) superfamily plays a critical role in multidrug efflux systems that transport a wide range of compounds, including fatty acids, antiseptics, detergents, virulence factors, toxins, and antibiotics [8]. The *acrAB-tolC* system belongs to the RND family and is one of the most important multidrug efflux pump systems protecting Gram-negative bacteria against toxic compounds [8,9]. RND-type multidrug efflux pumps typically consist of 3 components: an inner membrane transporter, a periplasmic adaptor protein, and an outer membrane channel [9]. Additionally, clustered regularly interspaced short palindromic repeats (CRISPR) loci have been reported to contribute to multidrug resistance in bacteria [10]. Clinical isolates of *K. pneumoniae* have frequently been reported to overexpress RND-type efflux pumps, particularly *acrAB* and *tolC*, which substantially contribute to multidrug resistance [8,9]. Such overexpression may increase the risk of severe clinical outcomes, including higher morbidity and mortality [11]. Consequently, the increasing global prevalence of antimicrobial-resistant *K. pneumoniae* has led the World Health Organization to classify this organism as a critical priority pathogen.

Given the urgency of this issue, generating data on the presence and genetic characteristics of efflux pump genes is essential for improving our understanding of resistance patterns and supporting antimicrobial stewardship strategies. However, molecular data from low- and middle-income regions, including Indonesia, remain limited, particularly regarding efflux-mediated resistance mechanisms. These gaps highlight the need to characterize efflux pumps, especially through genomic profiling of *tolC* diversity in *K. pneumoniae* isolates. *TolC* is the major outer membrane efflux channel in Enterobacterales and facilitates the extrusion of a wide range of molecules exported by bacteria [12,13].

HIGHLIGHTS

- The *tolC* gene was detected in all clinical *Klebsiella pneumoniae* isolates, indicating its widespread distribution.
- Sequence analysis revealed genetic variation in the *tolC* among the isolates, suggesting molecular diversity.
- Phylogenetic analysis highlights that isolate KP-2T shares sequence similarity with the hypervirulent reference strain KP 52.145 and isolate KP-38T with ATCC 43816.
- These findings highlight the potential contribution of the *tolC*-associated efflux system to antimicrobial resistance and warrant further surveillance.

To the best of our knowledge, this study represents the first report of genomic characterization of the *tolC* efflux pump gene in clinical *K. pneumoniae* isolates from Indonesia. These findings provide region-specific molecular data on multidrug resistance mechanisms.

Materials and Methods

Study Population

A cross-sectional study was conducted from June to November 2025. A total of 70 clinical specimens from patients diagnosed with *K. pneumoniae* infection at Dr. Soedarso Hospital in Pontianak, West Kalimantan, Indonesia, were collected during the study period. Multiple specimens from the same patient, subcultures not consistent with the phenotypic characteristics of *K. pneumoniae*, and isolates negative on molecular detection were excluded. Of these, 62 isolates were included in the final data analysis.

Specimens

Samples were collected from various sources, including blood, pus, tissue swabs, tissue, sputum, central venous catheters, feces, endotracheal tubes, pharyngeal swabs, and bronchoalveolar lavage, depending on the participant's diagnosis. All specimens were placed in sterile containers and transported to the laboratory at 4 °C within a maximum of 1 hour. The samples were processed within 2 hours of collection [14].

Phenotypic Identification

Specimens were inoculated onto blood agar (Merck, USA) and MacConkey agar (Merck, USA) and then incubated at 37 °C for 18 hours. Single colonies grown on blood agar or MacConkey agar were characterized by Gram staining to determine bacterial morphology and Gram reaction. Gram-negative rods were subsequently subjected to species identification

using the BD Phoenix system (Becton Dickinson, Canada). A 0.5 McFarland bacterial suspension was prepared and inoculated into the BD identification panel according to the manufacturer's protocol, followed by incubation at 37 °C for 18 hours. *K. pneumoniae* isolates with an identification probability of 80% or higher were included in the study.

Antibiotic Susceptibility Test

A 0.5 McFarland bacterial suspension was inoculated into the BD Antibiotic Susceptibility Test CPO panel and analyzed using EpiCenter ver. 6.61A (BD Diagnostic Systems). The antibiotics tested represented 11 antibiotic classes and included ampicillin, cefazolin, colistin, gentamicin, amikacin, cefepime, ertapenem, imipenem, meropenem, sulfamethoxazole-trimethoprim, ceftazidime, cefotaxime, minocycline, piperacillin-tazobactam, tigecycline, ampicillin-sulbactam, and ceftazidime-avibactam. In addition to classifying isolates as susceptible, intermediate, or resistant, the system also identified extended-spectrum β-lactamase (ESBL) production and carbapenem-resistant Enterobacterales (CRE).

Efflux Pump Gene Detection and Sequencing

Identification of the *acrAB* and *tolC* genes was performed using polymerase chain reaction (PCR). The *khe* gene was used as a housekeeping gene for *K. pneumoniae*. *K. pneumoniae* isolates were processed for DNA extraction according to the manufacturer's protocol (Presto Mini gDNA Bacteria Kit; GeneAid). PCR conditions were as follows: initial denaturation at 94 °C for 5 minutes, followed by 40 cycles for *khe* and *tolC* and 35 cycles for *acrAB*. Each cycle consisted of denaturation at 94 °C for 1 minute, annealing for 30 seconds (*acrAB*, 58 °C; *tolC*, 51 °C; khe, 55 °C), and extension at 72 °C for 60 seconds. Final extension was performed at 72 °C for 10 minutes for *khe* and *tolC* and for 7 minutes for *acrAB*. PCR products were subjected to 3% agarose gel electrophoresis and visualized using a gel documentation system. Primers used to detect efflux pump and housekeeping genes are listed in Table 1 [15,16]. All isolates positive for efflux pump genes were subjected to Sanger sequencing. Sequencing results were analyzed against reference sequences from *K. pneumoniae* ATCC 4386 (Gene Accession CP064352), *K. pneumoniae* KP 52.145 (Gene Accession FO834906), and *K. pneumoniae* HS21886 (Gene Accession YP_005228876.1).

Data Analysis

Descriptive statistical analysis was performed to summarize specimen sources, antibiotic resistance patterns, and the presence of efflux pump-related genes. Variables were presented as frequencies and percentages. Data were entered into Microsoft Excel ver. 16.91 (Microsoft Corp.), and the results were presented in tabular form. Phylogenetic analysis and visualization were performed using MEGA ver. 12 for Macintosh and Interactive Tree of Life (iTOL).

Ethics Approval

The study was approved by the Health Research Ethics Committee of Doctor Soedarso Regional General Hospital, Pontianak, Indonesia (No: 36/RSUD/KEPK/IV/2025).

Results

A total of 62 clinical isolates of *K. pneumoniae* were analyzed in this study from 70 patients clinically diagnosed with *K. pneumoniae* infection. The most common specimen source was sputum (19.4%), whereas tissue and pharyngeal swab specimens were the least common sources (1.6% each) (Table 2).

Table 2. Specimen source of *Klebsiella pneumoniae* clinical isolates (*n* = 62)

Specimen	Frequency (%)
Blood	10 (16.1)
Endotracheal tube	10 (16.1)
Tissue swab	7 (11.3)
Sputum	12 (19.4)
Bronchoalveolar lavage	11 (17.7)
Pus	5 (8.1)
Central venous catheter	3 (4.8)
Feces	2 (3.2)
Tissue	1 (1.6)
Pharyngeal swab	1 (1.6)

Table 1. Primer sequences used for the target genes

Target gene	Primer sequence (5' to 3')	Amplicon size (bp)	Reference
khe	F TGATTGCATTCGCCACTGG R GGTCAACCCAACGATCCTGG	428	[16]
acrAB	F ATCAGCGGCCGGATTGGTAAA R CGGGTTCGGGAAAATAGCGCG	312	[15]
tolC	F ATCAGCAACCCCGATCTGCGT R CCGGTGACTTGACGCAGTCCT	527	[15]

https://doi.org/10.24171/j.phrp.2025.0573

Phenotypic Detection of Antibiotic Resistance Characteristics

This study showed that 41 of 62 clinical isolates (66.1%) were classified as ESBL producers, whereas 4 of 62 (6.5%) were classified as CRE. An additional 4 isolates (6.5%) were identified as both ESBL and CRE, and 13 isolates (21.0%) were classified as non-resistant (Table 3). Antimicrobial resistance patterns were further characterized on the basis of antibiotic susceptibility results, as shown in Table 3. We found that 98.4% (61/62) of isolates were resistant to ampicillin. In addition, high proportions of isolates were resistant to cefazolin (90.3%, 55/62), ceftazidime (88.7%, 55/62), ceftriaxone (85.5%, 53/62), ciprofloxacin (83.9%, 52/62), and cefepime (83.9%, 52/62).

Table 3. Resistance pattern of *Klebsiella pneumoniae* clinical isolates by BD antibiotic susceptibility method (*n* = 62)

	Resistance frequency (*n*/isolates, %)[a]
Antibiotics	
Penicillin	
Ampicillin	61/62 (98.4)
Cephalosporin (first generation)	
Cefazolin	55/62 (90.3)
Cephalosporin (third generation)	
Ceftriaxone	53/61 (86.9)
Ceftazidime	55/62 (88.7)
Cephalosporin (fourth generation)	
Cefepime	52/62 (83.9)
Carbapenem	
Ertapenem	31/61 (50.8)
Imipenem	18/62 (29.03)
Meropenem	21/61 (34.4)
Beta-lactam and anti-beta lactamase	
Piperacillin-tazobactam	19/62 (30.6)
Ceftazidime-avibactam	26/60 (43.3)
Ampicillin-sulbactam	48/62 (77.4)
Tetracycline (30 S)	
Minocycline	25/61 (41.0)
Fluoroquinolone	
Ciprofloxacin	52/62 (83.9)
Sulfonamide and DHFR inhibitors	
Sulfamethoxazole-trimethoprim	39/61 (63.9)
Aminoglycoside (30 S)	
Gentamicin	32/62 (51.6)
Amikacin	7/62 (11.3)
Polymyxin	
Colistin	5/62 (8.1)
Drug resistance classification	
ESBL	41 (66.1)
CRE	4 (6.5)
ESBL+CRE	4 (6.5)
Not Detected	13 (21.0)

DHFR, dihydrofolate reductase; ESBL, extended-spectrum β-lactamase; CRE, carbapenem-resistant Enterobacterales.
[a]Intermediate results are not included.

Efflux Pump Gene Detection and Sequencing

All 62 isolates (100%) were positive for the *acrAB* operon and *tolC* gene. Isolates positive for *tolC* were subsequently subjected to Sanger sequencing and analyzed using phylogenetic methods. The circular phylogenetic tree illustrates the genetic relatedness of the *tolC* gene among *K. pneumoniae* clinical isolates (labeled KP-xT) in comparison with the reference strains KP ATCC 43816, KP 52.145, and KP HS11286 (Figure 1). The phylogenetic tree demonstrated that the clinical isolates clustered into distinct clades. One isolate (KP-2T) showed similarity to the *tolC* gene sequence of the hypervirulent KP 52.145 strain, whereas the remaining clinical isolates formed separate clusters, suggesting possible local genetic variation.

Discussion

Antibiotic Susceptibility Test

In this study, all *K. pneumoniae* isolates were obtained at Dr. Soedarso Hospital, Pontianak, from a diverse range of clinical specimen types. The antimicrobial resistance patterns observed in these isolates underscore the substantial burden posed by multidrug-resistant *K. pneumoniae* in the hospital setting. Nearly all isolates were resistant to ampicillin (98.4%), which is expected given the intrinsic resistance of *K. pneumoniae* mediated by chromosomal sulfhydryl variable (SHV) β-lactamase [17,18]. High resistance rates across first-, third-, and fourth-generation cephalosporins, ranging from 83.9% to 90.3%, further suggest a widespread presence of ESBL-producing isolates. Similar findings have been reported globally, particularly in Southeast Asia, where the prevalence of ESBL-producing organisms continues to rise [19,20].

In this study, carbapenem resistance, although less common than cephalosporin resistance, remains a significant clinical concern. Resistance to ertapenem (50.8%, 31/61), meropenem (33.9%, 21/62), and imipenem (29.0%, 18/62) may reflect the circulation of carbapenemase-producing strains or synergistic mechanisms involving ESBL production combined with porin loss. The relatively higher rate of ertapenem resistance is characteristic of porin-associated changes, whereas resistance to imipenem and meropenem may more strongly suggest carbapenemase activity. Ertapenem monoresistance, defined as resistance to ertapenem while retaining susceptibility to imipenem and meropenem, is a common phenotype in strains producing ESBLs or AmpC

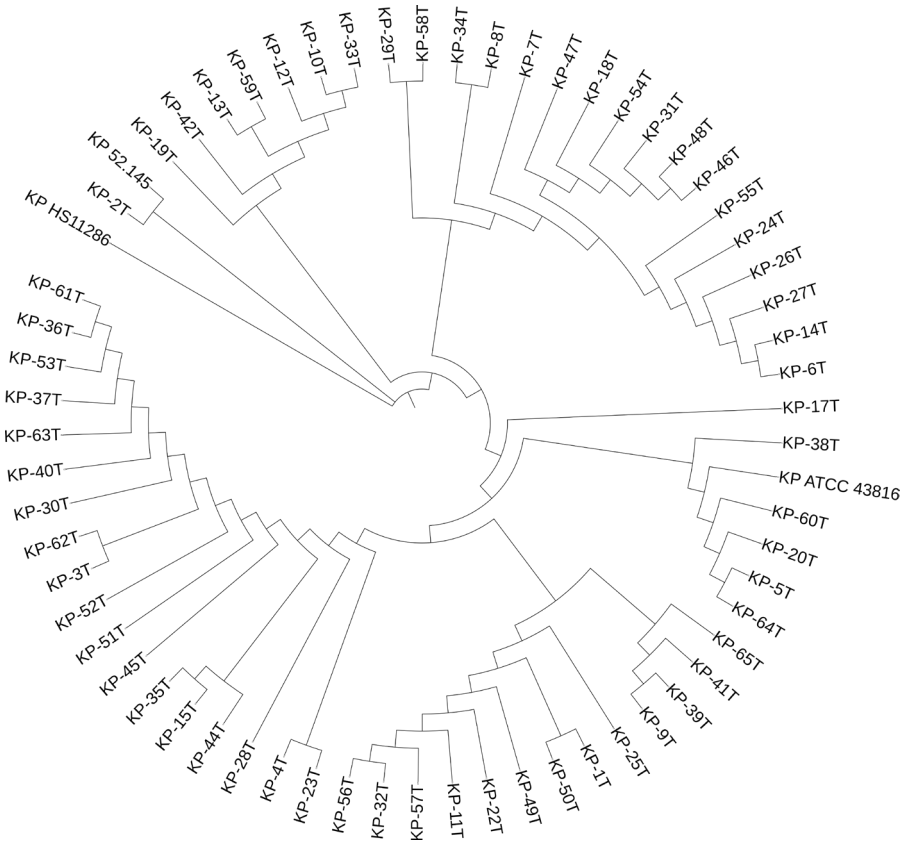


Figure 1. Phylogenetic tree. KP-xT: samples; *Klebsiella pneumoniae* reference using KP ATCC 43816, KP 52.145, and KP HS11286.

enzymes in combination with porin mutations, often in the absence of a carbapenemase [21,22]. These findings are consistent with regional surveillance data showing increasing carbapenem non-susceptibility among Enterobacterales in Indonesia [23]. Other reports have also highlighted a high proportion (74.6%) of carbapenem-resistant isolates among non-Enterobacterales, suggesting a broader global increase in carbapenem-resistant bacteria [24].

The β-lactam/β-lactamase inhibitor combinations tested showed variable activity. Resistance to ampicillin-sulbactam was high (77.4%), consistent with the limited efficacy of this agent against ESBL- and AmpC-producing strains. Resistance to piperacillin-tazobactam was lower (30.65%), although its clinical effectiveness in severe ESBL-associated infections remains uncertain, as highlighted by evidence from the MERINO trial demonstrating inferior outcomes compared with carbapenems [25]. Ceftazidime-avibactam showed greater activity, with 43.4% resistance, which is consistent with its retained efficacy against KPC- and OXA-48-producing strains; however, the presence of metallo-β-lactamases may explain resistance in nearly half of the isolates [26].

Resistance to non-β-lactam agents was also substantial. Fluoroquinolone resistance was high (83.9%), and trimethoprim-sulfamethoxazole resistance was also considerable (63.9%), limiting the utility of these agents for empirical therapy. These trends mirror global reports attributing fluoroquinolone resistance to plasmid-mediated *qnr* genes and chromosomal *gyrA*/*parC* mutations [27]. Aminoglycosides showed variable activity, with gentamicin resistance at 51.6%, whereas amikacin remained relatively active, with a resistance rate of 11.3%, consistent with its stability against most aminoglycoside-modifying enzymes [28]. These findings support amikacin as a valuable treatment option for multidrug-resistant *K. pneumoniae* infections.

Colistin resistance remained low (8.1%), indicating retained susceptibility despite global concerns regarding plasmid-mediated *mcr* genes [29]. Although colistin remains a last-resort therapy for carbapenem-resistant strains, the relatively low resistance observed in this study suggests that it may still have clinical utility, provided that it is used cautiously to minimize nephrotoxicity and limit further resistance emergence.

Overall, the resistance profile identified in this study

https://doi.org/10.24171/j.phrp.2025.0573

reflects the broader challenges posed by multidrug-resistant *K. pneumoniae* worldwide. The high prevalence of cephalosporin and fluoroquinolone resistance, together with notable carbapenem resistance, substantially limits available therapeutic options.

Genetic Profiling

Our study showed that all 62 clinical isolates were positive for the *acrAB* operon and *tolC* gene. The detection rates of the *acrAB* operon and tolC in this study suggest that the *acrAB-tolC* system is a dominant efflux mechanism associated with multidrug resistance. These findings are consistent with our previous study and with recent reports showing high detection rates of these genes in clinical isolates [7,8,30]. *acrAB* and *tolC* are part of the RND superfamily, which is associated with antibiotic resistance and also contributes to environmental adaptation, pathogenicity, and biofilm formation [12]. Notably, isolates harboring these genes may export several classes of antibiotics, including β-lactams, macrolides, fluoroquinolones, and tetracyclines [31]. This observation is consistent with our findings, in which most *K. pneumoniae* isolates showed high resistance to β-lactams and fluoroquinolones (Table 3). However, gene expression analysis was not performed, which should be considered a limitation of this study.

The importance of this study lies in the sequencing of *tolC*, which revealed genetic variation among clinical *K. pneumoniae* isolates. The phylogenetic tree showed several major clusters, indicating genetic diversity in the *tolC* gene sequences across the isolates. Sequence comparisons demonstrated nucleotide variation among isolates relative to the reference strains. However, comprehensive single-nucleotide polymorphism and mutation analyses were not performed and should be recognized as a limitation of this study.

Isolate KP-38T clustered closely with the KP ATCC 43816 reference strain, suggesting genetic similarity between them (Figure 1). This finding may indicate that they share a relatively recent common ancestor, as reflected by the short branch length in the phylogenetic tree. KP-2T showed an identical *tolC* sequence to that of the reference strain KP 52.145 (Figure 1). *K. pneumoniae* strain 52.145 originated in Indonesia and was isolated in 1935. The KP 52.145 strain is considered a hypervirulent *K. pneumoniae* strain and is frequently used in comparative studies of virulence factor detection in *K. pneumoniae* [32]. The phylogenetic tree further showed that KP-2T clustered closely with the hypervirulent reference strain KP 52.145, suggesting the presence of shared genetic features potentially related to virulence. This clinical isolate was obtained from sputum and was resistant to ampicillin, ampicillin-sulbactam, cefazolin, cefepime, ceftazidime, ceftriaxone, and ciprofloxacin and was identified as an ESBL producer. Hypervirulent *K. pneumoniae* strains possess plasmids encoding genetic factors that enable evasion of host immune defenses and the development of severe infections [33]. We also compared our sequencing results with those of the KP HS21886 strain. *K. pneumoniae* KP HS21886 is a multidrug-resistant strain primarily resistant to carbapenems. In our study, no clinical isolates clustered with KP HS21886 (Figure 1), despite the detection of 4 CRE isolates. However, this finding does not exclude the possibility that some isolates may share antimicrobial resistance mechanisms with KP HS21886.

Phylogenetic analysis of *tolC* among the clinical *K. pneumoniae* isolates revealed substantial genetic diversity. The reference strains were distributed across different clusters. This variability may indicate that the clinical isolates included in this study were derived from multiple ancestral lineages rather than from a single clonal population. This interpretation is consistent with the known genomic plasticity of *K. pneumoniae*. *K. pneumoniae* is recognized as a pathogen capable of adapting to diverse environments, largely because its genomic plasticity facilitates the acquisition and dissemination of antimicrobial resistance determinants through plasmids and other mobile genetic elements [34,35].

Because *tolC* is associated with efflux pump activity that contributes to antibiotic resistance, genetic variation in this gene may be associated with differences in phenotypic resistance profiles. In the phylogenetic tree, branch length reflects relatedness within subclusters, and longer branches may indicate greater sequence divergence in *tolC*. The observed diversity supports the need for ongoing molecular surveillance to identify emerging lineages of potential clinical importance [36].

Conclusion

This study provides the first report of *tolC* sequencing in clinical *K. pneumoniae* isolates from Indonesia and highlights this gene as an important component associated with efflux pump mechanisms. Understanding *tolC* gene evolution is important because this gene may contribute to antibiotic resistance mechanisms and may have implications for antimicrobial stewardship and the development of future therapeutic strategies. Further studies examining the functional and genomic variation of *tolC* are needed to clarify its role in multidrug resistance and its epidemiological significance.

Notes

Ethics Approval

This study was approved by the Institutional Review Board of Doctor Soedarso Regional General Hospital, Pontianak, Indonesia (No: 36/RSUD/KEPK/IV/2025) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained for publication of this study and accompanying images.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This study was supported in part by a grant from the Ministry of Higher Education, Science, and Technology, Indonesia, under the Fundamental Research program (No. 115/C3/DT.05.00/PL/2025).

Availability of Data

All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Authors' Contributions

Conceptualization: MMar, SEP, DFL; Data curation: MMar, MMah, RA; Formal analysis: MMar; Funding acquisition: MMar, SEP, DFL; Investigation: MMar, DFL, SEP, MMah, RA; Methodology: MMar, MMah; Project administration: MMar; Resources: MMar, SEP, DFL, MMah, RA; Software: MMah; Supervision: DFL; Validation: SEP; Visualization: MMar; Writing–original draft: MMar; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0493
Osong Public Health Res Perspect 2026;17(3):262-271

Original Article

The clonality and dissemination of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Malaysia: a retrospective analysis from 2022 to 2023

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Received: November 10, 2025
Revised: January 19, 2026
Accepted: January 28, 2026

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ABSTRACT

Objectives: Cases of nosocomial carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have been increasing steadily since the 1990s. In this study, we sought to assess CRKP clonal diversity and patterns of dissemination at Hospital Canselor Tuanku Muhriz (HCTM), a tertiary university hospital located in Kuala Lumpur, Malaysia.
Methods: From January 2022 to December 2023, all CRKP isolates from HCTM were included in the investigation. Associated patient demographic data and clinical histories were retrieved from hospital records. Antibiotic susceptibility data for the collected isolates were obtained from the HCTM diagnostic laboratory. Molecular typing was performed using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) to identify genetic clusters. Based on ERIC-PCR clustering results and epidemiological investigations, selected CRKP isolates were subjected to whole-genome sequencing (WGS) to validate clonal relatedness, identify antimicrobial resistance genes, and establish phylogenomic relationships.
Results: During the study period, a total of 147 CRKP isolates were recovered from various wards across HCTM. All isolates exhibited resistance to imipenem, meropenem, or ertapenem, with 124 isolates confirmed as carbapenemase producers. ERIC-PCR identified 24 genetic clusters disseminated across 23 wards within HCTM. Twelve CRKP isolates were selected for WGS based on integrated epidemiological investigation and ERIC-PCR genotyping; sequence typing revealed ST17 as the dominant circulating CRKP lineage in the intensive care unit, ward 6E, and possibly across the hospital. The carbapenemase gene *bla_NDM* was detected in all WGS-analyzed CRKP isolates.

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Conclusion: Integrating epidemiological investigation, phenotypic testing, molecular typing, and WGS is essential to understand resistance dynamics, map transmission, and guide hospital infection prevention strategies.

Keywords: Carbapenem; Carbapenem-resistant Enterobacteriaceae; *Klebsiella pneumoniae*; Molecular typing; Polymerase chain reaction; Whole genome sequencing

Introduction

In both global and Malaysian contexts, *Klebsiella pneumoniae* is the predominant species among carbapenem-resistant Enterobacterales (CRE). Carbapenem-resistant *K. pneumoniae* (CRKP) represents a major global public health challenge, particularly in healthcare-associated settings [1]. Surveillance data from Malaysia, as reported by the country's National Health Institute, underscore the growing threat posed by CRKP [2,3]. The resistance rate of *K. pneumoniae* to meropenem increased from 2.3% in 2018 to 5.0% in 2022, indicating a concerning upward trend. This increase is largely driven by the dissemination of carbapenemase-producing genes such as *bla_NDM* and *bla_OXA-48*, with *bla_NDM* being the most frequently identified among Malaysian CRE isolates [3].

CRKP poses a substantial clinical threat, driven primarily by the production of carbapenemase enzymes that render most β-lactam antibiotics ineffective and severely limit available treatment options. To elucidate the transmission dynamics of a prolonged CRKP outbreak at Hospital Canselor Tuanku Muhriz (HCTM), Universiti Kebangsaan Malaysia, a tertiary university hospital located in Cheras, Kuala Lumpur, we conducted a genomic epidemiology investigation integrating epidemiological data, enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping, and whole-genome sequencing (WGS) of circulating CRKP strains isolated from the hospital.

HIGHLIGHTS

- A total of 147 carbapenem-resistant *Klebsiella pneumoniae* isolates were analyzed from a tertiary university hospital between 2022 and 2023.
- Enterobacterial repetitive intergenic consensus polymerase chain reaction enterobacterial repetitive intergenic consensus polymerase chain reaction suggested clonal relatedness among isolates, which was confirmed using whole-genome sequencing (WGS).
- *bla_NDM* was identified in all WGS isolates, underscoring the role of mobile genetic elements in resistance spread.
- The findings highlight the need for a combined approach to infection control in critical care settings.

Materials and Methods

Study Setting and Data Collection

This retrospective study was conducted at HCTM. The hospital has an infection prevention and control unit (IPCU) responsible for routine surveillance, infection prevention activities, staff training, and outbreak investigations. Routine IPCU surveillance data collected between January 1, 2022, and December 31, 2023, were reviewed. For each CRKP infection, information on patient demographics, ward or unit of admission, clinical diagnosis, and specimen type was retrieved.

Bacterial Identification and Antimicrobial Susceptibility Testing

CRKP identification was performed by HCTM's Department of Diagnostic Laboratory Services (JPMD) using conventional biochemical tests and API 20E (bioMérieux) prior to the implementation of the automated VITEK 2 Compact system (bioMérieux) in July 2023, which utilized AST-N374 cards for Gram-negative organisms. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar for selected agents from the penicillin, cephalosporin, carbapenem, aminoglycoside, and fluoroquinolone classes and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) M100 guidelines [4]. Multidrug resistance was defined as non-susceptibility to at least 1 agent in 3 or more antimicrobial classes. For isolates with reduced susceptibility to carbapenems, minimum inhibitory concentrations for imipenem, meropenem, and ertapenem were determined using Etest gradient strips (bioMérieux).

Carbapenemase Detection and Classification

Carbapenemase production was assessed using the modified carbapenem inactivation method (mCIM) and, where applicable, the ethylenediaminetetraacetic acid-modified

carbapenem inactivation method (eCIM), in accordance with CLSI guidelines [4]. eCIM testing was performed for isolates that tested positive by mCIM.

ERIC-PCR and Cluster Identification

ERIC-PCR genotyping was performed for all 147 CRKP isolates collected during the study period using ERIC primers (forward: 5'-ATG TAA GCT CCT GGG GAT TCAC-3' and reverse: 5'-AAG TAA GTG ACT GGG GTG AGC G-3') [5]. The PCR protocol consisted of an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 48 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. PCR products were resolved on 1.5% agarose gels at 70 V for 1 hour and visualized under ultraviolet light. DNA fingerprint patterns generated by ERIC-PCR were analyzed using GelCompar II software to construct dendrograms based on unweighted pair group method with arithmetic mean clustering and dice similarity coefficients. Clinical and epidemiological data were cross-referenced to identify genetic clusters.

WGS, Genome Assembly, and Annotation

Selected strains representing ERIC-PCR clusters were subjected to further analysis using WGS. Genomic DNA extraction and purification were performed by bead homogenization using the ZymoBIOMICS DNA Extraction Kit (Zymo Research), according to the manufacturer's instructions. For library preparation, 100 ng of genomic DNA was fragmented to approximately 350 bp using a Bioruptor and processed with the NEB Ultra II Library Preparation Kit (New England BioLabs) following the manufacturer's protocol. Sequencing was conducted on an Illumina NovaSeq 6000 platform using a 2×150 bp paired-end configuration, generating approximately 1 Gb of sequencing data per isolate. Genome assembly was performed using SPAdes v4 [6]. Genome annotation was carried out using Bacterial Annotation Toolkit (BAKTA) v1.9.3 with its standard database [7], in which protein-coding genes were predicted using Prodigal v2.6.0 [8] and annotated against multiple databases, including National Center for Biotechnology Information (NCBI) RefSeq, UniProt, Pfam, Rfam, KEGG, and Gene Ontology. Protein sequences were additionally submitted to the eggNOG-mapper and KofamKoala web servers [9,10].

High-Resolution Taxonomic Classification, Single-Nucleotide Polymorphism-Tree Construction, Multi-Locus Sequence Typing, and Antimicrobial Resistance Gene Prediction

For taxonomic classification, pairwise average nucleotide identity was calculated between genome assemblies and reference genomes in the GTDB r220 database using FastANI v1.33 [11,12]. Genome assemblies were further used to construct a single-nucleotide polymorphism (SNP)-based phylogenetic tree using maast v1.0.8 with default parameters [13], in which SNPs were identified through pairwise comparisons to generate concatenated allele alignments. These nucleotide alignments were used as input for maximum-likelihood phylogenetic tree construction with FastTree 2 v2.1.11 [14]. CRKP sequence types (STs) were assigned according to the PubMLST database using the standard 7-locus multi-locus sequence typing (MLST) scheme (gapA, infB, mdh, pgi, phoE, rpoB, and tonB), as implemented in the mlst tool [15]. To identify antimicrobial resistance (AMR) genes, assembled genomes were searched against the NCBI AMRFinder, ResFinder, and CARD databases using Abricate v1.0.0 [16–18].

Integrated Epidemiological, Genotyping, and Genomic Analyses for CRKP Transmission Identification

Clinical and epidemiological data for WGS-selected isolates, including patient medical history, diagnosis, specimen type, ward or unit, and collection date, were reviewed in conjunction with ERIC-PCR genotyping and genomic findings (MLST, SNP, and AMR analyses) to infer potential transmission trajectories. Cases associated with strains exhibiting similar ERIC-PCR genotypes, STs, SNP profiles, and AMR gene content that also shared temporal overlap (ward admission periods) and spatial proximity (patient ward movement) were considered indicative of transmission events. In contrast, genetic or genomic dissimilarity among isolates with epidemiological overlap suggested independent introductions rather than direct transmission. This integrated analytical framework enabled higher-resolution mapping of CRKP transmission and provided critical insights into both clonal persistence and the coexistence of genetically diverse strains within HCTM.

Ethics Approval

This study was approved by the Medical Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2023-869).

Results

Most CRKP Isolates Were Multidrug-Resistant and Carbapenemase Producers

Thirty-eight and 109 cases of CRKP infections were recorded at HCTM in 2022 and 2023, respectively, resulting in the

https://doi.org/10.24171/j.phrp.2025.0493

isolation of 147 CRKP isolates (Table S1). The median age of patients from whom CRKP was isolated was 51.5 years (range, 14–89 years), and most patients were admitted to the general intensive care unit (GICU) (n = 46). Most CRKP isolates were recovered from blood specimens (n = 50) (Table S1).

All study isolates were multidrug resistant, with susceptibility largely restricted to gentamicin and amikacin (81.5% and 82.1% susceptibility to gentamicin in 2022 and 2023, respectively; 78.4% and 92.2% susceptibility to amikacin in 2022 and 2023, respectively). A total of 124 isolates (84.4%) were carbapenemase producers. Ten isolates (6.8%) were mCIM-negative and classified as non-carbapenemase-producing CRKP. Twelve isolates (8.2%) were not tested because of the unavailability of mCIM reagents at the time of analysis. Among the carbapenemase-producing isolates, 87 (70.2%) were identified as metallo-β-lactamase producers (eCIM positive), whereas 5 (4.0%) were classified as serine carbapenemase producers (eCIM negative). eCIM testing was not performed for 32 isolates (25.8%), as testing was conducted for epidemiological surveillance rather than therapeutic guidance.

Diverse CRKP Genotypes Identified by ERIC-PCR with Inter-Ward Distribution

ERIC-PCR dendrogram analysis revealed 24 clusters at a 50% similarity threshold (Figures S1, S2). Strains within clusters 10, 11, 22, and 24 demonstrated closer similarity (> 60%). No cluster was confined to a single ward, indicating the dissemination of multiple CRKP genotypes across hospital wards. Several isolates were further analyzed based on genotype similarity and epidemiological investigation (Figure 1).

Improved Detection of CRKP Transmission Using Combined Epidemiological, Clinical, ERIC-PCR, and Genomic Approaches

Twelve CRKP isolates were selected for WGS based on ERIC-PCR-defined clustering and epidemiological linkage. Genome assembly statistics and genome completeness information are provided in Table S2 and Figure S3.

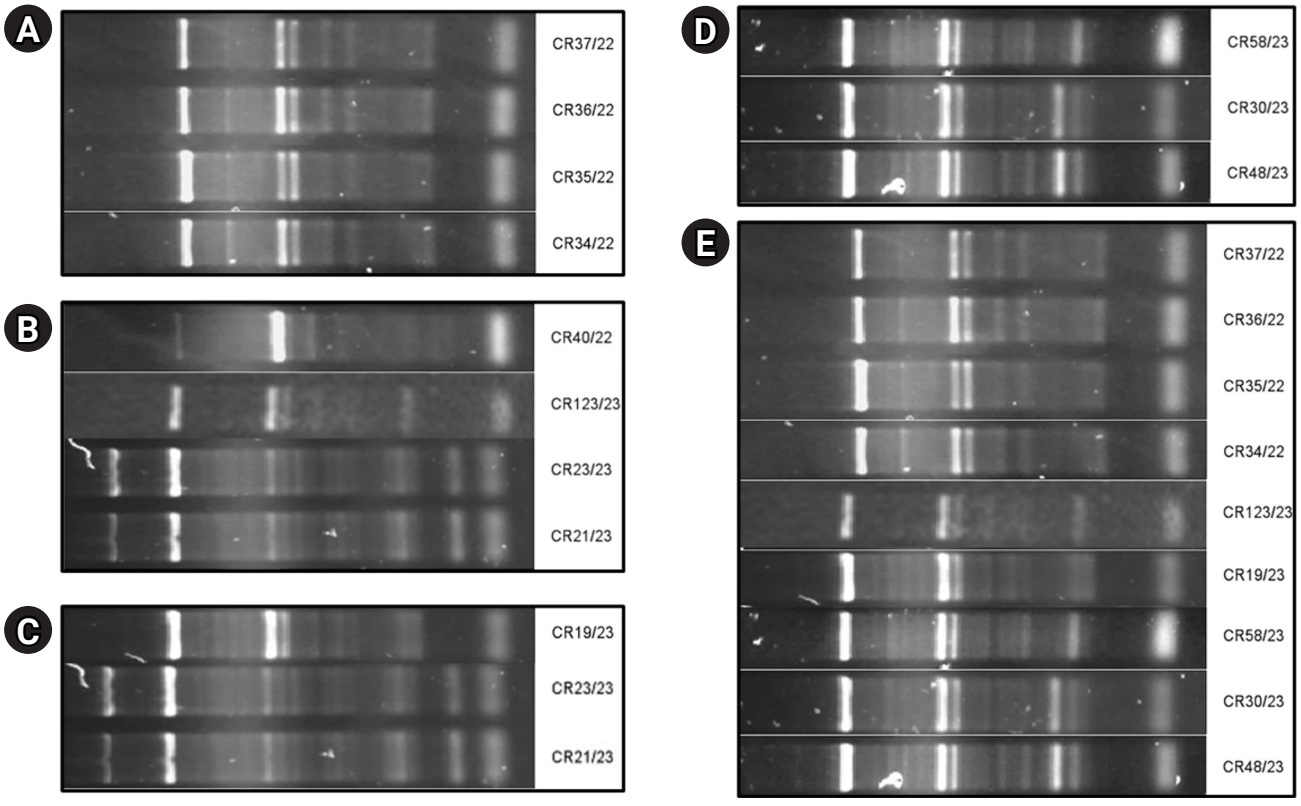


Figure 1. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) dendrograms of selected carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates investigated in this study. (A) A cluster comprising isolates CR34/22, CR35/22, CR36/22, and CR37/22. (B) Selected isolates from the general intensive care unit. (C) CR19/23 exhibited a different ERIC-PCR profile from CR21/23 and CR23/23, despite close contact between the patients associated with these isolates. (D) A cluster isolated from ward 6E, comprising CR58/23, CR30/23, and CR48/23. (E) All ST17 isolates were found to share the same ERIC-PCR profile. ST, sequence type.

First, 4 isolates from ERIC-PCR cluster 22 (CR34/22, CR35/22, CR36/22, and CR37/22), collected from a single intensive care unit (ICU) patient in 2022 (Table 1; Figure 1A), were investigated. In addition to sharing similar ERIC-PCR genotypes, WGS revealed that these 4 isolates shared identical STs and highly similar AMR gene profiles (Figure 2). This finding was consistent with persistent CRKP infection or colonization in a single patient during a prolonged ICU stay from late August to mid-September 2022, with multiple clinical specimens yielding isolates that were highly similar at the whole-genome level (Figure 3).

Next, several ERIC-PCR genotypes (that of CR40/22, CR21/23, CR23/23, CR123/23) (Table 1; Figure 1B) corresponding to CRKP isolates recovered from the GICU over the study period (2022–2023) were analyzed. The ERIC-PCR profile of CR40/22 was initially unclear; however, WGS identified this isolate as ST4276 with a distinct AMR gene profile compared with other sequenced CRKP isolates (Figure 2). In contrast, ERIC-

Table 1. Epidemiological, clinical, ERIC-PCR genotyping, and genomic data for WGS isolates

Sample ID	Patient ID	Close contact (if any)	Specimen type	Collection date	Ward of CRKP isolation	Patient ward movement prior to CRKP isolation	Patient medical history	ERIC-PCR cluster	ST
CR34/22	A		Blood	August 21, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR35/22	A		Urine	August 24, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR36/22	A		Urine	August 27, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR37/22	A		Urine	September 7, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR40/22	B		Blood	September 23, 2022	GICU	22/7 (GICU)	Septic shock secondary to HAP	9	4,276
CR19/23	C	Close contact in ICU	Tracheal aspirate	April 11, 2023	GICU	30/3 (4H) 2/4 (GICU)	Severe pancreatitis with multiorgan failure	11	17
CR21/23	D		Rectal swab (routine screening)	April 17, 2023	GICU	13/4 (GICU)	Severe pancreatitis with multiorgan failure	10	11
CR23/23	D		Tracheal aspirate	April 20, 2023	GICU	13/4 (GICU)	Severe pancreatitis with multiorgan failure	10	11
CR123/23	E		Urine	October 9, 2023	GICU	13/9 (GICU)	Extensive left cerebellar infarct	10	17
CR30/23	F	Close contact with D in ICU	Tracheal aspirate	May 5, 2023	Ward 6E	14/4 (7C) 17/4 (COVID ICU) 20/4 (GICU) 29/4 (6E)	Post COVID-19 category 5	10	17
CR48/23	G		Rectal swab	June 7, 2023	Ward 6E	17/4 (5C) 22/4 (6E) 19/5 (RICU) 23/5 (6E)	Sepsis secondary to HAP	10	17
CR58/23	H		Blood	June 16, 2023	Ward 6E	7/6 (4F)	Proximal myositis	10	17

ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; WGS, whole-genome sequencing; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; ST, sequence type; GICU, general intensive care unit; ICU, intensive care unit; COVID-19, coronavirus disease 2019; HAP, hospital-acquired pneumonia; RICU, respiratory intensive care unit.

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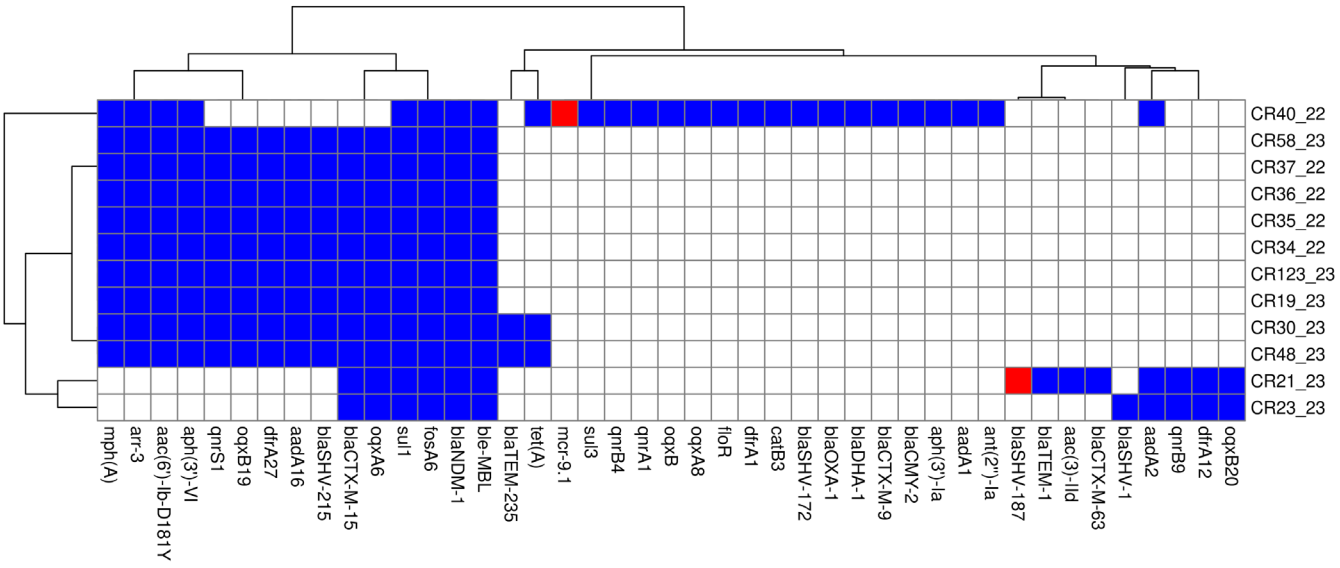


Figure 2. Heatmap of antimicrobial resistance (AMR) gene profiles of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates subjected to whole-genome sequencing. Each cell represents the presence or absence of a specific AMR gene in each genome. Dark blue indicates genes detected with 100% sequence identity, red indicates genes detected with <100% sequence identity (partial variant matches), and white indicates absence of the gene. All sequenced isolates carried the *bla_NDM* gene, confirming their classification as CRKP. While *bla_NDM* was universally present, other resistance determinants such as *aac(6')-Ib* and *oqxA* were variably detected, reinforcing that the CRKP isolates were not derived from a single clone.

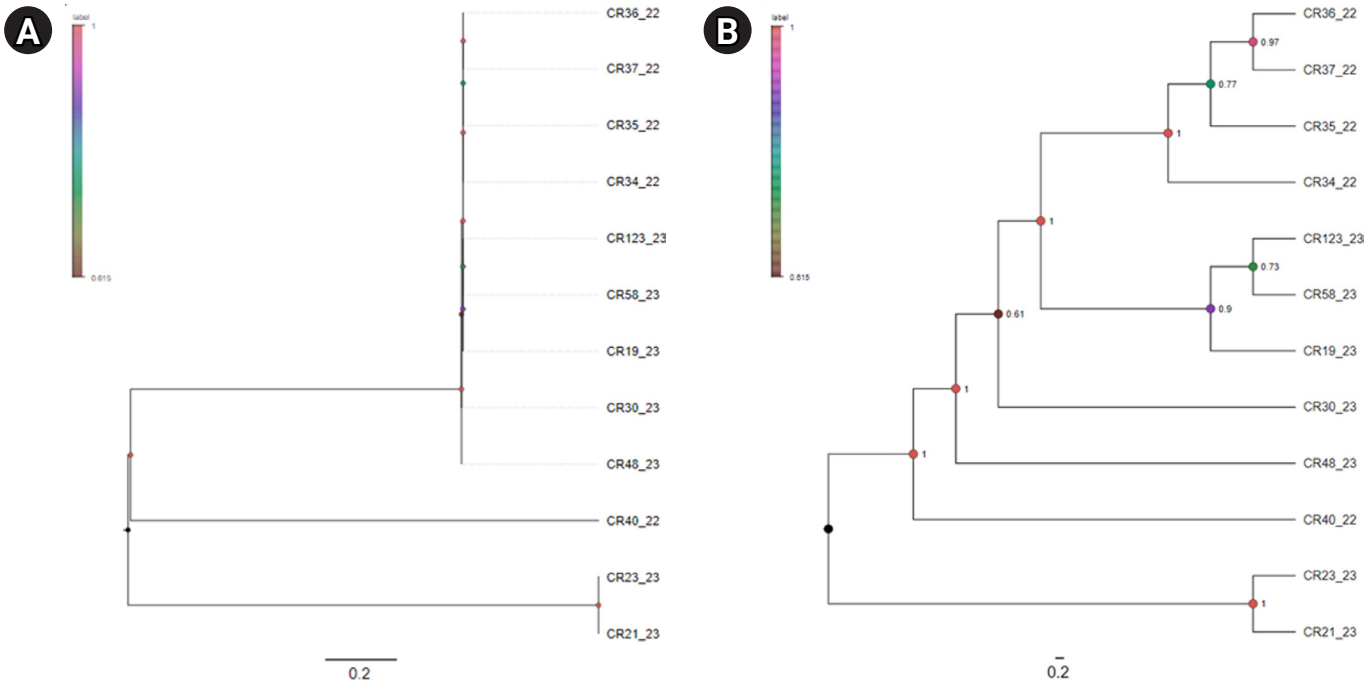


Figure 3. Maximum-likelihood phylogenomic analysis demonstrated that carbapenem-resistant *Klebsiella pneumoniae* isolates subjected to whole-genome sequencing did not belong to a single clone. (A) Although the majority were classified as ST17, genetically distinct ST4276 and ST11 lineages were also identified. (B) Isolates recovered in 2022 appeared to have diverged more recently from a ST17 ancestor than those recovered in 2023. These findings underscore the complexity of transmission dynamics in high-risk hospital settings, where clonal evolution and expansion may coexist with sporadic introductions, complicating transmission tracking efforts. ST, sequence type.

PCR genotypes were indistinguishable for isolates CR21/23 and CR23/23 (Figure 1B). These isolates were recovered from the same patient (patient D) in April 2023 and were later confirmed by WGS to belong to ST11 and to cluster on the same branch of the SNP-based phylogenomic tree (Figure 3A), differing by only a small number of SNPs (Figure 3B). Intriguingly, CR23/23, which was isolated 3 days later than CR21/3, did not carry *blaCTX-M-63*, *blaTEM-1*, *aac(3)-IId*, or *bla-SHV-187*, but instead harbored *blaSHV-1* (Figure 2). Based on epidemiological investigation, the IPCU included the ERIC-PCR genotype of CR19/23, an isolate from patient C (a close contact of patient D in the ICU), for further ERIC-PCR and WGS analysis (Figure 1C). CR19/23 was found to exhibit a different ERIC-PCR genotype from CR21/23 and CR23/23 and was confirmed by WGS to be phylogenomically distant from these isolates (Figure 3). Further epidemiological investigation revealed that CR21/23, the first isolate recovered from patient D, originated from routine rectal swab screening and belonged to ERIC-PCR cluster 10 (ST11). This isolate likely originated from the community, was not hospital-associated, and did not appear to spread beyond its initial host (patient D). In contrast, CR19/23 and CR123/23 were identified as ST17 and shared a common ancestor with CRKP isolates recovered in 2022 (Figure 3B). Overall, ST17 CRKP isolates recovered between August 2022 and October 2023 belonged to closely related ERIC-PCR clusters (clusters 10, 11, and 22) (Table 1; Figure S2) and were predominantly isolated from the ICU, suggesting a prolonged outbreak of ST17 CRKP in this setting.

CR30/23, CR48/23, and CR58/23 from ERIC-PCR cluster 10 (Table 1; Figure 1D) were isolated from patients F, G, and H, respectively, during an outbreak in ward 6E in May and June 2023. Epidemiological contact tracing indicated that patient F (CR30/23) had close contact with patient D (CR21/23 and CR23/23) during their ICU stay, initially raising suspicion of CRKP transmission from the ICU to ward 6E. However, WGS demonstrated that CR30/23, CR48/23, and CR58/23 were ST17 and shared a more recent phylogenomic ancestor with each other than with CR21/23 and CR23/23 (Figure 3), an indication that the hypothesis of transmission from patient D to patient F was incorrect. Indeed, all ST17 CRKP isolates subjected to WGS exhibited similar ERIC-PCR genotypes and were assigned to clusters 10, 11, or 22, which shared >60% similarity (Figure 1E), highlighting the utility of ERIC-PCR as a screening tool for WGS strain selection in resource-limited hospital settings. Chronologically, patients F (CR30/23) and G (CR48/23) may have acquired ST17 CRKP in the ICU and subsequently transmitted the organism to patient H (CR58/23) during their stay in ward 6E. However, CR30/23 and CR48/23 were the only WGS isolates that carried *tet(A)* and *blaTEM-235* (Figure 2), suggesting independent acquisition of these AMR determinants. In addition, CR58/23 appeared to have diverged more recently from the ST17 ancestor than CR30/23 and CR48/23 (Figure 3B). Sequencing additional isolates across the study period would allow a more comprehensive understanding of the evolutionary and transmission dynamics of ST17 CRKP.

Discussion

This study demonstrates the complementary yet critical roles of ERIC-PCR and WGS in the investigation of a CRKP outbreak in our university hospital. ERIC-PCR provided a rapid and cost-effective means of clustering isolates, making it a practical frontline screening tool in resource-limited settings [19–21]. However, poor gel image resolution, as observed for isolate CR40/22, may result from uneven PCR amplification of specific genomic regions and can complicate genotype assignment. In such situations, WGS offers substantially higher resolution and reveals genetic differences that ERIC-PCR cannot capture, consistent with previous reports highlighting the superiority of WGS for accurately delineating transmission events [22,23]. In our study, there was initial suspicion of CRKP transmission between patients C (CR19/23, ST17) and D (CR21/23 and CR23/23, both ST11), and subsequently between patients D (CR21/23 and CR23/23) and F (CR30/23) based on epidemiological investigation; notably, WGS demonstrated that these transmission events had not occurred. This finding further strengthened the confidence of our IPCU in applying WGS for future close-contact assessments and outbreak investigations.

A notable finding of this study was the persistence of CRKP ST17 across multiple time points during the study period, with possible horizontal acquisition of AMR mobile genetic elements in some isolates (CR30/23 and CR48/23). This persistence suggests that ST17 did not represent a short-lived outbreak but rather an endemic lineage entrenched within the ICU environment, with possible subsequent spread to ward 6E. Its prolonged survival points toward maintenance through environmental reservoirs and gaps in infection control measures [24]. In contrast, lineages such as ST4276 and ST11 were detected only once, suggesting sporadic introductions rather than sustained persistence. The contrast between these 2 patterns illustrates the dual challenge faced by infection prevention teams: controlling both endemic high-risk clones and newly introduced strains.

The ICU setting plays a central role in facilitating both the persistence and spread of CRKP. Patients frequently undergo invasive procedures, receive broad-spectrum antibiotics, and rely on multiple medical devices, all of

https://doi.org/10.24171/j.phrp.2025.0493

which increase susceptibility to colonization and infection. The coexistence of clonally related isolates such as ST17 with strains that appear to have independently acquired AMR determinants (e.g., CR30/23 and CR48/23 harboring *tet(A)* and *bla_TEM-235*) reflects the complex interplay between clonal expansion and repeated acquisition events, a pattern also observed in other high-risk settings [25,26]. These findings highlight the limitations of traditional epidemiological contact tracing. While epidemiological links provide valuable insights, they cannot fully capture the complexity of transmission dynamics, particularly in the presence of independent introductions. By integrating molecular tools such as ERIC-PCR and WGS into outbreak investigations, transmission pathways can be more accurately resolved, allowing infection prevention and control (IPC) teams to distinguish between clonal dissemination and multiple acquisition events [27–29].

The dissemination of ST17 from the ICU to ward 6E underscores the importance of hospital-level, rather than ward-specific, IPC strategies. Shared healthcare personnel, contaminated equipment, and patient transfers create opportunities for inter-ward spread, consistent with previous reports describing cross-unit dissemination of multidrug-resistant organisms [30,31]. Measures such as strict adherence to hand hygiene, environmental decontamination, and the use of dedicated medical devices for high-risk wards should therefore be prioritized [32–36]. In addition, inappropriate or excessive use of carbapenems may select for diverse resistant lineages, indicating that antimicrobial stewardship programs tailored to local resistance patterns are essential [37,38].

An important limitation in low-resource settings is the inability to perform WGS on all isolates because of financial and logistical constraints [39]. In such contexts, ERIC-PCR serves as a valuable complementary method for rapidly and cost-effectively screening large numbers of isolates, thereby prioritizing representative samples for sequencing [40]. This tiered approach enables a balance between resolution and feasibility, ensuring that outbreak investigations can still generate meaningful insights even when resources are constrained [41–43].

Overall, the persistence of ST17 in the ICU in our study illustrates how high-risk clones can establish long-term reservoirs in critical care environments, where they function as both amplifiers and sources of hospital-wide dissemination and potentially environmental contamination [30,31]. Sustained control of CRKP will require continuous genomic surveillance and bacterial characterization, ideally using WGS [44,45], integration of ERIC-PCR and WGS into routine workflows, and alignment of IPC and antimicrobial stewardship interventions [46]. Future studies should focus on real-time sequencing and prospective epidemiological investigations to better elucidate the contributions of asymptomatic carriers and environmental reservoirs to the persistence of high-risk clones such as ST17.

Conclusion

This study demonstrates the value of combining ERIC-PCR with WGS to refine investigations of CRKP outbreaks in our hospital. While ERIC-PCR offers a rapid and inexpensive means of clustering isolates, WGS provides high-resolution typing that distinguishes clonal spread from independent acquisition events. Nonetheless, ERIC-PCR remains a valuable frontline tool in low-resource settings for prioritizing isolates for WGS. Adoption of such a tiered approach allows hospitals to balance feasibility with analytical precision, enabling earlier detection, more accurate outbreak mapping, and targeted interventions. Integrating molecular epidemiology with robust infection control and antimicrobial stewardship efforts is essential to contain the threat of CRKP and mitigate its long-term impact on patient safety.

Supplementary Material

Table S1. Patient demographics and information on the 147 carbapenem-resistant *Klebsiella pneumoniae* isolates included in this study; **Table S2.** Genome assembly statistics for 12 carbapenem-resistant *Klebsiella pneumoniae* isolates subjected to whole-genome sequencing; **Figure S1.** Enterobacterial repetitive intergenic consensus polymerase chain reaction genotyping for all 147 carbapenem-resistant *Klebsiella pneumoniae* isolates of the study; **Figure S2.** Clustering of enterobacterial repetitive intergenic consensus polymerase chain reaction genotypes for study isolates. A total of 24 clusters were identified as having >50% similarity. Strains in clusters 10, 11, 22, and 24 appeared to have closer similarity (>60%). No cluster was distinct to a single ward, signifying transmission of various carbapenem-resistant *Klebsiella pneumoniae* genotypes across wards of the hospital; **Figure S3.** Benchmarking Universal Single-Copy Orthologs (BUSCO) completeness assessment of the assembled genomes using BUSCO v6 with the Enterobacterales_odb12 lineage dataset (n=874 orthologs). Bars indicate the proportion of single-copy complete (S, light blue), duplicated complete (D, dark blue), fragmented (F, yellow), and missing (M, red) BUSCOs. All genomes showed very high completeness (>98% complete BUSCOs), with only a small fraction of fragmented or missing orthologs, reflecting the overall high quality and completeness of the genome assemblies. Supplementary data are available at

https://doi.org/10.24171/j.phrp.2025.0493.

Notes

Ethics Approval

This study was approved by the Medical Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2023-869) and conducted at Hospital Canselor Tuanku Muhriz in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived due to the retrospective nature of the study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This study was supported by the Faculty of Medicine Fundamental Grant (GFFP) (code: FF-2024-016), Universiti Kebangsaan Malaysia. No additional external funding was received.

Availability of Data

The whole-genome sequencing (WGS) project for *Klebsiella pneumoniae* isolates analyzed in this study has been deposited in the DDBJ/ENA/GenBank under the BioProject accession PRJNA1333618. Individual genome sequences are available under accession numbers JBROFO000000000–JBROFZ000000000 (BioSamples SAMN51830973–SAMN51830984). The version described in this paper is version JBROFO010000000–JBROFZ010000000.

Raw sequencing data and genome assemblies generated from this study have been submitted under the BioProject PRJNA1333618.

Authors' Contributions

Conceptualization: UAZ, PP, SAS, HN; Data curation: UAZ, PP, XKC, NK, CLL, SAS, SS, NAAF, MFNY, HMG, HN, SRY; Formal analysis: UAZ, PP, HN, SAS, HMG; Funding acquisition: UAZ, PP, HN; Investigation: SAS, SS, UAZ, HN; Methodology: UAZ, HN; Project administration: all authors; Resources: all authors; Software: UAZ, HMG, SAS, HN; Supervision: PP, SAS, HN; Validation: PP, SAS, HMG, HN; Visualization: UAZ, HN; Writing–original draft: UAZ, HN; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0440
Osong Public Health Res Perspect 2026;17(3):272-281

Original Article

Factor structure and measurement invariance of the EuroQol 5-dimensional questionnaire: a secondary analysis of the Korea Health Panel survey

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ABSTRACT

Objectives: This study evaluated the EuroQol 5-dimensional questionnaire, 3-level version (EQ-5D-3L), using Korea Health Panel (KHP) data by examining its factor structure, measurement invariance across gender and age groups, and longitudinal measurement invariance.
Methods: Panel 1 data from the second survey year (2009), when the EQ-5D-3L was first introduced in the KHP, through the 12th year (2017) were analyzed, along with panel 2 data from 2019 to 2021. Confirmatory factor analysis and measurement invariance tests by gender and age groups were conducted within each period. Longitudinal measurement invariance was also evaluated for each period.
Results: A 1-factor model demonstrated good fit for the EQ-5D-3L. In panel 1, full measurement invariance across gender and age groups was supported. In panel 2, partial invariance was achieved after relaxing constraints on item 5. Longitudinal measurement invariance was supported over 5- and 10-year intervals in panel 1 and over a 3-year interval in panel 2, indicating temporal stability of the measurement model.
Conclusion: The EQ-5D-3L used in the KHP panel 1 and panel 2 datasets demonstrates a stable 1-factor structure and acceptable measurement invariance across key subgroups and over time. These findings support the use of the EQ-5D-3L as an appropriate instrument for assessing health-related quality of life among Korean adults and for longitudinal analyses within large-scale panel surveys.

Keywords: Confirmatory factor analysis; EuroQol five-dimensional questionnaire; Health-related quality of life; Korea Health Panel; Measurement invariance

Received: October 13, 2025
Revised: February 4, 2026
Accepted: March 9, 2026

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Introduction

Background/Rationale

Health-related quality of life (HRQOL) is widely recognized as a multidimensional construct

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reflecting an individual's perceived health status. The EuroQol 5-dimensional questionnaire (EQ-5D) is one of the most widely used instruments for measuring HRQOL because of its simplicity and international comparability [1]. The scale has been translated into more than 130 languages and is applied across a wide range of research and policy contexts owing to its cross-cultural validity, ease of administration, and strong psychometric performance [2,3]. The EQ-5D assesses 5 dimensions of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression [4]. Respondents indicate their health status using 3 response levels (1 = no problems, 2 = some problems, 3 = extreme problems), producing a 5-item health profile [2].

The 3-level version of the EQ-5D (EQ-5D-3L) is easy to understand and requires minimal time to complete, making it widely applicable in both clinical and population-based studies. However, because of its well-documented ceiling effect, the instrument may have limited sensitivity for detecting subtle differences in health status or changes over time, particularly in general population samples [5–7]. To address this limitation, the EuroQol Group developed the 5-level version of the EQ-5D (EQ-5D-5L), which retains the same 5 dimensions but expands the response scale to 5 severity levels [8]. Consequently, a growing body of research has examined the psychometric properties and practical applications of both the EQ-5D-3L and EQ-5D-5L instruments [2,4,9,10].

In the Republic of Korea, the EQ-5D-3L is widely used to measure HRQOL. In particular, it is included in the Korea Health Panel (KHP), a nationally representative panel survey that collects detailed information on health status, health care utilization, and medical expenditures [11]. The KHP has provided data from panel 1 covering the period from 2008 to 2018, and panel 2 has been conducted since 2019. Although the EQ-5D-3L is routinely used in the KHP to measure quality of life, the factor structure and measurement invariance of the instrument have not been systematically evaluated using these panel data.

Measurement invariance refers to the degree to which a measurement instrument assesses the same construct in the same way across groups or time points, thereby enabling valid comparisons [12]. When comparing scores across groups or across time using the same scale, it is necessary to assume that the measurement properties (e.g., structure and meaning) of the instrument remain equivalent across those contexts [13]. For example, when comparing EQ-5D responses across languages—as in studies examining equivalence between English and Chinese versions—the stability and comparability of the measurement instrument must first be established [14]. Although several studies have investigated the measurement invariance of the EQ-5D-3L [2,4,9,14,15], evidence regarding longitudinal measurement invariance (LMI) remains limited.

HIGHLIGHTS

- Using Korea Health Panel data, we evaluated the EuroQol 5-dimensional questionnaire, 3-level version and confirmed a robust 1-factor structure with good model fit.
- Measurement invariance held across gender and age in panel 1; in panel 2, partial invariance was achieved when relaxing constraints on item 5.
- Longitudinal invariance was supported over 5–10 years (panel 1) and 3 years (panel 2), supporting valid group comparisons and longitudinal use in Korean adults.

Objectives

In this context, the objectives of this study were threefold. First, using panel 1 and panel 2 data from the KHP, we examined the factor structure of the EQ-5D-3L and tested measurement invariance across gender and age groups. Second, using panel 1 data, for which the panel survey has been completed, we evaluated short-term LMI over 5 years and long-term LMI over 10 years. Third, using panel 2 data that are currently being collected, we examined LMI across available survey waves. Through these analyses, we aimed to provide empirical evidence supporting the validity and applicability of the EQ-5D-3L for comparative and longitudinal research on HRQOL using KHP data.

Materials and Methods

Study Design and Setting

This study was a cross-sectional observational analysis based on secondary data from the KHP.

Setting and Participants

In this study, data from panel 1 (2008–2018) and panel 2 (2019–2021) of the KHP were used for analysis. The KHP is a nationally representative panel survey that provides data for detailed analyses of health care utilization, medical expenditures, and related factors [11]. It serves as an important source of evidence for establishing health and medical policy in the Republic of Korea. The survey was conducted using probability-proportional-to-size 2-stage stratified cluster sampling and included approximately 8,000 households nationwide.

The data used in this study included panel 1 data from the second survey year (2009), when the EQ-5D-3L was first introduced, through the 12th survey year (2017), and panel 2 data from the first survey year (2019) through the third survey year (2021), which were the most recent data released at the time of analysis. The analytic sample comprised 12,606 participants based on the second year of panel 1 and 11,593 participants based on the first year of panel 2.

Variables

The EQ-5D-3L, a measure of HRQOL, comprises 5 items: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each item is rated on a 3-point scale: 1=no problems, 2=some or moderate problems, and 3=extreme problems or unable to perform the activity. Higher scores indicate poorer quality of life [2,16].

Cronbach's α values for the EQ-5D-3L across survey years were as follows: panel 1, 2009 (0.704), 2010 (0.701), 2011 (0.728), 2012 (0.757), 2013 (0.760), 2015 (0.787), and 2017 (0.790); Panel 2, 2019 (0.762), 2020 (0.769), and 2021 (0.760).

Data Analysis

Participants and data collection

Confirmatory factor analysis (CFA) and measurement invariance analyses were conducted to evaluate the validity and measurement invariance of the EQ-5D-3L used in the KHP. The analyses used data from both panel 1 (2008–2018) and panel 2 (2019–2021), in which the EQ-5D-3L was administered. For panel 1, data from 12,606 individuals were analyzed using responses anchored to the second survey year (2009), when the EQ-5D-3L was first introduced. For panel 2, data from 11,593 individuals were analyzed using responses from the first survey year (2019).

Confirmatory factor analysis

CFA was conducted to examine the factor structure of the EQ-5D-3L. Specifically, we compared alternative factor structure models discussed in previous studies and selected the model judged to provide the most appropriate representation of the data.

Measurement invariance analysis

To evaluate measurement invariance across gender and age groups, we first examined the fit of the factor structure model within each group and then performed multi-group confirmatory factor analysis. Invariance testing proceeded sequentially through configural, weak, strong, and strict invariance [12,17–19]. For group comparisons, participants were categorized by gender as male or female and by age as younger than 65 years or 65 years and older. This approach allowed us to examine whether the EQ-5D-3L functioned consistently across gender and age groups.

Longitudinal measurement invariance

LMI was tested to assess the stability of measurement over time. For the factor structure model, all latent factors and observed indicators across the time points included in each analysis were specified simultaneously, and invariance testing proceeded through configural, weak, strong, and strict models [13,20–22].

Statistical Methods

IBM SPSS ver. 24.0 (IBM Corp.) was used to analyze the demographic characteristics of the study participants and the characteristics of the main variables. Mplus ver. 8.4 (Muthén & Muthén) was used to evaluate the factor structure model and measurement invariance. Because the EQ-5D-3L items are categorical, the weighted least squares mean and variance adjusted (WLSMV) estimator was used. Missing data were handled using the default WLSMV procedure in Mplus, which applies pairwise deletion for the estimation of polychoric correlations and thresholds. This approach uses all available pairs of observations; however, because pairwise deletion may still be sensitive to the missing-data mechanism, we additionally report the proportion of missing responses for each EQ-5D-3L item at each wave and interpret the longitudinal findings cautiously. Model fit was evaluated using the comparative fit index (CFI) and Tucker-Lewis index (TLI), with values of 0.90 or higher indicating acceptable fit, and the standardized root mean square residual (SRMR) and root mean square error of approximation (RMSEA), with values of 0.08 or lower indicating acceptable fit [23]. For model comparisons, invariance (or negligible differences) was considered supported when the decrease in CFI was no greater than 0.010 ($\Delta CFI \geq -0.010$), the increase in RMSEA was no greater than 0.015 ($\Delta RMSEA \leq 0.015$), and the increase in SRMR was no greater than 0.010 ($\Delta SRMR \leq 0.010$), which is appropriate for large-sample settings [24].

Ethics Statement

This study used publicly available secondary data without any personally identifiable information. As such, it does not constitute human subjects research and was exempt from Institutional Review Board (IRB) approval. All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

https://doi.org/10.24171/j.phrp.2025.0440

Results

Participants

Table 1 presents the demographic characteristics of participants from the 2 baseline periods, 2009 and 2019, including gender, age, marital status, and educational level. The gender distribution was similar across periods, with the proportion of male participants increasing slightly from 44.0% in 2009 to 44.6% in 2019. The mean age increased from 48.82 years (standard deviation [SD], 16.1) to 56.48 years (SD, 16.4). Marital status showed a decrease in the proportion of married participants, from 72.5% to 71.2%, and increases in the proportions of divorced participants, from 8.9% to 11.1%, and separated participants, from 2.4% to 5.4%. Educational level remained relatively consistent, with approximately one-third of participants having a university degree or higher in both periods.

Confirmatory Factor Analysis

Previous studies have proposed several factor structure models for the EQ-5D-3L, including a 1-factor model [2,4] and a 2-factor model that distinguishes activities (mobility, self-care, and usual activities) from symptoms (pain/discomfort and anxiety/depression). Accordingly, we compared these alternative structures to identify the most appropriate factor structure for the present data (Figure 1). The main results showed that all tested models demonstrated acceptable fit in both panel 1 and panel 2. Detailed factor loadings for all evaluated models are presented in Table S1. Although the χ^2 statistic was lower

Table 1. Participants' demographic characteristics

Characteristic	Panel 1, 2nd data (2009) (*n* = 12,606)	Panel 2, 1st data (2019) (*n* = 11,593)
Sex		
Male	5,544 (44.0)	5,170 (44.6)
Female	7,062 (56.0)	6,423 (55.4)
Age (y)	48.82 ± 16.1 (18, 98)	56.48 ± 16.4 (19, 99)
Marital status		
Married	9,142 (72.5)	8,250 (71.2)
Bereaved	69 (0.5)	74 (0.6)
Divorced	1,117 (8.9)	1,289 (11.1)
Separated	302 (2.4)	627 (5.4)
Single	1,976 (15.7)	1,352 (11.7)
Educational background		
Below elementary school graduation	2,792 (22.1)	2,578 (22.2)
Middle school graduation	1,470 (11.7)	1,593 (13.7)
High school graduation	4,102 (32.5)	3,498 (30.2)
University graduation or higher	4,242 (33.7)	3,924 (33.8)

Data are presented as *n* (%) or mean ± standard deviation (minimum, maximum).

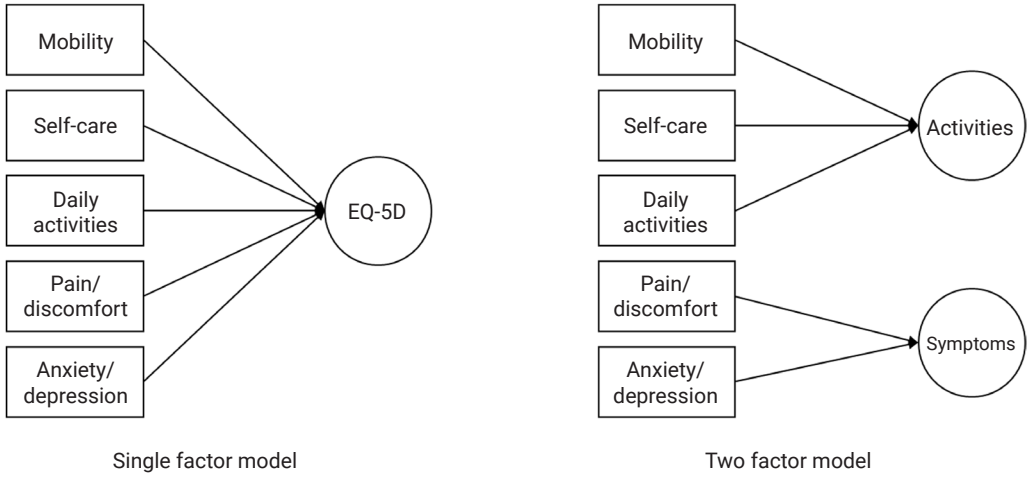


Figure 1. Factor structure model of EuroQol 5-dimensional questionnaire, 3-level version (EQ-5D-3L).

for the 2-factor model than for the 1-factor model, this difference is likely attributable to the sensitivity of the χ^2 test to trivial deviations in very large samples (panel 1, n=12,606; panel 2, n=11,593). In such settings, it is generally more informative to evaluate changes in incremental fit indices, such as the CFI and TLI, and absolute fit indices, such as the RMSEA, than to rely solely on χ^2 differences [24]. When comparing the 2 models, the ΔCFI, ΔSRMR, and ΔRMSEA values remained within the recommended cutoffs shown in Table 2, indicating that the difference in fit between the models was negligible. Under the principle of parsimony, simpler models are preferred when they explain the data comparably well. Because the 1-factor model already demonstrated excellent fit (CFI > 0.99, TLI > 0.99, RMSEA < 0.06), adopting a more complex 2-factor model was not necessary. In addition, the strong fit of the 1-factor model supports the interpretation that the 5 items reflect a common latent construct. Given the minimal gain in model fit and the consistent replication of the 1-factor structure across both periods, the 1-factor model was selected as the final model.

Validation of the Group-Specific Baseline Model

Before testing measurement invariance across gender and age groups, we examined the fit of the baseline model separately within each group. The main results are presented in Table 3. For the second survey year of panel 1 and the first survey year of panel 2, the CFA results indicated that all group-specific models demonstrated adequate fit.

Measurement Invariance

Measurement invariance across gender and age groups was evaluated using 2 datasets. First, Table 4 presents the results based on the second survey year of panel 1. In this analysis,

Table 2. Factor structure analysis of the EQ-5D-3L

	χ^2	df	CFI	TLI	SRMR	RMSEA (90% CI)
Panel 1, 2nd data in 2009 (n = 12,606)						
One factor	187.412	5	0.996	0.991	0.035	0.054 (0.047–0.061)
Two factors	122.743	4	0.997	0.993	0.025	0.049 (0.041–0.056)
Panel 2, 1st data in 2019 (n = 11,593)						
One factor	179.536	5	0.998	0.995	0.027	0.055 (0.048–0.062)
Two factors	123.406	4	0.998	0.996	0.019	0.051 (0.043–0.059)

EQ-5D-3L, EuroQol5-dimensional questionnaire,3-level version; df, degrees of freedom; CFI, comparative fit index; TLI, Tucker-Lewis index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval.

Table 3. Results of analysis of the baseline model for data by gender and age groups of EQ-5D-3L

	Model fit index					
	χ^2	df	CFI	TLI	SRMR	RMSEA (90% CI)
Panel 1, 2nd data in 2009 (n = 12,606)						
Sex						
Male (n = 5,544)	53.428	5	0.997	0.994	0.027	0.042 (0.032–0.052)
Female (n = 7,062)	109.636	5	0.996	0.992	0.038	0.054 (0.046–0.064)
Age						
Under 65 years (n = 9,979)	88.177	5	0.994	0.988	0.035	0.041 (0.034–0.049)
Older 65 years (n = 2,627)	109.212	5	0.992	0.985	0.045	0.089 (0.075–0.104)
Panel 2, 1st data in 2019 (n = 11,593)						
Sex						
Male (n = 5,170)	55.660	5	0.998	0.997	0.025	0.044 (0.034–0.055)
Female (n = 6,423)	132.613	5	0.997	0.994	0.029	0.063 (0.054–0.073)
Age						
Under 65 years (n = 7,388)	82.525	5	0.994	0.989	0.039	0.046 (0.037–0.055)
Older 65 years (n = 4,205)	109.946	5	0.997	0.994	0.027	0.071 (0.060–0.082)

EQ-5D-3L, EuroQol5-dimensional questionnaire,3-level version; df, degrees of freedom; CFI, comparative fit index; TLI, Tucker-Lewis index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval.

https://doi.org/10.24171/j.phrp.2025.0440

strict invariance was supported across both gender groups and age groups, indicating that measurement invariance was well maintained in panel 1.

Next, Table 5 presents the results for the first wave of panel 2 (2019). The configural model showed acceptable fit in both the gender-group and age-group analyses, indicating that the overall 1-factor structure was comparable across groups. However, when factor loadings were constrained to equality, corresponding to weak (metric) invariance, the increase in RMSEA relative to the configural model exceeded the recommended threshold (ΔRMSEA > 0.015), suggesting that full metric invariance was not supported in panel 2 [24].

Accordingly, we proceeded to test partial measurement

Table 4. Results of measurement invariance verification for gender and age groups of EQ-5D-3L (panel 1 data)

Invariance model	Model fit index					Model comparison		
	χ^2	df	CFI	SRMR	RMSEA (90% CI)	ΔCFI	ΔSRMR	ΔRMSEA
1st period 2nd data (2009)								
Sex group								
Configural	167.031	10	0.996	0.033	0.050 (0.043–0.057)			
Weak	138.746	14	0.997	0.034	0.038 (0.032–0.043)	0.001	0.001	−0.012
Strong	170.025	18	0.996	0.034	0.037 (0.032–0.042)	−0.001	0.000	−0.001
Strict	191.703	23	0.996	0.035	0.034 (0.030–0.039)	0.000	0.001	−0.003
Age group								
Configural	196.866	10	0.993	0.037	0.054 (0.048–0.061)			
Weak	152.500	14	0.995	0.038	0.040 (0.034–0.045)	0.002	0.001	−0.014
Strong	251.910	18	0.991	0.039	0.045 (0.041–0.050)	−0.004	0.001	0.005
Strict	371.193	23	0.987	0.040	0.049 (0.045–0.053)	−0.004	0.001	0.004

ΔCFI, ΔRMSEA, and ΔSRMR were computed as (fit index of the more constrained model) minus (fit index of the less constrained model). Thus, negative ΔCFI indicates a decrease in CFI under additional constraints.

EQ-5D-3L, EuroQol5-dimensional questionnaire, 3-level version; df, degrees of freedom; CFI, comparative fit index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval; ΔCFI, change in comparative fit index; ΔSRMR, change in standardized root mean square residual; ΔRMSEA, change in root mean square error of approximation.

Table 5. Results of measurement invariance verification for gender and age groups of EQ-5D-3L (panel 2 data)

Invariance model	Model fit index					Model comparison		
	χ^2	df	CFI	SRMR	RMSEA (90% CI)	ΔCFI	ΔSRMR	ΔRMSEA
2nd period 1st data (2019)								
Sex group								
Configural	194.145	10	0.997	0.028	0.056 (0.050–0.063)			
Weak	134.378	14	0.998	0.028	0.039 (0.033–0.045)	0.001	0.000	−0.017
Partial weak (with factor loading of item 5 free)	173.535	13	0.998	0.028	0.046 (0.040–0.052)	0.001	0.000	−0.010
Partial strong	190.764	17	0.997	0.028	0.042 (0.037–0.047)	−0.001	0.000	−0.004
Partial strict	192.380	22	0.998	0.029	0.037 (0.032–0.041)	0.001	0.001	−0.005
Age group								
Configural	189.829	10	0.996	0.036	0.056 (0.049–0.063)			
Weak	134.620	14	0.997	0.036	0.039 (0.033–0.045)	0.001	0.000	−0.017
Partial weak (with factor loading of item 5 free)	166.224	13	0.997	0.036	0.045 (0.039–0.051)	0.001	0.000	−0.011
Partial strong	250.564	17	0.995	0.037	0.049 (0.043–0.054)	−0.002	0.001	0.004
Partial strict	372.138	22	0.993	0.049	0.052 (0.048–0.057)	−0.002	0.012	0.003

ΔCFI, ΔRMSEA, and ΔSRMR were computed by comparing each model with the immediately preceding, less constrained model (e.g., metric vs. configural; strong vs. metric; strict vs. strong). For partial invariance models, changes were computed relative to the corresponding preceding model in the partial sequence (e.g., partial metric vs. configural; partial strong vs. partial metric; partial strict vs. partial strong). Negative ΔCFI indicates a decrease in CFI under additional constraints.

EQ-5D-3L, EuroQol5-dimensional questionnaire,3-level version; df, degrees of freedom; CFI, comparative fit index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval; ΔCFI, change in comparative fit index; ΔSRMR, change in standardized root mean square residual; ΔRMSEA, change in root mean square error of approximation.

invariance by relaxing equality constraints on items showing evidence of non-invariance [20,25–27]. Inspection of modification indices (MI) and expected parameter changes (EPC) identified item 5 (anxiety/depression) as the primary source of non-invariance in the weak invariance model, with substantial misfit observed in both the gender-group analysis (MI, 32.096; EPC, 0.262) and the age-group analysis (MI, 27.892; EPC, 0.213). On this basis, we relaxed the loading constraint for item 5 and evaluated partial invariance primarily using changes in approximate fit indices and substantive interpretability rather than relying solely on raw chi-square statistics under WLSMV estimation [23]. This adjustment also acknowledges the possibility that the anxiety/depression dimension was particularly sensitive to socioenvironmental changes during panel 2, including the coronavirus disease 2019 (COVID-19) pandemic.

Subsequent analyses showed that, after freeing item 5, the partial weak and partial strong invariance models were supported for both gender and age groups (Table 5). For gender groups, partial strict invariance was also supported. For age groups, however, the transition from the partial strong to the partial strict invariance model resulted in a change in SRMR that exceeded the recommended threshold (ΔSRMR > 0.010). Therefore, in panel 2, comparisons between age groups should be interpreted at the level of partial strong invariance, and strict invariance across age groups was not fully supported [24].

Validation of Baseline Models for Each Year for LMI

To evaluate LMI, analyses were conducted using data from both panel 1 and panel 2. For panel 1, short-term longitudinal invariance was examined using 5 waves from the second through the sixth survey years (LMI model 1), and long-term longitudinal invariance was examined using 5 waves from the second through the 10th survey years at 2-year intervals (LMI model 2). For panel 2, the analysis used the first 3 released waves, corresponding to the first through third survey years, to generate empirical evidence on the LMI of the EQ-5D-3L in the KHP.

Before testing LMI, CFA was performed to confirm that the annual datasets included in each analysis fit the selected EQ-5D-3L factor structure model. The results are presented in Table 6. All annual datasets demonstrated adequate fit to the study model.

Longitudinal Measurement Invariance

The results of the LMI analyses are presented in Table 7. In panel 1, both LMI model 1 and LMI model 2 were supported through the strict invariance level. In panel 2, LMI model 3 was also supported through the strict invariance level. These

Table 6. Baseline model analysis for each year's data to verify longitudinal measurement invariance

Division	Model fit index					
	χ^2	df	CFI	TLI	SRMR	RMSEA (90% CI)
LMI model 1						
Panel 1 2nd–6th data (5 wave) (*n* = 12,606)						
2nd (2009) (*n* = 12,606)	187.424	5	0.996	0.991	0.035	0.054 (0.047–0.061)
3rd (2010) (*n* = 10,719)	237.787	5	0.994	0.987	0.040	0.066 (0.059–0.073)
4th (2011) (*n* = 9,986)	235.915	5	0.994	0.988	0.038	0.068 (0.061–0.076)
5th (2012) (*n* = 9,186)	180.837	5	0.998	0.995	0.032	0.062 (0.054–0.070)
6th (2013) (*n* = 8,598)	159.142	5	0.997	0.994	0.029	0.060 (0.052–0.068)
LMI model 2						
Panel 1 2nd–10th data (5 wave) (*n* = 12,606)						
2nd (2009) (*n* = 12,606)	187.424	5	0.996	0.991	0.035	0.054 (0.047–0.061)
4th (2011) (*n* = 9,986)	235.914	5	0.994	0.988	0.038	0.068 (0.061–0.076)
6th (2013) (*n* = 8,598)	159.142	5	0.997	0.994	0.029	0.060 (0.052–0.068)
8th (2015) (*n* = 7,624)	106.522	5	0.998	0.997	0.022	0.052 (0.043–0.060)
10th (2017) (*n* = 7,086)	69.947	5	0.999	0.998	0.019	0.043 (0.034–0.052)
LMI model 3						
Panel 2 1st–3rd data (3 wave) (*n* = 11,593)						
1st (2019) (*n* = 11,593)	179.534	5	0.998	0.995	0.027	0.055 (0.048–0.062)
2nd (2020) (*n* = 10,034)	200.206	5	0.997	0.994	0.030	0.062 (0.055–0.070)
3rd (2021) (*n* = 9,091)	161.861	5	0.997	0.994	0.030	0.059 (0.051–0.067)

LMI, longitudinal measurement invariance; df, degrees of freedom; CFI, comparative fit index; TLI, Tucker-Lewis index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval.

https://doi.org/10.24171/j.phrp.2025.0440

Table 7. LMI verification and model comparison of the EQ-5D-3L scale

Invariance model	Model fit index					Model comparison		
	χ^2	df	CFI	SRMR	RMSEA (90% CI)	ΔCFI	ΔSRMR	ΔRMSEA
Panel 1 2nd–6th data								
Configural	1507.117	215	0.993	0.032	0.022 (0.021–0.023)			
Weak	1332.760	231	0.994	0.034	0.019 (0.018–0.020)	0.001	0.002	−0.003
Strong	1568.244	247	0.993	0.033	0.021 (0.020–0.022)	−0.001	−0.001	0.002
Strict	1514.836	267	0.993	0.034	0.019 (0.018–0.020)	0.000	0.001	−0.002
Panel 1 2nd–10th data								
Configural	1086.739	215	0.996	0.028	0.018 (0.017–0.019)	-	-	-
Weak	1099.223	231	0.996	0.032	0.017 (0.016–0.018)	0.000	0.004	−0.001
Strong	1199.627	247	0.995	0.030	0.017 (0.017–0.018)	−0.001	−0.002	0.000
Strict	1287.631	267	0.995	0.033	0.017 (0.016–0.018)	0.000	0.003	0.000
Panel 2 1st–3rd data								
Configural	667.491	72	0.996	0.027	0.027 (0.025–0.029)	-	-	-
Weak	539.324	80	0.997	0.028	0.022 (0.020–0.024)	0.001	0.001	−0.005
Strong	654.577	88	0.997	0.028	0.024 (0.022–0.025)	0.000	0.000	0.002
Strict	606.196	98	0.997	0.028	0.021 (0.020–0.023)	0.000	0.000	−0.003

LMI, longitudinal measurement invariance; EQ-5D-3L, EuroQol5-dimensional questionnaire,3-level version; df, degrees of freedom; CFI, comparative fit index; SRMR, standardized root mean square residual; RMSEA, root mean square error of approximation; CI, confidence interval; ΔCFI, change in comparative fit index; ΔSRMR, change in standardized root mean square residual; ΔRMSEA, change in root mean square error of approximation.

findings indicate that the EQ-5D-3L can be used appropriately in both short-term and long-term longitudinal studies of HRQOL.

Discussion

Key Results

This study examined the factor structure and measurement invariance of the EQ-5D-3L across time, gender, and age using nationally representative data from the KHP. The confirmatory factor analysis results consistently supported the original 5-item, 1-factor structure across all survey waves. This finding reinforces previous research suggesting that the EQ-5D descriptive system can be represented parsimoniously by a single latent construct [2,4]. Overall, the results indicate that the EQ-5D-3L maintains a stable and interpretable measurement structure over time while also revealing meaningful period- and group-specific differences in the degree of measurement invariance achieved. These findings highlight the importance of clearly specifying the conditions under which comparisons of HRQOL are interpreted across demographic groups and survey periods.

In panel 1 (2009–2017), strict measurement invariance was supported across both gender and age groups. This result indicates that the EQ-5D-3L functioned equivalently across these groups during this period, permitting valid comparisons of latent means, variances, and other structural parameters [23,24]. The robustness of measurement invariance observed in panel 1 is consistent with previous studies reporting stable psychometric properties of the EQ-5D-3L in general population samples [6,9,12]. Taken together, these findings support the use of the EQ-5D-3L for subgroup comparisons in Korean population health data collected prior to 2018 without substantial concern regarding measurement bias.

In contrast, the results for panel 2 (2019–2021) were more nuanced. Although configural invariance was supported across both gender and age groups, full metric invariance was not supported according to commonly recommended criteria based on changes in approximate fit indices [24]. Subsequent partial measurement invariance analyses identified item 5 (anxiety/depression) as the primary source of non-invariance. After relaxing the equality constraint for this item, the partial invariance models demonstrated acceptable fit. Specifically, partial strict invariance was supported for gender groups, whereas only partial strong invariance was supported for age groups. Accordingly, comparisons between age groups in panel 2 should be interpreted at the level of partial strong invariance rather than strict invariance, consistent with established methodological guidance for partial measurement invariance [20,25–27].

The differential performance of the anxiety/depression item in panel 2 suggests that this dimension may be particularly sensitive to temporal and contextual influences. The period after 2019 coincided with substantial socioenvironmental disruption, including the COVID-19 pandemic, which has been widely associated with changes in population mental health.

Compared with physical health domains such as mobility or self-care, mental health-related dimensions may be more responsive to external stressors and broader shifts in social context. Consequently, changes in social and psychological conditions during this period may have influenced how respondents across age groups interpreted and reported anxiety or depressive symptoms. These contextual effects could reduce measurement equivalence even when the overall factor structure remains stable. Importantly, this pattern should not be interpreted as a weakness of the EQ-5D-3L itself but rather as reflecting context-dependent variation in how mental health experiences are reported within population surveys.

With respect to longitudinal stability, the LMI analyses indicated that the measurement structure of the EQ-5D-3L remained stable across both short-term and longer-term intervals within each panel. These findings suggest that, despite the context-specific variation observed for the anxiety/depression dimension in panel 2, the underlying construct measured by the EQ-5D-3L remained largely consistent over time. As a result, the instrument appears suitable for longitudinal analyses of HRQOL in panel data when appropriate measurement invariance assumptions are verified.

From a methodological perspective, the present findings highlight the practical value of partial measurement invariance approaches when strict invariance assumptions are not fully satisfied. By identifying and freeing the parameter associated with item 5, the analysis preserved comparability for the remaining dimensions of the EQ-5D-3L while avoiding overly restrictive model constraints. This strategy aligns with contemporary recommendations in the structural equation modeling literature, which emphasize balancing statistical fit criteria with substantive interpretability when evaluating measurement invariance in large-scale survey data [20,23,25].

Several limitations should be considered when interpreting these findings. First, the EQ-5D-3L is known to exhibit ceiling effects, particularly in general population samples, which may limit its sensitivity to subtle changes in health status [6,9]. Such ceiling effects may partially contribute to the apparent stability observed in some invariance tests, particularly in panel 2. Second, although the present study focused on establishing measurement equivalence, it did not examine substantive differences in latent means or variances across groups. Future research should extend this work by investigating group differences in HRQOL once appropriate levels of measurement invariance have been established. Third, the age classification used in this study dichotomized respondents at 65 years to reflect policy-relevant definitions in the Republic of Korea. However, this approach may obscure heterogeneity within broader age categories. Future studies could adopt more granular age-group classifications or continuous measurement invariance approaches to better capture age-related variation in HRQOL. Finally, differences in sampling design and survey context between panel 1 and panel 2 of the KHP may have influenced response patterns. These structural differences should be considered when interpreting comparisons across survey periods.

In summary, the findings of this study demonstrate that the EQ-5D-3L generally maintains a stable measurement structure over time in the Korean population. At the same time, the results underscore the importance of careful, context-sensitive interpretation of group comparisons during periods characterized by substantial social and environmental change. Explicit consideration of partial measurement invariance is therefore essential for the valid use of the EQ-5D-3L in contemporary population health research and policy evaluation [23,24].

Conclusion

This study supports the validity and applicability of the EQ-5D-3L by examining its measurement invariance using KHP data. The findings provide empirical evidence that the instrument can be used appropriately in comparative and longitudinal studies of HRQOL across gender and age groups in the Republic of Korea.

Supplementary Material

Table S1. Participants' demographic characteristics. Supplementary data are available at https://doi.org/10.24171/j.phrp.2025.0440.

Notes

Ethics Approval

This study used de-identified secondary data from the KHP. Because the dataset is publicly available in anonymized form, IRB approval for this secondary analysis was waived in accordance with applicable regulations and institutional policy.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by the Ministry of Education of the Republic of Korea, the National Research Foundation of Korea (NRF-2023S1A5A2A01077879), and the 2024 Academic Research Support Program at Gangneung-Wonju National University.

Availability of Data

The data that support the findings of this study are available from the KHP. Access is subject to the KHP data access policy and may require an application; the authors did not receive special access privileges.

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phrp
OSONG PUBLIC HEALTH AND RESEARCH PERSPECTIVES

pISSN 2210-9099 eISSN 2233-6052
https://doi.org/10.24171/j.phrp.2025.0560
Osong Public Health Res Perspect 2026;17(3):282-285

Perspective

Integrating Ayush practitioners in outbreak investigations in India: a call for action

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Introduction

Public health surveillance, early detection, rapid response, and prevention of disease spread are essential components of any outbreak investigation [1]. Effective outbreak management in India's heterogeneous population requires both technical expertise and strong community engagement [2]. Strengthening outbreak detection and response remains a public health priority for India and the South-East Asia Region, and this effort requires the involvement of the country's diverse health systems. Over the past decade, the Ministry of Ayush has implemented a dedicated public health strategy through national and research programs, strengthened regulatory and quality standards, and expanded primary and tertiary care. Extensive research on Ayush medicines during the coronavirus disease 2019 (COVID-19) pandemic, along with population-level assessments, has contributed to the evidence base for traditional medicine [3]. During COVID-19, the Ministry of Ayush issued advisories and guidelines for each system of medicine, and Ayush practitioners participated in preventive care through the distribution of Kabasura Kudineer and Ayush-64 in communities. These preventive measures and immunity-enhancing advisories gained public trust and contributed to substantial behavioral change. However, Ayush practitioners were not formally included in outbreak investigation teams. This gap remained evident during the sudden hair-loss outbreak in rural Maharashtra. This manuscript aims to examine the potential for integrating Ayush practitioners into outbreak investigations, using a rural case study and international practices to support policy recommendations for India.

Received: December 10, 2025
Revised: March 3, 2026
Accepted: March 26, 2026

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Case Example: Sudden Hair Loss in Rural Maharashtra

A cluster of sudden hair loss was first reported in Bondgaon village in Shegaon Tehsil, Buldhana District, Maharashtra, in early January 2025. By February, more than 250 people from nearby villages had reported similar complaints. Affected individuals described itching of the scalp followed by rapid hair shedding, often progressing to complete loss of scalp and beard hair within 3 days. No other symptoms were reported. Biopsy findings did not indicate fungal infection or seborrheic dermatitis, although treatment was initially administered for suspected fungal disease. Villagers consumed mixed diets that included wheat, *bhajira*, *channa*, brinjal,

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fish, and prawns, and they relied on water from wells, rivers, and local dams for drinking. Some water samples showed elevated nitrate levels and increased total dissolved solids. The clinical presentation and clustering pattern were atypical compared with previously reported cases of alopecia areata in northern India.

In northern India, alopecia areata reportedly affects adult men younger than 40 years more often than females; however, onset during childhood is reportedly higher among women and girls. The incidence of severe alopecia is higher in males and boys at younger ages [4]. Severe alopecia is uncommon, and the most frequent forms of temporary hair loss include androgenetic alopecia, telogen effluvium, alopecia areata, and traction alopecia [5,6]. Acute telogen effluvium presents as sudden scalp hair loss occurring 2–3 months after a triggering event such as fever, surgery, starvation, or blood loss, with daily shedding ranging from fewer than 100 to more than 1,000 strands. It reduces hair density but does not cause complete baldness and usually resolves within several months [6,7].

No clear triggers were identified in this case. Therefore, it was considered an undiagnosed disease of unknown etiology. Many undiagnosed diseases occur in rural settings [8]. Outbreaks in rural settings have received limited attention in the scientific literature. Public health authorities and epidemiologists face particular challenges when investigating undiagnosed diseases of unknown etiology, especially in rural areas [9].

The Indian Council of Medical Research initiated an outbreak investigation and collected samples, including food, water, hair products, hair, and nails, to identify the possible cause. However, Dr Bawaskar, a renowned physician, collected water, soil, and food samples and conducted a study in Buldhana District. He found high levels of selenium in the wheat distributed through the Public Distribution System. The selenium content was estimated to be 600 times higher than the normal limit, and this was identified as the primary cause of baldness. Subsequently, the government withdrew the distributed wheat batch, and hair regrowth was observed among affected individuals [10].

The Integrated Disease Surveillance Program of the National Centre for Disease Control, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, has established a structured approach to outbreak detection and response, with designated officers at both the state and district levels responsible for monitoring and responding to potential threats [2]. However, Ayush is not included within this outbreak management framework. During coronavirus disease 2019 (COVID-19), rather than being involved in disease surveillance or contact tracing, Ayush practitioners were used primarily for the clinical management of mild-to-moderate cases [11]. There are multiple examples of Ayush systems being used for preventive care; for example, the Government of Tamil Nadu routinely distributes the Siddha medicine Nilavembu Kudineer during dengue and chikungunya outbreaks [12]. Likewise, Ayush medical camps were conducted in Buldhana District to alleviate hair loss, boost immunity, and reduce severity. However, the outcomes of these camps have not been systematically documented. Ayush practitioners contributed to supportive care but were not engaged in epidemiological assessment.

HIGHLIGHTS

- This Perspective highlights integrating Ayush practitioners into formal outbreak investigation systems. Despite their role during coronavirus disease 2019, they remain excluded from surveillance and rapid response teams. As an example, Ayush practitioners were utilized for the management of alopecia in Maharashtra rather than the outbreak investigations. Field work links the outbreak to selenium-contaminated Public Distribution System wheat, where withdrawal led to recovery. Evidence indicates that Complementary and Alternative Medicine providers can support surveillance and risk communication. Key constraints include limited epidemiological training, policy gaps, and weak coordination. Structured Ayush-focused Field Epidemiology Training, and defined roles could strengthen outbreak response in India.

Call for Action on Integrating Ayush Practitioners in Public Health

Several countries have involved complementary and alternative medicine (CAM) practitioners in outbreak investigations and public health emergencies. Traditional Chinese medicine practitioners were involved in COVID-19-related activities across hospital and community settings, working with allopathic teams in triage, surveillance, and patient management during both prevention and treatment phases [13,14]. In the Republic of Korea, Korean medicine practitioners participate in early triage, home-based monitoring, and teleconsultations through a dual system that places biomedicine and Korean medicine within the same public health framework [15]. Several African countries, including Uganda and Ghana, have involved local healers in community-level surveillance and risk communication during outbreaks such as Ebola, consistent with the World Health Organization (WHO) Traditional Medicine Strategy [16]. To address surveillance inequities, the WHO Workforce 2030 strategy promotes diverse workforce composition, including the

integration of community-based and mid-level practitioners into interprofessional monitoring systems.

The lack of formal epidemiology training for Ayush practitioners, the absence of a clear policy framework, and weak transdisciplinary coordination are key barriers to the inclusion of Ayush in outbreak investigations and public health emergencies. Ayush practitioners possess foundational qualifications relevant to outbreak investigations, yet they remain underutilized. Ayush graduate programs in India include basic training in public health, epidemiology, and community medicine. Ayush systems were involved during chikungunya, dengue, and COVID-19 outbreaks. Ayush practitioners were also deployed for health promotion and immunity-boosting interventions under the National Ayush Mission.

Targeted training programs could prepare Ayush professionals for defined outbreak-response roles such as contact tracing, syndromic surveillance, and risk communication. Establishing a dedicated Ayush track within India's Field Epidemiology Training Program and including Ayush practitioners in mock drills, rapid response exercises, and district-level teams could strengthen their readiness for field deployment. Training in the use of Integrated Disease Surveillance Programme reporting tools, together with the assignment of defined roles within rapid response teams, would create a structured pathway for their involvement in public health emergencies.

India has over 770,000 registered Ayush practitioners, many of whom already work in rural and underserved areas. These practitioners represent an underused but potentially valuable public health resource in India. Integration of traditional, complementary, and alternative medicine in 3 Indian states has shown that interpersonal trust, cooperation, and mutual recognition can be developed between Ayush and biomedical systems [17]. Lessons from global CAM integration suggest that the involvement of these practitioners in outbreak investigations is both feasible and potentially beneficial. With structured training, digital enablement, and policy inclusion, India could transform Ayush practitioners from peripheral care providers into frontline public health allies.

Notes

Ethics Approval
Not applicable.

Conflicts of Interest
The authors have no conflicts of interest to declare.

Funding
None.

Availability of Data
Not applicable.

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https://doi.org/10.24171/j.phrp.2025.0560

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Enacted: January 1, 2010
Last revision: June 1, 2024
Partial revision: October 21, 2025

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3 tables and figures combined is allowed. Authorship is generally limited to 1–2, and these articles are often commissioned by the Editorial Board.

Key features and limits of articles are summarized in Table 1 below.

Table 1. Key features and limits of articles

Type of article	Abstract (words)	Text (words)*	References	Tables and figures
Original article	Structured, 250	5,000	-	-
Review article	200	6,500	100	10 Tables 10 Figures
Data profile	200	4,000	40	7
Special article	200	3,500	40	7
Brief report	Structured, 250	2,000	-	-
Short communication	Structured, 250	3,000	-	-
Viewpoint	150	3,000	30	4
Editorial	Not required	1,000	20	-
Commentary	Not required	500	10	2
Correspondence	Not required	500	-	2
Perspective	Not required	2,000	20	3

*Excluding abstract, references, tables, and figure legends.

Title Page

Title page should include (1) the title of the article (less than 50 words); (2) name of the authors (first name, middle initial, last name in capitals) and institutional affiliation including the name of department(s) and institution(s) of each author; (3) name, full address (including the postal code) of the institutional affiliation, telephone and e-mail address of the corresponding author; (4) a running title of 50 characters or less including blank spaces; and (5) notes (disclaimers). Notes include ethics approval and consent to participate, conflict of interest, funding, availability of data, authors' contributions according to the CRediT taxonomy (https://credit.niso.org/), additional contributions, and ORCID of all authors. All contributors who do not meet the criteria for authorship as defined above should be listed in an additional contribution section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

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Main Body

- **Introduction** should provide concise yet sufficient background information about the study to provide the readers with a better understanding of the study, avoiding a detailed literature survey or a summary of the results.
- **Materials and methods** should contain detailed procedures of the study or experiment including investigation period, methods of subject selection, and information on subjects such as age, sex or gender, and other significant features, in order to enable the experiment to be repeated. A procedure that has been already published or standardized should be described only briefly using literature citations. Clinical trials or experiments involving laboratory animals or pathogens must elaborate on the animal care and use and experimental protocols, in addition to mentioning approval from the relevant committees. The sources of special equipment and chemicals must be stated with the name of the manufacturer. All statistical procedures used in the study and criteria for determining significance levels must be described. Ensure correct use of the terms "sex" (when reporting biological factors) and "gender" (identity, psychosocial or cultural factors). Unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population (only one sex, for example), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity, and justify its relevance. Institutional Review Board approval and informed consent procedures can be described as follows: The study protocol was approved by the Institutional Review Board of OOO (IRB No: OO-OO-OO). Informed consent was obtained by all participants (or the participant's legal guardian) / Informed consent was waived by the IRB.
- **Results** should be presented in logical sequence. Only the most important observations should be emphasized or summarized, and the main or the most important

findings should be mentioned first. Tables and figures must be numbered in the order they are cited in the text, kept to a minimum, and should not be repeated. Supplementary materials and other details can be separately presented in an appendix. The authors should state the statistical method used to analyze the results (statistical significance of differences) with the probability values given in parentheses.

- **Discussion** should contain an interpretation and explanation of the results and important aspects of the study, followed by the conclusions drawn from them. Information already mentioned in the Introduction or Results sections should not be repeated and the main conclusions of the study may be presented in the discussion.
- **Conclusion** (if any) must be linked with the purpose of the study stated in the abstract, and clearly supported by the data produced in the study. New hypotheses may be stated when warranted, but must be clearly labeled.

References

Authors are responsible for the accuracy and completeness of their references and for correct text citations.

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- **Journal articles**
  1. Park AK, Kim IH, Kim J, et al. Genomic surveillance of SARS-CoV-2: distribution of clades in the Republic of Korea in 2020. Osong Public Health Res Perspect 2021; 12:37-43.
  2. Hyun J, Lee JH, Park Y, et al. Interim epidemiological and clinical characteristic of COVID-19 28 cases in South Korea. Public Health Wkly Rep 2020;13:464-74. Korean.
  3. Gultekin V, Allmer J. Novel perspectives for SARS-CoV-2 genome browsing. J Integr Bioinform 2021 Mar 15 [Epub]. https://doi.org/10.1515/jib-2021-0001.
- **Books**
  1. Riffenburgh RH, Gillen DL. Statistics in medicine. 4th ed. Academic Press; 2020.
  2. Miller DD. Minerals. In: Damodaran S, Parkin KL, editors. Fennema's food chemistry. 5th ed. CRC Press; 2017. p. 627-80.
  3. Ministry of Employment and Labor (KR). Statistics on occupational injuries and illnesses, 2008. Ministry of Employment and Labor; 2009. Korean.
- **Websites**
  1. World Health Organization (WHO) (CH). COVID-19 vaccines [Internet]. WHO; 2021 [cited 2021 Mar 15]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines.
- **Conference papers**
  1. Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, IE. Springer; 2002. p. 182-91.
- **Dissertation**
  1. Park HY. The role of the thrombomodulin gene in the development of myocardial infarction [dissertation]. Yonsei University; 2000.

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If any materials are not enough to be included in the main text such as questionnaires, they can be listed in the Appendix. Any supplementary materials that help the understanding of readers or contain too great an amount of data to be included in the main text may be placed as supplementary data. Not only a recording of the abstract, text, audio or video files, but also data files should be added here.

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